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CO-INOCULATION EFFECT OF *BACILLUS PUMILUS* TUAT1 AND *BRADYRHIZOBIUM JAPONICUM* USDA110 ON GROWTH PARAMETERS OF SOYBEAN*

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Abstract

Enhancement of legume nitrogen fixation by co-inoculation of plant-growth-promoting rhizobacteria (PGPR) with rhizobia is an alternative way to improve the availability of nitrogen in a sustainable agricultural system. This study was conducted to evaluate the co-inoculation effect of *Bacillus pumilus* TUAT1 and *Bradyrhizobium japonicum* USDA110 on soybean (Glycine max L.) cv. Enrei by using the plant box experiment and examined the growth parameters (plant weight, root weight, nodules number and nodule weight). The experiment was performed in the Plant Microbiology Laboratory, Department of International Environmental and Agricultural Science, Tokyo University of Agriculture and Technology, Japan in 2018. The growth parameters were measured after 15 days, and 30 days of inoculation period and the results indicated an increase number for all parameters in the dual inoculation than the single inoculation. The plants inoculated with *B. pumilus* TUAT1, *B. japonicum* USDA110 and co-inoculation (TUAT1 + USDA110) promoted the plant weight and root weight of soybean compared to un-inoculated plants in both inoculation periods. The nodule formation was not observed in the plants inoculated with *B. pumilus* TUAT1 alone and un-inoculated (control) at 15 days post inoculation, when co-inoculated and USDA110 inoculated plants provided the highest number of nodules and nodules weight after 30 days. The results proved the potential benefits of increasing nodulation and promoting plant growth through the synergetic effect of TUAT1 and USDA110.

Keywords: *Bacillus pumilus* TUAT1, *Bradyrhizobium japonicum* USDA110, co-inoculation, soybean

Introduction

Soybean (*Glycine max* L. Merr.) is an important legume plant for human consumption, which has high protein content and rich nitrogen because it can fix nitrogen in the atmosphere. Some legumes that form nodules, such as soybean, peanut, and common bean are important crop plants that are difficult to grow under stress (Barea *et al*., 2005; Esitken *et al*., 2006). Likewise, the growth of soybean also has many limitations, which cause various problems and yield losses. The direct or indirect use of beneficial microorganisms such as plant growth-promoting rhizobia (PGPR) can promote plant growth because they have multiple mechanisms and play a key role in modern agriculture in developing countries (Glick, 1995).

The formation of symbioses with a variety of nitrogen-fixing soil bacteria (called rhizobia) makes its own fertilizers in the legumes. In agricultural production, the symbiotic relationship between legumes and rhizobia has become an important sector. Rhizobia are very important for crop production due to biological nitrogen fixation, wherein atmospheric elemental N_2 is converted to ammonia (NH_3), provides the required nitrogen to legumes because 65% of the nitrogen applied in agriculture (Matiru and Dakora 2004; Franche *et al*., 2009). In addition, it is a simple low-cost method to maintain soil fertility and increase crop yield through legume–rhizobium symbiosis. Therefore, inoculation with rhizobium has become a popular agronomic preparation, which can provide sufficient nitrogen for legumes instead of using nitrogen fertilizer.

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Bradyrhizobium japonicum is a gram-negative, rod-shaped bacterium that performs root nodulating and nitrogen-fixing in legume plants. In 2015, Hungria *et al*. reported that dual inoculation of nitrogen-fixing bacteria and plant growth-promoting bacteria (PGPB) is more popular than single inoculation because this combination increases soybean yield and improves agriculture development. The combination of *Bacillus* strains and *B. japonicum* provided the increased number of nodules, weight of nodule, shoot, and root, total nitrogen together with grain yield in soybean (Bai *et al*., 2003 and Elkoca *et al*., 2008). Therefore, the improvement of plant's performance in the agricultural system benefits crop growth when inoculated with PGPR and rhizobial strains.

Among PGPR strains, *Bacillus* species are gram-positive, endospore-forming bacteria, which competitively colonize the roots and supply crop productivity directly or indirectly. In this study, one PGPR strain, *B. pumilus* TUAT1 was selected, which was isolated from the field of Tokyo University of Agriculture and Technology (Fuchu, Japan) and it has the plant growth-promoting effect because it promoted the rice yield up to 20-30% (Khin Thuzar Win *et al*., 2018). The aim of the study was to evaluate the single-inoculation and co-inoculation effect of *B. pumilus* TUAT1 with *B. japonicum* USDA110 on some growth parameters of soybean.

Materials and Methods

(i) Bacterial strains and growth conditions

Bacillus pumilus TUAT1 strain was grown in Luria-Bertani (LB) medium (Green *et al*., 2012) at 28°C and *Bradyrhizobium japonicum* USDA110 (current name *B. diazoefficiens)* (Krause, 2002) strain was grown at 28°C in arabinose-gluconate (AG) medium (Sadowsky *et al*., 1987). Both strains were provided from the Plant Microbiology Laboratory, Tokyo University of Agriculture and Technology.

(ii) Inoculum preparation

B. pumilus TUAT1 strain was incubated in LB broth for 24 h and *B. japonicum* USDA110 strain was incubated in AG broth for 72 h at 28°C under shaker condition. Then, cell suspensions were prepared containing 1 x 10^7 CFU ml^{-1} for each isolate.

(iii) Experimental design and inoculation test for plant assay

The box experiment was conducted in the Plant Microbiology Laboratory, Department of International Environmental and Agricultural Science, Tokyo University of Agriculture and Technology, Japan. The soybean (*Glycine max* L.) cv. Enrei seeds were sterilized with 70% ethanol for 30 seconds (s) and then with 1% sodium hypochlorite for 30 s. Seeds were rinsed with sterilized distilled water for 10 times, placed the seeds on the plates containing the autoclaved tissue paper. Then, it covered with double layers aluminium foil and incubated at 25°C for 2 days under dark conditions. After 2 days of incubation, the germinated seedlings were transferred to plant box (CUL-JAR300; Iwaki, Tokyo, Japan) containing sterile vermiculite. Each plant box containing 1 seed of Enrei was inoculated with 1 ml/10^7 cells of bacterial strains and watering with B & D (Broughton and Dilworth, 1971) nitrogen-free plant nutrient solution. The plant boxes were cultivated in a plant growth cabinet (LPH-410SP; NK Systems Co. Ltd., Osaka, Japan) at 25 °C and 70% humidity under a 16/8h day/night cycle (Faruque *et al*., 2015). The four treatments were performed: 1. No inoculation (Control), 2. *B. pumilus* TUAT1 alone, 3. *B. japonicum* USDA110 alone, and 4. *B. pumilus* TUAT1 and *B. japonicum* USDA110 (co-inoculation) respectively with three replications per treatment. Plant weight, root weight, nodules number, and nodule weight were evaluated at 15 days and 30 days post-inoculation (dpi).

Results

Effect of single and co-inoculation on soybean growth

The evaluation of single inoculation and co-inoculation effect on soybean seedling growth was recorded at 15 days and 30 days after sowing. In 15 days, the plant weight and root fresh weight were not significantly different in all treatments, while it was moderately changed in these parameters at 30 days (Fig.1 and 2). Remarkably, no significant differences were observed in the plant weight of controls, TUAT1 and USDA110 inoculated plants, whereas it was slightly improved in co-inoculation treatment at 15 days (Fig.3A). Similarly, root fresh weight remained stable in all inoculated plants, while a small increase recorded in co-inoculated plants (Fig.4A). Interestingly, the co-inoculated and TUAT1 inoculated plants indicated the high branches of the leaf at 15 days post-inoculation (Fig.1).

In 30 days, the highest plant weight was obtained in the plants inoculated with USDA110 and co-inoculated plants with significantly different, whereas the same results were not recorded in control and TUAT1 inoculated plants (Fig.3B). The fresh weight increased in roots of co-inoculated plants as compared to other treatments, but no significant differences were observed between them (Fig.4B).

In nodulation performance, the number of nodules was variable based on each inoculates (Fig. 1 and 2). As expected, uninoculated plants did not show any nodules at both incubation period. In 15 days after sowing, no nodules were formed in TUAT1 treatment, but nodules appeared in the other two treatments (Fig. 5A and 6A). However, the number of nodules and nodule weight were significantly different in co-inoculation as compared with other treatments after 30 days post-inoculation (Fig. 5B and 6B). Small nodules were observed in the secondary root of TUAT1 while the big nodules were enhanced in the main root of USDA110 treatment. Both sizes of nodules were formed in co-inoculation treatment with the highest nodules number (Fig. 2).

Another interesting point was the color of leaves, which is one of the important parameters to determine the level of nitrogen fixation. The color of leaves was not shown significance in 15 days (Fig. 1), although it was changed into yellow color in control and TUAT1 treatments at 30 days post-inoculation. However, in the plants inoculated with USDA110 and co-inoculated ones, the color of the green leaves did not change. (Fig. 2). That means USDA110 affected the concentration of chlorophyll.

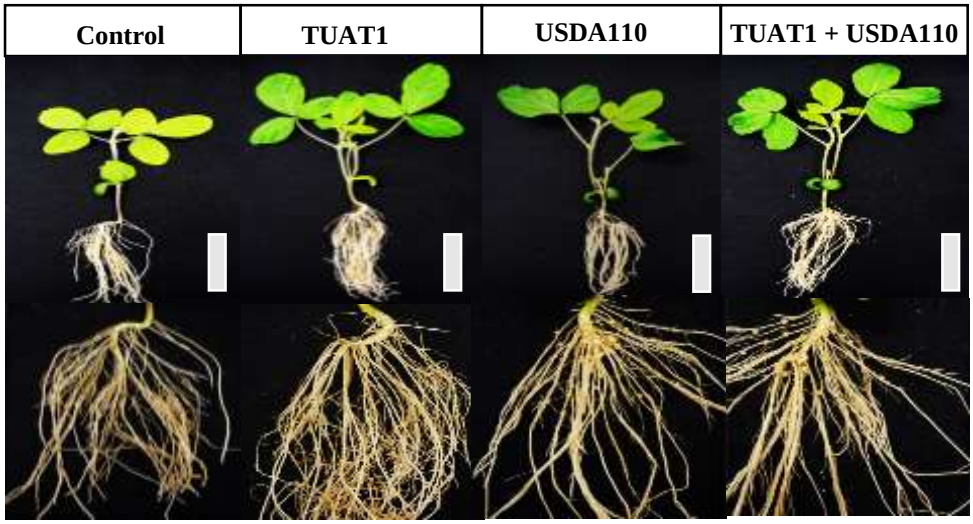

Figure 1 Comparison of plant growth and nodulation of *Glycine max* cv. Enrei plants, roots, and nodules inoculated with no-inoculation (control), *B. pumilus* TUAT1, *B. japonicum* USDA110, and *B. pumilus* TUAT1 and *B. japonicum* USDA110. Plants were photographed at 15 days post-inoculation (dpi).

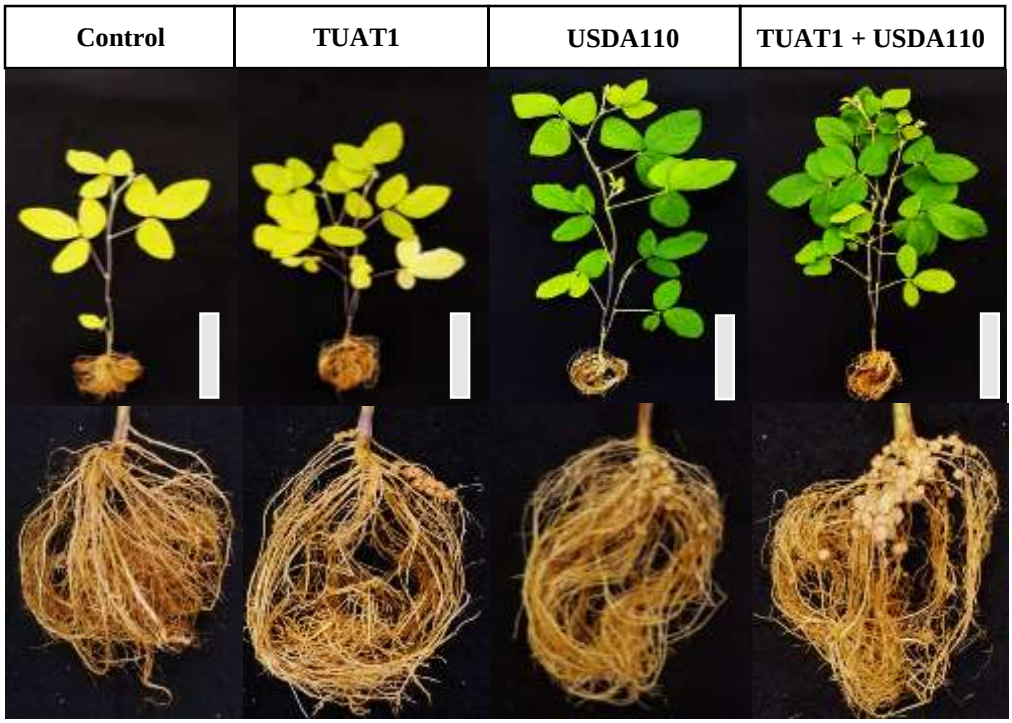

Figure 2 Comparison of plant growth and nodulation of *Glycine max* cv. Enrei plants, roots, and nodules inoculated with no-inoculation (control), *B. pumilus* TUAT1, *B. japonicum* USDA110, and *B. pumilus* TUAT1 and *B. japonicum* USDA110. Plants were photographed at 30 days post-inoculation (dpi).

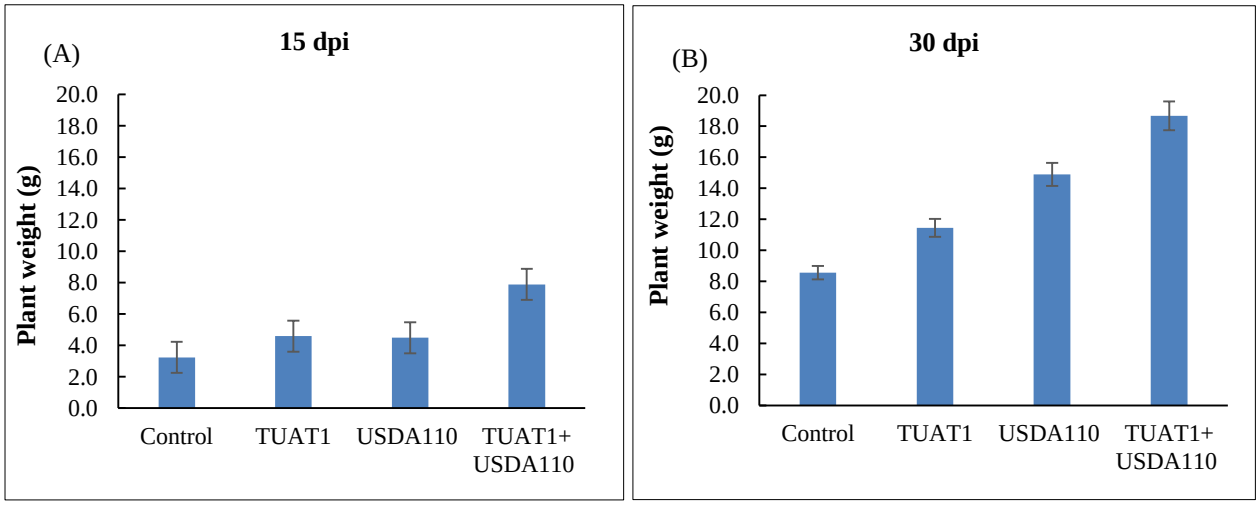

Figure 3 Fresh plant weight of soybean seedling at (A) 15 days and (B) 30 days after sowing. The histograms at each treatment are not significantly different at $P < 0.05$ (t-test). The bar on each histogram indicates standard deviation (SD).

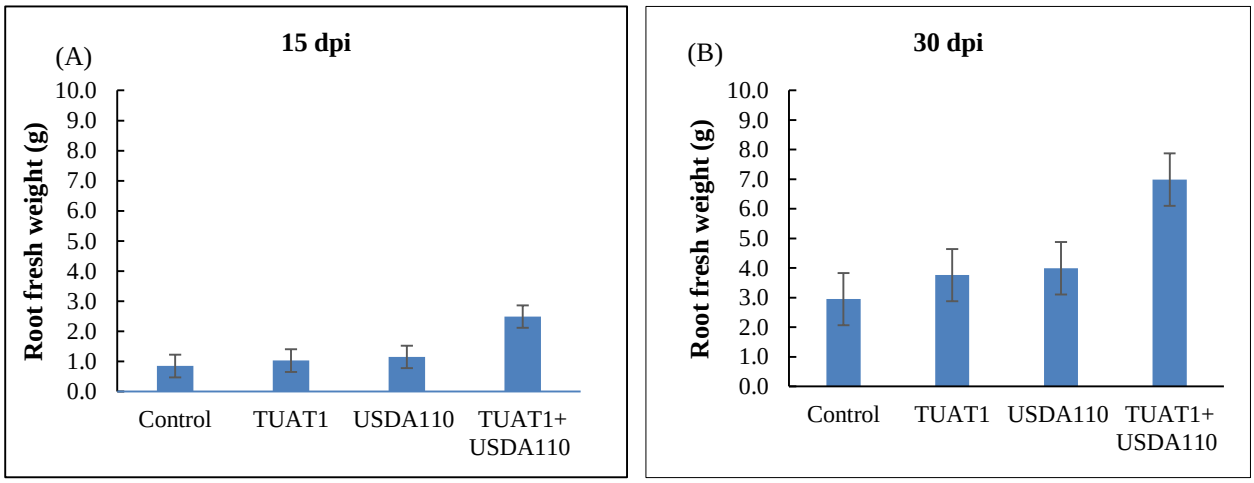

Figure 4 Root fresh weight of soybean seedling at (A) 15 days and (B) after sowing. The histograms at each treatment are not significantly different at $P < 0.05$ (t-test). The bar on each histogram indicates standard deviation (SD).

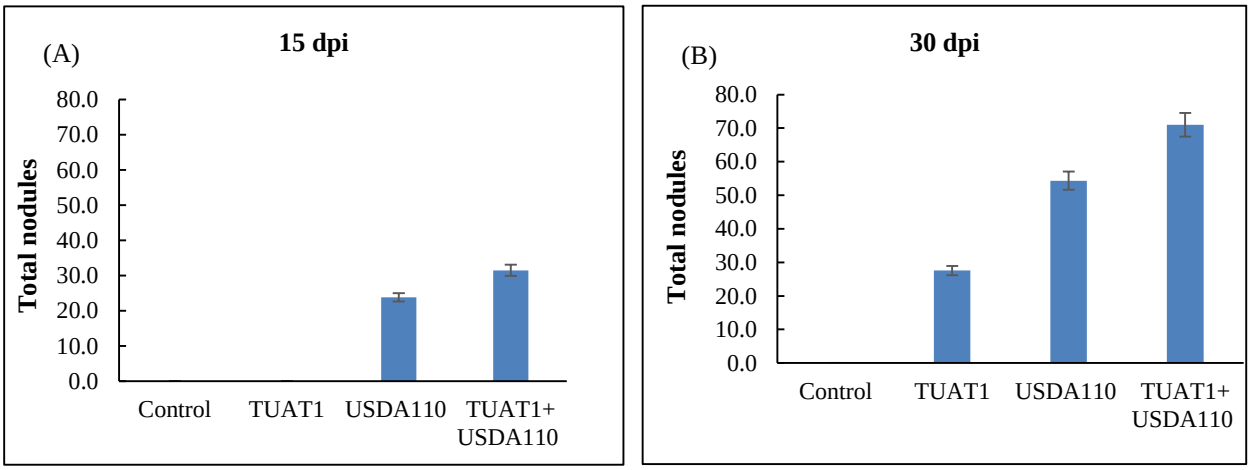

Figure 5 Number of nodules on principal, secondary root, and total of soybean seedling at (A) 15 days and (B) 30 days after sowing. The histograms in co-inoculation treatment are significantly different at 30 days, $P > 0.05$ (t-test). The bar on each histogram indicates standard deviation (SD).

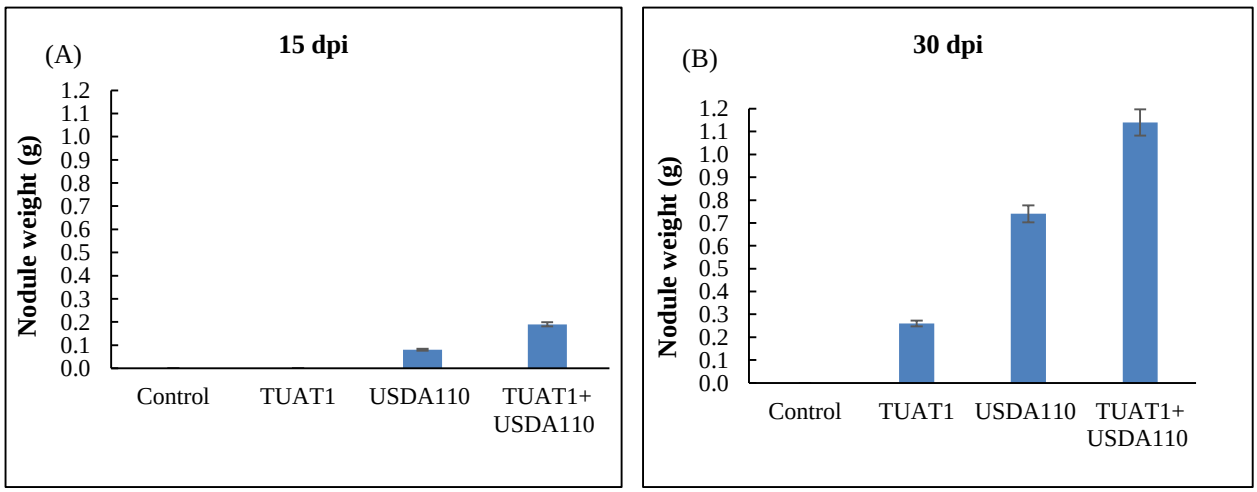

Figure 6 Nodule weight of soybean seedling at (A) 15 days and (B) 30 days after sowing. The histograms at each treatment are not significantly different at $P < 0.05$ (t-test). The bar on each histogram indicates standard deviation (SD).

Discussion

The dual inoculation of non-rhizobial bacterial strains improves the nodulation and N-fixing when combined with rhizobial strains. Plant growth promoting rhizobacteria (PGPR) plays an interesting role in the agricultural sector because it can improve soil fertility and increase crop productivity and nutrients. The beneficial effect of rhizobial inoculation in legumes has been the focus of biological nitrogen fixation, and it plays an important role in sustaining the fertility of a cropping system (Deshwal *et al*., 2003). In this research work, the results showed that *B. japonicum* USDA 110 with *B. pumilus* TUAT1 can increase plant weight, nodule number, nodule weight, and root weight by inoculation alone or in combination.

Soybean productivity can be determined by nodulation and subsequent nitrogen fixation, which is an important factor. According to the study of Wang and Martinez-Romero (2000), the nitrogen nutrition can be determined based on the number of nodules occupied by effective bacteria, and the symbiotic relationship of legume crops to achieve maximum nitrogen fixation. The current results indicated the ability to promote early soybean seedling growth and nodulation when the two strains are together in the plant box. The research conducted by Vessey and Buss (2002) showed that the co-inoculation of rhizobia with PGPR can improve nodules and nitrogen fixation. Reported by Tilak *et al.,* (2006) and Wani *et al.,* (2007) demonstrated that synergism between *Bacillus* and *Bradyrhizobium* improve forming nodules and plant biomass in the rhizosphere. Similarly, the increased nodulation was obtained in the current study at 30 days after sowing by the dual inoculation of TUAT1 and USDA110. A significant increase in the number of nodules by co-inoculation can be considered as an improvement in nitrogen fixation.

Although one PGPR strain increases the efficacy of the *Rhizobium* species in one legume, it does not perform the same in other legumes. Study by Camacho *et al*., (2001) clearly revealed that the dual inoculation of *Bacillus* sp. CECT 450 with *Rhizobium tropici* CIAT 899 improved nodules in common bean, although when co-inoculated with *B. japonicum* USDA 110 strain, it performed poorly in nodule formation in soybeans. However, the present study demonstrated that *B. pumilus* TUAT1 increases significantly nodulation in soybean when co-inoculated with *B. japonicum* USDA110.

The extensive reports of inoculating soybeans with suitable rhizobium strains have shown positive effects. Previous reported by Sajid *et al*., (2010) and Solomon *et al*., (2012) revealed that the significant increase of nodule number per plant was achieved by inoculation with Rhizobium or *B. japonicum* strains alone, although in the present study was not achieved the similar outcome by single inoculation. In addition, Rajendran *et al*., (2008) proved that the plant growth promoting bacterium *Bacillus* can promote plant weight. However, in the current study, TUAT1 alone did not support promoting plant weight.

Compared with *Bradyrhizobium* alone, the dual inoculation of *Bradyrhizobium* and PGPR microbes significantly increased the growth performance and nodulation improvement in soybean (Dubey, 1996, Wasule *et al*., 2007, and Abbasi *et al*., 2011). Interestingly, the present results recorded the high branches of leaf in the co-inoculated and TUAT1 inoculated plants after 15 days as well as the highest plant weight was obtained in co-inoculation than the USDA110 alone at 30 days post inoculation. The present investigation indicated the potential of non-rhizobial strain when combined with rhizobial strain enhance the growth and nodulation of cultivated legumes such as soybean. This approach meets the needs of modern agriculture, economy, and environmental sustainability.

Conclusion

The present study demonstrated that *Bacillus pumilus* TUAT1 was co-inoculated with *Bradyrhizobium japonicum* USDA110, enhanced the growth and nodulation of soybean cv. Enrei, under laboratory conditions. All treatments have a positive effect on soybeans, and co-inoculation with USDA110 has the largest increase at 30 days post-inoculation compared with single inoculation of TUAT1 and USDA110 alone. Therefore, it can be concluded that the combined use of TUAT1 and USDA110 will be helpful for soybean production by providing plant growth and nodulation. This combination can be used as an alternative to expensive inorganic fertilizers to provide farmers with product formulations, while reducing the excessive use of inorganic fertilizers and helping to alleviate environmental problems. Further studies under field conditions with a great number of soybean cultivars will be needed to corroborate the findings of this study.

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ISOLATION OF FUNGI FROM SOIL SAMPLES AND THEIR ANTIMICROBIAL ACTIVITIES

Phu Htet*

Abstract

Ten soil samples were collected from ten different places of Waing Maw areas, Kachin State. 22 fungi were isolated from these ten soil samples. The isolated soil fungi were checked the antimicrobial activity by paper disc diffusion assay method. PPK-01, PPK-03, PPK-04, PPK-10, PPK-14, PPK-18 and PPK-20 showed antibacterial activities. The fungus PPK-10 and PPK-14 showed the activity on Agrobacterium tumefaciens IFO543, Bacillus subtilis KY-327, Micrococcus luteus NITE83297, Pseudomonas fluorescens IFO94307. Among them, PPK-10 showed the highest antibacterial activities (PPK-10, 29.62 mm inhibitory zone) on Agrobacterium tumefaciens IFO543. This fungus PPK-10 was isolated from the soil sample collected from Nawng Hee. Therefore, fungus PPK-10 was selected for further investigations based on the results of antimicrobial activity.

Keywords: isolation, antimicrobial activity of soil fungi

Introduction

Microorganisms are the important sources of bioactive compounds with enormous potential to be developed as new molecules for drug discovery. Microorganisms grow in unique and extreme habitats that provide them the capability to produce unique and unusual metabolites (Thamilvanan, Ram kumar, Ramesh, Balakumar and Kumaresan,2018). Soil are highly complex systems, with many components playing diverse functions mainly due to the activity of soil organisms. Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth. Microorganisms are beneficial in increasing the soil fertility and plant growth as they are involved in biochemical transformation and mineralization activities in soil (Gaddeyya, Shiny Niharika, Bharathi and Ratna Kumar, 2012).

Fungi are microscopic cells that usually grow as long threads or strands called hyphae. Hyphae interact with soil particles, roots, and rocks forming a filamentous body that promotes foraging for food.

These networks release enzymes into the soil and break down complex molecules that the filaments then reabsorb. Fungus act like natural recyclingbins, reabsorbing nutrients in the soil. Hyphae are usually only several thousandths of an inch (a few micrometers) in deameter. Single hyphae can span in length from a few cells to many yards. Hyphae sometimes group into masses called mycelium or thick, cord-like rhizomorphs that look like roots.

Mushrooms are a special type of fungus with special features (spores, gills, fruiting bodies). A single individual fungus can include many fruiting bodies scattered across a large area (as big as a baseball diamond). They generally make up 10-20% of the total microorganisms in the soil rhizosphere. Fungus generally have a lower number of individuals in a healthy soil, however they dominate the soil biomass due to their larger size fungus biomass in the soil ranges from the equivalent of two to six cows in a healthy soil. There are at least 70,000 different species of fungus but it is estimated that there are at least 20 times that number worldwide. See Understanding Soil Microbes and Nutrient Recycling for more information on microbial numbers.

Fungi perform important services related to water dynamics, nutrient cycling, and disease suppression. Along with bacteria, fungi are important as decomposers in the soil food web,

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converting hard to digest organic material into usable forms. In no-till, fungal population dominate the soil food web (although they are less in number than the bacteria).

Fungi have 40-55% carbon use efficiency so they store and recycle more carbon (C) compared to bacteria. Bacteria are less efficient at retaining C and release more into the air as carbon dioxide. Fungi have higher C content (10:1 C:N ratio) and less nitrogen (N=10%) in their cells than bacteria. Fungus help recycle both N and phosphorus (P) to plants. Due to their smaller size and much greater surface area, fungus can efficiently scavenge for N and P better than plant root hairs and greatly increase the plant root nutrient extraction efficiency. Many plants cultivate certain species of both bacteria and fungus to increase nutrient extraction from the soil.

Genetically, fungi are closely related to plants and animals. Membrane bound organelles present in each cell are similar to insects, plants, and animals. They evolved about a billion years ago and are equal in rank to plants and animals. In fact, fungi have 80% or more of the some genes as humans. They generally reproduce by spores (microscopic parts similar to plant seeds). The longevity of fungus has not been measured in many species but their open ended growth suggests that they have a longevity measured in millions of years, because they are basically the same organism. For example, fairy fungal rings grow in ever widening circles, much like rings on a tree, and are measured in decades and centuries instead of days and weeks for most microbes (James. Hoorman, 2011).

The aim and objectives of this study were to isolate the growth of fungi occurring inactively in the soil, to study the morphology of isolated fungi and to observe the preliminary study of antimicrobial activity.

Materials and Methods

Collection of soil samples

Ten different soil samples were collected from Waing Maw areas (Nang War, Aung Myay-1, Ma Hkan Tee, San Ka, Nawng Hee, Mai Na, Nawng Ta Law, Mading, Wu Yang, Inn Lay) in Kachin state during 2018. (Table 1) (Fig. 1).

Table 1 Soil samples collected from different places of Waing Maw areas

Soil No.	Collected place		Collected Date	pH	Soil type
S-1	Nang War	N 25° 22' 37.340" E 097° 33' 30.361"	27.7.2017	6.04	Sandy loam
S-2	Aung Myay-1	N 25° 14' 32. 857" E 097° 26' 13.489"	27.7.2017	5.76	Sandy loam
S-3	Ma Hkan Tee	N 25° 19' 32.186" E 097° 22' 50.874"	27.7.2017	5.26	Sandy loam
S-4	San Ka	N 25° 18' 59 337" E 097° 19 10.735"	27.7.2017	5.62	Sandy loam
S-5	Nawng Hee	N 25° 21' 01.656" E 097° 20' 49.448"	27.7.2017	4.53	Clay loam
S-6	Mai Na	N 25° 24' 56.400" E 097° 25' 48.221"	27.7.2017	5.59	Loam
S-7	Nawng Ta Law	N 25° 23' 18.045" E 097° 25' 29.132"	27.7.2017	4.62	Silt loam
S-8	Mading	N 25° 21' 18.149" E 097° 27' 19.737"	27.7.2017	5.75	Loam
S-9	Wu Yang	N 25° 21' 51.369" E 097° 29' 33.962"	27.7.2017	5.88	Sandy loam
S-10	Inn Lay	N 25° 17' 19.245" E 097° 26' 30.820"	27.7.2017	6.04	Loamy sand

Source: Department of Geography Pathein University

Figure 1 Map of soil samples collected area

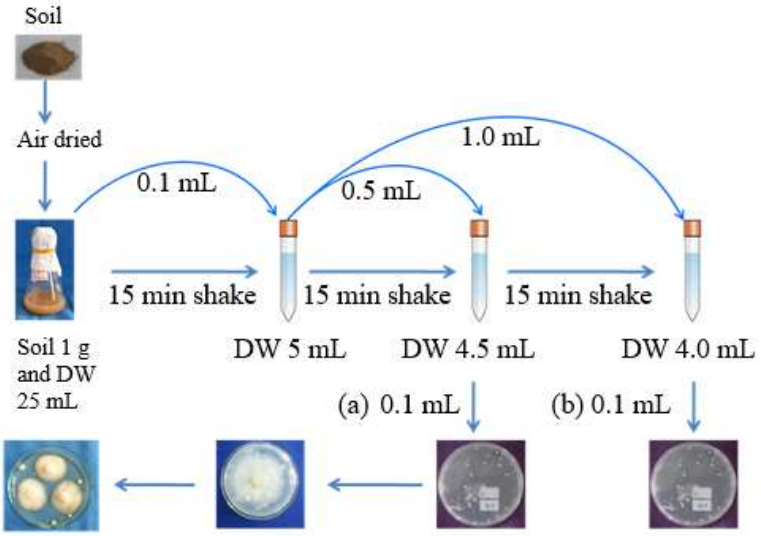

Figure 2 Dilution method (Phay and Yamamura, 2005)

Dilution method (Phay and Yamamura, 2005)

Soil was air dried at room temperature for three days. Grounded and sieved of 1 mm. 1 g of soil sample put into 25 mL of sterilized water. 0.1 mL of diluted soil suspension was put into 5 mL of sterilized water. 0.5 mL of diluted soil suspension was put into 4.5 mL of sterilized water. 1.0 mL of diluted soil suspension was put into 4.0 mL of sterilized water. Cultured onto PGA medium. Transferred onto GYP medium.

Media used for the isolation of soil fungi (Ando, 2004)

Glucose Yeast Peptone Medium (GYP medium)

Glucose	1.3 g
Yeast extract	0.7 g
Peptone	0.7 g
K_2HPO_4	0.05 g
$MgSO_4$	0.01 g
Agar	1.8 g
DW	100 mL

Potato Glucose Agar Medium (PGA medium)

Components per liter

Potato	20 g
Glucose	2 g
Agar	1.8 g
DW	100 mL

Seed Medium (Components of per liter)

Glucose	1.0 g
Yeast extract	0.7 g
K_2HPO_4	0.01 g
$MgSO_4$	0.01 g
DW	100 mL

Fermentation Medium (Components of per liter)

Glucose	1.5 g
Yeast extracta	0.5 g
Peptone	0.5 g
K_2HPO_4	0.01 g
$MgSO_4$	0.01 g
DW	100 mL

After autoclaving, chloramphenicol 25mL/100mL was added to this medium.

Assay medium

Glucose	0.8 g
Yeast extract	0.4 g
Peptone	0.4 g
KNO_3	0.1 g
Agar	1.6 g

Table 2 Test Organisms utilized for antimicrobial activities

No.	Test Organisms	Diseases
1	*Aspergillus flavus* IFO3290	Fruits diseases
2	*Bacillus subtilis* KY-327	Fever
3	*Micrococcus luteus* NITE83297	Skin disease
4	*Escherichia coli* AHU5436	diarrhea
5	*Pseudomonas fluorescens* IFO94307	Rice pathogen
6	*Salmonella typhi* AHU7943	Typhoid fever
7	*Agrobacterium tumefaciens* IFO543	Tumour cell in plant

Results

Table 3 Fungi isolated from different soil samples by physical treatment methods

Soil No.	Collected place	Total Isolation fungi
S-1	Nang War	PPK-01
S-2	Aung Myay-1	PPK-02
S-3	Ma Hkan Tee	PPK-03, PPK-04, PPK-05
S-4	San Ka	PPK-06, PPK-07
S-5	Nawng Hee	PPK-08, PPK-09, PPK-10,
S-6	Mai Na	PPK-11, PPK-12
S-7	Nawng Ta Law	PPK-13, PPK-14, PPK-15
S-8	Mading	PPK-16
S-9	Wu Yang	PPK-17, PPK-18, PPK-19,
S-10	Inn Lay	PPK-20, PPK-21, PPK-22

Table 4 Morphological color of isolated fungi

No.	Isolated fungi	Character			No.	Isolated fungi	Character	
		Surface color	Reverse color				Surface color	Reverse color
1	PPK-01	White	Cream		12	PPK-12	Yellowish white	Yellowish
2	PPK-02	White	White		13	PPK-13	Greenish white	Yellow
3	PPK-03	White	Cream		14	PPK-14	Greenish white	Cream
4	PPK-04	Gray	Cream		15	PPK-15	Greenish white	Cream
5	PPK-05	White	Yellow		16	PPK-16	White	White
6	PPK-06	White	Cream		17	PPK-17	Greenish white	Cream
7	PPK-07	White	Cream		18	PPK-18	Greenish white	Cream
8	PPK-08	White	Cream		19	PPK-19	White	Cream
9	PPK-09	White	Cream		20	PPK-20	Greenish white	Cream
10	PPK-10	Greenish	Cream		21	PPK-21	Greenish white	Cream
11	PPK-11	Greenish	Yellow		22	PPK-22	White	Cream

Table 5 Preliminary studies of antimicrobial activities

No.	Isolated fungi	Test organisms and Inhibitory zone (mm)						
		A. tumefaciens	***B. subtilis***	***M. luteus***	***E. coli***	***P. fluores***	***S. typhi***	***A. flavus***
1	PPK-01	19.00	-	-	-	18.40	-	-
2	PPK-02	-	-	-	-	-	-	-
3	PPK-03	20.00	17.44	18.44	-	18.44	-	-
4	PPK-04	-	17.18	17.18	-	-	-	-
5	PPK-05	-	-	-	-	-	-	-
6	PPK-06	-	-	-	-	-	-	-
7	PPK-07	-	-	-	-	-	-	-
8	PPK-08	-	-	-	-	-	-	-
9	PPK-09	-	-	-	-	-	-	-
10	PPK-10	29.62	27.04	27.06	-	20.00	-	-
11	PPK-11	-	-	-	-	-	-	-
12	PPK-12	-	-	-	-	-	-	-
13	PPK-13	-	-	-	-	-	-	-
14	PPK-14	27.62	27.04	27.00	-	23.03	-	-
15	PPK-15	-	-	-	-	-	-	-
16	PPK-16	-	-	-	-	-	-	-
17	PPK-17	-	-	-	-	-	-	-
18	PPK-18	-	-	-	-	19.44	-	-
19	PPK-19	-	-	-	-	-	-	-
20	PPK-20	16.08	-	-	-	20.00	-	-
21	PPK-21	-	-	-	-	-	-	-
22	PPK-22	-	-	-	-	-	-	-

(-) no activity

Table 6 Preliminary studies of antimicrobial activities

No.	Isolated fungi	Test organisms and Inhibitory zone (mm)						
		A. tumefaciens	***B. subtilis***	***M. luteus***	***E. coli***	***P. fluores***	***S. typhi***	***A. flavus***
1	PPK-01	19.00	-	-	-	18.40	-	-
2	PPK-03	20.00	17.44	18.44	-	18.44	-	-
3	PPK-04	-	17.18	17.18	-	-	-	-
4	PPK-10	29.62	27.04	27.06	-	20.00	-	-
5	PPK-14	27.62	27.04	27.00	-	23.03	-	-
6	PPK-18	-	-	-	-	19.44	-	-
7	PPK-20	16.08	-	-	-	20.00	-	-

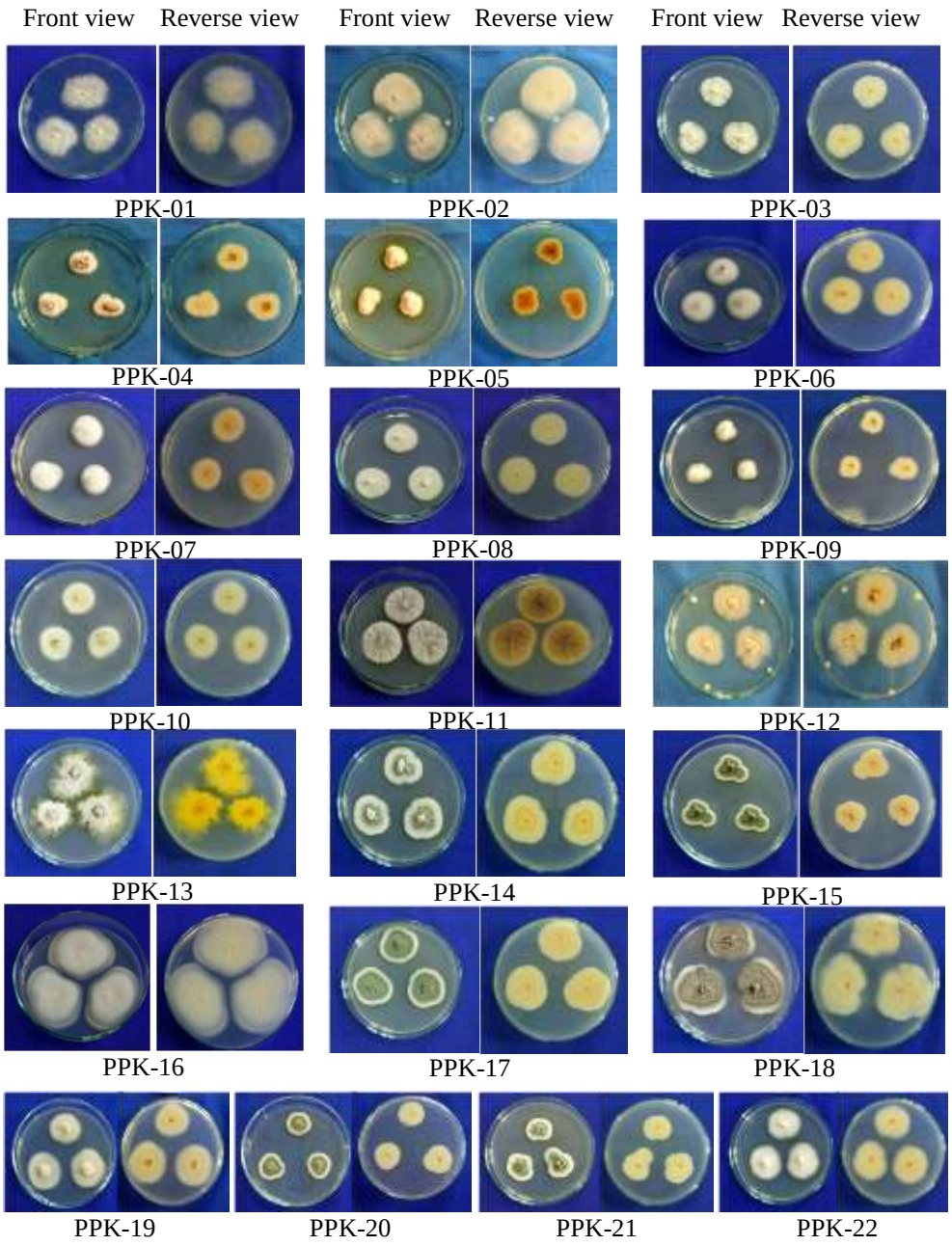

Figure 3 Morphologies of soil fungi (7 days after culture)

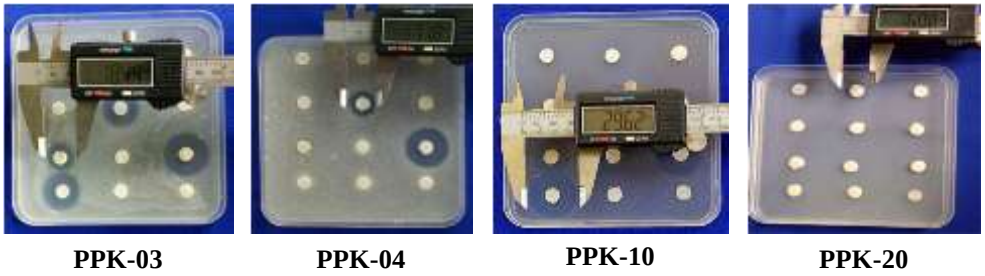

Figure 4 Antimicrobial activity of isolated fungi against *Agrobacterium tumefaciens*

PPK-10
Antimicrobial activity of isolated fungi against *Micrococcus luteus*

PPK-14
Antimicrobial activity of isolated fungi against *Bacillus subtilis*

PPK-14
Antimicrobial activity of isolated fungi against *Pseudomonas fluorescens*

Figure 5 Antimicrobial activity of isolated fungi against *Micrococcus luteus, Bacillus subtilis* and *Pseudomonas fluorescens*

Discussion and Conclusion

Fungi grow on diverse habitats in nature and are cosmopolitian in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmosphere gas mixture (Northolt and Bullerman, 1982).

In this study, 22 fungi were isolated from ten soil samples collected at Waing Maw areas, Kachin State. It was observed that one fungus (PPK-01) was isolated from the soil sample No.1, one (PPK-02) from soil sample No.2, three (PPK-03-05) from soil sample No.3, two (PPK-06,07) from soil sample No.4, three (PPK-08-10) from soil sample No.5, two (PPK-11,12) from soil sample No.6, three (PPK-13-15) from soil sample No.7, one (PPK-16) from soil sample No.8, three (PPK-17-19) from soil sample No.9 and three (PPK-20-22) from soil sample No.10 receptivity.

In studying the morphological colours of fungi, the front colours of PPK-01, 02, 03, 05, 06, 07, 08, 09, 16, 19 & 22 were the same white. The reverse colours of PPK-02,16 were the same white. The reverse colours of PPK-01,03, 06, 07, 08, 09, 19 & 22 were the same cream. The revese colour of PPK-05 was yellow. The front colour of PPK-04 was gray and it reverse colour was cream. The front colour of PPK-10, 11, 13, 14, 15, 17, 18, 20 & 21 were the same greenish white. The reverse colours of PPK-10, 14, 15, 17, 18, 20 & 21 were the same cream. The reverse colours of PPK-11,13 were the same yellow. The front colour of PPK-12 was yellowish white and its reverse colour was yellowish cream.

In the investigation of antimicrobial activities, soil fungi were tested with seven test organisms by using paper disc diffusion assay method. Seven fungi (PPK-01, PPK-03, PPK-04, PPK-10, PPK-14, PPK-18 and PPK-20) showed the antimicrobial activity. All of them, PPK-10 showed the highest activity (29.62mm) followed by PPK-14 (27.62mm),PPK-03 (20.00mm), PPK-01 (19.00mm) and PPK-20 (16.08mm) in 5 days old culture. Among them, the fungus PPK-10 showed the highest activity on Agrobacterium tumefaciens IFO543 (29.62 mm inhibitory zone). Therefore, fungus PPK-10 was selected for further investigations.

In the investigation, soil fungus PPK-10 showed the antibacterial activity on Agrobacterium tumefaciens. PPK-10 was isolated from the S-5 (Loamy sand, pH- 6.04). This soil samples was collected from Nawng Hee Village, Waing Maw areas, Kachin State. Soil fungus

PPK-10 will further studies to clarify the fermentation optimization, identification of isolated fungus up to species level and to find out the nature of metabolites those can kill the test organism.

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Appendix

DEPARTMENT OF AGRICULTURE (LAND USE)
SOIL ANALYTICAL DATA SHEET

Division - ကချင်ပြည်နယ်
Township - ဗိန်းမော်

Sheet No. 1
Sr No. S 1-10 / 17-18

Sr No.	Sample plot	Moisture %	pH (Soil : Water 1 : 2.5)	Texture				SOIL INTERPRETATION OF RESULTS	
				Sand %	Silt %	Clay %	Total %	pH (Soil : Water 1 : 2.5)	Texture
1	Nang War	4.27	5.59	46.75	36.50	15.10	98.35	Moderately acid	Loam
2	Aung Myay-1	6.80	4.62	19.30	54.30	24.90	98.50	Strongly acid	Silt Loam
3	Ma Hkan Too	4.33	5.75	47.35	42.50	8.40	98.25	Moderately acid	Loam
4	San Ka	4.40	5.88	54.05	38.30	5.70	98.05	Moderately acid	Sandy Loam
5	Naung Hee	2.70	6.04	75.05	21.25	2.10	98.40	Slightly acid	Loamy Sand
6	Mai Na	4.01	6.04	60.35	31.90	6.15	98.40	Slightly acid	Sandy Loam
7	Naung Ta Law	3.25	5.76	67.85	18.20	12.55	98.60	Moderately acid	Sandy Loam
8	Mading	3.93	5.26	51.80	39.75	6.65	98.20	Strongly acid	Sandy Loam
9	Wu Yang	3.53	5.62	51.40	22.75	24.35	98.50	Moderately acid	Sandy Clay Loam
10	Inn Lay	6.35	4.53	25.75	38.40	34.30	98.45	Strongly acid	Clay Loam

MORPHOLOGICAL AND HISTOLOGICAL STUDIES OF *HYPSELANDRA VARIABILIS* (Collett & Hemsl.) Pax & K. Hoffm.

Lai Lai Win[1], Yee Yee Thu[2], Aye Kyi[3]

Abstract

Hypselandra variabilis (Collett & Hemsl.) Pax & K.Hoffm., locally known as Thamon, belongs to the family Capparaceae (Capparidaceae). It is indigenous to Myanmar, especially found in Mandalay, Sagaing and Magway Regions. The specimens were collected from Pakokku Township in Magway Region. The present work deals with the morphological and anatomical characters of both vegetative and reproductive parts of the plant. In morphological study, *Hypselandra variabiis* (Collett & Hemsl.) Pax & K.Hoffm is a perennial, medium-sized tree that bears greyish brown to dark brown bark, the branches are slightly pubescent when young. The leaves are simple and variable in shape and size. Inflorescences are terminal or axillary corymbose racemes. Flowers are bisexual, actinomorphic, hypogynous and leafy bracts. The outstanding characters are the presence of the androgynophore and the absence of corolla. The ovary is superior and parietal placentation. The histological study showed that stomata were present only on lower epidermis and anomocytic type. Uniseriate, multicellular, basal-celled trichomes were present on the upper surface of epidermal cells. Vascular bundles were found in the form of a collateral, closed type and crescent shape in midrib. The petiole, stem and gall were observed in the form of a collateral, closed type and circular in shape but the root was found in radial type. These characters presented in this research could be used as standardization in traditional medicine.

Keywords: *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm., Morphology, Histology

Introduction

Capparaceae or Capparidaceae of some authorities, the Caper family, derives its name and importance from Kapparis, the Greek name used by Dioscoides for *Capparis spinosa.* The Capper family consists of 42 genera and 725 tropical and warm temperate flowering plant species with strong drought resistant tendencies (Anon, 1950).

Hypselandra variabilis (Collett & Hemsl.) Pax & K. Hoffm. is a medicinal plant that belongs to the family Capparaceae (Capparidaceae) in the order Brassicales. This plant is a tropical species that widely distributed in Pakokku Township, Magway region. Pakokku is located at the bank of the west of the Ayeyawaddy River. It lies between 21˙35′ and 21˙55′ north latitude and between 95˙08′ and 95˙40′ east longitude and about 40 meters elevation above the sea level.

Capparaceae is mainly tropical family, consists of herbs, shrubs and small trees, while a few genera are included in Cadaba, Capparis and other genera. The stomata are ranunculaceous and variously shaped sclerenchymatous cells frequently present in the mesophyll of certain species of *Hypselandra.* Sclerenchymatous cells are sometimes extending between the epidermal cells and thus coming into contact with the leaf surface in *Capparis* and *Hypselandra* spp. (Metcalfe & Chalk, 1950).

Successive cambia have been reported in *Hypselandra* (*Boscia)* Lam. by Adamson (1935) and *Nieburia* DC. (*Hypselandra variabilis* (Collett & Hemsl.) Pax & K.Hoffm., by Hansen 1977), but some species of *Hypselandra* and *Maerua* as presently sampled do not have successive cambia (Metcalfe and Chalk 1950). Pandey and Chadha (1998) stated that anomocytic type is also called irregular-celled type where the subsidiary cells are indistinguishable from other epidermal cells. Such cases are found in members of Capparaceae.

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The leaves and roots of this plant are used in aching, sedema, cold extremities and also used as stomachic, expectorant and counter irritant (San and Han, 1998). The bark is used for eye disease, and the flowers are famous for making salad. The gall of "Thamon" is used to relieve eye sore. In Myanmar, it is believed that eating the buds and flowers of "Thamon" once a year can keep the good health throughout the lifespan (Ashin-nargathein, 1978).

In this study, an attempt was made to determine the morphological and histological characters of this plant. This plant has been chosen for this research because it has high medicinal value and there is no the previous records on its histological characters.

Materials and Methods

Collection, classification and identification of plant samples

The plant samples of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm. were collected from Pakokku Township, Magway Region in January, 2019. After collection, all vegetative and reproductive parts of the fresh specimens were identified with the help of available literatures Hooker (1879), Hundley and Chit Ko Ko (1987), Kurz. (1877).

The collected specimens were washed with water and then air dried at room temperature. When constant weight of the sample was obtained, the dried samples were pulverized by grinding machine and stored in air-tight bottles for further use.

Histological examination of different plant parts

The fresh specimens of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm. were examined by cutting free hand sections and studied under microscope. The microchemical tests for the presence of lignin were made according to the methods and reagents given in Esau (1953), Trease and Evans (1978, 2002). The following reagents were used for microchemical test examination:

(1) Chloral hydrate solution B.P as clearing reagent

(2) Safranin for lignified and cutinized cell wall

(3) Solution of Phloroglucinol B.P, followed by concentrated Hydrochloric acid for testing lignin

Results

Morphological characters of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm.

Perennial, xerophytic, medium-sized tree, unarmed, about 10 m in height; **Stems** greyish brown to dark brown bark, branches slightly pubescent when young, bear numerous galls. **Leaves** simple, oblong to elliptic; stipules minute; petioles 1.0 to 2.5 cm long. The leaves are light green to dark green and variously shape and size. The midrib is sunken at the upper surface and raised at the below (Fig. 1a). **Inflorescences** are axillary or terminal, corymbose racemes. Flowers bisexual, regular, actinomorphic, hypogynous, apetalous, 3.0-3.5 cm long, 2-2.8 cm wide; bracts leafy, creamy-white (Fig. 1b). **Calyx** campanulate, three to four partite. **Androecium** six to thirteen stamens, filaments filiform, 0.7-1.5 cm long, androphore 0.2-0.3 cm long, anthers dithecous, dorsifixed, longitudinal dehiscent. **Gynoecium** carpel one, unilocular or bilocular due to false septum, many ovules in the locule, parietal placentation; gynophore 0.5 to 1.0 cm long, ovary superior; style very short, stigma discoid (Fig. 1c). **Fruits** berry, ovoid or sub-globoid (Fig. 1c). **Flowering period** is January to March as shown in Fig. 1a, b and c.

Habit

Branches with galls

Branches with flowers

Arrangement of leaves

Upper surfaces of leaves

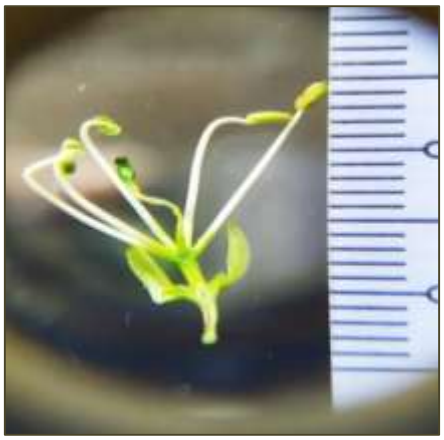
Lower surface of leaves

Figure 1(a) Morphological characters of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K.Hoffm.

Leaves with Galls

Galls

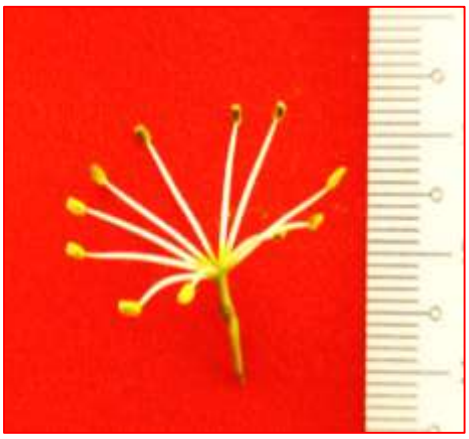
Flower



L.S of flower

Inflorenscence

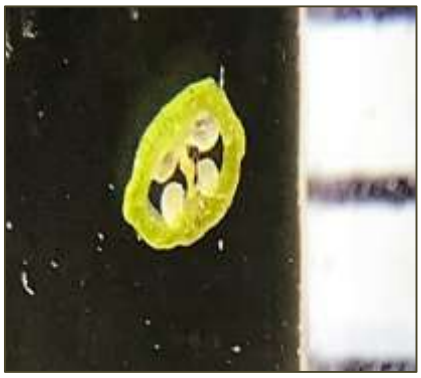
Figure 1(b) Morphological characters of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K.Hoffm.



Androecium

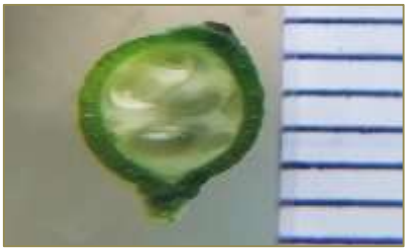
Carpel with androgynophore

L.S of ovary



T.S of ovary

Fruit



L.S of fruit

T.S of fruit

Figure 1c. Morphological characters of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K.Hoffm.

Histological characters of leaves, stems and roots of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm.

Lamina

In the surface view, the cuticle is striated on both surfaces. The epidermal cells are polygonal in shape, thick walled parenchymatous cells with distinctly wavy. Trichomes are uniseriate, multicellular, basal-celled, two or three cells in each trichome. Stomata are present only on lower surface and anomocytic (ranunculaceous) type (Fig. 2).

In transverse section, the cuticles are present on both surfaces. The epidermal cells are only one-layered and barrel-shaped. Mesophyll is differentiated into palisade and spongy. The palisade layer is composed of compactly arranged columnar cells. They are of two layers thick. The spongy layer consists of many layers of isodiametric cells arranged in loosely. Mesophyll cells contain abundant chloroplasts. Vascular bundles are collateral and closed type (Fig. 2).

Midrib

In the surface view, the epidermal cells are thick-walled and rectangular in shape, elongated along the axis. In transverse section, midrib is arranged in an arch with the convex surface towards the lower side. Trichomes are observed on both upper and lower surfaces. These are uniseriate, multicellular and basal-celled. Epidermal cells are one layered and barrel shaped. Collenchyma cells are rounded to polygonal in shape. Vascular bundle are with a massive fibrous or sclerenchymatous sheath, crescent shaped, collateral, closed type and endarch. Fibers are present in the cortical region (Fig. 3).

Petiole

In the surface view, the epidermal cells are polygonal in shape and thick walled parenchymatous cells. Stomata are also found. In transverse section, petioles are more or less rounded in shape and covered with cuticles. Many trichomes are present in the form of uniseriate, multicellular and basal celled. In cortical region, 2-3 layers of collenchyma cells are found towards the peripheral region. The parenchyma cells are 3-6 layered in thickness. These cells are rounded to oval in shape. Variously sclerenchymatous cells were observed. Vascular bundles are circular in shape, collateral, closed type and endarch. Pith is composed of parenchymatous cells (Fig. 4).

Stem

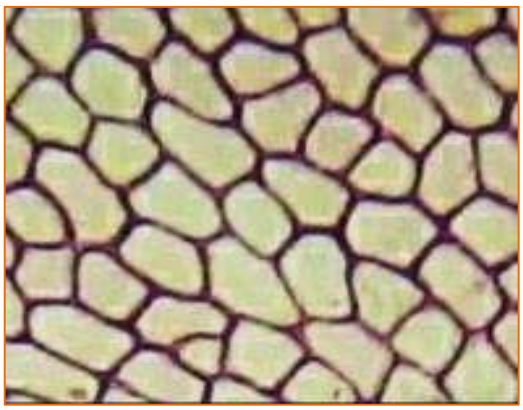
In the surface view, the epidermal cells are thick-walled, rectangular in shape, compact, anticlinal walls and elongated along the axis. Stomata are also found. In transverse section, it is more or less circular in outline. The epidermal cells are one layered, barrel shape, cuticle and trichomes present. Cortex layer consists of 4 - 6 layers of collenchymatous cells and 3 - 7 layers of parenchymatous cells. Many fibers are also found in the cortex. Vascular bundles are arranged in a ring, collateral closed and endarch. Pith composed of thin-walled, rounded parenchymatous cells (Fig. 5).

Root

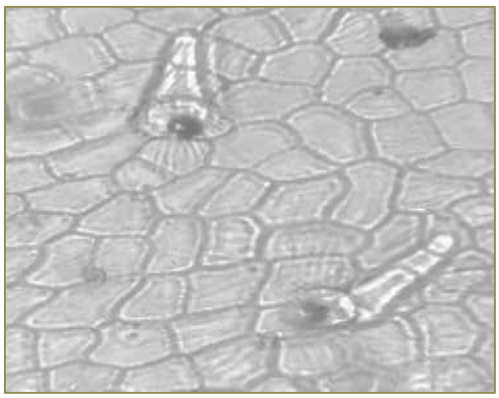
In the surface view, the epidermal cells are polygonal in shape and thick walled parenchymatous cells. Stomata are absent. In transverse section, it is more or less circular in outline. In transverse section, the epidermal cells are 3- layered and barrel shaped and cuticles present. Cortex consists of 3-6 layers, collenchymatous cells, oval shaped and compact. Endodermis composed of many layers, parenchymatous cells. Vascular bundles are radial type (Fig. 7).

Galls

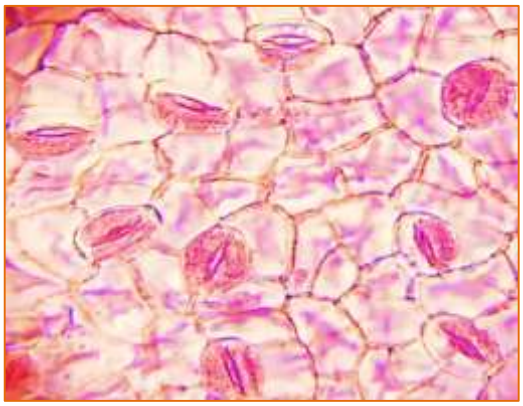
In the surface view, the epidermal cells are parenchymatous, thick-walled and polygonal in shape. In transverse section, vascular bundles are radial type, collateral, closed type and endrach. Pith consists of parenchymatous cells (Fig. 6).



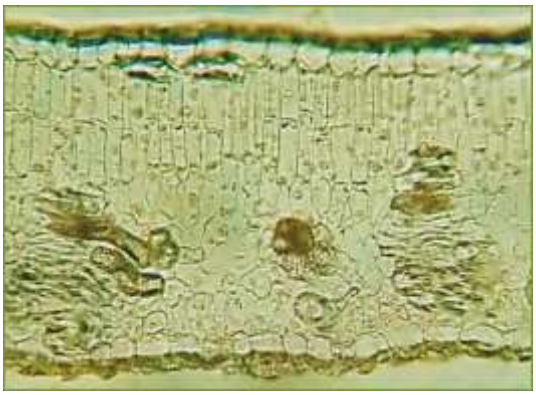
Upper surface of lamina (x400)



Upper surface of lamina with trichomes (x400)



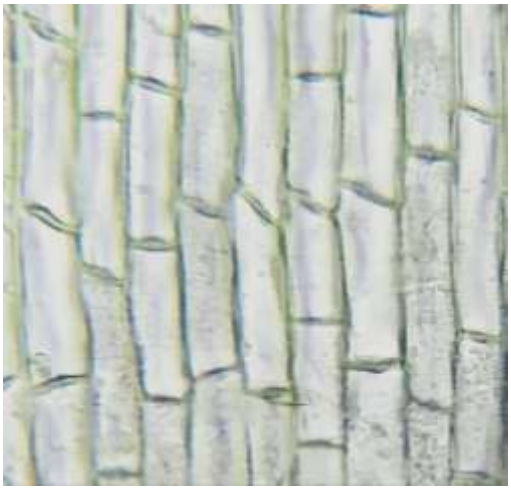
Lower surface of lamina (x400)



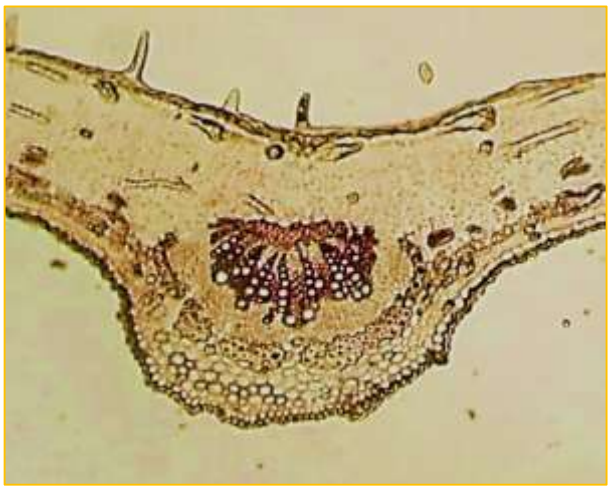
T.S of Lamina (x100)

Close up view of vascular bundle (x400)

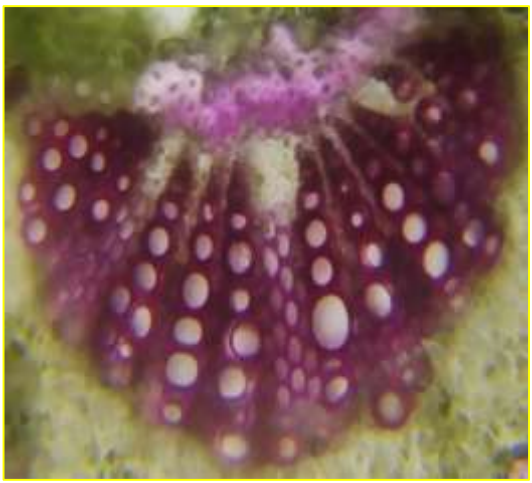
Figure 2 Microscopical characters of lamina of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm.



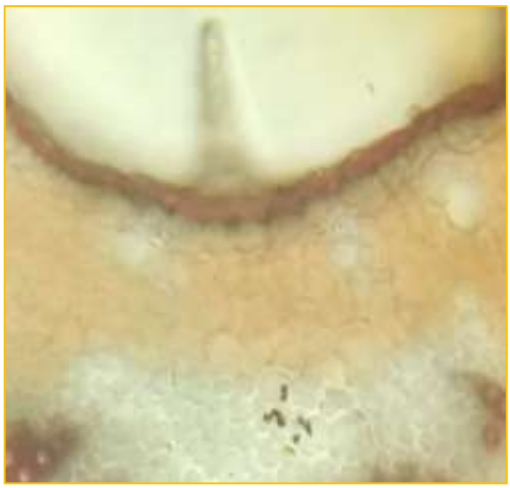
L.S of madrib (x400)



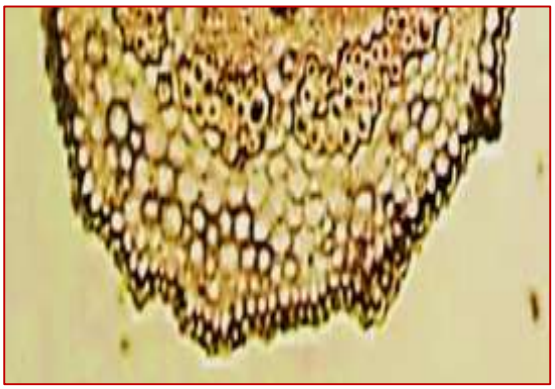
T.S of madrib (x400)



Close up view of vascular bundles (x400)

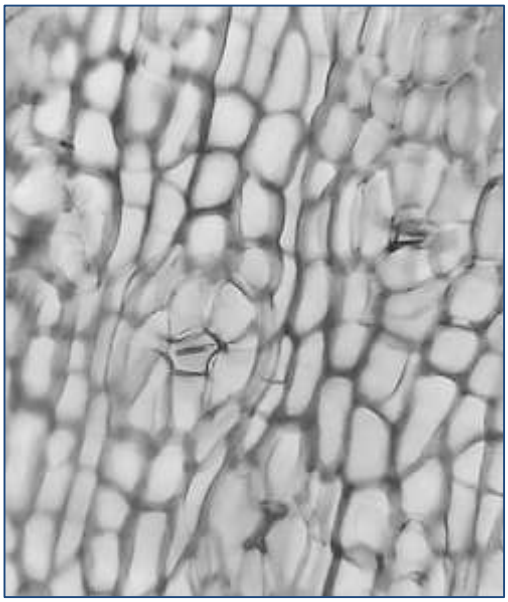


T.S of upper surface of midrib (x400)

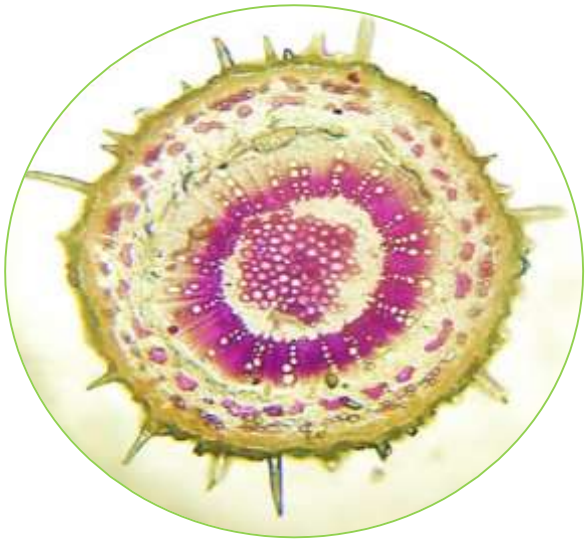


Transverse section of lower surfaces of midrib (x400)

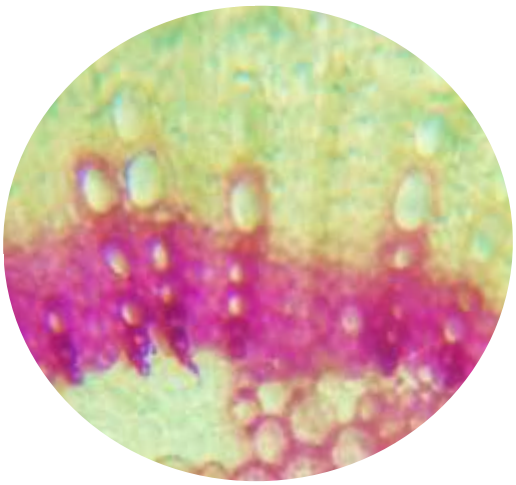
Figure 3 Microscopical characters of midrib of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm



Surface view of petiole (x400)



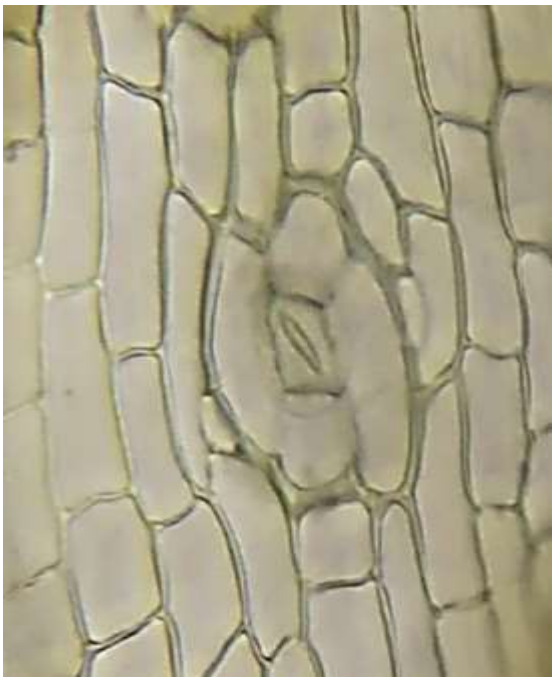
T.S of petiole (x100)



Close up view of vascular bundles (x400)

Trichome (x400)

Figure 4 Microscopical characters of petiole of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K.Hoffm



Surface view of stem (x400)

T.S of stem (x100)

Close up view of vascular bundle (x400)

Figure 5 Microscopical characters of stem of *Hypselandra variabilis* Pax & K. Hoffm.

Surface view of gall

T.S of gall

Figure 6 Microscopical characters of gall of *Hypselandra variabilis* Pax & K. Hoffm.

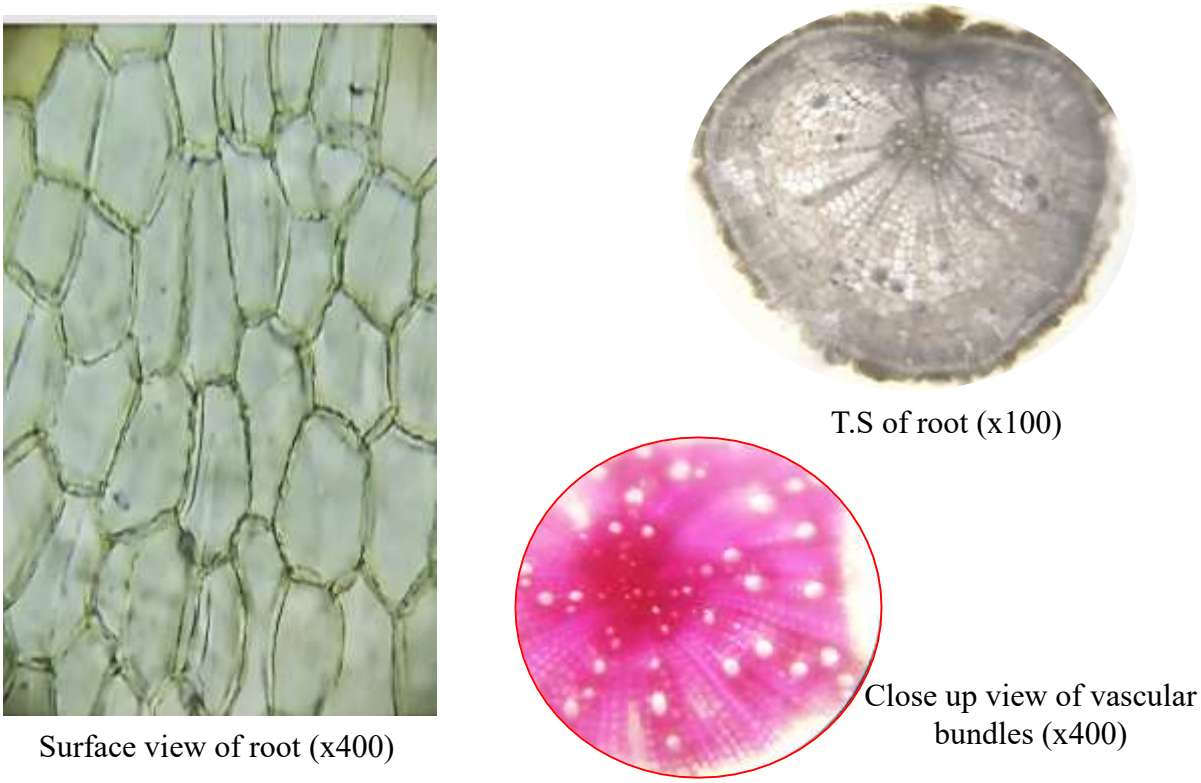


Figure 7 Microscopical characters of root of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm.

Discussion and Conclusion

Hypselandra variabilis (Collett & Hemsl.) Pax & K. Hoffm. is a perennial, xerophytic plant that belongs to the family Capparaceae. In the present investigation, the morphological and histological characters of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm. were performed. Metcalfe & Chalk (1950) have reported that Capparaceae is mainly tropical family consists of herbs, shrubs and small trees, while a few genera are included in Cadaba, Capparis and other genera. Morphological studies were in agreement with the statements of Dassanayake (1996) and Kurz. (1877). This plant was tree; the stem color was found as grayish brown to dark brown; branches bear numerous galls, flowers bisexual, actinomorphic; sepals 3-4, united at the base into a funnel; petals none; stamens 6 to numerous; gynophore elongated; parietal placentae. These features were in agreement with Metcalfe and Chalk, 1950 and Trease and Evans, 2002.

In histological study, the anomocytic types of stomata were found only on lower surface of the leaves but on upper surfaces were not found. Two layers of palisade parenchymatous cells were found under the upper epidermis of leaves, and three to six layers of spongy parenchymatous cells above the lower epidermis. Variously sclerenchymatous cells are frequently present in the mesophyll. Sclerenchymatous cells are sometimes extending between the epidermal cells and thus coming into contact with the leaf surface. Midribs were found in an arc with the convex surface towards the lower side. These histological characters agreed with the statements of Metcalfe and Chalk, 1950.

Adamson (1935) stated that successive cambia have been reported in *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm. by Hansen (1977). But, Metcalfe & Chalk (1950) have reported that some species of *Hypselandra* do not have successive cambia. Pandey and Chadha (1998) stated that anomocytic type also called irregular-celled type where subsidiary cells

are indistinguishable from other epidermal cells. Such cases are found in members of Capparaceae. The diagnostic characters of powdered leaves, stems and roots of this plant were also found the presence of the fragments of tracheids, vessels and fibres.

In this research *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm. was chosen because it has high medicinal value. San and Han (1998) stated that its leaves and roots are used to treat aching, sedema, cold extremities and also used as stomachic, expectorant and counter irritant. Its galls are used to relieve eye sored. Local people believed that taking its buds and flowers once a year can keep the good health throughout the lifespan in Mzanmar (Ashin-nargathein, 1978). It is concluded that the histological characters and the diagnostic characters of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm. have not been conducted by other scientists in Myanmar before. These characters presented in this research could be used as standardization in traditional medicine.

Acknowledgements

We would like to express our sincere gratitude to Chairperson and all Professors from Department of Botany, University of Yangon for their kind permission and suggestion to present this paper on the Conference for Myanmar Academy of Arts and Science.

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MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF *CURCUMA AROMATIC* SALISB.

Moe Moe Myint Aung[1], Htay Htay Lwin[2], Yee Yee Thu[3], Aye Kyi[4]

Abstract

The plant *Curcuma aromatica* Salisb. locally known as Taw-Sanwin, belongs to the family Zingiberaceae. It is collected from Hpa-an Township, Kayin State during the flowering and rhizomes period in 2019. In this paper, the morphological and histological characters of the leaves, midrib, petiole, leaf sheath, tuber, root, and rhizomes of *Curcuma aromatic* Salisb. were studied at Department of Botany, Hpa-an University. Verification of plant was carried out by available literatures. In histological study, free hand sections of leaves, midrib, petiole, leaf sheath, tuber, root, and rhizome were studied and examination of powdered samples was also carried out for standardization of drug. In morphological study, the plant is perennial herb with tubers aromatic rhizome. Leaves are distichous with open sheath. Inflorescences are terminal a leafy shoot directly from the rhizome. Flower bisexual, zygomorphic and epigynous. Calyx (3), synsepalous. Corolla (3), synpetalous. Androecium of two whorls, fertile stamen and staminodes. Ovary tricarpellary, axile placentation and discs are present. The fruits are fleshy and dehiscent. Seeds round, mostly covered with a large divided aril endosperm abundant, white, hard or mealy. In histological study, the anticlinal walls in the epidermal cell of both surfaces are straight. Tetracytic stomata are present on both surfaces but more abundant on lower surface. Oil cells contain parenchymatous layer of midrib, petiole, leaf sheath, tuber, root and rhizome. Secretory cells and starch grains are present in tuber, root and rhizome. Vascular bundles of lamina, midrib, petiole, leaf sheath, tuber, root and rhizome are collateral type and closed type.

Keyword: *Curcuma aromatica* Salisb., morphological and histological characters

Introduction

The terms of medicinal plants include various types of uses in herbalism and some of these plants have medicinal activities. These medicinal plants consider as a rich resources of ingredients that can be used in drug development and synthesis. Moreover, some plants consider as important source of nutrition and as a result of these plants recommended for their therapeutic values (Hassan, 2012; and Revathy, *et al.*, 2013).

Curcuma is an aromatic rhizomatous herbs belonging to family Zingiberaceae. Zingiberaceae is a group of monocotyledonous plant that is economically important (Bhattacherijee, 2000; Heywood, *et al.*, 2007 and Sikha, *et al.*, 2015). It is used due to the secretory structure inside the plant's organs that produce metabolites that are used as medicine (Dassanayake, 1983; Charles, *et al.*, 1992 and Zhoa, *et al.*, 2001).

The plant has been in traditional use and in medicinal values it is mentioned as a remedy for various diseases. *Curcuma aromatic* is already known in India as a tonic, carminative, as an antidote to snake bites and astringent. It is used for bruises, corn, sprains, snake bite and is a well-known for enhancing complexion, for skin infections, eruption. Paste of rhizome with milk is used for dysentery and gastric ailments.

The leaves are distichous with open sheath (Narayan, *et al*., 2006 and Heywood, *et al.*, 2007). The inflorescences are terminal spikes (Judd, *et al*., 2006 and Promod, *et al*., 2018). Flowers fragrant are shorter than the bracts (Kirtikar and Basu, 1935 and Pandey, 2008). It is pinkish-white with an orange lip (Polunin *et al.*, 1997 and Narayan *et al*., 2006). Flowers are zygomorphic (Judd,

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[4] Professor and Head (Retired), Department of Botany, University of Yangon

et al., 2006 and Heywood, *et al.*, 2007; Pandey, 2008). Fruits are capsule and dehiscent. Seeds are arrillated (Heywood, *et al*., 2007 and Pandey, 1998).

In this plant, anticlinal walls of both upper and lower surfaces of lamina are straight. Tetracytic stomata are present on both surfaces. Vascular bundle of lamina is collateral and closed type. Vascular bundle of midrib, petiole and leaf sheath are arranged in three rows. The main vascular bundles are between alternating with air canals. Oil cells are embedded in parenchymatous layers of midrib and leaf sheath (Pandey, 1998 and Ravindran, *et al*., 2007).

In root, cortex layer lie below epiblema layer. Only one layer of endodermis and 1–2 layers of pericycle are present. Vascular bundle is polyarch in root. Oil cells, secretory cells and starch grains are scattered in the ground tissues. In rhizome, periderm layers present. Vascular bundles are scattered throughout the ground tissue. Oil cells, secretory cells and starch grains are scattered in the ground tissues (Tomlinson, 1956 and Shantha, *et al*., 1991).

The objectives of this study are to verify the plant *Curcuma aromatic* Salisb. by using vegetative and floral parts, to investigate the histological characters of leaves, leaf sheath, midrib, petiole, tuber, root and rhizome of this plants and to examine the leaves, tuber, root and rhizome powdered for standardization in traditional medicine of plant *Curcuma aromatic* Salisb.

Materials and Methods

Morphological study

The specimens of *Curcuma aromatic* Salisb. were collected from Hpa-an township, Kayin State, during May to October, 2019. It is situated between latitude 16° 47′ 29 N and longitude 97° 38′ 43 E, 56 feet above sea level. The collected specimens were made careful notes and recorded by taking photographs to classify and identify systematically. The morphological study of plant was undertaken with the help of available literatures such as (Wealth of India, 1951; Kirtikar and Basu, 1975; Dassanayake, 1983; Hundley and Chit Ko Ko, 2003 and Kress, *et al*., 2003).

Histological study of *Curcuma aromatic* Salisb.

In histological study, free hand section of lamina, midrib, leaf sheath, stem, root and rhizome from the fresh specimens were prepared by using chloral hydrate solution for clearing reagents, safranin for testing lignin and iodine solution B.P for testing starch and sudan III for oil cells. These characters were determined according to the literature of Tomlinson (1956), Wallis (1967) and Pandey (1998).

Results

Morphological Characters of *Curcuma aromatic* Salisb.

Scientific Name - ***Curcuma aromatic* Salisb.**

family - **Zingiberaceae**

Myanmar Name - **Taw-sanwin**

Common Name - **Wild turmeric, Yellow zedoary**

The plant is perennial herb with tubers aromatic rhizome 70-75 cm high, rhizome thick, more than 10.0 - 14.0 cm long and 1.0 – 1.5 cm wide, tubers thick, 3.0-3.5 cm long and 2.5 - 3.0 cm wide, the fresh and aromatic, light yellow. Leaves are distichous with open sheath

17.5 – 32 cm long and 6.5 – 13.7 cm wide, uniformly green, the lamina is acuminate, pubescent below, with a prominent midrib and parallel, pinnate, lateral veins diverging obliquely from the midrib; petiole as long as the lamina, 9.5 - 25.0 and 3.0 – 4.0 cm wide; a distinct ligules, 1.0 – 1.4 cm long and 8.0 – 15.0 mm wide, present at junction of leaf blade and leaf sheath, glabrous. Inflorescences terminal spikes, 10.0 – 12.0 cm long and 4.0 – 5.0 cm wide, it comes out from the dominant underground rhizomes, the cymose units in the axile of usually conspicuous bracts and compact spike, flower bract, 2.5 – 3.5 cm and 1.5 – 2.0 cm wide, ovate, recurved, cymbiform, rounded at the tips, pale green, connate below forming pouches for the flowers; comma bract, 2.5 – 3.5 cm long and 1.0 – 1.5 cm wide, tinge with pink. Flower yellow, 4.0 – 4.5 cm long and 1.5 – 2.0 cm wide, sessile, complete, bisexual, zygomorphic, trimerous, fragrant, epigynous; calyx (3), synsepalous, tubular, spathaceous splitting above, 0.6 – 0.8 cm long and 0.2 – 0.3 cm wide, valvate, white; corolla (3), synpetalous, the tube cylindrical at the lower part, 2.0 – 2.5 cm long and 0.5 – 0.7 cm wide, the three linear lobes at the apex, 1.5 – 2.0 cm long and 0.5 – 0.7 cm wide, white, membranous, reflexed; androecium of two whorls, stamens and staminodes, only one stamen of inner whorl is fertile, the outer whorls are modified into large petaloid staminodes, within the three petaloid staminodes, two petal-like lateral staminodes, broadly lanceolate to oblong-ovate, 0.5 – 0.8 cm long and 0.2 – 0.3 cm wide, the tips obtuse, remain separate, petal-like staminodes which are united to form a bilobed labellum, 2.0 – 2.3 cm long and 0.5 – 0.7 cm wide, slightly longer with a yellowish or dull white patch, in the center fertile stamen erect, epipetalous, the filament short, 0.4 – 0.6 cm long and about 0.1 cm wide, white, the anther ellipsoidal, 1.4 – 1.5 cm long and 3.0 – 4.0 mm wide, dithecous, dorsifixed, introrse, pollen sacs usually longitudinal dehiscence; ovary inferior, globoids-oblongoid, about 0.5 – 1.0 cm long and 0.5 – 0.7 cm wide, tricarpellary, syncarpous, trilocular, axile placentation, white, pubescent, many ovules in each locule, the style 3.5 – 4.0 cm long and about 0.5 cm wide, passing through the groove of the fertile stamen, white, the stigma capitate and ciliate, white, about 1.0 mm long and 2.0 mm wide, yellowish discs are 0.5 – 0.7 cm long and about 1.0 mm wide (Figure 1).

Histological characters of *Curcuma aromatic* Salisb.

Lamina

In surface view, the cuticle is thin and smooth. The anticlinal walls in the epidermal cell of both surfaces are straight. The cells are polygonal in shape, thin- walled, parenchymatous. Tetracytic stomata (each stoma with a pair of lateral subsidiary cells and a pair of terminal subsidiary cells) are present on both surfaces but more abundant on lower surface. The stomata are oval in outline with two reniform-shaped guard cells and contain abundant chloroplasts. Unicellular trichomes are present on both upper and lower surfaces.

In transverse section, cuticle layers are thick and smooth on both surfaces. Epidermal cells are barrel shaped. 1 – 2 layers of hypodermis are present in abaxial. These cells are polygonal in shape. The abaxial hypodermis is interrupted by large substomatal chambers. These cells are irregular in shape. The mesophyll cells composed of palisade parenchymatous and spongy mesophyll cells. Palisade parenchymatous layers are vertically elongated, tightly packed with one another and contained many chloroplasts. The spongy mesophyll cells are three-five layers the cells are loosely arranged, irregular in shape, thin walled parenchymatous cells and intercellular spaces. Vascular bundles are embedded in the mesophyll cells and are collateral and closed type. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem tissue composed of sieves tube elements, companion cells, phloem fibres and phloem parenchyma (Figure 2).

Midrib

In surface view, the epidermal cells are thin- walled, parenchymatous cells, polygonal in shape, tetracytic stomata and oil cells are present (Figure 3).

In transverse section, the midrib is V-shaped in outline. Both upper and lower surfaces of epidermal cells are barrel shaped, thin-walled, parenchymatous cells. Lower epidermal cells are smaller than upper ones in width. Below the epidermis, both upper and lower collenchymatous cells are 2 – 3 layers, polygonal in shape. 4 – 10 layers of parenchymatous cells are found above main vascular bundles. These cells are polygonal in shape and thin-walled. Vascular bundles are arranged in three rows, developing unequally at different levels. The main vascular bundles are between alternating with air canals and embedded in chlorenchyma. The abaxial conducting system consists of an arc of vascular bundles of different sizes that are circular in outline. The adaxial conducting system consists of 1 – 3 vascular bundles (subsidiary vascular bundle) that are similar in appearance to the main vascular bundles but are smaller in size. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Abaxial bundles are enveloped within almost a complete fibrous sheath. Air canals contain a loose network of lobed cells. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibres and phloem parenchyma. Oil cells are embedded in parenchymatous layers (Figure 3).

Petiole

In surface view, the epidermal cells of both upper and lower surfaces are thin-walled, parenchymatous cells, polygonal in shape and thin walled parenchymatous cells. Tetracytic stomata and unicellular trichome are present (Figure 4).

In transverse section, the petiole is semi-circular in outline. Both upper and lower epidermal cells are barrel shaped and tightly arranged, thin-walled, parenchymatous cells. Lower epidermal cells are smaller than upper ones in width. Collenchymatous cells are 2-3 layers in upper epidermis and 3-5 layers in lower ones and polygonal in shape. Both upper and lower parenchymatous cells 6 – 10 layers .These cells are irregular and polygonal in shape and thin-walled. Vascular bundles are arranged in three rows, developing unequally at different levels. The main vascular bundles are between alternating with air canals and embedded in chlorenchyma. The abaxial conducting system consists of an arc of vascular bundles of different sizes that are circular in outline. The adaxial conducting consists of 1 – 3 (subsidiary vascular bundle) that are similar in appearance to the main vascular bundles but are smaller in size. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Abaxial bundles are enveloped within almost a complete fibrous sheath. Air cannals contain a loose network of lobed cells. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibres and phloem parenchyma. Oil cells are embedded in parenchymatous layers (Figure 4).

Leaf sheath

In surface view, the epidermal cells are polygonal in shaped, thin walled parenchymatous cells, tetracytic stomata and unicellular trichome (Figure 5).

In transverse section, both adaxial and abaxial epidermal cells are barrel shaped, thin-walled, parenchymatous cells. The abaxial epidermal cells are smaller than adaxial cells. Above the abaxial epidermal layer, 1 – 3 layers of collenchymatous cells are polygonal in shape. Parenchymatous cells are 4 – 5 layers in abaxial region and 7 – 10 layered in adaxial region. Both are polygonal in shape. Vascular bundles are arranged in three rows, developing unequally at different levels. The

main vascular bundles are between alternating with air canals and above the chlorenchyma. The abaxial conducting system consists of an arc of vascular bundles of different sizes that are circular in outline. The adaxial conducting system consists of vascular bundles (subsidiary vascular bundle) that are similar in appearance to the main vascular bundles but are smaller in size. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Abaxial bundles are enveloped within almost a complete fibrous sheath. Air canals contain a loose network of lobed cells. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibres and phloem parenchyma. Oil cells are embedded in parenchymatous layers (Figure 5).

Tuber

In surface view, the epidermal cells are polygonal in shaped and anticlinal walls are straight. Oil cells, secretory cells and starch grains are present (Figure 6).

In transverse section, periderm consists of 7-10 layers, thin-walled, parenchymatous cells and rectangular to irregular in shape. Periderm composed of phellem or cork, phellogen or cork cambium and phelloderm or secondary cortex. Cortex 35 – 45 layers, thin-walled, parenchymatous cells and polygonal in shape. The endodermis is one layer, lie the inner region of cortex layer, barrel-shaped, thin-walled parenchymatous cells. Pericycle layer lie below endodermal layers are only one layer, barrel shaped, thin-walled, parenchymatous cells. Vascular bundles are collateral below endodermis cells. Vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem composed of sieve tube, companion cells, phloem fibres and phoem parenchyma. Oil cells, secretory cells and starch grains are scattered in the ground tissues (Figure 6).

Root

In surface view, epiblemal cells are polygonal shaped, thin-walled parenchymatous cells (Figure 7). In transverse section, the root is more or less circular in outline. The roots hairs are present. The epiblemal layer is only one layer, barrel shaped and thin-walled parenchymatous cells. Periderm consists of 5-7 layers and composed of phellem or cork, phellogen or cork cambium and phelloderm or secondary cortex. Below the priderm, 5 – 6 layers of outer cortex which are polygonal in shape. In the middle cortical layers composed of 6-7 layers of aerenchymatous cells. Inner cortical layers composed of 4-6 layers polygonal in shaped. The endodermis is only one layer, barrel-shaped, thin-walled parenchymatous cells. Pericycle layer lie below endodermal layers are 1 – 2 layers, barrel shaped, thin-walled parenchymatous cells. Vascular bundle is polyarch. Bundles of the xylem are exarch i.e. the metaxylem towards the central and protoxylem towards the periphery. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibres and phloem parenchyma. Oil cells, secretory cells and starch grains are scattered in the ground tissues (Figure 7).

Rhizome

In surface view, the epidermal cells are polygonal in shape, and anticlinal walls are straight. Oil cells, secretory cells and starch grains are present (Figure 8).

In transverse section, periderm consists of 5–7 layers, thin-walled, parenchymatous cells, rectangular to irregular in shape. Hypodermis consists of 2 – 4 layers and polygonal to irregular in shape. Periderm consists of 5-7 layers and composed of phellem or cork, phellogen or cork

cambium and phelloderm or secondary cortex. Cortex 35 – 45 layers, thin-walled, parenchymatous cells and polygonal in shape. Endodermal cells lie the inner region of cortex layer, only one layer, thin-walled parenchymatous cells. Vascular bundles are collateral and all scattered throughout the ground tissue. Vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem composed of sieve tube, companion cells, phloem fibres and phoem parenchyma. Oil cells, secretory cells and starch grains are scattered in ground tissues (Figure 8).

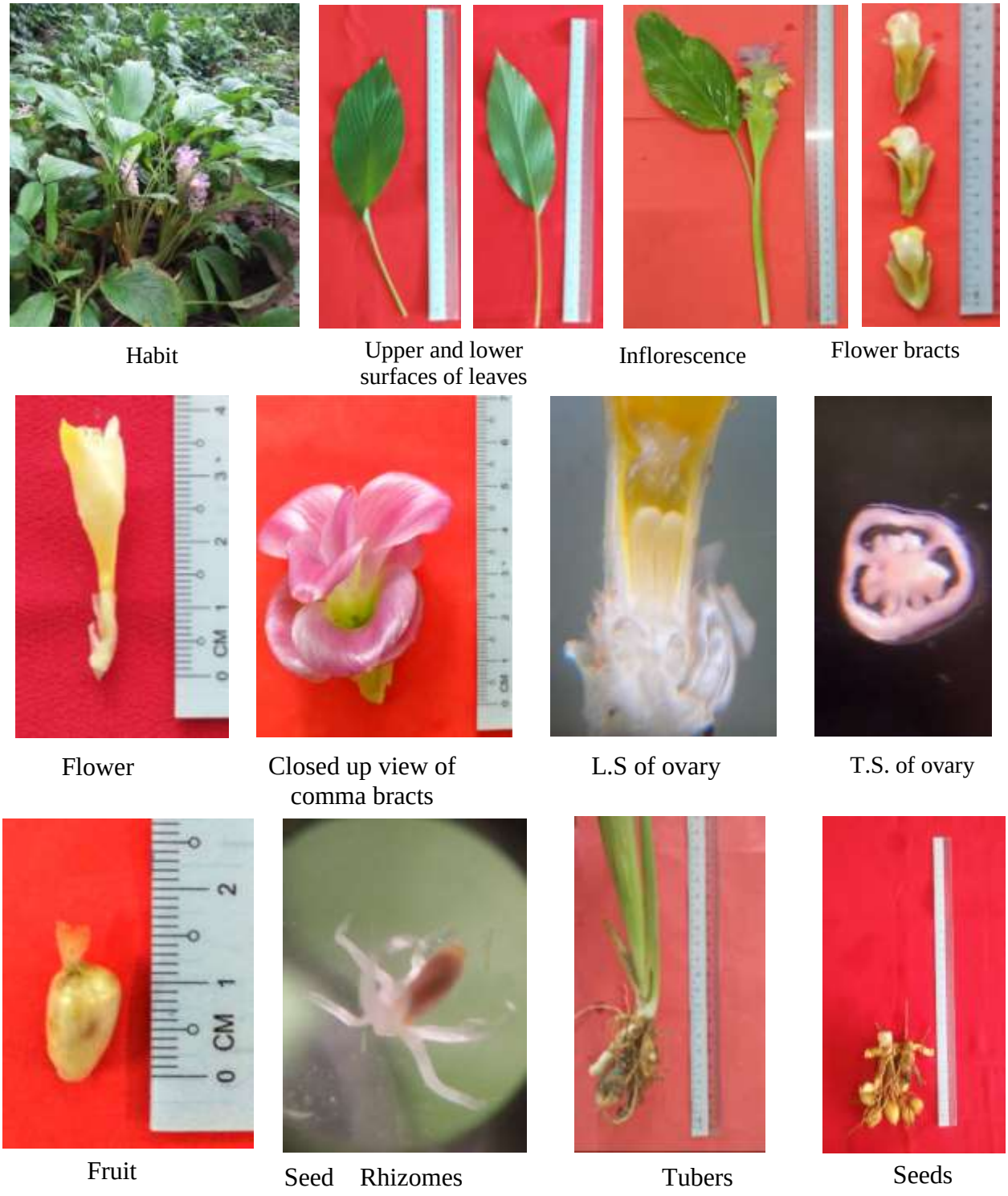
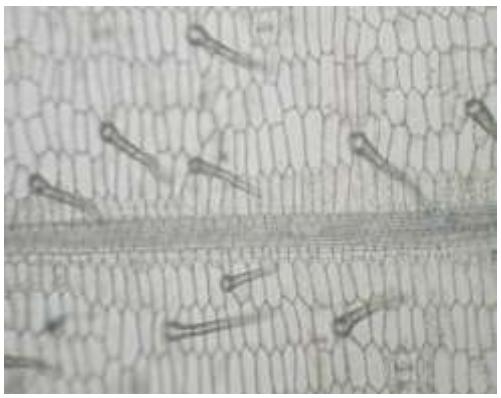
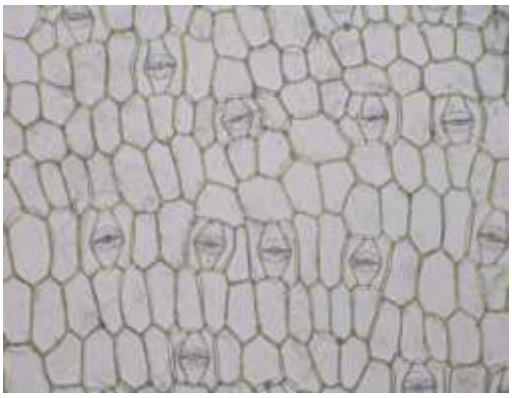


Figure 1 Morphological characters of *Curcuma aromatic* Salisb.

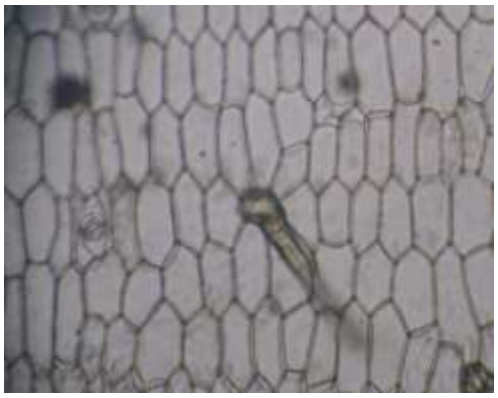
Surface view of upper epidermis of lamina showing stomata (100x)



Surface view of upper epidermis of lamina showing trichomes (100x)

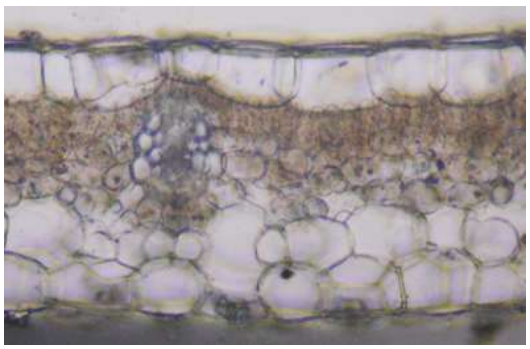


Surface view of lower epidermis of lamina showing tetracytic stomata (100x)



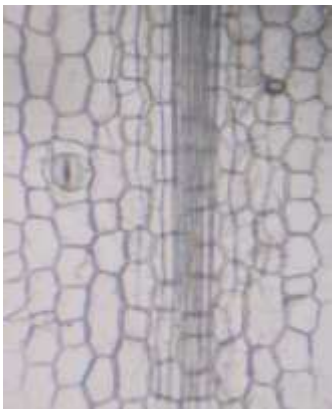
Surface view of lower epidermis of lamina showing unicellular trichome (100x)

Transverse section of lamina showing mesophyll layer (400x)



Transverse section of lamina showing vascular bundle (400x)

Figure 2 Histological characters of lamina of *Curcuma aromatic* Salisb.



Surface view of midrib showing tetracytic stomata and oil cells (400x)

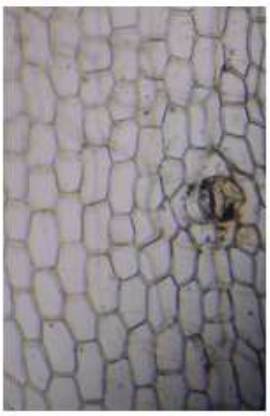
Transverse section of midrib in outline (100x)

Figure 3 Histological characters of midrib of *Curcuma aromatic* Salisb.

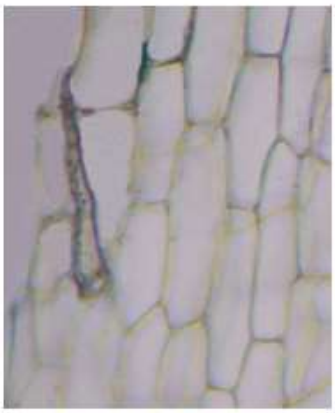
Surface view of epiderma cell of petiole (400x)

Transverse section of petiole showing in outline (400x)

Figure 4 Histological characters of petiole of *Curcuma aromatic* Salisb.



Surface view of leaf sheath showing stomata (400x)



Surface view of unicellular trichome (400x)

T.S. of leaf sheath in outline (400x)

Figure 5 Histological characters of leaf sheath of *Curcuma aaromatica* Salisb.

Surface view of epidermal cells of tuber (400x)

Transverse section of tuber in outline (100x)

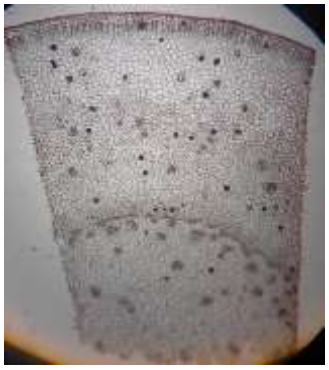
Figure 6 Histological characters of tuber of *Curcuma aromatic* Salisb.

Surface view of epiblema cells of root (400x)

Transverse section of root in outline (400x)

Figure 7 Histological characters of root of *Curcuma aromatic* Salisb.

Surface view of epiblema cells of rhizome (400x)



Transverse section of rhizome showing vascular bundle (200x)

Figure 8 Histological characters of rhizome of *Curcuma aromatic* Salisb.

Discussion and Conclusion

In this research, morphological and histological characters of *Curcuma aromatic* Salisb. were carried out. Myanmar name Taw-Sanwin (Hundley and Chit Ko Ko, 2003 and Kress, *et al.*, 2003) and Na-Nwinherbaceous perennials, sympodially branched rhizomes covered and scales leaves (Burkill, 1935; Tomlinson, 1969; Dassanayake, 1983; and Kress, *et al*., 2003) and tubers yellow and aromatic inside (Dassanayake, 1983 and Narayan, 2006).

The leaves are distichous with open sheath (Narayan, *et al*., 2006 and Heywood, *et al.*, 2007). The inflorescences are terminal spikes (Medicinal plant of India, 1987; Judd, *et al*., 2006 and Promod, *et al*., 2018) and open raceme (Cronquist, 1981 and Heywood, *et al*., 2007). Flowers fragrant are shorter than the bracts (Kirtikar and Basu, 1935 and Pandey, 2008). It is pinkish-white with an orange lip (Medicinal plants of India, 1987; Polunin *et al.*, 1997 and Narayan, *et al*., 2006). Flowers are zygomorphic (Judd, *et al*., 2006 and Heywood, *et al.*, 2007; Pandey, 2008). Calyx (3), synsepalous, are united into a tube with valvate estivation (Judd, *et al*., 2006; Heywood, *et al*., 2007 and Pandey, 1998). Corolla tube cylindrical, the lobes linear and reflexed (Judd, *et al.*, 2006 and Heywood, *et al.*, 2007).

Fertile stamen one, staminodes three (Cronquist, 1981; Heywood, *et al.*, 2007 and Pandey, 2008). Ovary tricarpellary, axile placentation, many ovules in each locule (Heywood, *et al.*, 2007 and Pandey, 2008). The stigma capitate (Pandey, 2008). Fruits are capsule and dehiscent. Seeds are arrillated (Bhattacharj, 2000; Heywood, *et al*., 2007 and Pandey, 1998).

In histological study, anticlinal walls of both upper and lower surfaces of lamina are straight. Tetracytic stomata are present on both surfaces but lower surface is more abundant than upper ones. Vascular bundle of lamina is collateral and closed type. Vascular bundle of midrib, petiole and leaf sheath are arranged in three rows. The main vascular bundles are between alternating with air canals. The abaxial conducting system consists of an arc of vascular bundles of different sizes that are circular in outline. The adaxial conducting system consists of vascular bundles that are similar in appearance to the main vascular bundles but are smaller in size. Oil cells are embedded in parenchymatous layers of midrib and leaf sheath (Esau, 1953; Tomlinson, 1956; Pandey, 1998 and Ravindran, *et al*., 2007).

In tuber, periderm layers present. Only one layer of endodermis is present. Vascular bundles are collateral types and below the endodermis. Oil cells, secretory cells and starch grains are scattered in the ground tissues. In root, cortex layer lie below epiblema layer. Only one layer of endodermis and 1 – 2 layers of pericycle are present. Vascular bundle is polyarch in root. Oil cells, secretory cells and starch grains are scattered in the ground tissues. In rhizome, periderm layers present. Vascular bundles are scattered throughout the ground tissue. Oil cells, secretory cells and starch grains are scattered in the ground tissues (Tomlinson, 1956 and Shantha, *et al*., 1991).

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STUDY OF MOUNTAIN WILD GRASSES IN LOILEM DISTRICT, SOUTHERN SHAN STATE

Lwin Mar Saing[1]

Abstract

Grass belong to family Poaceae (Gramineae) and the one of the largest family in monocotyledon gramineous flowering plants. Grass taxonomy is very differ and difficulty from other flowering plants. Altogether 15 species, 13 genera, 8 tribes, 4 subfamilies in family Poaceae were collected from Loiem district of Southern Shan State of Myanmar, flowering time from 2018 to 2019.The habitat, underground portions, vegetative and reproductive parts of collected species were presented with photograph records. The whole plant portions are classified, identified, verified and evolutionary status by principles author citations of vegetative and reproductive characters. This paper highlights the modified differences characteristics and evolutionary trends of wild mountain grasses in Loilem district of Southern Shan State.

Keywords: Grasses taxonomy, evolution, main fodder, natural resources, soil stabilization

Introduction

The grass family Poaceae represent the fifth largest of flowering plant families. The study species were collected in some area of Southern Shan State, especially the study area was divided into Pang Long, Laichia, Loiliem, Nansann and Sannin. The study area are located between 21º11′20″ N and 97º54′42″ E longitude at an elevation about 2147 m (6444 ft.). According to Halfliger and Scholz's classification (1981), grasses are divided into 5 subfamilies; Bambusoideae, Pooideae, Panicoideae, Chloridoideae and Oryzoideae based on the morphological characters of spikelet (flowers) and vegetative structures. Among them 4 subfamilies are recorded, except subfamily Bamboosoideae.

Subfamily Pooideae comprises 6 species, 4 genera and 4 tribes. Genus *Arundinella* in Tribe Arundinellae, *genus Koeleria* in Tribe Aveneae, Genus *Cortaderia* in Tribe Arundineae and genus *Poa* in Tribe Festuceae. Genus *Poa* is type genus of Family Poaceae (Bor 1960). 3 species of *Arundinella hirmanica* Hook, *A. pumila* (Hochst) Steut and *A. setosa* Trin in genus *Arundinella* in Tribe Arundinellae. 1 species of *Koeleria* spp. in genus *Koleria* in Tribe Aveneae, 1 species of *Cortaderia selloana* in genus *Cortaderia* of Tribe Arundineae and 1 species of *Poa annua* L. in genus *Poa of* Tribe Festuceae. The distinct characters of subfamily Pooideae is 1 to many flowers and usually empty glume provide many flowering glumes (lemma, palea)

Subfamily Panicoideae includes 7 species, 7 genera and 2 Tribes. 3 genera of *Themeda*, *Imperata* and *Schizachyrium* belong to Tribe Andropogoneae. 1 species of *Themeda villosa* (Poir.) Camus in genus *Themeda*, 1 species of *Imparata cylindrica* (Linn.) P.Beauv. in genus *Imperata* and 1 species of *Schizachyrium scoparium* (Michx.) Nash in genus *Schizachyrium*. 4 genera of *Echinochloa*, *Urochloa*, *Rhynchelytrum* and *Axonopus* in Tribe Paniceae. 1 species of *Echinochloa crus- pavonis* (H.B.K.) in genus *Echinochloa*, 1 species of *Urochloa panicoideae* P.B. in genus *Urochloa*, 1 species of *Rhynchelytrum repens* (Willd.) Hubb.in genus *Rhynchelytrum* and 1 species of *Axonopus affins* Chase in genus *Axonopus*. This subfamily distinct characters are 1 to 2 exactly flowered numbers.

1 genus of *Cynodon dactylon* (Linn.) Pers. includes in Tribe *Eragrosteae* of subfamily Chloridoideae. This subfamily distinct characters are 1 to many and usually dwarf tuft culm. 1 genus of *Leersia hexandra* Swartz comprises in Tribe Oryzeae of subfamily Oryzoideae. The distinct characters of this family is always hard crustaceous flowering glumes with awn or awn

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less. These 15 species of wild grass were used in various purposes especially main fodder for cattle in survey region. Wild grasses provide for soil stabilization in survey area as destroy soil erosion by various process. This research highlights differences morphological characters of mountain wild grasses, uses for cattle fodder, soil stable condition, and it is using in various material for native region, Myanmar.

Materials and Methods

Collection Procedure

Specimens were collected from some area of Southern Shan State during flowering time from 2018 to 2019.

Classification, Identification, Verification and Evolutionary Trends

The morphological of grass was classified according to Halfliger and Scholz's classification (1981) that based upon the morphological characters. The identification, verification and evolutionary trends were done by using keys, principles of many author citations; Hooker; 1897, Rhind, 1945; Stebbin, 1956; Bor, 1960; Clayton, 1977; Halfliger, 1981; Hundley, 1987; Willis, 2002, APG III, 2014.

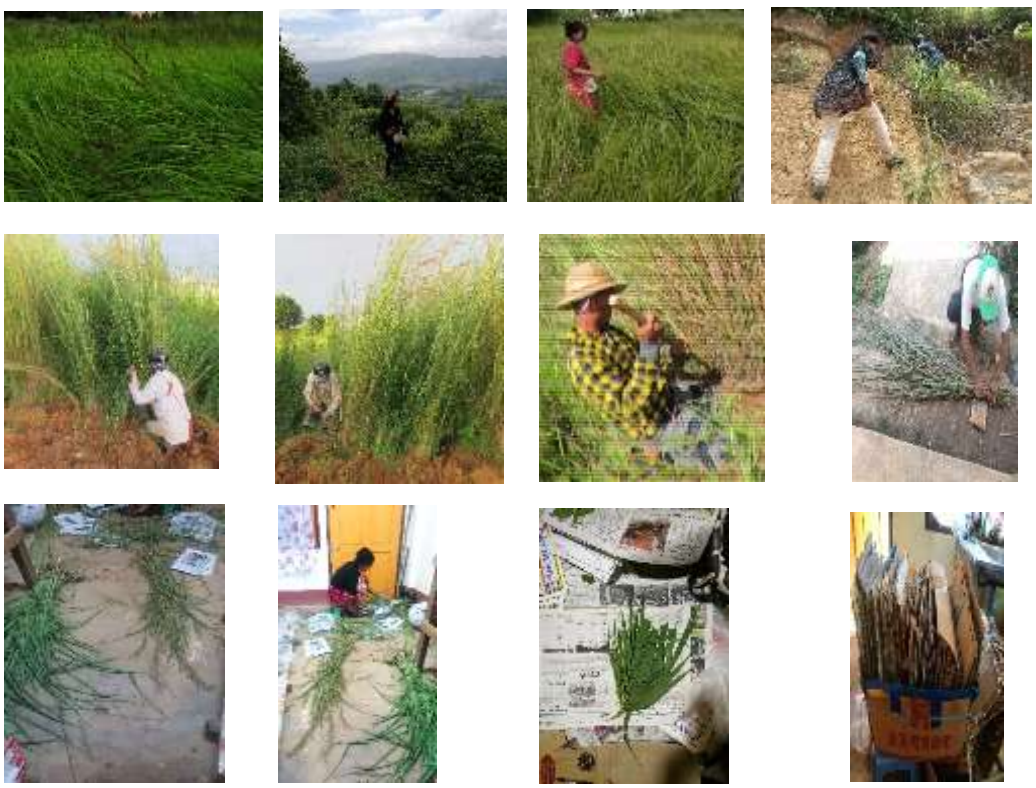


Figure 1 Collection procedure

Results

Morphology of Grass

Spikelet structure

The spikelet is the unit of inflorescences. It can be differentiated into 1 to 2 flowered and 1 to many flowered spikelet. Spikelet comprises glumes.The basally outher 2 glumes is lower empty

glume and upper empty glume.The floering glumes; outer lemma and inner palea arrange the above of empty glumes. All glumes may be various modified characters etc. texture, silkly hairs, bristles and awns.

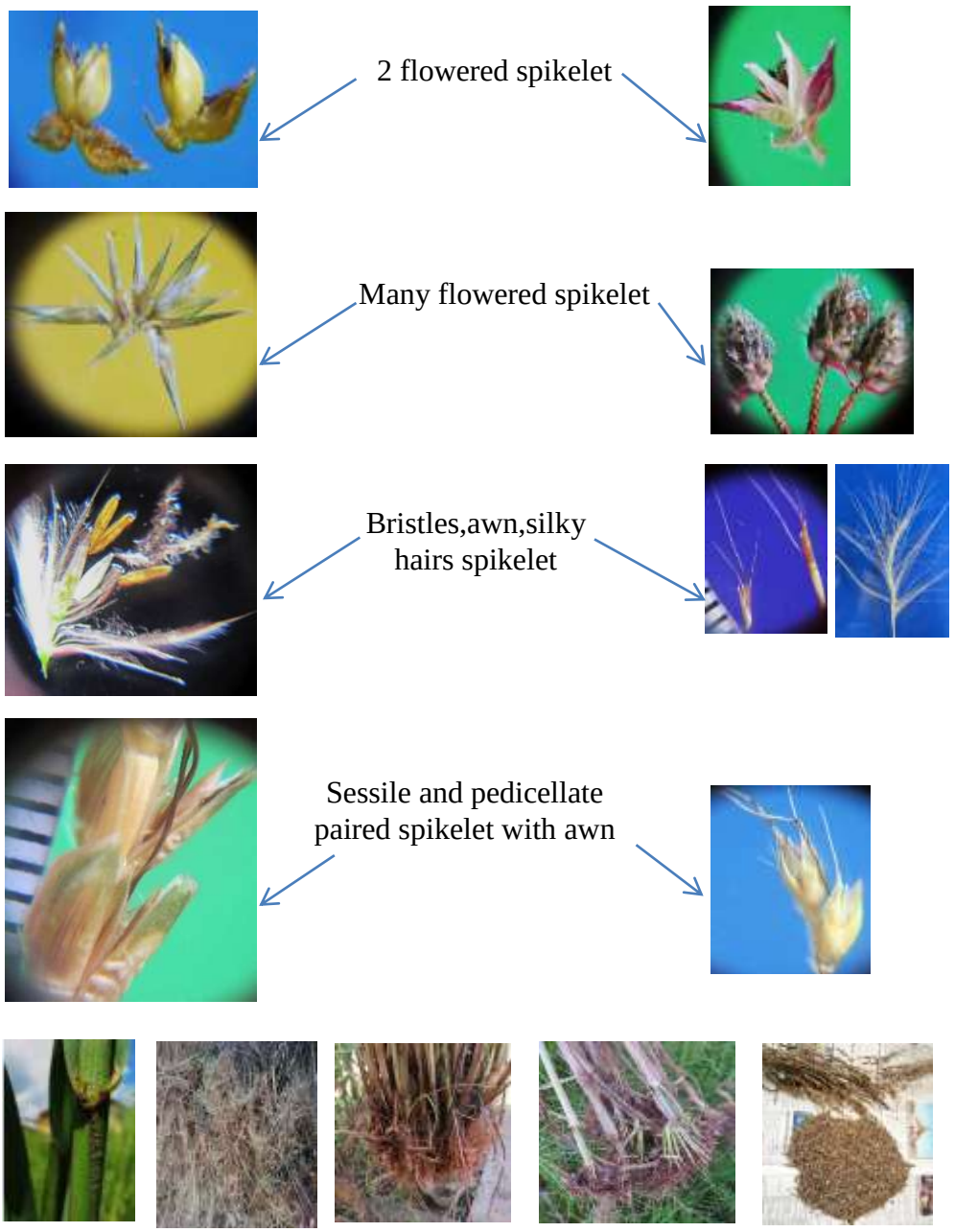


Figure 2 Basic morphology of grass

I. Subfamily - Pooideae

1. Scientific Name - *Arundinella hirmanica* Hook.

Myanmar Name - Kyu – yaing

Habit, prop root,

Inflorescence, spikelet, grain

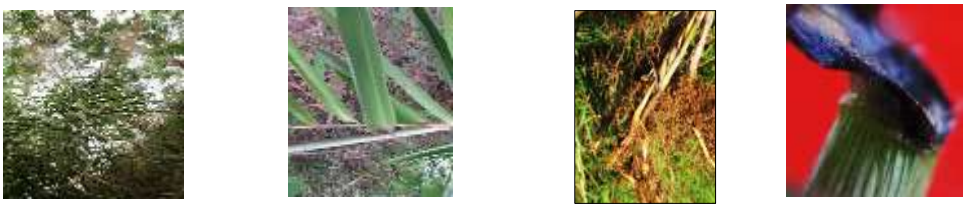
Florets

Figure 3 *Arundinella hirmanica* Hook.

Distinct characters: Strongly tall over 1m , culm like small bamboo,inflorescence very large open panicle, spikelet cluster on rachis ,readily fragile, florets 2; lower neuter; upper bisexual, grain likely to rice, grain are used for important fodder for cattle in army of survey area. (Southern Shan State)

2. Scientific Name - *Arundinella pumila* (Hochst) Steut

Myanmar Name - Kaing



Habit, rhizome, ligule

Inflorescences, spikelets, florets

Figure 4 *Arundinella pumila* (Hochst) Steut

Distinct characters: strongly reed like tall culm, rhizome system, inflorescences very large open plumose panicle, spikelet cluster , very small, bristle numerous, florets 4 - 6 , lowest to middle floret perfect, the upper most neuter, inflorescences are used for cleaning material. (Southern Shan State)

3. Scientific Name - *Arundinella setosa* Trin.

Myanmar name - Kyu

Habit inflorescences rhizome ligule Florets

Figure 5 *Arundinella setosa* Trin.

Distinct characters: Strongly reed tall culm, rhizome system, inflorescences very large plumose open panicle, spikelet paired, slender, florets 3-5, lowest fertile, middle to upper most sterile, inflorescences are used for cleaning material. (Southern Shan State)

4. Scientific Name - *Koeleria* spp.

Myanmar Name - Nil

Habit, root, inflorescences

Figure 6 *Koeleria* spp.

Distinct characters: strongly grow on hilly side culm, roots firmly to soil, inflorescences dense like racemes, spikelet dense on rachis, florets 14 - 23. Widely distributed on hilly side, so they prevent for hilly road erosion condition. (Southern Shan State)

5. Scientific Name - *Cortaderia selloana* (Schult.) Asschers et Graebn

Myanmar Name - Kaing

Habit, rhizome

Inflorescences, florets

Figure 7 *Cortaderia selloana* (Schult.) Asschers *et* Graebn

Distinct characters: Reed - like culm, distinctly wide lanceolate – ovate leaves, inflorescences large loosely open panicle, spikelet crowed at the rachis node, spikelet paired or more, florets 5- 6; lowest to middle fertile and the upper most usually neuter. This species is used for washing material in region. (Southern Shan State)

6. Scientific Name - *Poa annua* L.

Myanmar Name - Myet- mwer

Habit roots draft culm inflorescences

Spikelet florets

Figure 8 *Poa annua* L.

Distinct characters: culm very small and up to 16 cm high, inflorescences densely narrow racemes, spikelet densely alternate on narrow rachis, florets 18 - 23 ; all flowers usually fertile.(Southern Shan State)

II. Subfamily Panicoideae

7. Scientific Name - *Themeda villosa* (Poir.) Camus

Myanmar Name - Myet – sawe- lai

Habit, rhizome

Bulb, inflorescences

Figure 9 *Themeda villosa* (Poir.) Camus

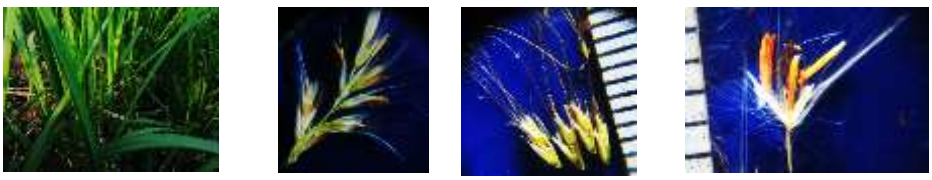
Distinct characters: strongly tall culm,rhizome and bulb distnct, inflorescences large spike like receme and down ward to ground, invoulcure bract strongly cover to spikelet, spikelet paired, florets 2, lower and upper fertile. Underground part used in traditioanl medicine.

(Southern Shan State).

8. Scientific Name - *Imperata cylindrica* (Linn.) P. Beauv.

Myanmar Name - Kyet - mei, Dawn-mei-pyan

Habit inflorescences rhizome



Sharply leaves spikelet florets

Figure 10 *Imperata cylindrica* (Linn.) P. Beauv

Distinct characters: medium tall culm, very stronly rgizome and invasic alien species, inflorescence densely silkly cylindrical panicle, spikelet very long lightly with silkly bristles, florets 2, lower male or neuter ; upper fertile, very exotic species and especially readily invasive to surrounding species by their rhizome and lightly flowers.(Southern Shan State)

9. Scientific Name - *Echinochloa crus – pavonis* (H.B.K.)

Myanmar Name - Myet –let –thae




Habit roots inflorescences



Spikelet florets

Figure 11 *Echinochloa crus – pavonis* (H.B.K.)

Distinct characters: Aquatic invasive species, mediate tall, rigid culm, inflorescences densely spike like receme, awn like empty and flowering glumes, florets 2, lower male and upper fertile. This species is very distrub to rice field. (Southern Shan State)

10. Scientific Name - *Urochloa panicoides* P.B.

Myanmar Name - Nil






Habit inflorescences rhizome ligule spikelet florets

Figure 12 ***Urochloa panicoides*** P.B.

Distinct characters: Aquatic spongy tall culm, rhizomatous to stoloniferous, inflorescences spike like raceme, dense alterately arrange spike , spikelet sunken in rachis, spikelet paired or triad, florets 2, lower male; upper fertile.This species is very useful for cattle fodder and it is buying in raining season for fodder.(Southern Shan State)

11. Scientific Name - *Rhynchelytrum repens* (Willd.) Hubb.

Myanmar Name - Nil




Habit Stoloniferous roots inflorescences

Figure 13 *Rhynchelytrum repens* (Willd.) Hubb

Distinct characters: Small stoloniferous roots culm, inflorescences raceme like plumose panicle, pinkish to red spikelets cluster; spikelets 6 - 15 florets, lowest to uppermost fertile with plumose bristles, readily broken on rachis. This species is so graceful and growing on hilly side reddish color muddy soil.(Southern Shan State)

12. Scientific Name - *Schizachyrium scoparium* (Michx.) Nash

Myanmar Name - Yasa- myet

Habit, inflorescences, rhizome

Spikelet, floret

Figure 14 *Schizachyrium scoparium* (Michx.) Nash

Distinct characters: reed like culm, strongly roots, inflorescences spike like raceme with distinct invoulcure bract, spikelet paired, flotets 2; lower and upper florets fertile with invoulcure bracts.This species leaves is aromatic and used for fodder. (Southern Shan State)

13. Scientific Name - *Axonopus affinis* Chase

Myanmar Name - Nyet-daw –ni

Habit inflorescences rhizome spikelet florets

Figure 15 *Axonopus affinis* Chase

Distinct characters: small lanscaping grass, strogly rhizome, inflorescences digitately arrange, spikelet alterately arranged sunken in flattened rachis, florets 2; lower floet neuter, upper fertile. This grass is important as a landscaping lawngrass in Myanmar.(Southern Shan State)

III. Subfamily Chloridoideae

14. Scientific Name - *Cynodon dactylon* (Linn.) Pers

Myanmar Name - Myesa- myet

Figure 16 *Cynodon dactylon* (Linn.) Pers

Distinct characters: culm small stoloniferous, inflorescences digitate, spikelet sunken in rachis, floret 1 : fertile, glumes crustaceous. This species is land habitat and if they grow near the aquatic is used as traditional species in spirit . (Southern Shan State)

IV. Subfamily - Oryzoideae

15. **Scientific Name - *Leerasia hexandra* Swartz**

Myanmar name - Thaman-myet

Figure 17 Oryzoideae

Distinct chatracters: Aquatic small culm, mix grow in rice field, inforescences spike like raceme, spikelet alterately arrange sunken in rachis, floret 1, fertile, very closely affinity to *Oryza*. This species is eaten by birds but this mature ovary like *Oryza*. (Southern Shan State)

Evolutionary Trends Based on Principles Morphology Characters of Grasses for 4 Subfamilies

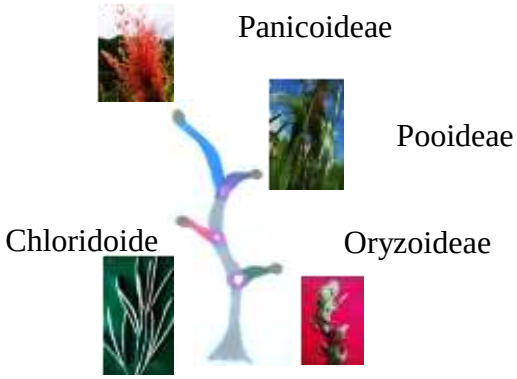
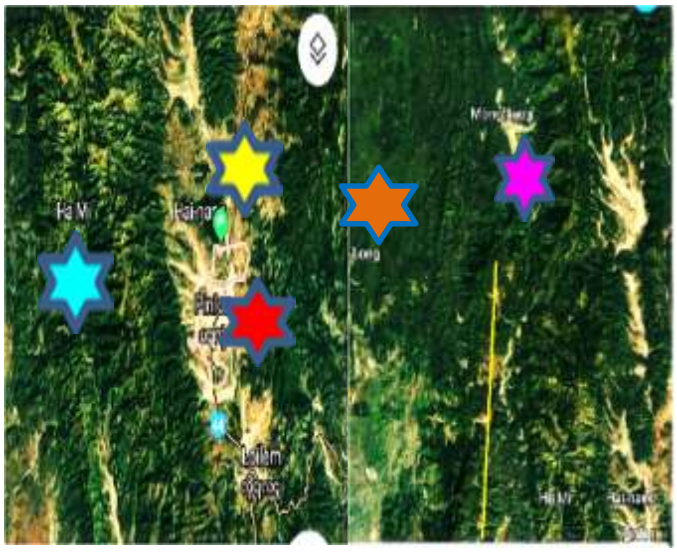


Figure 18 Evolutionary Trends Based on Principles Morphology Characters of Grasses for 4 Subfamilies

Study Areas **Collection Map**



Pang Long Loiliem Sannin Nansann Laichia

Figure 19 Collection Map

Useful of Wild Grasses for Main Natural Recesources Fodder and Status of Survey Areas

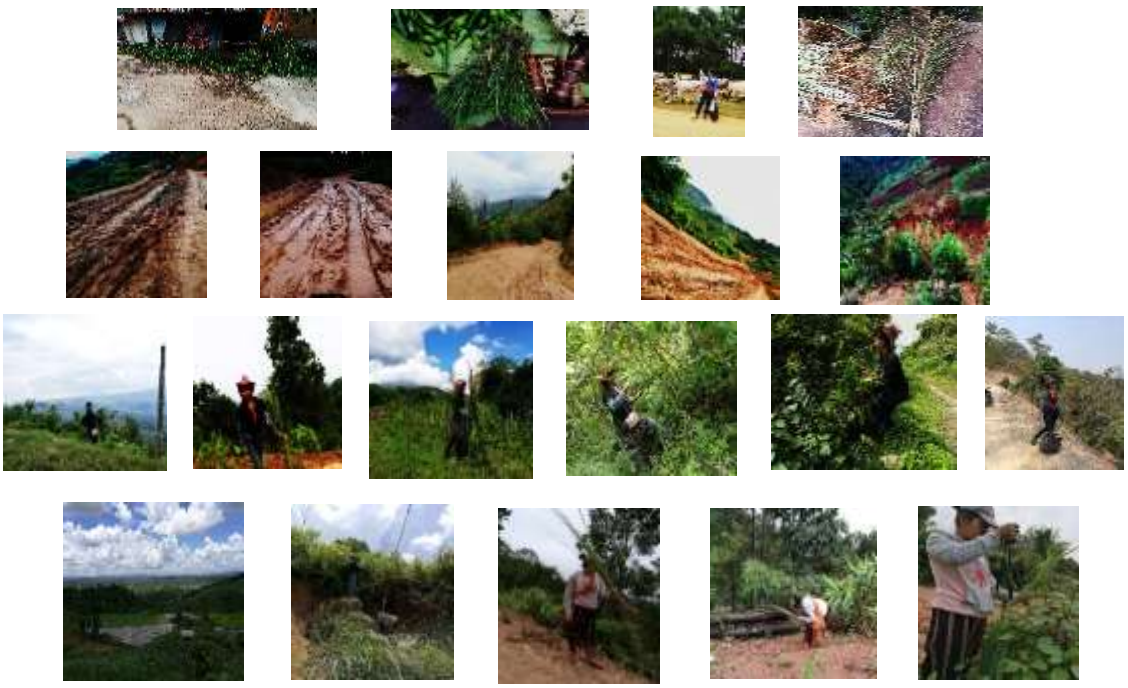


Figure 20 Useful of Wild Grasses for Main Natural Recesources Fodder and Status of Survey Areas

Discussion and Conclusion

All grasses are belong to the family Poaceae (graminaae) in Order Poales (Bor 1960). In this present study 15 species, 13 genera, 8 tribes and 4 subfamily are systematicaally arranged according to Halfliger and Scolz's classification (1981). The most distributed number of species included in subfamily Panicoideae.Tribe Paniceae is more abundant than tribe Andropogoneae in this subfamily.Genera of *Echinochloa, Urochloa, Rhynchelytrum* and *Axonopus* in trbe Paniceae are usually perfect fertile florets with modified glume texture and rhizamatous advance characters. *Echinochloa crus – pavonis* (H.B.K.) comprises awned flowering glume. *Rhynchelytrum repens* (Willd.) Hubb. possess bristles like advance features for species distribution. *Urochloa panicoides* P.B. have densely stiff hairs with leaf-sheath, all perfet florets fertile. *Axonopus affinis* Chase. comprises firmly strong rhizomatous with stoloniferous culm and it has exactly upper fertile floret. These are gradually adaptation from simplicity to complexity of modified characters and more resistent to all natural conditions. In tribe Andropogonneae, Genus *Themeda* and *Schizachyrium* are modified very distict invoulcure bracts with their inflorescences and each floret. The floret glumes with densely bristles in *Themeda villosa* (Poir.) Camus while *Schizachyrium scoparium* (Michx.) Nash with silkly like hairs. Both genera with long distint awns in flowering glumes and empty glumes. Moreover all glumes texture are crustaceous that is advance modification for adaptation in reproductive structures covering for sexual organs. Genus *Imperata cylindrica* (Linn.) P. Beauv.is strongly dominant in survey area as their lightly spikelet with dense silkly hairs and this genus is invasive alien species in Myanmar. All species of subfamily Panicoideae are mainly for fodder and *Axonopus affinis* Chase. is widely useful for lawngrass. All of modified advance characters are more occur in their including respectivelly species of this subfamily Panicoideae.

The second large number subfaily Pooideae include reed - like arborescent characters to small dwarf culm habit. Genus *Arundinella* in tribe Arundinellae is more number than other genus. *Arundinella hirmanica* Hook.is very arborescent tall grass and up to 1m inflorescences with very perfect spikelet and cultivated for cattle fodder in army for domatic animals. *Arundinella pumila* (Hochst) Steut. grow on hilly side and very distint bristle of flowering glumes. These bristles are advance mechanisms for spikelet distribution. *Arundinella setosa* Trin. is growing on hilly side to downward side position and their spikelet comprises many floret with awns.This genus is very firmly for to native soil stabilization of hilly side and control adaptation to environmet status. All of 3 species are widely used in cleaning material for buying economic market in region, Myanmar. Genus *Koeleria* spp. in tribe Aveneae grow hilly side region and their distribution by densely arrange fertile spikelet structures. Genus *Cortaderia selloana* (Schult.) Asschers et Graebn in Tribe Arundineae grow on hilly downside and very plumose infloresence It is possess broader leaf blade arrange in based of culm and it's inflorescceces are very useful for cleaning material for daily uses in region. Genus *Poa* in tribe Fescuteae is type genus of family Poaceae and dense strongly taft dwarf habit. Their florets are many fertile and distrbuted by fertile mature seeds as their flowering time is very quickly and early mature. Advance modifiy characters of subfamily Pooideae is sencond evolutionary status in survey region. Therfore subfamily Panicoideae are more advance taxonomic charcters than subfamily Pooideae.

Genus *Cynodon dactylon* (Linn.) Pers includes in tribe Eragrosteae of subfamily Chloridoideae is exactly 1 fertile floret with advance their strongly stroloniferous culm structure. This genus has all spikelets are fertile with advance all glumes crustaceous characters and third evolutionary status modify structures species. This is uesed in traditional spirit plant in some native Myanmar.

Genus *Leersia hexandra* Swartz comprises in tribe Oryzeae of subfamily Oryzoideae is very affinity to genus *Oryza* by their crustaceous texture flowering glumes and stamens number 6.

But *Leersia* have not empty glumes while *Oryza* with empty glumes. The rest 3 subfamlies have 3 stamens. 6 stamens occur in subfamily Oryzoideae. This is the most primitive stamen characters than 3 stamens subfamilies. Subfamily Oryzoideae is the most primitive status than the rest 3 subfamilies. According to conclusion ,the most advance evolutinory status is subfamily Panicoideae the second is subfamily Pooideae and then the subfamily Chloridoideae. The most primitive is subfamily Oryzoideae. Therfore, this research hilghts the significance role of grsses are important formain natural resources of fodder livestock, daily usees material for humanity, provide soil stabilization and balance environment status, evolutionaty trends in region, Myaanmar.

Acknowledgements

I would like to express my deepest gratitude to DR. Aung Aung Min, Rector – in – charge, Pyay University, and Dr. Thwe Linn Ko, Pro – rector, Pyay University, for their encouragement to do this research. I am grateful Dr. Nyo Nyo Thaung, Professor and Head, Department of Botany , Pyay University, for her encouragement in writing up this research. I would like to express deepest gratitude tu U Nyo Maung , Professor (Rtd.) , Advisor, Department of Botany, Yangon Universiry, for his effective comments and priceless guidance in writing this research.

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THE EFFECTS OF CARBON AND NITROGEN SOURCES FOR THE GROWTH OF SOIL FUNGUS MPF- 7 AND ITS FERMENTATION OPTIMIZATION

Myat Myat Phyo[1], Zar Zar Yin[2] and Marlar Aung[3]

Abstract

In this research work, selected fungal strain collected from Mudon Township. The present study was focused on the utilization of carbon and nitrogen sources for the growth and fermentation conditions of soil fungus MPF-7 on *Escherichia coli*. In the growth effect of the carbon and nitrogen, the excellent growth of MPF-7 was found on glucose and peptone. In the study of fermentation conditions, MPF-7 showed the highest antibacterial activity (22.06 mm) by using 15% seed culture while the best inoculum age was found at 108 hrs on *E. coli*. In addition of glucose as the carbon source and peptone as nitrogen source in the fermentation, MPF-7 showed the highest antibacterial activity (21.05 mm) and (30.78 mm) on *E. coli*. Maximum antibacterial activity was observed at pH 6 (23.36 mm). In the temperature effect, the strong activity was obtained at 25ºC (23.18 mm). In the comparison effect of static and shaking culture of MPF-7, the maximum antibacterial metabolite was observed under shaking condition (20.00 mm) and the static culture of MPF-7 showed the activity (17.87 mm) on *E. coli* respectively.

Keywords: Soil fungi, antimicrobial activity, fermentation optimization

Introduction

The large number of known bioactive compounds (primary and secondary metabolites) of microbial origin are currently produced by fermentation (Gaden, 1959). Therefore the fermentation conditions such as substrates inoculum cultivation and transfer have to optimize for the production of primary and secondary metabolites (Dale, 1984). Carbon and nitrogen sources together with fermentation time have been described to play significant roles in the determination of the final morphology of the culture (Papagianni, 2004). The nature of the nitrogen source has a prominent effect on the production of the antimicrobial metabolite. High nitrogen levels have been noted to repress idiophase production of antibiotics (Spizek J *et al.*, 1995).

Control of ammonia concentration through the mid-cycle was create to be important in the optimization of idiophase secondary metabolite production (Junker *et al*., 1998), though this may reveal the role of nitrogen in growth promotion.

Antibiotic formation usually follows during the late growth phase of the producing microorganism. Microorganisms have developed different mechanisms for uptake and assimilation of mineral and organic forms of N, enabling them to utilize a wide range of organic and mineral compounds (see reviews by Merrick, 1995; Marzluf, 1997).

The production of antimicrobial ingredients be partial by upon the substrate medium for their best growth, temperature, pH and the concentration of nutrients in the medium (Leifert *et al*., 1995). Incubation age and temperature are vital factors that modulate lab growth and significantly affect the amounts of antimicrobial metabolites produced. Production of antibiotics occurs during a distinct idiophase of culture growth phase (Thaer, 2017).

Microbial production of antibiotics is one of the rapidly increasing branches of industrial microbiology. The investigation of new habitats plays a pivotal role in search of new microbes

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possessing potentials to produce novel metabolites, and is urgent to counter the threats posed by the fast developing phenomenon of antibiotic resistance (Shiburaj, 2003).

The aim and objectives of this research were to investigate the utilization of carbon and nitrogen sources of the fungal growth and to optimize the fermentation conditions.

Materials and Methods

Preliminary study for antimicrobial activity

The selected fungus (MPF- 7) was grown of BMEA medium for 3 days. The selected fungus was inoculated into 25 mL seed medium and incubated at room temperature. After 3 days, 25 mL seed culture was transferred into the 25 mL of fermentation medium and incubated at room temperature. Fermentation was carried out for 3-7 days.

Medium used in Antimicrobial activity test (Ando *et al,* 2004)

(**Seed medium** Glucose 20.0 g, Sucrose 3.0 g, Yeast Extract 3.0 g, $KNO_3$1.0 g, K_2HPO_4 0.1 g, Distilled Water 1000 mL, pH 6.5), (**Fermentation medium** (Glucose 20.0 g, Yeast Extract 3.0 g, K_2HPO_4 0.01g, $MgSO_4$. H_2O 0.01 g, $CaCO_3$ 1.0 g, Distilled Water 1000mL, pH 7) and (**Assay (GYP) medium** Glucose 50.0 g, Yeast Extract 30.0 g, Peptone 30.0 g, Agar 14.0 g, Distilled Water1000 mL, pH 6.5).

Agar well Method (Collins, 1965)

Selected strain was tested by agar well method for the antimicrobial activities. One day old culture test (0.1 mL) was added to 100 mL of assay medium and thoroughly mixed and poured into plate. After solidification, cork borer was left to set. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3-7 days old culture fermented broth (20 µL) was carefully added into the wells and incubated at room temperature for 24 to 48 hours. Therefore, the diameter of the zones had been observed as potent activity as shown by respective strain. Clear zones surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Table 1 Test Organism used for Antimicrobial Activity

Test No	Test Organism	Diseases
1	*Escherichia coli* AHU5436	Diarrhea, pneumonia, abdominal pain

The Utilization of Carbon and Nitrogen Sources for the Fungal Growth

The optimal fermentations are very important for maximal productivity metabolites. To determine the effect of carbon sources on antimicrobial metabolite production from MPF-7, different carbon sources such as glucose, fructose, lactose, xylose, potato, soluble starch, sucrose, corn and tapioca were used. Nitrogen sources such as peptone, polypeptone, casein, yeast, gelatin, malt, potassium nitrate, sodium nitrate, ammonium nitrate, ammonium sulfate, ammonium chloride and fish cake were also used.

Study on the effects of age and size of inoculum for the fermentation

The proper cultivation (age) and transfer (size) of the inoculum is crucial for the production of metabolites (Crueger & Crueger, 1989; Emily, 2009). In these studies, the seed culture - 5%, 10%, 15%, 20%, 25% and 30% were employed for the fermentation and age of seed culture was employed at 72, 84, 96, 108, 120 and 132 hrs.

Study on different carbon sources utilization for the fermentation

Carbon sources (each 1.0 g or 1.0 mL) such as glucose, fructose, lactose, xylose, potato, soluble starch, sucrose, corn and tapioca were used. Fermentations were incubated at 25ºC for 6 days.

Study on different nitrogen sources utilization for the fermentation

Nitrogen sources (each 1.0 g or 1.0 mL) such as peptone, polypeptone, casein, yeast, gelatin, malt, potassium nitrate, sodium nitrate, ammonium nitrate, ammonium sulfate, ammonium chloride and fish cake were used. Fermentations were incubated at 25ºC for 6 days.

Effect of pH

The optimum pH of the fermentation medium for antibacterial metabolite production was done by carried out the fermentation at seven different pH values such as 4, 5, 6, 7, 8, 9 and 10. For each pH values, 25 mL of fermentation medium (adjusted to desired pH by using either 1 N NaOH or 0.1 N HCl) was taken in 100 mL conical flasks and autoclaved at 121ºC for 45 minutes. The inoculated flasks were incubated at 25ºC.

Effect of temperature

The optimization temperature for antibacterial metabolite production was undertaken. MPF-7 was incubated at five different temperatures 20, 25, 30, 35, 40 and 45ºC. The fermented broths were tested by using ager well diffusion assay for antibacterial activity against *E.coli*.

Effect of agitation

The antibacterial activity of MPF-7 was studied at two conditions such as agitation (shaking) and stationary (static) conditions.

Results

In the present study, the growth of soil fungus MPF -7 was studied on various carbon and nitrogen sources. Among the carbon sources, the excellent growth of MPF -7 was found on glucose (47.57-49.19 mm) followed by fructose (41.50-43.34 mm) and potato (39.89-41.56 mm). Among the carbon sources, the excellent growth of MPF -7 was found on peptone (49.67-51.98 mm) followed by polypeptone (47.45-49.34 mm), fish cake (45.34-47.37 mm), KNO_3 (41.54-43.86 mm) and Malt extract (37.56-40.32 mm) respectively.

In the size of inoculum, MPF-7 showed the highest antibacterial activity showed at 15% seed culture followed by 10% seed culture (20.79 mm) In the age of inoculum, MPF-7 showed the best activity (18.64 mm) at 108 hrs on *E. coli*. In addition of glucose as the carbon source, MPF-7 showed the highest antibacterial activity (21.05 mm*)* followed by fructose, lactose and soluble starch.

The effect of nitrogen sources were also used and the best result was found in peptone (30.78 mm). Maximum antibacterial activity was observed at pH 6 (23.36 mm). In the temperature effect, the strong activity was obtained at 25ºC (23.18 m) on *E. coli*. In the comparison effect of static and shaking culture of MPF-7, the maximum activity was observed under shaking condition (20.00 mm) and the static culture of MPF-7 showed activity (17.87 mm) on *E. coli* respectively.

Table 2 Colony character and growth of MPF-7 on various carbon sources

Sr. No	Carbon sources	Surface colour	Reverse colour	Size (mm)	Growth
1	Glucose	Brownish green	Brownish cream	**47.57-49.19**	**Excellent**
2	Lactose	Brownish green	Brown	37.35-39.49	Good
3	Sucrose	Pale green	Cream	35.34-37.39	Good
4	Xylose	Brownish green	Brownish cream	37.56-39.23	Good
5	Fructose	Brownish green	Brownish cream	41.50-43.34	Excellent
6	Soluble starch	Brownish green	Brownish cream	37.35-39.37	Good
7	Potato	Brownish green	Brownish cream	39.89-41.56	Excellent
8	Tapioca	Brownish green	Brownish cream	36.56-38.34	Good
9	Corn	Green	Cream	35.50-37.34	Good

20-30 mm = Moderate growth, 30-40 mm = Good, 40 to above = Excellent

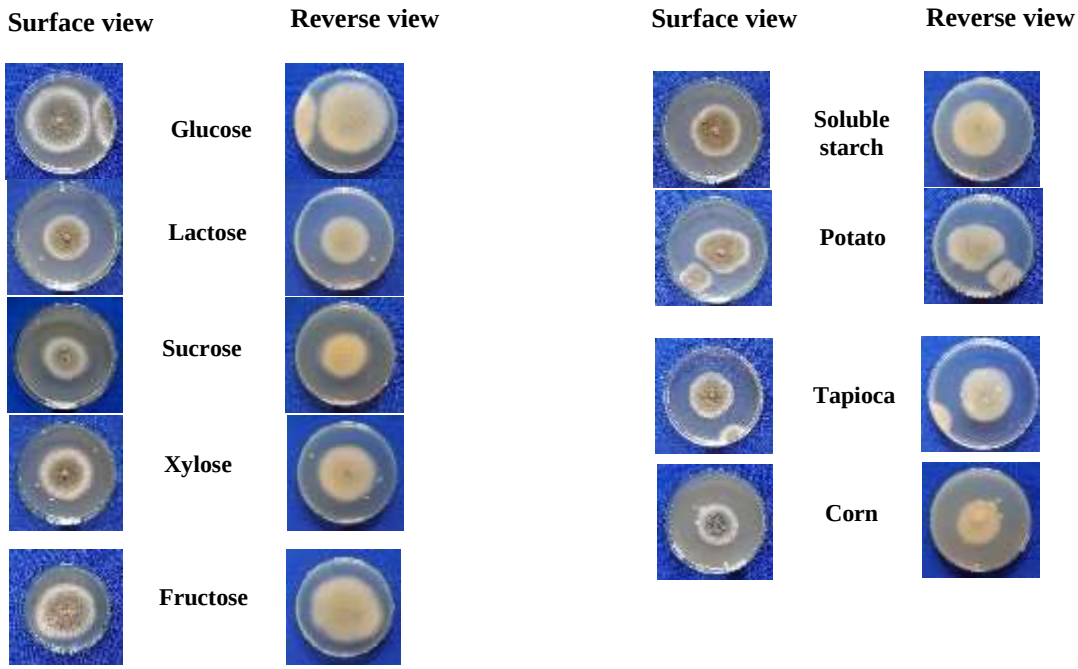

Figure 1 Colony character and growth of MPF-7 on various carbon sources

Table 3 Colony character and growth of MPF-7 on various nitrogen sources

Sr. No	Nitrogen sources	Surface colour	Reverse colour	Size (mm)	Growth
1	Peptone	Brownish cream	Greenish cream	**49.67-51.98**	**Excellent**
2	Polypeptone	Brownish cream	Greenish cream	47.45-49.34	Excellent
3	Casein	Greenish cream	Cream	37.56-39.67	Good
4	Yeast	Cream	cream	35.12-37.56	Good
5	Gelatin	Brownish cream	cream	29.56-33.13	Good
6	Malt	Pale green	Cream	37.56-40.32	Excellent
7	KNO_3	Brownish cream	Greenish cream	41.54-43.86	Excellent
8	$NaNO_3$	Brownish cream	cream	25.21-27.98	Moderate
9	NH_4NO_3	Brownish cream	cream	25.67-26.87	Moderate
10	$(NH4)_2SO_4$	Cream	White	23.34-25.67	Moderate
11	NH_4Cl	White	White	21.66-25.98	Moderate
12	Fish cake	Brownish green	Green cream	45.34-47.37	Excellent

20-30 mm = Moderate growth, 30-40 mm = Good, 40 to above= excellent

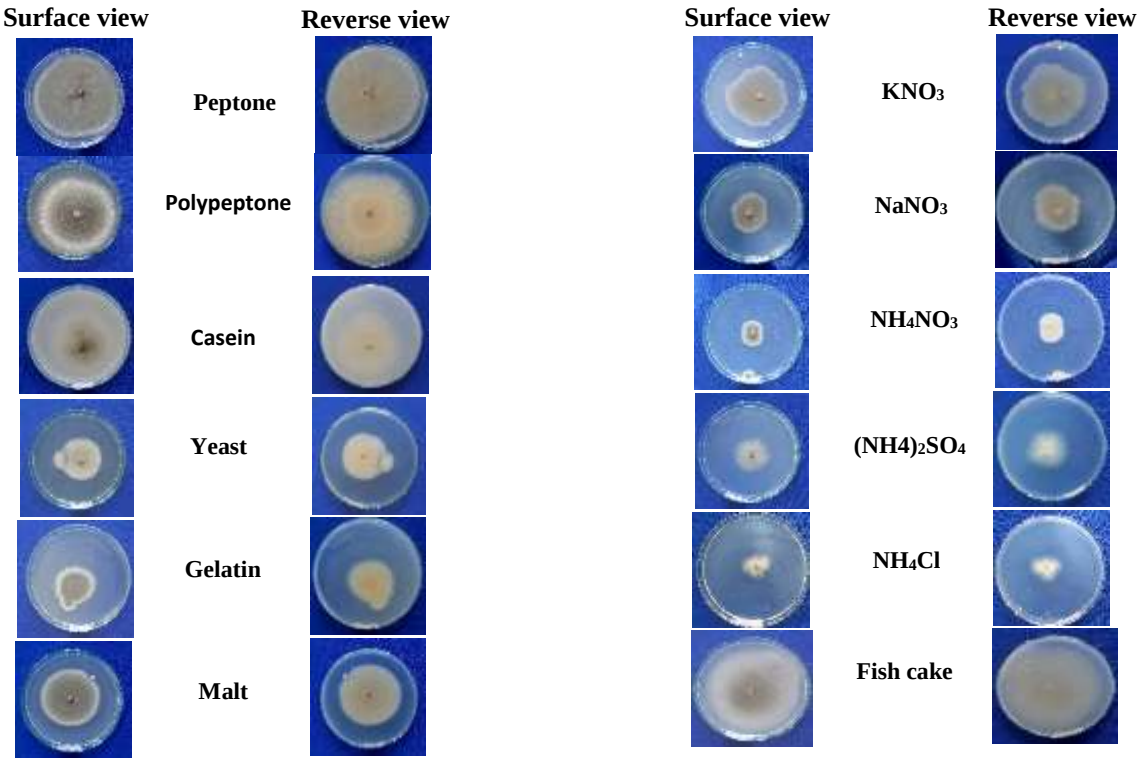

Figure 2 Colony character and growth of MPF-7 on various nitrogen sources

Table 4 The effect of sizes of inoculums of MPF-7 against *E. coli*

Sr. No	Sizes of inoculums (%)	Test organism and Inhibition Zone (mm) *E. coli*
1	5	15.54
2	10	20.79
3	**15**	**22.06**
4	20	21.71
5	25	20.15
6	30	17.12

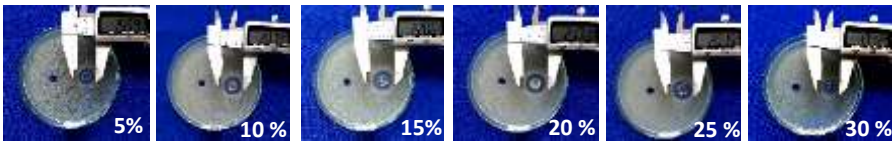

Figure 3 The effect of sizes of inoculums of MPF-7 against *E. coli*

Table 5 The effects of ages of inoculum MPF-7 against *E. coli*

Sr. No	Age of culture (hrs)	Test organism and Inhibition Zone (mm) *E. coli*
1	72	**14.34**
2	84	15.25
3	96	15.53
4	**108**	**18.64**
5	120	16.58
6	132	15.30

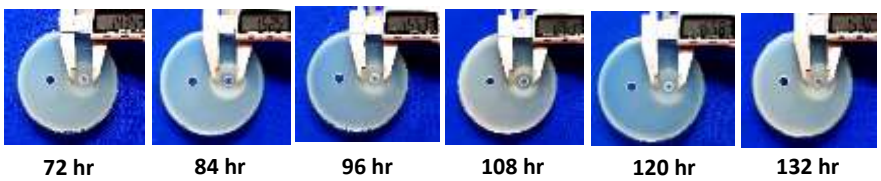

Figure 4 The effect of ages of inoculums of MPF- 7 against *E. coli*

Table 6 Effect of carbon sources on the fermentation of selected MPF- 7 against *E. coli*

Sr. No	Carbon source	Test organism and Inhibition Zone (mm) *E. coli*
1	**Glucose**	**21.05**
2	Fructose	20.50
3	Lactose	20.36
4	Soluble Starch	20.16
5	Sucrose	19.39
6	Xylose	17.99
7	Tapioca	17.54
8	Corn	17.34
9	Potato	15.96

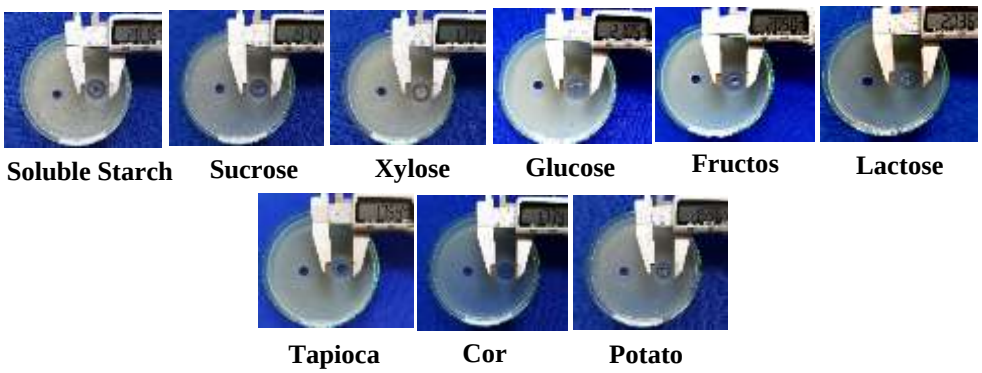

Figure 5 The effect of carbon sources of MPF-7 against *E. coli*

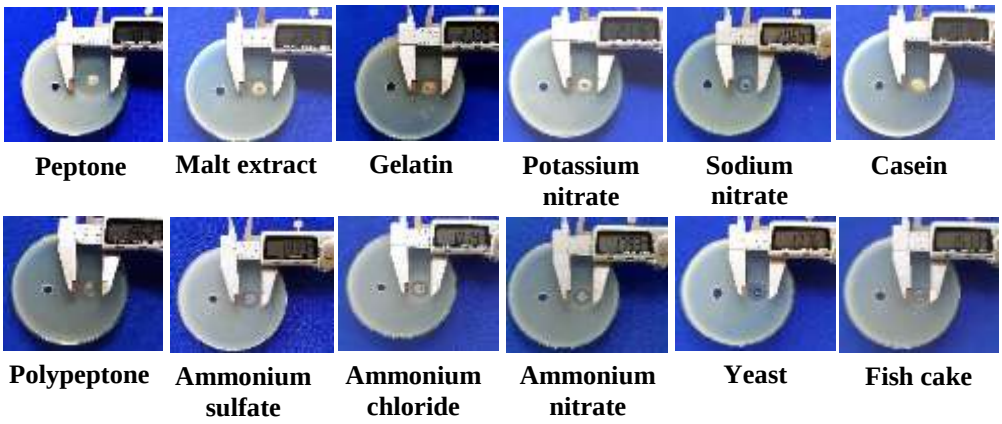

Figure 6 The effect of nitrogen sources of MPF-7 against *E. coli*

Table 7 Effect of nitrogen sources on the fermentation of selected fungi MPF- 7 against *E. coli*

Sr. No	Nitrogen sources	Test organism and Inhibition Zone (mm) *E. coli*
1	**Peptone**	**30.78**
2	Malt extract	22.98
3	Gelatin	21.39
4	Potassium nitrate	21.16
5	Sodium nitrate	20.64
6	Casein	20.18
7	Polypeptone	19.62
8	Ammonium sulfate	19.33
9	Ammonium chloride	18.14
10	Ammonium nitrate	17.93
11	Yeast	17.76
12	Fish cake	15.13

Table 8 Effect of pH on the fermentation of selected fungus MPF-7 against *E. coli*

Sr. No	pH	Test organism and Inhibition Zone (mm) *E. coli*
1	4	20.78
2	5	20.91
3	**6**	**23.36**
4	7	20.53
5	8	20.34
6	9	19.84
7	10	17.90

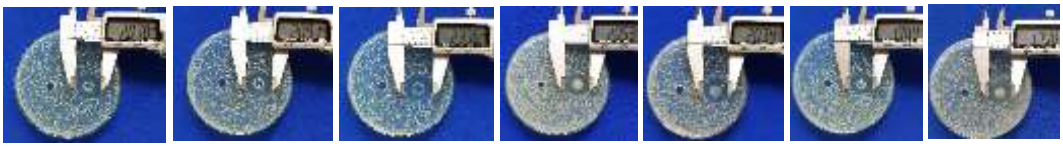

Figure 7 The effect of pH on the fermentation of MPF-7 against *E. coli*

Table 9 Effect of temperature on the fermentation of selected fungus MPF-7 against *E. coli*

Sr. No	Temperature (ºC)	Test organism and Inhibition Zone (mm) *E. coli*
1	20	22.02
2	**25**	**23.18**
3	30	22.81
4	35	18.91
5	40	18.88
6	45	16.22

Figure 8 The effect of temperature on the fermentation of MPF-7 against *E. coli*

Table 10 Effect of agitation on the fermentation of selected fungus MPF-7 against *E. coli*

Sr. No	Agitation	Test organism and Inhibition Zone (mm) *E. coli*
1	**Shaking**	**20.00**
2	Static	17.87

Figure 9 The effect of agitation on the fermentation of MPF-7 against *E. coli*

Discussion and Conclusion

In current research work, the growth effect and optimum fermentation of soil fungus MPF-7 was observed on *E. coli*.

While the effect of sizes of inoculums were considered by using 5%, 10%, 15%, 20 %, 25 % and 30 % inoculums, and the best antibacterial activity (22.06 mm*)* was perceived at 15% seed culture. Consistent with this study my research similar has shown to be similar with the research conducted by Tomita (1988).

In the study of age of inoculum, the best antibacterial activities were gained at 108 hrs. This result campare with Reddy *et al*., 1985 discovered that maximal production of antibiotic substances occurred after 96 hrs.

In this investigation work, the various carbon sources were used for the growth morphology of MPF-7. MPF-7 was excellent growth on glucose,

potato and fructose. Besides the various nitrogen sources were also used and excellent growth was found on peptone, polypeptone, malt, KNO_3 and fish cake.

Furthermore glucose as the carbon source, MPF-7 showed the highest activity (21.05 mm*)* whereas use as nitrogen sources. This results are in agree with (Buchanan *et al*., 1984) and (Calvo *et al.,* 2002). The best results was found in peptone (30.78 mm) on *E. coli*.

El-Tayeb *et al*., 2004; Rizk *et al.,* 2007 have been studied on different aspects of microbial media for example carbon and nitrogen sources, minimal salts, trace elements, vitamins and pH.

And then, maximum antibacterial activity was observed at pH 6 (23.36 mm) on *E. coli*. pH 6.0 is the greatest for the production of antimicrobial metabolite by *A. terreus*. Like result had been stated previous by Nishihara *et al.* (2001) during the production of FR198248, a new anti-influenza agent at pH value among 6.3 to 6.4 from *A. terreus*.

In the current study, the temperature effect, the strong activity was gained at 25ºC (23.18 mm on *E. coli*). Rizk *et al*., 2007 described that physical features such as incubation temperature can apply diverse effects on the growth and production phases of secondary metabolism. Pandey *et al*., 2005 also described that temperature is an important parameter that controls the overall growth and development of the microorganisms.

The fermentation broth was planned at two conditions such as agitation (shaking) and stationary (static) conditions. In the contrast effect of static and shaking culture of MPF-7. The maximum of antibacterial metabolite was detected under shaking condition (20.00 mm) and the static culture (17.87 mm) on *E. coli* singly. Tani *et al*, 2004 described well produce of antibiotics in shake culture fermentation condition.

It can be concluded that the optimal fermentation conditions necessary for further research plan and current detail characterization of bioactive compounds.

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EFFECT OF ETHYLENE ON FEMALE FLOWERS, FRUIT YIELD AND FRUIT QUALITY OF *TRICHOSANTHES CUCUMERINA* L.

Khin Thet Oo*

Abstract

The experiment was conducted at the field of VFRDC (Vegetable and Fruit Research and Development Center) Yemon, Hlegu Township, Yangon Region from June 2018 to September 2018. The Key point of the experiment was the effect of ethylene treatments on female flowers emergence and the fruit production of *Trichosanthes cucumerina* L. (Bon-Lon) plants. The experiment was set up in a randomized complete block design (RCBD) with three treatments (1.5ml, 1.0ml, 0.5ml) and each replicated by four. The control plants received none of ethylene treatments. In the experiment, ethylene solutions were sprayed to the plants in alternate weeks, before flower bud emergence. The ethylene solutions applied two times during the life cycle. The statistical results showed that the plant height of ethylene treated plants were reduced than control plants and also the stem girth, number of nodes, internode length, leaf length and leaf width as well. However, the reverse effect of ethylene was observed in the female flower emergence and the fruit yield. Among treatments, T2 (1.0ml) treated plant had higher female flowers and fruit yield. Regarding to the fruit quality, the most sweetness fruit were obtained from T2 (1.0ml). The experiment showed that T2 (1.0ml) ethylene was the proper dosage for female flower, fruit quality and fruit production in Bon-Lon plants.

Keywords: Effect of Ethylene on female flower emergence and the fruit production of Bon-Lon (various concentrations of ethylene 0.5ml, 1.0ml, 1.5ml)

Introduction

Trichosanthes cucumerina L. (Bon-Lon) is the largest genus of the family Cucurbitaceae. It's commonly called as snake gourd, viper gourd, snake tomato or long tomato. Snake gourd are native to southeast Asia, including India, Myanmar, Indonesia, Sri lanka and neighbouring countries (Website. 1). The fruit is a good source of vitamin A, B and C. It is very rich fiber to keep digestive system it will be useful for aiding constipation. It is used in the treatment of headache, fever, malaria, laxative, bronchitis, diarrhoea and skin allergy (Website. 2). January and July are the best time for snake gourd cultivation. Snake gourd grows very well in hot and warm climate (Website No.3). Ethylene can be naturally produced by any part of a plant, but can also be stimulate from other plant hormones such as auxins, gibberellins, abscisic acid and cytokinins (Yang 1969, Woeste *et al*., 1999). Ethylene can promote flowering, inhibit flowering or change the sex expression of monoecious plants such as Cucurbits (Abeles, 1971). Ethylene is also used to promote female sex expression in Cucumber, to prevent self-polination and in increase yield and to inhibit terminal growth of some plant in order to promote lateral growth and compact flowering stem (Yang, 1969). Ethylene changes sex expression also in unisexual plants, increase female flowers in several members of Cucurbitaceae. It also induces male sterility in cucurbits and wheat (Srivastava, 2012). Ethylene has been used in this liquid form to effect seed germination and bulb sprouting, to retard growth, to induce, promote or delay flowering, to alter sex expression in cucumber. (Abeles *et al*., 1971 and Saltveit, 1998). In general, the number of male flowers is produced more than the female flowers in Cucurbits (Website No.4). Ethylene is a gaseous hormone produced naturally by plants. However, the amount is low. The consequence, fruit production is low (Website No.4). The application of ethylene to seedlings would dramatically change the ratio of the female flowers in members of Cucurbitaceae. Ethylene inhibits linear growth of stem, increases diameter and the number of female flowers (Verma, 2003). The present

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study aimed at generally to compare the effect of different applications of ethylene on vegetative growth, female flowers and fruit yield of Bon-Lon plants.

Materials and Methods

Botanical studies: Plant identification was done by the references such as The Flora of British India (1879), Taxonomy of Vascular Plants (1951), The Classification of Flowering Plants (1952), List of Trees, Shrubs, Herbs and Principal Climbers etc. (1961), Flora of Java Vol. I (1963), The genera of Flowering Plants Vol. II (1967). Flora of Ceylon; Vol. XI (1997).

Source of plant materials and seed germination: The seeds of *Trichosanthes* (Bon-Lon) were collected from VFRDC. Full cheek and vigorous seeds were selected for seed germination. The seeds were soaked in pure water for 4 hours.

Transplanting of seedlings: When the seedlings possessed 2-true leaves were transplanted to prepare the experimental field.

Soil preparation for cultivation: The cultivation field was thoroughly crushed and removed stones, hard soil balls and garbage. The soil was mixed with cow dung (12.5kg/bed) as basal fertilizer.

Preparation of ethylene solutions: The different concentrations of liquid ethylene 1.5ml, 1.0ml, 0.5ml were used in this experiment. Each concentration of ethylene solution was diluted in 1000 ml of distilled water.

Experimental layout: Randomized completely block design (RCBD) with four replicates were used in this experiment. There were four treatments of ethylene solution as control, 1.5 ml, 1.0 ml, 0.5 ml.

Ethylene treatment: When the plants become 30cm height, the plants were sprayed with various concentrations of ethylene by using hand spray. Ethylene solutions were sprayed to the plants, before flower bud emergence in alternate weeks, applied two times during the life cycle.

Fertilizer application: 15 days after cultivation, the compound fertilizer NPK (15:15:15) were applied as 10g / plants.

Weeding: Weeding was done every week by manually.

Pets and Disease control: The fungicide (sinagi), co-oxide and pesticides (neem) were sprayed to the plants when it was necessary.

Data collection and statistical analysis: The data for horticultural characters such as plant height, number of lateral branches, number of male and female flowers, number of fruit/plant and etc. were collected. The single fruit quality such as fruit weight, fruit length, fruit width, flesh thickness, sweetness and acidity were also collected. All record data were statistically analyzed using CROPSTAT software.

Results

The statistically analyzed result of the plant height, stem girth, number of node/plant, inter node length, number of leaf/plant, petiole length, petiole girth, leaf width, leaf length, number of branch were shown in the following figures (1 to 10) and tables (1 to 10). Moreover, the single

fruit characters, mean value of yield and yield components, number of male and female flowers/plant shown in table (11 to 14) and figure (11 to 13).

Table 1 Effect of ethylene solution on plant height in *Trichosanthes cucumerina*

Treatment	Plant height (cm)							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	34.46	41.95	44.57	46.25	49.95	73.50	108.72	57.06
T 2(1.0 ml)	45.63	54.70	67.20	73.90	76.42	106.07	133.33	79.61
T 3(0.5 ml)	42.44	54.55	73.30	76.05	78.95	135.05	155.50	87.98
T4 (control)	54.27	79.30	93.55	111.00	122.20	145.95	164.37	110.09
F-test	ns	*	ns	*	**	**	ns	-
5 % LSD	13.8	22.25	37.9	39.18	35.15	37.5	46.5	-
cv %	19.6	24.1	34.1	31.9	26.8	20.4	20.7	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

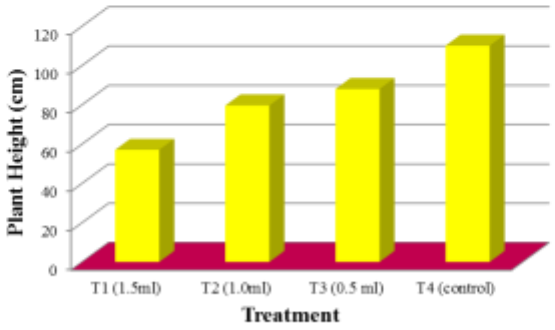

Figure 1 Different doses of ethylene solution on the plant height in *Trichosanthes cucumerina*

Table 2 Effect of ethylene solution on the stem girth in *Trichosanthes cucumerina*

Treatment	Stem girth (cm)							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	3.75	4.13	4.90	5.42	5.60	5.72	5.97	5.07
T 2(1.0 ml)	3.05	3.80	5.07	5.60	5.95	6.15	5.80	5.06
T 3(0.5 ml)	3.40	3.70	5.27	5.65	5.77	5.97	6.17	5.13
T4 (control)	3.45	4.90	5.05	5.32	5.57	6.05	6.40	5.25
F-test	ns	ns	ns	ns	ns	ns	ns	-
5 % LSD	1	0.88	0.66	0.76	0.85	0.86	1.21	-
cv %	18.4	13.4	8.2	8.7	9.3	9.1	12.4	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

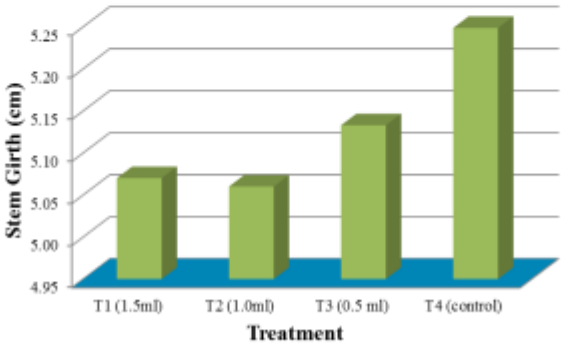

Figure 2 Different doses of ethylene solution on the stem girth in *Trichosanthes cucumerina*

Table 3 Effect of ethylene solution on the number of node/ plant in *Trichosanthes cucumerina*

Treatment	Number of node/ Plant							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	6.90	7.45	7.60	8.00	8.62	10.95	14.67	9.17
T 2(1.0 ml)	8.20	8.75	9.60	10.15	10.90	12.01	19.27	11.27
T 3(0.5 ml)	8.30	8.60	9.80	10.70	10.90	15.25	20.75	12.04
T4 (control)	8.70	11.20	12.45	14.40	15.00	18.50	26.50	15.25
F-test	ns	**	*	*	*	*	**	-
5 % LSD	1.96	1.87	2.91	3.27	3.30	4.96	5.44	-
cv %	15.3	13.0	18.5	18.9	18.2	21.9	16.8	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

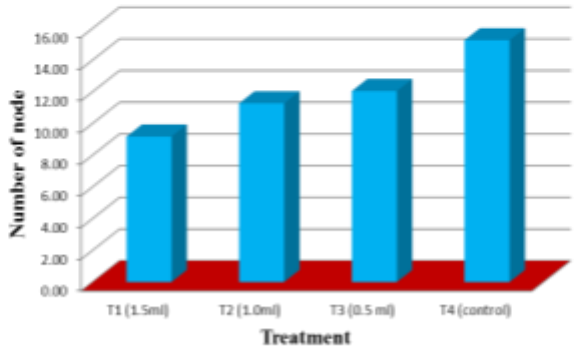

Figure 3 Different doses of ethylene solution on the number of node / plant in *Trichosanthes cucumerina*

Table 4 Effect of ethylene solution on internode length in *Trichosanthes cucumerina*

Treatment	Internode length (cm)							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	4.27	4.55	5.64	5.85	6.16	5.52	8.75	5.82
T 2(1.0 ml)	4.80	5.27	6.23	6.56	7.01	7.60	8.20	6.52
T 3(0.5 ml)	5.52	5.85	6.68	7.60	8.20	8.65	12.62	7.87
T4 (control)	6.27	6.91	7.73	8.45	9.80	10.05	10.75	8.57
F-test	ns	*	ns	*	**	**	ns	-
5 % LSD	1.96	1.6	1.7	1.47	1.64	1.78	4.8	-
cv %	18.3	17.8	16.2	12.9	13.2	13.6	30	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

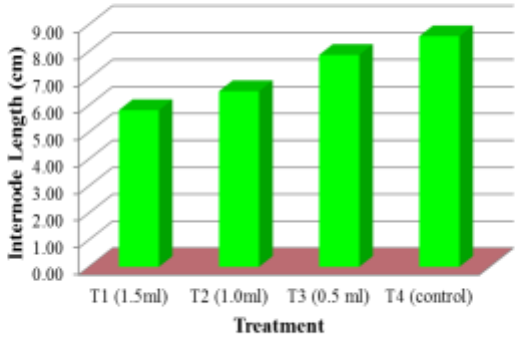

Figure 4 Different doses of ethylene solution on the internode length in *Trichosanthes cucumerina*

Table 5 Effect of ethylene solution on the number of leaf/ plant in *Trichosanthes cucumerina*

Treatment	Number of leaf /plant							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	5.05	5.35	6.43	7.50	8.75	10.35	12.37	7.97
T 2(1.0 ml)	5.75	6.85	10.45	11.75	12.70	17.05	21.67	12.32
T 3(0.5 ml)	6.35	7.25	8.35	10.25	11.95	19.10	22.90	12.31
T4 (control)	6.85	8.05	8.35	9.70	10.70	17.80	24.10	12.22
F-test	ns	ns	ns	ns	ns	ns	*	-
5 % LSD	1.57	2.39	4.58	5.12	5.30	6.89	7.38	-
cv %	16.4	21.8	34.1	32.7	30	26.8	22.8	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

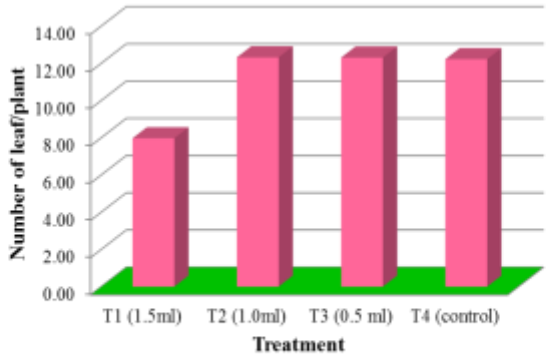

Figure 5 Different doses of ethylene solution on the number of Leaf /plant in *Trichosanthes cucumerina*

Table 6 Effect of ethylene solution on petiole length in *Trichosanthes cucumerina*

Treatment	Petiole length(cm)							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	4.00	4.30	4.73	5.65	6.46	6.87	7.40	5.63
T 2(1.0 ml)	4.16	5.77	6.31	6.83	7.57	8.03	8.52	6.74
T 3(0.5 ml)	5.10	6.00	6.89	7.34	7.07	8.32	9.37	7.16
T4 (control)	3.52	4.94	5.38	5.95	6.46	7.26	8.75	6.04
F-test	ns	ns	*	**	ns	*	ns	-
5 % LSD	1.94	1.66	1.46	0.79	1.22	1.02	1.9	-
cv %	29	19.9	15.8	7.7	10.9	8.4	14	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

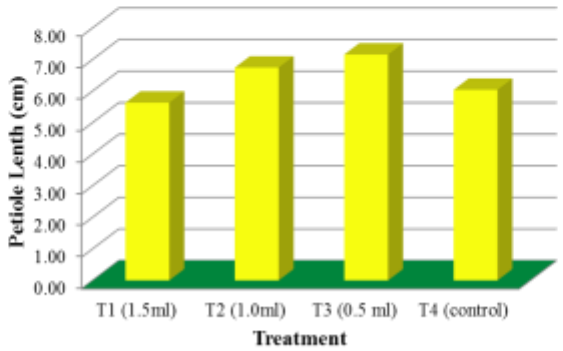

Figure 6 Different doses of ethylene solution on the petiole length in *Trichosanthes cucumerina*

Table 7 Effect of ethylene solution on the petiole girth in *Trichosanthes cucumerina*

Treatment	Petiole girth(cm)							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	2.00	2.27	2.47	2.67	2.75	3.02	3.22	2.63
T 2(1.0 ml)	1.72	2.45	2.80	2.92	3.17	3.37	3.68	2.87
T 3(0.5 ml)	1.97	2.25	2.77	2.95	3.12	3.55	3.70	2.90
T4 (control)	2.25	2.62	3.00	3.50	3.70	3.83	4.06	3.28
F-test	*	*	*	*	*	*	ns	-
5 % LSD	0.33	0.26	0.28	0.52	0.52	0.51	0.63	-
cv %	10.5	6.9	6.4	11	10.3	9.4	10.8	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

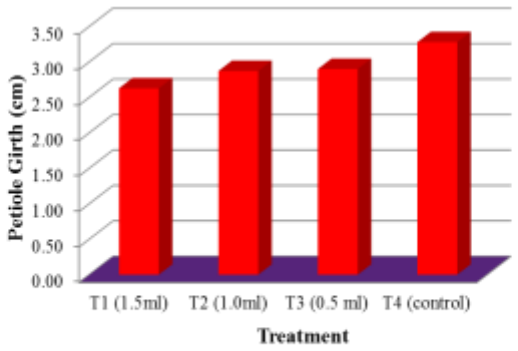

Figure 7 Different doses of ethylene solution on the petiole girth in *Trichosanthes cucumerina*

Table 8 Effect of ethylene solution on the leaf length in *Trichosanthes cucumerina*

Treatment	Leaf length (cm)							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	3.43	3.94	4.50	5.29	5.62	5.95	6.73	5.07
T 2(1.0 ml)	4.40	4.85	5.48	5.97	6.53	7.01	8.15	6.06
T 3(0.5 ml)	5.26	5.63	6.00	6.26	6.67	7.03	8.84	6.53
T4 (control)	5.15	6.05	7.33	7.47	7.91	8.31	8.80	7.29
F-test	*	*	**	**	*	**	ns	-
5 % LSD	1.05	1.32	0.91	0.88	1.34	1.15	2.4	-
cv %	14.5	16.2	9.9	8.9	12.5	10.2	18.5	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

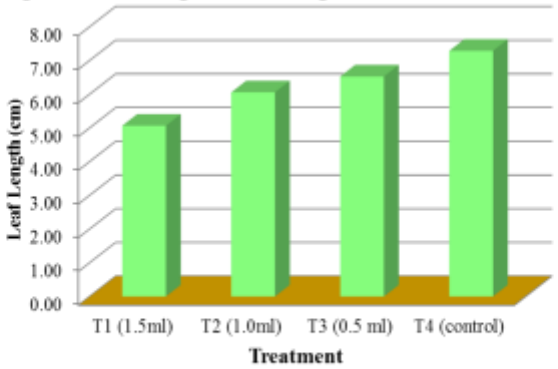

Figure 8 Different doses of ethylene solution on the leaf length in *Trichosanthes cucumerina*

Table 9 Effect of ethylene solution on Leaf width in *Trichosanthes cucumerina*

Treatment	Leaf width (cm)							
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	60 DAS	Mean
T 1(1.5 ml)	4.51	5.52	5.61	6.31	6.60	7.73	8.42	6.39
T 2(1.0 ml)	6.24	6.59	7.36	7.64	7.79	9.37	11.43	8.06
T 3(0.5 ml)	7.08	7.23	7.32	7.59	8.11	9.90	10.75	8.28
T4 (control)	7.30	7.86	8.99	9.38	10.01	11.17	11.30	9.43
F-test	*	*	**	**	**	**	*	-
5 % LSD	1.64	1.5	1.45	1.44	1.16	1.56	2.07	-
cv %	16.4	13.8	12.4	11.7	8.9	10.2	12.4	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

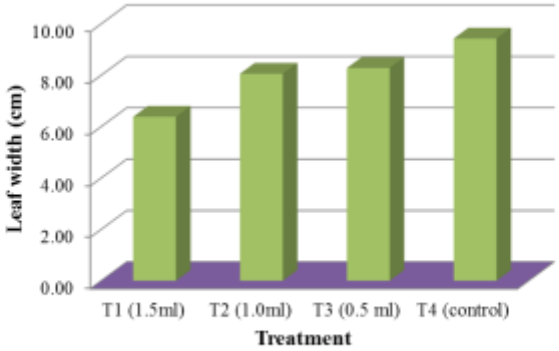

Figure 9 Different doses of ethylene solution on leaf width in *Trichosanthes cucumerina*

Table 10 Effect of ethylene solution on the number of branches /plant

Treatment	Number of branches /plant						
	18 DAS	25 DAS	32 DAS	39 DAS	46 DAS	53 DAS	Mean
T 1(1.5 ml)	0.75	0.87	0.95	1.22	1.47	1.95	0.75
T 2(1.0 ml)	1.25	1.55	1.65	1.90	2.15	2.35	1.25
T 3(0.5 ml)	1.20	1.40	1.65	2.05	2.25	2.55	1.20
T4 (control)	0.80	0.90	0.95	1.25	1.40	1.50	0.80
F-test	ns	ns	ns	ns	ns	ns	-
5 % LSD	0.69	0.77	0.8	0.85	1.03	1.37	-
cv %	43.5	41.0	38.5	33.4	35.6	41.1	-

DAS = days after sowing, ns = non significant *significant P<0.05 **highly significant <0.01

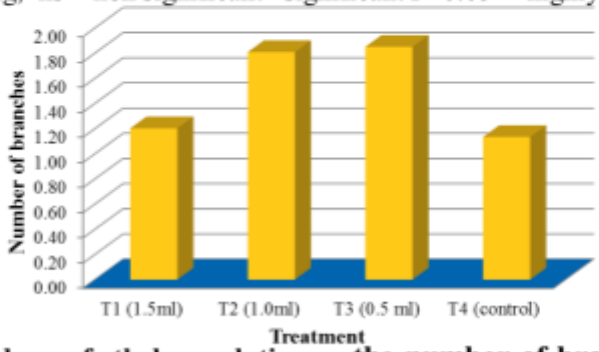

Figure 10 Different doses of ethylene solution on the number of branches /plant in *Trichosnathes cucumerina*

Table 11 A single fruit characters in *Trichosanthes* from different ethylene treatments

Treatment	Fruit length(cm)	Fruit width(cm)	Flesh Thickness(mm)	Sweetness(%)	Acidity(%)
T1 (1.5ml)	56.56	23.43	3.75	3.81	1.68
T2 (1.0ml)	56.87	27.25	4.87	4.37	1.36
T3 (0.5ml)	60.37	33.50	5.37	3.37	1.40
T4 (control)	59.50	29.45	5.12	3.05	1.15
F-test	ns	ns	ns	ns	ns
5 % LSD	21.46	8.45	1.47	1.80	0.65
cv %	23.0	18.6	19.3	30.9	29.4

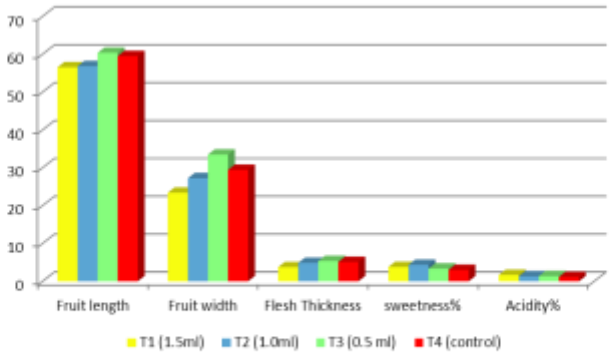

Figure 11 A single fruit characters in *Trichosanthes* from different ethylene treatments

Table 12 Number of fruit/plants and seed/fruit of *Trichosanthes cucumerina* L.

Treatment	Number of fruit/plants	Number of seed/fruit
T1 (1.5ml)	1.5	13
T2 (1.0ml)	3.5	18
T3 (0.5 ml)	2.25	13.5
T4 (control)	2.75	18.5
F-test	ns	ns
5 % LSD	1.92	12.65
cv %	48.1	50.2

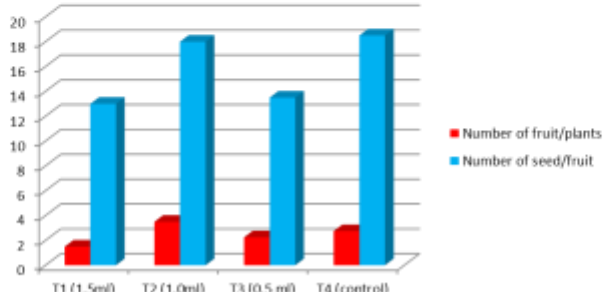

Figure 12 Number of fruit/plants and seed/fruit of *Trichosanthes cucumerina* L.

Table 13 Mean values of Yield components of *Trichosanthes cucumerina* L.

Treatment	Fruit weight(g)	Total seed weight(g)	10,seed weight(g)
T1 (1.5ml)	129.13	4.67	3.47
T2 (1.0ml)	175.87	6.12	3.37
T3 (0.5 ml)	273.17	4.57	3.45
T4 (control)	197.02	3.95	2.25
F-test	ns	ns	*
5 % LSD	132.76	3.75	1
cv %	42.8	48.6	20.1

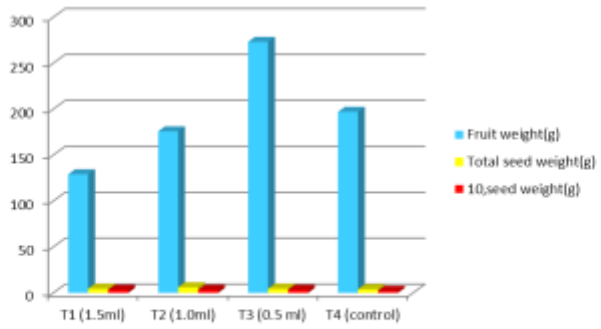

Figure 13 Mean values of Yield components of *Trichosanthes cucumerina* L.

Table 14. Effect of ethylene solution on the number of male and female flower/plant in *Trichosanthes cucumerina*

Treatment	Number of male flower/plant	Number of female flower/plant
T1 (1.5ml)	7.0	4.8
T2 (1.0ml)	5.8	10.0
T3 (0.5 ml)	4.0	4.4
T4 (control)	55.4	7.4

Discussion and Conclusion

In this study, three treatments of ethylene were tested on *Trichosanthes cucumerina* (Bon-Lon) plants in order to determine their effects on female flowers, fruit yield and fruit quality. The control plants did not receive any treatments except regular watering. The result showed that the control plant (T4) were increased in plant height, internode length, stem girth, number of node/plant, petiole girth, leaf length and width than the treatments of ethylene solution. This observation was in agreement with Pratt *et al*., (1969) who mentioned that ethylene is a natural growth regulator has been implicated in several developmental processes of plants. Moreover, Verma (2003) agreed that ethylene inhibited linear stem growth but increased in stem diameter and number of female flower. Saltveit (1998) mentioned that ethylene has been used in this liquid form to effect seed germination and bulb sprouting, to retard growth. The present study examined plant height, number of node, internode length, number of leaf/plant, petiole length and girth, leaf length and width, number of branch/plant have reduced at high concentration of ethylene solution (1.5ml) T1. Abeles (1971) stated that the presence of high level of ethylene prevents the inhibition of internode elongation and leaf expression play a primary role. Ethylene treatments showed that reduced the vegetative growth than control plants. These observation was in agreement with Verma (2003) reported that ethylene has the role in increasing in stem girth. Salisbury and Ross (1992) stated that the ethylene inhibited stem elongation, increased in stem diameter and horizontal growth habit. Ethylene has ability to alter sex expression in number of cucurbits (Warner *et al*., 1969). The statistical analysis of the present study revealed that female flowers were significantly increased in ethylene concentration at (1.0ml) T2 than (control) T4. This result was in agreement to Srivastava (2012) Saltveit (1998) reported that ethylene changes sex expression in unisexual plants. It increases female flowers in several members of cucurbitaceae. In the present study, T2 (1.0ml) treatment of plants gave the highest number of female flowers. Abeles (1971) reported that ethylene can promote flowering, inhibit flowering or change the sex expression of monoecious plants such as cucurbits.T2 (1.0ml) treated plants in all treatments also gave the maximum yield in this investigation.T2 (1.0ml) was the sweetest in all treatments, when compared to the sweetness of fruits.

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MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF *PHYSALIS MINIMA* L.

Myint Myint San*

Abstract

In this paper, the morphology and histological characters of *Physalis minima* L. have been carried to understand its cell structure and medicinal values. A medicinal plant of *Physalis minima* L. belonged to the family Solanaceae. It was commonly known as Bauk-pin or sunberry in Myanmar, Country gooseberry in English. This plant was collected from Tharketa Township, Yangon Region during the flowering and fruiting period. Then, the collected specimen was classified and identified to confirm the morphological and histological characters with the help of available literatures. In the study of morphological characters, the plant was herb, leaves alternate, cymose inflorescence, the flowers cream colour; bisexual; pentamerous; hypogynous, fruits globose enclosed by the calyx and seeds endospermic. In the anatomical study, the anisocytic type of stomata was present on both surfaces of leaves. The mesophyll cells were made up of palisade and spongy mesophyll cells in lamina. In the transverse section of midrib, it was nearly circular in outline. The vascular bundles were collateral type, tanniferous cells, prismatic crystals found in the parenchyma cells and unicellular hairs present on the epidermis. In transverse section of petiole, it was semicircular in outline, crescent shaped vascular bundles and unicellular hairs present on the epidermis. In transverse section of stem, more or less circular or irregularly quadrangular in outline, vascular bundles were collateral type, unicellular hairs present on the epidermis. In transverse section of roots, circular in outline, the cortex made up of parenchymatous cells and the vascular bundles were pentarch. In addition, the microscopical characters of powdered of the plant were also investigated for their standarization used in medicine. According to the result, vessels, tracheids, fibers, unicellular hairs and calcium oxalate crystals were observed in the powder sample.

Keywords: morphological and histological characters

Introduction

Many varieties of medicinal plants were rich in Myanmar. These plants were widely distributed in different climate zones. These were 12,000 different plants growing in Myanmar and most of them have been regarded as medicinal plants (Kress, 2003).

The medicinal plant of *Physalis minima* L. belonged to the family Solanceae. It is commonly known as Bauk-pin or sunberry in Myanmar, Country gooseberry in English. (Hundley and Chit Ko Ko, 1961; San Khin, 1970 and Kress, 2003).

The Solanceae family occurred throughout the world, especially in tropical and temperate regions. This family included 90 genera and between 2000 and 3000 species (Backer, 1963; Dassanayake, 1987). The genus *Physalis* contained 45 species mostly in the warmer part of North and South America (Rendle, 1967); 50 species found in cosmopolitan (Kirtikar & Basu, 1935) and 30 species distributed throughout India, in the tropical region common in tropical Asia, Africa and Australia (Hooker, 1885).

Physalis minima L. has grown in arable lands, dry rice-fields, gardens and waste places. Its edible fruits have vitamin C which was a good quality for antioxidant activities (Backer, 1963; https://indiabiodiversity.org. and https://en.m.wikipedia.org).

Physalis minima L. used as diuretic, laxative, expectorant, appetizing and tonic, burning sensation, colic, ulcers, cough bronchitis, pruritus, erysipelas and ingredient of the medicinal oil which is given for spleen. Fruits taste like cherry tomato and used as tonic, diuretic, relieve pain

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analgesic and anti-inflammatory. The calyx was toxic and should not be eaten. Extracts from the plant have shown anticancer activity. The juice of the leaves mixed with mustard oil and water has been used as a remedy for earache (Kirtikar & Basu, 1935; Chopra, 1956; Prajapati, 2003; https://pfaf.org>user>plant and https://hort.purdue.edu).

The ethanolic extract of the whole plant was devoid of antibacterial, antiprotozoal, anthelmintic, antiviral, hypoglycaemic, respiratory and anticancer activities (Medicinal plants of India, 1987).

The medicinal plants were used throughout the world and the regulations defining their proper use such as identification of the correct species and verification of the presence, purity and concentration of the required chemical compounds were widely recognized (https://ncbi.nlm.nih.gov).

Most plants were classified based on the morphological characters of plants, flowers and fruits. These characters were not enough to classify. Therefore, in the classification of plant need to be involved the anatomical studied especially of leaves (Jibril & Jakada, 2016).

The aims and objectives of this research were to identify the morphological and microscopical characters of *Physalis minima* L., to study the sensory characters of powdered of this plant for standardization, to understand its medicinal values and to improve the wide application of traditional medicine.

Materials and Methods

Collection and Identification of *Physalis minima* L.

The specimens were collected from wild areas of Thaketa Township, Yangon Division during the flowering and fruiting period, October - December, 2018. After collection, the identification of the morphological and histological characters was carried out at the Department of Botany, East Yangon University. Then, the collected plants of vegetative and reproductive parts were recorded with photo images. The specimens were identified with the help of available literatures such as Hooker, 1885; Kirtikar & Basu, 1935; Chopra, 1956; Hundley & Chit Ko Ko, 1961; Backer, 1963; San Khin, 1970; Dassanayake, 1987; Kress, 2003 and Prajapati, 2003.

Histological characters of *Physalis minima* L.

In the microscopical study, free hand sections of fresh specimens were prepared by using the razor blade and examined by the help of microscope. The sections of each sample were cleared by with chloralhydrate solution. Then, the powdered samples were examined for the diagnostic characters and used in standardization of plant material for medicinal purposes. The microscopical characters of leaves, stems, roots were examined and confirmed by the available literatures of Medcalfe & Chalk, 1950; Pandey, 1998 and Trease & Evans, 2002.

Results

Morphological characters of *Physalis minima* L.

Scientific Name - *Physalis minima* L.

Myanmar Name - Bauk-pin, sunberry

English Name - Country gooseberry

Family - Solanaceae

The plant is herb; stem with angular-ribbed above, solid with narrow cavity, erect and branches. Leaves are simple, alternate, with hairs, ovate-oblong, the tip acuminate, the margin shallowly repand-dentate, tapering at the base, deciduous, petiolate, stipulate. Inflorescences are cymose, terminal and axillary. Flowers are cream colour, bracteates, ebracteolate, solitary, nodding, bisexual, complete, actinomorphic, pentamerous, hypogynous; sepals (5), synsepalous, campanulate, valvate, sepaloid, persistent, inferior; petals (5), synpetalous, with a brown spot, hairs on the margin, corolla tube widely campanulate, valvate, petaloid (cream colour), inferior; stamens 5, epipetalous, alternating with the petals, anthers dithecous, basifixed, dehiscence by apical pores;carpel (2), bicarpellary, syncarpous, bilocular, many ovules in each locule; style filiform; stigma capitates; ovary superior. Fruits are berry, pendulous, globose, enclosed by calyx. Seeds are numerous, compressed and endospermic.

Microscopical characters of *Physalis minima* L.

Lamina

In surface view, the epidermal cells of both surfaces were parenchymatous and thin-walled. The cell walls of upper surfaces were slightly wavy and lower surfaces were more wavy. Stomata were present on both surfaces and more abundant in the lower surface. They were anisocytic type, oval in outline. The guard cells were reniform in shape and contained abundant chloroplasts.

In transverse section of leaves, the cuticle layers of the upper and lower surfaces were thick and slightly wavy. The upper and lower epidermal cells were rectangular to barrel in shape. The dorsiventral structure of mesophyll layer made up of palisade and spongy parenchyma cells. The palisade mesophyll cells made up of one layer. They were vertically elongated cylindrical cells which were closely packed with one another and contained numerous chloroplasts. The spongy mesophyll cells made up of 2-3 layers which were irregular in shape, thin wall and loosely arranged with intercellular spaces. They were contained numerous chloroplasts.

The vascular bundle of lateral veins embedded in the mesophyll layers. It consisted of xylem lies towards the upper and phloem towards the lower, these were arrangement collateral type. The xylem tissues consisted of vessels and tracheids. Phloem tissues composed of sieve tube elements, companion cells, phloem fibers and phloem parenchyma. Phloem cells were very small.

Midrib

In surface view of the midrib, the epidermal cells of both surfaces were rectangular in shape. Unicellular hairs and calcium oxalate crystals were present on the surface.

In transverse section of the midrib, it was nearly circular in outline. The cuticle layer was thin and slightly wavy. The upper and lower epidermal cells were rectangular to barrel shaped. Unicellular hairs were present on the upper and lower epidermal cells. The cortex was made up of collenchyma cells and thin-walled parenchymatous cells. Collenchyma cells were beneath the upper epidermal cells and above the lower epidermal cells, 2-3 layers. They were oval to rounded in shape. The upper side and lower side of the parenchymatous cells were 4-6 layers. They were thin-walled and rounded to polygonal in shape.

Vascular bundles were collateral type and crescent-shaped. Xylem strands were made up of 2-4 cells in each row. Xylem cells were towards the upper surface. They were hexagonal in shape. Xylem consisted of vessels and tracheids. Phloem cells made up of 2-4 layers. Phloem tissues consisted of sieve tube elements, companion cells and phloem fibers.

Petiole

In surface view of petiole, the epidermal cells of both surfaces were thin-walled, parenchymatous and rectangular to polygonal in shape and unicellular hairs and calcium oxalate crystals were present.

In transverse section of petiole, it was semicircular in outline. The cuticle layer was thick and slightly wavy. The epidermal cells were rectangular to barrel in shape. The cortex was made up of collenchyma cells and thin-walled parenchymatous cells. The collenchyma cells were beneath the upper and lower epidermal cells, 2-3 layers. They were oval to rounded shape. The upper sides of the parenchyma cells was 7-9 layers and lower one were 5-7 layers. They were thin walled and rounded to polygonal in shape. Calcium oxalate crystals were present in the parenchyma cells.

The vascular bundles were collateral type and crescent-shaped. The vascular bundles embedded in the parenchyma cells. Xylem strands were made up of 3-5 cells in each row. Xylem cells were towards the upper surface and hexagonal in shape. Xylem composed of vessels, tracheids and xylem fibers. Phloem cells made up of 2-4 layers. Phloem tissues consisted of sieve tube, companion cells, phloem parenchyma and phloem fibers.

Stem

In surface view, the epidermal cells were parenchymatous, thin-walled and rectangular to polygonal shape, elongated lengthwise. Unicellular hairs and stomata were present on the surface. Stomata were anisocytic type.

In transverse section of primary stem, it was more or less circular or irregularly quadrangular in outline with 4-prominent ridges. The cuticle layers thick and slightly wavy. The epidermal cells were parenchymatous, rectangular to barrel-shaped. Unicellular hairs were present on the epidermis. The cortex was made up of outer collenchymatous and inner parenchymatous cells, the collenchymatous cells toward the peripheral region and parenchymatous cells toward the inner region. The collenchymatous cells consisted of 2 - 4 layers. They were oval to rounded in shape. The parenchymatous cells consisted of 3-5 layers. They were rounded to polygonal in shape. Pith parenchymatous cells consisted of 9-12 layers, irregularly rounded to polygonal in shape. In mature stem, the cells of pith were broken and disorganized leaving a hollow pith cavity in the center. The endodermis was made up of one layer, barrel shaped with starch sheath. Prismatic calcium oxalate crystals were present in the cortex and pith.

The vascular bundles were circular ring and 7-10 numbers. They were collateral type. The bundles were embedded in the parenchymatous tissues. The sclerenchymatous bundle caps were 3 - 5 layers and compactly, thick-walled, rounded to polygonal in shape. Xylem consisted of pitted and scalariform vessels, tracheids and fibres. The xylem cells were hexagonal in shape. The phloem tissues consisted of sieve tube elements and companion cells. Phloem tissues were 2 - 4 layers. Phloem cells were compactly arranged.

Roots

In surface view, epidermal cells were compactly and rectangular in shape. In transverse section of root, it was circular in outline. The epidermis was single layer and rectangular to polygonal in shape. Root hairs were present on the epidermis.

The exodermis was 1-2 layers. They were rounded in shape. The cortex was below the exodermis layer. The cortical cells made up of parenchymatous cells. They were rounded to polygonal in shape and 3 - 5 layers. Tanins were present in the cortical cells. Endodermis was

single layer. They were barrel shaped and parenchymatous. Pericycle was 2 - 3 layers. Pith present in the center of the root.

The vascular bundles were pentarch. Xylem strands consisted of 2 - 3 cells. Xylem composed of pitted and scalariform vessels, pitted, annular and spiral tracheids and fibres. Phloem tissues composed of sieve tubes and companion cells.

Diagnostic characters of the powdered *Physalis minima* L.

The powdered of *Physalis minima* L. was yellowish green colour and slightly aromatic, slightly bitter taste and fibrous in texture as shown in table (1).

In the investigation of powder sample, the epidermal cells, unicellular hairs, vessels, tracheids, fibres and calcium oxalate crystals were observed. The epidermal cells were parenchymatous, thin walled and wavy in surface view. Covering unicellular hairs were present. The vessels were simple pitted and scalariform with perforation plate. Tracheids were thick walled. Fibres were thick walled and the end walls tapering acute. Prismatic crystals were present.

Table 1 Sensory characters of powdered of *Physalis minima* L.

No.	Sensory character	Sample
1.	Colour	Yellowish green
2.	Odour	aromatic
3.	Taste	Slightly bitter
4.	Texture	Fibrous

Morphological Characters of *Physalis minima* L.

Figure 1 Habit of the plant

Figure 2 Inflorescences

Figure 3 Leaves

Figure 4 flowers

Figure 5 L.S of flower

Figure 6 T.S of ovary

Figure 7 Fruits

Figure 8 Seeds

Microscopical Characters of *Physalis minima* L.

Figure 9 Surface view of upper epidermal cell (x 100)

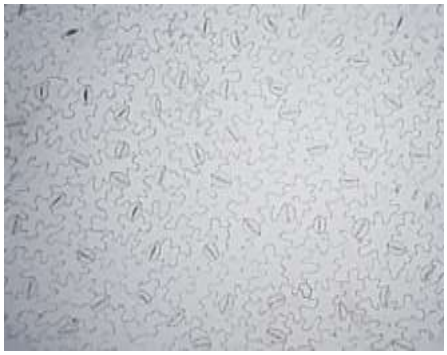

Figure10 Surface view of lower epidermal cells (x 100)

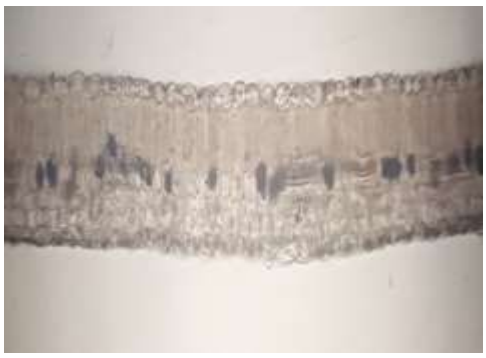

Figure11 T.S of lamina (x 100)

Figure 12 Surface view of (x 100)

Figure 13 T.S of midrib midrib (x100)

Figure 14 Surface view of petiole (x 100)

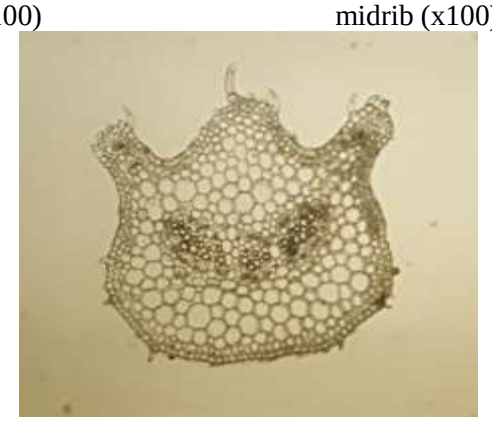

Figure 15 T.S of petiole (x 100)

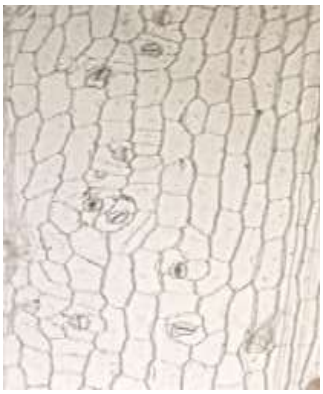

Figure 16 Surface view of stem (x 100)

Figure 17 T.S of young stem (x 100)

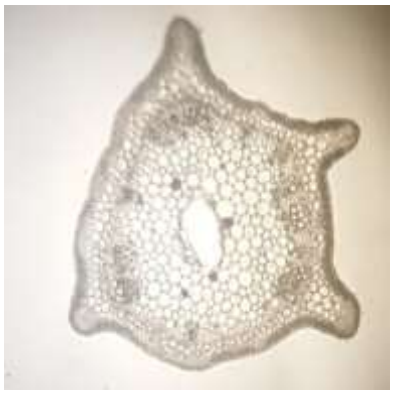

Figure 18 T.S of mature stem (x 100)

Figure 19 Surface view of root (x 100)

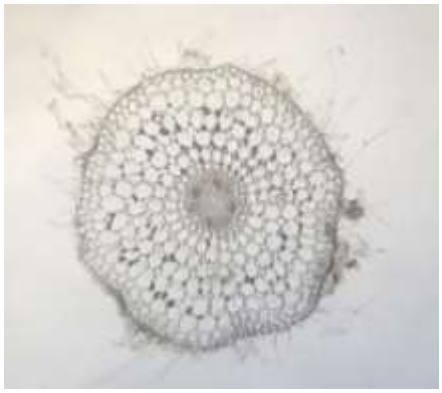

Figure 20 T.S of young root (x 400)

Diagnostic characters of powdered of *Physalis minima* L.

Figure21 powder of the plant

Figure 22 vessels (x 40)

Figure 23 Vessel (x 100)

Figure 24 Tracheid (x 400)

Figure 25 Tracheid (x 100)

Figure 26 Tracheids (x 40)

Figure 27 Fiber (x 40)

Figure 28 Crystals (x 100)

Figure 29 Hairs (x 100)

Discussion

In this research, the morphological characters and histological characters of *Physalis minima* L. have been described.

Physalis minima L. belonged to the family Solanceae. It was commonly known as Bauk-pin, sunberry in Myanmar, Country gooseberry in English. These characters were agreement with those described by Hundley and Chit Ko Ko, 1961; San Khin, 1970 and Kress, 2003.

Physalis minima L. was annual herb, stem with angular-ribbed. Leaves were simple, alternate, shallowly toothed margin, pubescent, petiolate. Flowers were cream colour, solitary in the axil, pedicels filiform, nodding; sepals (5), synsepalous, campanulate, valvate; petals (5), with a brown spot, hairs on the margin; stamens 5, epipetalous, alternating with the petals, anthers dithecous, basifixed, dehiscence by apical pores; ovary ovoid, style glabrous. Fruits were berry, pendulous, globose, entirely enveloped in the enlarged calyx. Seeds were numerous and discoid. These characters were agreement with those mentioned by Hooker, 1885; Kirtikar & Basu, 1935; *Backer, 1963;* Dassanayake, 1987; Prajapati, 2003 and https://indiabiodiversity.org.

Stem of the Physalis minima L. *has angular ribbed, subterete below solid with narrow cavity. Stem, petiole and pedicel cover with hairs.* These were agreement with those described by *Backer, 1963.*

In the microscopical study of *Physalis minima* L., stomata were present on both surfaces, more abundant and more wavy in the lower surface. They were anisocytic, oval in outline. The guard cells were reniform in shape and contain abundant chloroplasts. In transverse section of leaf, the dorsiventral structure of mesophyll layer made up of 1- layer of palisade mesophyll cells and 2-3 layers of spongy mesophyll cells. In transverse section of the midrib, it was semicircular in outline. The cortex was made up of collenchymas cells and parenchyma cells. Vascular bundles of lamina, midrib and petiole were collateral type and crescent-shaped in midrib and petiole. Unicellular hairs present on the epidermis of midrib and petiole. The transverse section of primary stems was more or less circular or irregularly quadrangular in outline with 4-prominent ridges. Unicellular hairs were present. Pith parenchymatous cells consisted of 9-12 layers, irregularly rounded to polygonal in shape. In mature stem, the cells of pith were broken and disorganized leaving a hollow pith cavity in the center. The vascular bundles were circular ring and collateral type. In transverse section of roots were circular in outline, epiblema cells were compactly and rectangular in shape. The vascular bundles were pentarch.

Pitted vessels, scalariform vessels, tracheids, fibers, fiber-tracheids, anisocystic stomata and calcium oxalate crystals were observed in powdered of the plant. These characters were agreement with those mentioned by Metcalf and Chalk, 1950 and Trease and Evans, 2002.

Conclusion

The classification of the plant based on the morphological and histological characters. The research will support to the traditional use of the plant for the treatment of various diseases. The extracts of the *Physalis minima* L. should be further study in phytochemical constituents and antimicrobial activity for medicinal purpose.

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AGRONOMICAL CHARACTERS OF *Triticum aestivum* L. CULTIVAR KYONE PHYU FROM SIX DIFFERENT COLLECTION SITES

Thida Oo[1] Soe Soe Hlaing[2], Tin Tin Maw[3]

Abstract

Wheat is grown on more land area than any other commercial crop and continues to be the most important food grain source for human. *Triticum aestivum* L. cultivar Kyone Phyu have been cultivated in Myanmar as a source of bread wheat since 1890. Spike morphology, seed fertility of these varieties were also studied in an attempt to determine the relationship between external appearances. Cultivars Kyone Phyu from six different collection sites such as Sintkaing, Wundwin, Sagaing, Chaung Oo, Monywa and Pindaya Township were collected and studied their morphological and yield characters. However, environmental factors, the degree day temperature, rainfall and relative humidity were also strongly affected on the formation of branching spikelets. It was observed that the above mentioned characters were varied among the samples collected from six growing different places.

Keywords: Morphological, yield, *Triticum aestivum*

Introduction

Wheat is a major diet component because of the wheat plant's agronomic adaptability, ease of grain storage and ease of converting grain into flour for making edible, interesting and satisfying foods. The cultivation of wheat (*Triticum* spp.) reaches far back into history. Nowadays, more than 200 cultivars of hexaploid wheat (i.e. including, pure line, hybrid line, induced mutation line, introducing cultivars from many research center (especially from CIMMYT), various selection line from various sources were cultivated on many sown acres of Myanmar Land. Spike morphology and yield characters were also studied in an attempt to find out the relationship between yield characters and their genetic resources. Kalsikes and Lec (1972) stated that genotype and environmental conditions are also important for wheat and triticale as it is in other crops, to other cultivated cereals, Sears (1956) and to all cultivated from species Shigenaga (1987). Several studies have been made by Coutinho (1936), Camara (1944), Riley *et al*., (1958), Tsunewaki (1963), Upadhya and Swaminathan (1963), Morris and Sears (1967), and Larsen and Kimber (1973). Although many papers concerning about the agronomy, yield characters, disease and pest resistant, biochemistry and adaptability were available, the information concerning with genetic resources of each and individual cultivars or varieties that grown in Myanmar were still far left behind. By knowing this information, it can be explored from every point of view to improve the wheat cultivars with desired characteristics. In the present study, the information of where wheat grown, the cultivars that the farmers chosen depending on their growing field and its environmental conditions. The yield characters that changes according to their growing land were also investigated.

Materials and Methods

Materials The hexaploid wheat cultivars namely Kyone Phyu, (cultivated in most parts of the wheat growing regions as commercial crops) were collected from six various parts of the Upper Myanmar wheat growing regions. The information concerning with the materials used were described in Table: 1 respectively.

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[2] Dr, Associate Professor, Department of Botany, Kalay University
[3] Dr, Associate Professor, Department of Botany, Taung Gote Degree College

Table 1 ***Triticum aestivum*** **L. cultivar Kyone Phyu growing regions in upper Myanmar and cultivars that growing in that region**

No.	State (Division)	Township cultivated	Name of cultivars	Purpose of cultivation	Ploidy level	Genome constitution
1.	Mandalay Division	Sintkaing Township	Kyone Phyu	Commercially	6x	AABBDD
2.		Wundwin Township	Kyone Phyu	Commercially	6x	AABBDD
3.	Sagaing Division	Sagaing Township	Kyone Phyu	Commercially	6x	AABBDD
4.		Chaung Oo Township	Kyone Phyu	Commercially	6x	AABBDD
5.		Monywa Township	Kyone Phyu	Commercially	6x	AABBDD
6.	Southern Shan State	Pindaya Township	Kyone Phyu	Commercially	6x	AABBDD

Methods

Samples and data of wheat that studied were collected from government experimental farms and from farmers in the field. Samples were collected from Southern Shan State, Mandalay Division and Sagaing Division. Spikes and seeds were collected for further study. Their morphological characters and seed characters were recorded with photographs.

Studies on spike morphology and yield characters

When the plants were fully matured, thirty spikes from each of the cultivar collected randomly from studying the spike characters as well as yield characters. Thirty spikes for each cultivar Kyone Phyu from different collection sites were examined. Spike length, number of spikelets per spike, density of spikelets, number of florets per spike, fertility of first and second florets (gene control character) number of seeds per spike, number of tiller per plants were measured and recorded.

Statistical analysis

Equal student ‘t’ test that stated by Steel and Torrie (1960) was used to compare the differences of the yield characters studied in this research works.

Results

Morphological characters of *Triticum aestivum* L. cultivar Kyone Phyu

Plant 20-90cm tall, forming (2-6 seminal roots and many secondary roots), often strongly tillering (up to 8 tillers per plant, depending on cultivar and environment, but normally 2-5). Stem smooth. Leaf blade long and 2-3cm wide glabrous or pubescent. Spike 4-15cm long. Caryopsis ventrally with a central groove, reddish brown, yellow, white or intermediate hues. Seed color is white creamy color (Plate: 1).

Plant characters

Most plants (i.e. Kyone Phyu) cultivated in various parts of the studied areas, exhibited healthy plants. The leaf characters showed somewhat waxy, (the characters of rye) which is

resistant to leaf transpiration and give rust resistant. The Kyone Phyu wheat grown in Monywa township have been observed that it have moderately sensitive (i.e. susceptible to) leaf rust.

Cultivar Kyone Phyu from Pindaya have strong resistance to leaf and stem rust compare to Kyone Phyu from the other sites. Although this cultivar was less interest by the farmers in Mandalay and Sagaing division farmers. It was widely cultivated in Southern Shan State both in Southern and in Northern. Because of its stiff awn characters, it can defend from the birds (Plate: 2, 3).

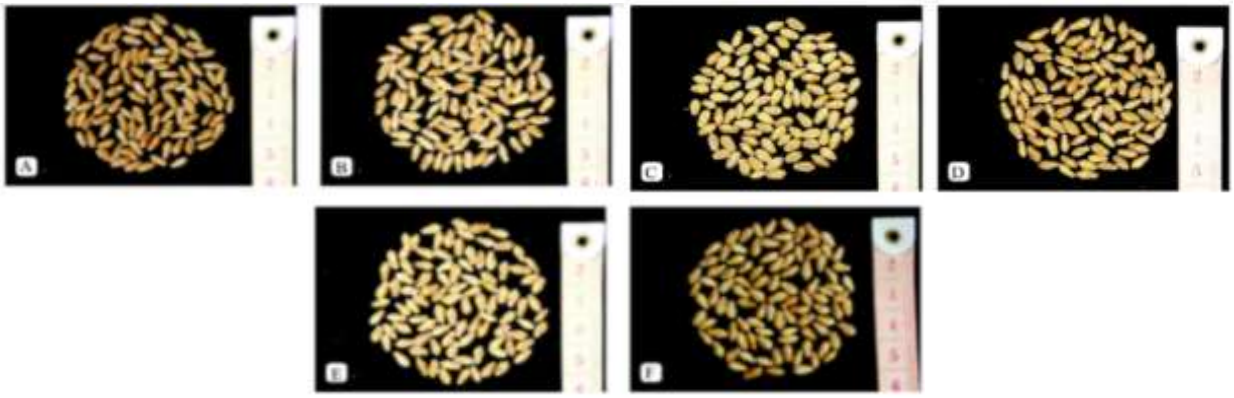

Plate 1 Seeds character of *Triticum aestivum* L. cultivar Kyone Phyu A. Sintkaing; B. Wundwin C. Sagaing; D. Pindaya E. Chaung Oo; F. Monywa

Originally, Kyone Phyu is almost awnless cultivars. After planting for more than a century in Myanmar, some possessed awnless, some with short awn and some exhibited moderately awn. The seeds obtained from six various wheat growing region are grown in Men Chumary II of Mandalay University Campus field. The morphological characters exhibited that some are similar to Kyone Phyu some little showed compactoid spike character and some exhitited similar to triticale (hybrid of wheat and rye also known as first man made cereal) spikes.

For the seed characters, all the seeds obtained from the present research showed that they are all like the wheat seed characters i.e. short and plump, colouring from white to moderately brown. Kyone Phyu possess white to creamy white in seed colour (Plate: 1).

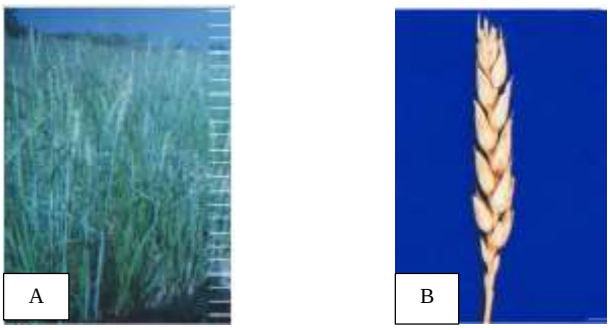

Plate 2 A. Habit of *Triticum aestivum* L. Cultivar Kyone Phyu from Sintkaing
B. Spike of cultivar Kyone Phyu from Wundwin

Spike Characters

Spike length

Pindaya Kyone Phyu resulted the longest spike length among the studied hexaploid cultivar Kyone Phyu while Monywa Kyone Phyu have the shortest length (Fig: 1).

Plate 3 A. Habit of Cultivar Kyone Phyu from Sagaing B. Spike of Cultivar Kyone Phyu from Pindaya C. Habit of cultivar Kyone Phyu from Chaung Oo D. Spike of cultivar Kyone Phyu from Monywa

When tested with 't' test for each collecting sites, comparison between Sintkaing and Wundwin Kyone Phyu, Sintkaing and Sagaing Kyone Phyu, Sintkaing and Chaung Oo Kyone Phyu, Wundwin and Sagaing Kyone Phyu and Pindaya and Chaung Oo Kyone Phyu showed no significant differences (Table: 2). Similarly student 't' test with among the cultivars that Sintkaing and Pindaya, Wundwin and Chaung Oo, all the later two have significantly longer in spike length characters at 5% level (Table: 2; Fig: 1).

Number of spikelets per spike

The highest number of spikelets per spike was observed on Kyone Phyu from Sintkaing (Table: 2; Fig: 2). Comparison made between Sintkaing and Sagaing, Sintkaing and Pindaya, Sintkaing and Monywa, Wundwin and Monywa, Chaung Oo and Monywa all the formers were significantly different at 1% level respectively and comparison between Wundwin and Sagaing, Pindaya and Monywa all the formers were significantly different at 5% level respectively (Table: 2). No significant differences were observed from the rest comparison (Table: 2; Fig: 2).

Density of spikelet

Pindaya Kyone Phyu have the poorest density of spikelet among the six studied cultivar Kyone phyu collected from different sites, while Monywa Kyone Phyu have the highest density of spikelet (Table: 2). when comparison made among Kyone Phyu collected from six different sites, Sintkaing showed significantly superior than Pindaya and Chaung Oo, Sagaing than Pindaya, Wundwin than Pindaya and Chaung Oo at 5% and 1% level respectively (Table: 2; Fig: 3).

Number of florets per spike

The range between 70 and 80 florets number per spike was observed on cultivars Chaung Oo Kyone Phyu while range between 50 and 60 was observed on Monywa Kyone Phyu (Fig: 4). Although these two kinds of Kyone Phyu showed the highest and lowest number of florests per spike comparison between Sintkaing and Sagaing, Sintkaing and Pindaya, Sagaing and Pindaya, Sagaing and Monywa Kyone Phyu didn't exhibit significantly differences from one to another (Table: 3; Fig: 4).

Table 2 Comparison between the spike length, number of spikelets per spike and density of spikelet of *Triticum aestivum* L. cultivar Kyone Phyu from six different collection sites.

No.	Comparison	Spike Length		Number of Spikelet per spike		Density of spikelet	
		Mean ± S.E	't' Value	Mean ± S.E	't' Value	Mean ± S.E	't' Value
1	SK-WD	9.167 ± 0.898 8.850 ± 1.089	1.209^{ns}	17.867 ± 1.454 17.267 ± 1.153	1.739^{ns}	1.966 ± 0.238 1.984 ± 0.308	-0.250^{ns}
2	SK-SG	9.167 ± 0.898 8.683 ± 1.012	1.927^{ns}	17.867 ± 1.454 16.367 ± 1.722	3.580**	1.966 ± 0.238 1.916 ± 0.358	0.625^{ns}
3	SK-PDY	9.167 ± 0.898 9.817 ± 1.242	-2.285*	17.867 ± 1.454 16.767 ± 1.453	2.880**	1.966 ± 0.238 1.730 ± 0.234	3.806**
4	SK-CO	9.167 ± 0.898 9.450 ± 0.943	-1.171^{ns}	17.867 ± 1.454 17.067 ± 1.590	2.000^{ns}	1.966 ± 0.238 1.824 ± 0.253	2.185*
5	SK-MY	9.167 ± 0.898 7.583 ± 0.708	7.472**	17.867 ± 1.454 15.933 ± 1.031	5.843**	1.966 ± 0.238 2.118 ± 0.236	-2.452*
6	WD-SG	8.850 ± 1.089 8.683 ± 1.012	0.605^{ns}	17.267 ± 1.153 16.367 ± 1.722	2.338*	1.984 ± 0.308 1.916 ± 0.358	0.773^{ns}
7	WD-PDY	8.850 ± 1.089 9.817 ± 1.242	-3.150**	17.267 ± 1.153 16.767 ± 1.453	1.453^{ns}	1.984 ± 0.308 1.730 ± 0.234	3.528**
8	WD-CO	8.850 ± 1.089 9.450 ± 0.943	-2.239*	17.267 ± 1.153 17.067 ± 1.590	0.548^{ns}	1.984 ± 0.308 1.824 ± 0.253	2.162*
9	WD-MY	8.850 ± 1.089 7.583 ± 0.708	5.257**	17.267 ± 1.153 15.933 ± 1.031	4.648**	1.984 ± 0.308 2.118 ± 0.236	-1.861^{ns}
10	SG-PDY	8.683 ± 1.012 9.817 ± 1.242	-3.818**	16.367 ± 1.722 16.767 ± 1.453	-0.957^{ns}	1.916 ± 0.358 1.730 ± 0.234	2.325*
11	SG-CO	8.683 ± 1.012 9.450 ± 0.943	-2.984**	16.367 ± 1.722 17.067 ± 1.590	-1.609^{ns}	1.916 ± 0.358 1.824 ± 0.253	1.136^{ns}
12	SG-MY	8.683 ± 1.012 7.583 ± 0.708	4.803**	16.367 ± 1.722 15.933 ± 1.031	1.164^{ns}	1.916 ± 0.358 2.118 ± 0.236	-2.525*
13	PDY-CO	9.817 ± 1.242 9.450 ± 0.943	1.270^{ns}	16.767 ± 1.453 17.067 ± 1.590	-0.750^{ns}	1.730 ± 0.234 1.824 ± 0.253	-1.469*
14	PDY-MY	9.817 ± 1.242 7.583 ± 0.708	8.430**	16.767 ± 1.453 15.933 ± 1.031	2.520*	1.730 ± 0.234 2.118 ± 0.236	-6.258**
15	CO-MY	9.450 ± 0.943 7.583 ± 0.708	8.525**	17.067 ± 1.590 15.933 ± 1.031	3222**	1.824 ± 0.253 2.118 ± 0.236	-4.594**

S.E = Standard Error; Ns = Nonsignificant; * , ** = significantly different at 5% and 1% level respectively
S.K = Sintkaing; WD = Wundwin; SG = Sagaing; PDY = Pindaya; CO = Chaung Oo; MY = Monywa.

Table 3 Comparison between the number of florets per spikelet, number of florets per spike and fertility of 1st and 2nd florets of *Triticum aestivum* L. cultivar Kyone Phyu from six different collection sites.

No	Comparison	Numbers of florets per spikelet		Number of florets per spike		Fertility of 1st and 2nd floret	
		Mean ± S.E	't' Value	Mean ± S.E	't' Value	Mean ± S.E	't' Value
1	SK-WD	3.422 ± 0.535 4.135 ± 0.287	-6.310**	56.567 ± 9.222 62.567 ± 7.352	-2.740*	26.800 ± 3.655 26.967 ± 5.480	-0.137^{ns}
2	SK-SG	3.422 ± 0.535 3.516 ± 0.374	-0.777^{ns}	56.567 ± 9.222 54.667 ± 8.183	0.830^{ns}	26.800 ± 3.655 29.167 ± 4.906	-2.084*
3	SK-PDY	3.422 ± 0.535 3.682 ± 0.379	-2.131*	56.567 ± 9.222 55.200 ± 7.521	0.619^{ns}	26.800 ± 3.655 27.900 ± 3.953	-1.100^{ns}
4	SK-CO	3.422 ± 0.535 4.230 ± 0.318	-6.966**	56.567 ± 9.222 71.200 ± 8.199	-6.387**	26.800 ± 3.655 30.667 ± 5.198	-3.277**
5	SK-MY	3.422 ± 0.535 3.351 ± 0.220	0.664^{ns}	56.567 ± 9.222 51.300 ± 6.334	2.535*	26.800 ± 3.655 27.300 ± 5.928	-0.387^{ns}
6	WD-SG	4.135 ± 0.287 3.516 ± 0.374	7.034**	62.567 ± 7.352 54.667 ± 8.183	3.867**	26.967 ± 5.480 29.167 ± 4.906	-1.611^{ns}
7	WD-PDY	4.135 ± 0.287 3.682 ± 0.379	5.148**	62.567 ± 7.352 55.200 ± 7.521	3.772**	26.967 ± 5.480 27.900 ± 3.953	-0.797^{ns}
8	WD-CO	4.135 ± 0.287 4.230 ± 0.318	-1.188^{ns}	62.567 ± 7.352 71.200 ± 8.199	-4.222**	26.967 ± 5.480 30.667 ± 5.198	-2.637*
9	WD-MY	4.135 ± 0.287 3.351 ± 0.220	11.701**	62.567 ± 7.352 51.300 ± 6.334	6.252**	26.967 ± 5.480 27.300 ± 5.928	-0.222^{ns}
10	SG-PDY	3.516 ± 0.374 3.682 ± 0.379	-1.677^{ns}	54.667 ± 8.183 55.200 ± 7.521	-0.258^{ns}	29.167 ± 4.906 27.900 ± 3.953	1.026^{ns}
11	SG-CO	3.516 ± 0.374 4.230 ± 0.318	-7.846**	54.667 ± 8.183 71.200 ± 8.199	-7.686**	29.167 ± 4.906 30.667 ± 5.198	-1.130^{ns}
12	SG-MY	3.516 ± 0.374 3.351 ± 0.220	2.037^{ns}	54.667 ± 8.183 51.300 ± 6.334	1.752^{ns}	29.167 ± 4.906 27.300 ± 5.928	1.307^{ns}
13	PDY-CO	3.682 ± 0.379 4.230 ± 0.318	-5.957**	55.200 ± 7.521 71.200 ± 8.199	-7.744**	27.900 ± 3.953 30.667 ± 5.198	2.281^{ns}
14	PDY-MY	3.682 ± 0.379 3.351 ± 0.220	4.068**	55.200 ± 7.521 51.300 ± 6.334	2.136*	27.900 ± 3.953 27.300 ± 5.928	0.454^{ns}
15	CO-MY	4.230 ± 0.318 3.351 ± 0.220	12.208**	71.200 ± 8.199 51.300 ± 6.334	10.343**	30.667 ± 5.198 27.300 ± 5.928	2.300*

S.E = Standard Error; Ns = Nonsignificant; * , ** = significantly different at 5% and 1% level respectively
S.K = Sintkaing; WD = Wundwin; SG = Sagaing; PDY = Pindaya; CO= Chaung Oo; MY = Monywa.

Table 4 Comparison between the number of seeds per spikelet, number of seeds per spike, fertility of seeds per spike and number of tillers per plant of *Triticum aestivum* L. cultivar Kyone Phyu from six different collection sites.

No.	Comparison	Number of seeds per spikelet		Number of Seeds per spike		Fertility of Seeds per spike		Number of tillers per plant	
		Mean ± S.E	't' Value	Mean ± S.E	't' Value	Mean ± S.E	't' Value	Mean ± S.E	't' Value
1	SK-WD	2.019 ± 0.375 2.533 ± 0.206	-6.425**	26.900 ± 5.031 35.500 ± 3.234	-8.125**	25.500 ± 4.849 34.667 ± 3.534	-8.229*	3.033 ± 1.278 4.233 ± 1.257	-3.604**
2	SK-SG	2.019 ± 0.375 2.813 ± 0.248	-9.452**	26.900 ± 5.031 40.467 ± 3.490	-9.441**	25.500 ± 4.849 39.500 ± 3.677	-12.389**	3.033 ± 1.278 3.967 ± 1.251	-2.813**
3	SK-PDY	2.019 ± 0.375 3.483 ± 0.352	-15.250**	26.900 ± 5.031 54.233 ± 4.161	-22.965**	25.500 ± 4.849 53.433 ± 4.287	-23.339**	3.033 ± 1.278 4.700 ± 1.754	-4.136**
4	SK-CO	2.019 ± 0.375 3.350 ± 0.257	-8.067**	26.900 ± 5.031 55.933 ± 4.711	-23.071**	25.500 ± 4.849 54.400 ± 4.439	-23.669*	3.033 ± 1.278 4.733 ± 1.632	-4.416**
5	SK-MY	2.019 ± 0.375 2.319 ± 0.230	-3.659**	26.900 ± 5.031 32.767 ± 4.161	-5.253**	25.500 ± 4.849 32.033 ± 4.154	-5.508**	3.033 ± 1.278 4.200 ± 1.275	-3.484**
6	WD-SG	2.533 ± 0.206 2.813 ± 0.248	-4.667**	35.500 ± 3.324 40.467 ± 3.490	-5.550**	34.667 ± 3.534 39.500 ± 3.667	-5.103**	4.233 ± 1.257 3.967 ± 1.251	0.809^{ns}
7	WD-PDY	2.533 ± 0.206 3.483 ± 0.352	-12.500**	35.500 ± 3.324 54.233 ± 4.161	-18.941**	34.667 ± 3.534 53.433 ± 4.287	-18.184**	4.233 ± 1.257 4.700 ± 1.754	-1.165^{ns}
8	WD-CO	2.533 ± 0.206 3.350 ± 0.257	-5.237**	35.500 ± 3.324 55.933 ± 4.711	-19.078**	34.667 ± 3.534 54.400 ± 4.439	-18.722**	4.233 ± 1.257 4.733 ± 1.632	-1.309^{ns}
9	WD-MY	2.533 ± 0.206 2.319 ± 0.230	3.754**	35.500 ± 3.324 32.767 ± 4.161	2.763**	34.667 ± 3.534 32.033 ± 4.159	2.600*	4.233 ± 1.257 4.200 ± 1.275	0.099^{ns}
10	SG-PDY	2.813 ± 0.248 3.483 ± 0.352	-8.375**	40.467 ± 3.490 54.233 ± 4.161	-13.657**	39.500 ± 3.677 53.433 ± 4.287	-13.282**	3.967 ± 1.251 4.700 ± 1.754	-1.833^{ns}
11	SG-CO	2.813 ± 0.248 3.350 ± 0.257	-3.442**	40.467 ± 3.490 55.933 ± 4.711	-14.202**	39.500 ± 3.677 54.400 ± 4.439	-13.925**	3.967 ± 1.251 4.733 ± 1.632	-2.005^{ns}
12	SG-MY	2.813 ± 0.248 2.319 ± 0.257	7.841**	40.467 ± 3.490 32.767 ± 4.161	7.639**	39.500 ± 3.677 32.033 ± 4.159	7.242**	3.967 ± 1.251 4.200 ± 1.275	-0.702^{ns}
13	PDY-CO	3.483 ± 0.352 3.350 ± 0.257	0.816^{ns}	54.233 ± 4.161 55.933 ± 4.711	-1.457^{ns}	53.433 ± 4.287 54.400 ± 4.439	-0.844^{ns}	4.700 ± 1.754 4.733 ± 1.632	-0.074^{ns}
14	PDY-MY	3.483 ± 0.352 2.319 ± 0.230	16.394**	54.233 ± 4.161 32.767 ± 4.161	19.640**	53.433 ± 4.287 32.033 ± 4.159	19.797**	4.700 ± 1.754 4.200 ± 1.275	1.241^{ns}
15	CO-MY	3.350 ± 0.257 2.319 ± 0.230	11.988**	55.933 ± 4.711 32.767 ± 4.161	19.851**	54.400 ± 4.439 32.033 ± 4.159	19.794**	4.733 ± 1.632 4.200 ± 1.275	1.384^{ns}

S.E = Standard Error; Ns = Nonsignificant; * , ** = significantly different at 5% and 1% level respectively
S.K = Sintkaing; WD = Wundwin; SG = Sagaing; PDY = Pindaya; CO= Chaung Oo; MY = Monywa.

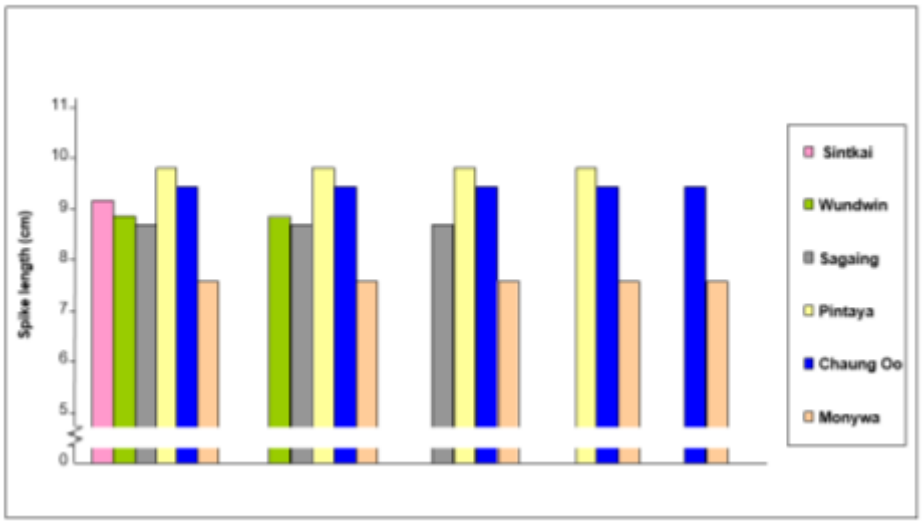

Figure 1 Comparison on spike length of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

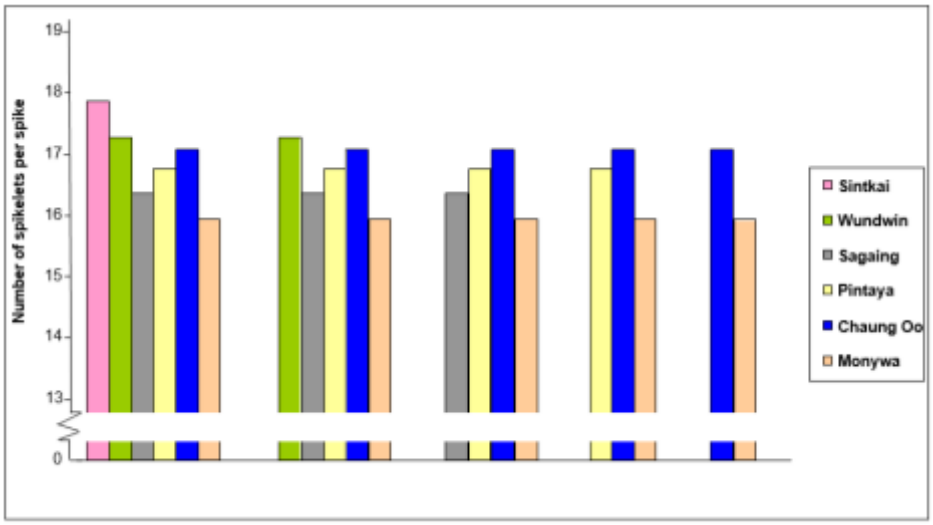

Figure 2 Comparison on number of spikelets per spike of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

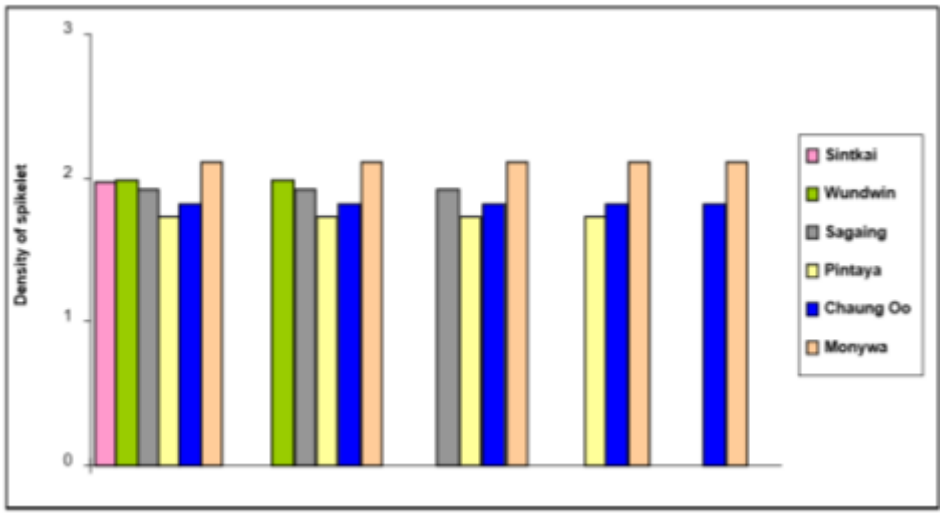

Figure 3 Comparison on density of spikelet of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

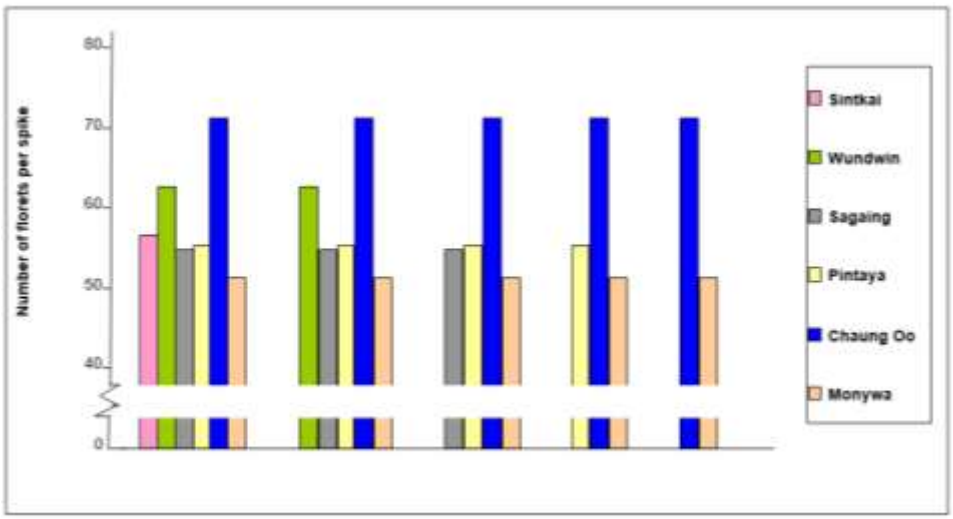

Figure 4 Comparison on number of florets per spike of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

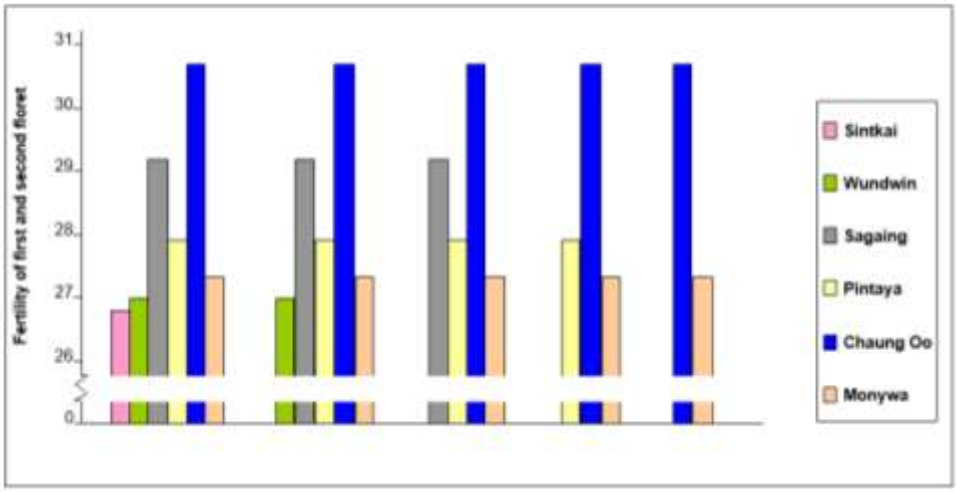

Figure 5 Comparison on fertility of 1st and 2nd floret of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

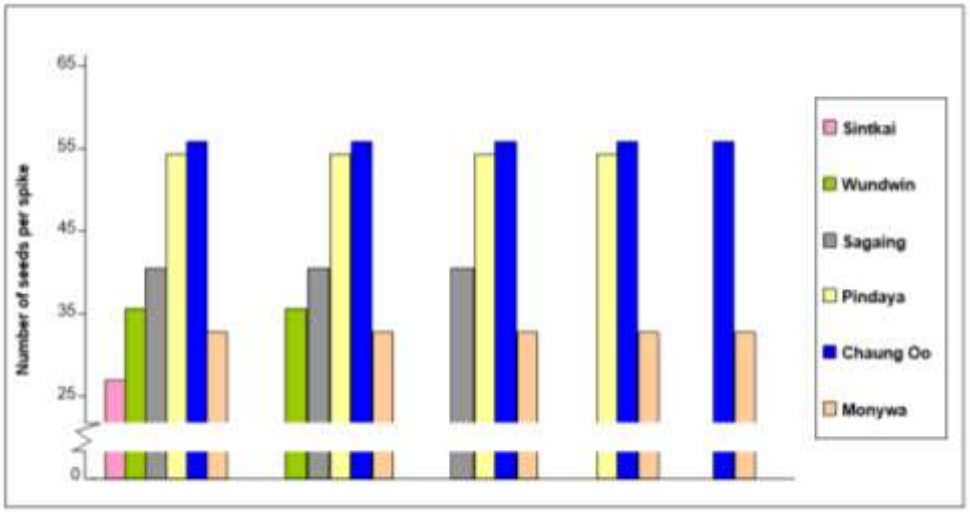

Figure 6 Comparison on number of seeds per spike of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

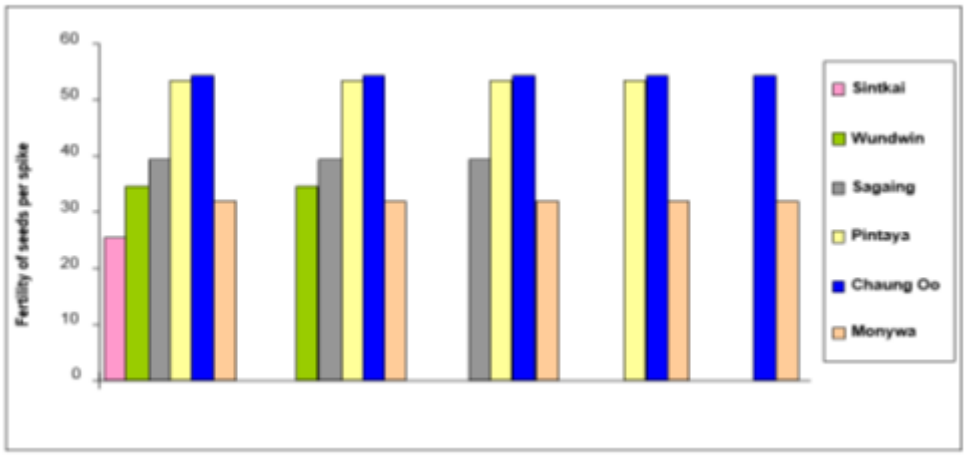

Figure 7 Comparison on fertility of seeds per spike of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

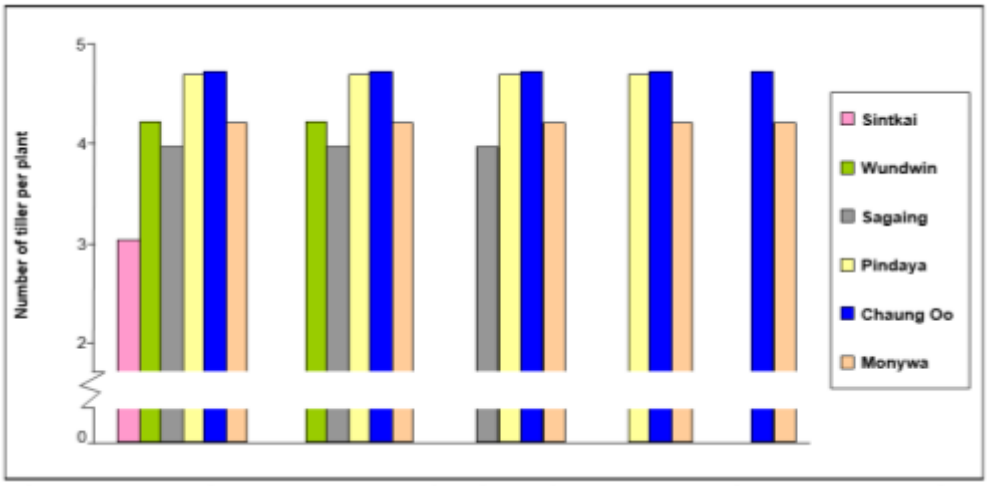

Figure 8 Comparison on number of tiller per plant of *Triticum aestivum* L. cultivar Kyone phyu from six different collection sites.

Fertility of 1st and 2nd florets

Chaung Oo Kyone Phyu have the highest fertility of 1st and 2nd florets have 30.667 in fertility while Sintkaing Kyone Phyu resulted the lowest fertility having only26.80 (Table: 3). Comparison between Kyone Phyu of Sintkaing with Sagaing, Wundwin with Chaung Oo, Chaung Oo with Monywa exhibited significantly differences at 5% level, while comparison of Sintkaing with Chaung Oo Kyone Phyu showed significantly difference at 1% level of fertility on 1st and 2nd florets, the other comparison did not result any differences respectively (Table: 3; Fig: 5).

Number of seeds per spike

Sintkaing Kyone Phyu have the lowest mean number of seeds per spike which followed by Monywa Kyone Phyu (Table: 4). When comparison made among the cultivars Kyone Phyu from six different collection sites, only the comparison between Kyone Phyu of Pindaya with Chaung Oo did not show significantly different from one to another while the rest fourteen comparisons resulted significantly differences at 1% level respectively (Table:4; Fig: 6).

Fertility of seeds per spike

Kyone Phyu of Chaung Oo and Sintkaing have the highest and lowest fertility of seeds per spike among the cultivars Kyone Phyu from six different collection sites studies for the fertility of

seeds per spike character showed that Kyone Phyu as similar number of seeds per spike characters, except comparison between Pindaya with Chaung Oo Kyone Phyu that they didn't show any significant differences from one to another and the other comparison were observed significantly differences at 1% level (Table: 4; Fig: 7).

Number of tillers per plant

Chaung Oo Kyone Phyu have 4.733 tillers per plant, the highest mean number and Sintkaing Kyone Phyu have 3.033 tillers per plant, which is the smallest number among the cultivars Kyone Phyu studied from six different collection sites. By using equal student 't' test and compared among the cultivars Kyone Phyu from six different collection sites, it was observed that except Sintkaing Kyone Phyu resulted significantly inferior than the rest cultivars Kyone Phyu of five different collection sites, the rest cultivars Kyone Phyu of five different collection sites exhibit that they haveno significantly differences from one to another at 5% and 1% level respectively (Table: 4, Fig: 8).

Discussion

Transformation in morphological characters as well as chromosomal characters in number of speciesof Family Graminae have been demonstrated by Lorz *et al*., (1985), Hsan (1990) stated that this transformation in characters is largely influenced by the environmental condition such as light, temperature, availability of water an available of nutrition in the cultivated soils. There are many research works with environment related to wheat culms, tillers, spike, ear, and yield characters and many reliable data concerning with wheat and the concepts and facts are available.

In the present study it was also observed that cultivar Kyone Phyu grown in Pindaya exhibited more resistant to leaf rust showed that the environmental condition is also one of the Key factor of controlling leaf rust. The present data also exhibit that cultivar Kyone Phyu have wide range of adaptability.

Khin Than Htwe (1997) stated that some morphological characters on hexaploid wheat cultivar Kyone Phyu have been studied in Myanmar. Khin Mg Oo (1980) stated that the local wheat variety Kyone Phyu that have low spike density and lesser number of florets per spike and chromosome diminution (i.e. a shorter chromosome complement) have somewhat influence by the environment.

Spike length, number of spikelet per spike, density of spikelet, number of florets per spike, fertility of first and second florets, number of seeds per spike, fertility of seed per spike are the factors that have a key role in yield characters (Sears & Sears, 1978). These characters is mainly found superior in wheat plants with branching characters compared to those with no branching characters (Hsan, 1990, Hla Myint Than, 1997).

In the present investigation, it was observed that Kyone Phyu of Monywa have significantly superior in spike length than the other five cultivars Kyone Phyu (Table: 2). Number of spikelet per spike of Monywa Kyone Phyu have significantly the highest numbers that followed by Wundwin Kyone Phyu. For fertility of 1st and 2nd florets, Chaung Oo Kyone Phyu showed significantly superior than Sintkaing Kyone Phyu and first and second florets of Sintkanig and Sagaing Kyone Phyu (Table: 3).

Tiller number of individual plants of hexaploid wheat cultivar Kyone Phyu i.e. Sintkaing with Wundwin, Sagaing, Pindaya, Chaung Oo and Monywa Kyone Phyu were significantly differences at 5% and 1% level respectively. The rest of all cultivars Kyone Phyu did not exhibit significantly difference. Spike characters of cultivars Kyone phyu from six different collection sites were described in plate (Plate: 2 and 3).

In the present investigation, it was observed the spike and seed characters, of even a cultivar have been varied slightly from one to another collection sites. It showed that it may be the effect of the seasonal condition i.e. soil, elevation as well as available water. Awn character that is one of the spike characters seems to be due to the adaptability of the cultivar. This research is carried out to growth character of the some cultivars cultivated in different places.

Thus, the present results showed that the present finding will be useful to create good future outcome for the cultivated wheat in both cultivation as well as in improvement process. The result of present finding will serve as an important informations for those who are going to carried out their further research works with local cultivar Kyone phyu in Myanmar.

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MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF *BOESENBERGIA ROTUNDA* (L.) MANSF.

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Abstract

Boesenbergia rotunda (L.) Mansf. is locally known as Seik-Phoo belongs to the family Zingiberaceae. These specimens were collected from Shwe-Thin-Phyu Village, Ye-Zin, Mandalay Region, in 2018. These plants have been used as a remedy in traditional medicine and also as vegetables and spices in the preparation of appetizing meal by local people in these collected areas. This study was conducted to identify the morphological and histological characters of *Boesenbergia rotunda* (L.) Mansf. In morphological studies, it is an aromatic perennial herb which has bright yellow rhizomes and finger-like tuberous roots; leaves alternate, biseriate; inflorescences spike, terminal on a leafy shoot; flowers pinkish purple, bisexual, irregular, epigynous. In histological studies, tetracytic stomata are present on both surfaces of lamina, midribs, petioles and leaf sheaths. The three types of vascular bundles such as main arc, abaxial arc and adaxial arc are also found in midribs, petioles and leaf sheaths. Prismatic calcium oxalate crystals and oil cells are found in lamina, midribs, petioles and leaf sheaths. Starch grains and oil cells are abundantly found in tuberous roots and rhizomes. In diagnostic characters, oil cells, fibers, fiber-tracheids and fragments of vessels (pitted, spiral thickening, reticulate thickening and scalariform thickening) are found in both powdered of aerial shoots and subterranean organs. Moreover, fragments of epidermal cells with stomata and calcium oxalate crystals (prism) are also found in aerial shoot while starch grains in subterranean organs.

Keywords: *Boesenbergia rotunda*, Prismatic Calcium oxalate crystals, tetracytic

Introduction

Boesenbergia rotunda is a ginger species belonging to the family Zingiberaceae that grows in Southeast Asia, India, Srilanka, and Southern China. (Baker, 1890). In Myanmar, it grows wild in Bago Region, Mandalay Region, Yangon Region, Sagaing Region and Chin State. It is a very common plant growing naturally in damp, shaded parts of the low-land or hill slopes.

Boesenbergia rotunda (L.) Mansf. is commonly known as Seik-Phoo in Myanmar and fingerroot in English (Hundley and Chit Ko Ko, 1987 and Kress et al., 2003). It is known as fingerroot because the plant consists of a small globular shaped central rhizome with fleshy long and thick tubers sprout all in the same direction like fingers. (Yaya Rukauadi, 2015).

Boesenbergia rotunda is small herbaceous plant with slender rhizomes and few leaves. The fresh rhizomes have a characteristic aroma and a slightly pungent taste. It is commonly used in Southeast Asia as a food ingredient, a folk medicine for the treatment of several diseases such as aphthous ulcer, dry mouth, stomach discomfort, leucorrhoea and dysentry. (Burkill, 1935)

The species taken into study are widely spread in moist and shady places of some areas in Myanmar. Aerial shoots and subterranean organs of Seik-Phoo have been used as a condiment in food such as curry and soup due to its aromatic flavour, which promotes appetite and also used as traditional medicines for dysentry and stomach discomfort by local people in this collected area. Therefore, *Boesenbergia rotunda* (L.) Mansf. was chosen and studied due to the poor data available in this plants.

The aim and objectives of this chapter is to identify the vegetative and reproductive parts and to characterize the histological characters of leaves, rhizomes and roots of the plant *Boesenbergia rotunda* (L.) Mansf.

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Material and Methods

1. Collection, Classification and Identification

Collection

The specimens were mainly collected from Shwe-Thin-Phyu Village, Ye-Zin, Mandalay Region, during the month of September, 2018.

Classification and Identification

Morphological characters of vegetative and reproductive parts of the specimens were identified by using literatures of key to the families of Myanmar Flowering plants, 1994; Flora of Java, Backer, 1968; Flora of Ceylon, Dassannayake, 1983 and Flora of China, Wu-Te-lin, 2000. Herbarium of *Boesenbergia rotunda* (L.) Mansf. was deposited in the Herbarium of Botany Department, Bago University.

2. Histological examination of different plant parts and powders.

Microscopical examination

Different plant parts such as aerial parts and underground parts were washed, dried, powdered and kept in an airtight bottle for microscopical examination. The fresh different plant parts were examined by the free hand sections. Powders were examined to get standardization for medicine. The free hand sections and powdered samples were analyzed by using the following reagents.

1. Chloral hydrate solution B.P. as clearing reagent.
2. Dilute glycerine B.P. for mounting reagent.
3. Phloroglucinol HCL B.P. for lignin test.
4. Iodine solution B.P. for testing starch.
5. Sudan III solution B.P. for testing oils and
6. Conc: H_2SO_4 for testing calcium oxalate crystals.

Results

Morphological Characters of *Boesenbergia rotunda* (L.) Mansf.

Scientific Name - *Boesenbergia rotunda* (L.) Mansf.

Family Name - Zingiberaceae

Myanmar Name - Seik-Phoo

English Name - Finger Root

Flowering Period - July to September

Aromatic Perennial herbs; leafy shoot 3.5-8.5 m in height. Rhizomes globose with several finger-like tuberous roots. Leaves alternate, simple, biseriate, aromatic; leaf sheath reddish green; ligules triangular; petioles long, channeled. Inflorescences spike, terminal on a leafy shoot. Flowers pinkish purple, bisexual, zygomorphic, 3-merous, epigynous; calyx 3, synsepalous, tubular, bifid, pinkish white; corolla 3, synpetalous, tubes long and white, lobes pink, oblong, incurved, the posterior lobe larger than the lateral twos; staminodes are broadly obovate, molted purple; the labellum oblong-obovate, apex crenate, undulate, plicate, the upper half pink and the lower half

pale pink with red-violet dots within it. Fertile stamen one, epipetalous. Pistils 3, tricarpellary, syncarpous, tri-locular, axile placentation, ovary ovoid, inferior; the styles filiform, passing through the channel along the fertile stamen, the stigma funnel-shaped. Fruits are not seen (Fig.1-4).

1.4.2 Histological characters of Leaves, Roots and Rhizomes of *Boesenbergia rotunda* (L.) Mansf.

Lamina

In surface view, upper epidermal cells are transverse extended and hexagonal, more or less isodiametric. Anticlinal walls are straight. Lower epidermal cells are polygonal-shaped, anticlinal walls are straight. Prismatic calcium oxalate crystals and oil cells are found in lower surface. Tetracytic stomata are present in both surfaces but more numerous in the lower surface (Figure 5-6).

In transverse section, the cuticles are present on both surfaces. The upper epidermal cells are rectangular-shaped. The lower epidermal cells are rectangular to polygonal-shaped. The epidermal cells of both surfaces are thin-walled, compactly arranged.

The hypodermal cells are one-layered, colourless and found on both sides. The mesophyll composed of palisade parenchyma and spongy mesophyll cells. Below the upper hypodermis is one-layered thick, short conical palisade cells which are compactly arranged. The spongy mesophyll cells are two layers of irregular-shaped, thin-walled parenchymatous cells. Prismatic calcium oxalate crystals are present in the spongy mesophyll cells.

The vascular bundles are collateral and closed type. Xylem lies towards the upper epidermis and composed of vessels, tracheids, fibers, fiber-tracheids and xylem parenchyma. Phloem lies towards the lower epidermis and consists of sieve tube, companion cells and phloem parenchyma cells. Each bundle is surrounded by sclerenchymatous sheath, which is distinct from the neighbouring cells. (Figure. 7).

Midribs

In surface view, the epidermis of both surfaces are made up of thin-walled, parenchymatous cells. The upper epidermal cells are rectangular to polygonal-shaped. The lower epidermal cells are rectangular and elongated. Prismatic calcium oxalate crystals, oil cells and tetracytic stomata are present on both surfaces but more numerous in the lower surface. (Figure 8 - 9).

In transverse sections, the adaxial surfaces of midrib are concave and abaxial surfaces are convex in basal region, middle region and apical region. Epidermal cells are single-layered, barrel-shaped, parenchymatous cells, compactly arranged. Under the epidermis, collenchymatous cells are 1-2 layers in thickness on both sides. They are polygonal-shaped. Parenchymatous cells are thin-walled, irregular, polygonal-shaped, compactly arranged. The large air canals are abundantly present. Prismatic calcium oxalate crystals are present in the parenchymatous cells that surround the air canal.

The vascular bundles of midrib are oval-shaped, collateral and closed type. The vascular tissue of middle and basal regions consists of main vascular bundles, abaxial bundles and adaxial bundles. The apical region consists of only one main vascular bundle. Xylem composed of vessels, tracheids, fibers, fiber tracheids and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma cells. (Figure 10)

Leaf sheaths

In surface view, epidermal cells are rectangular to polygonal shaped, thin-walled, parenchymatous cells. The upper epidermal cells are rectangular and elongated. Prismatic calcium oxalate crystals, oil cells and tetracytic stomata are present on both surfaces but more numerous on the lower surface. (Figure 11-12)

In transverse section, the leaf sheaths are overwapping. The cuticle is thin, the epidermal cells are barrel-shaped, parenchymatous, compactly arranged. Below the epidermis are the 1-2 layers collenchymatous cells on both sides. The parenchymatous cells are rounded to polygonal-shaped. The vascular bundles of leaf sheaths are oval-shaped, collateral and closed type. The vascular tissue consists of main vascular bundles, abaxial bundles and adaxial bundles. The adaxial bundles are divided into center arc and upper arc. Xylem composed of vessels, tracheids, fiber, fiber-tracheids and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma cells. (Figure 13)

Petioles

In surface view, the epidermal cells of both surfaces are made up of parenchymatous cells. The upper epidermal cells are rectangular to polygonal-shaped. The lower epidermal cells are rectangular and elongated. Prismatic calcium oxalate crystals, oil cells and tetracytic stomata are present on both surfaces but more numerous on the lower surface. (Figure 14 - 15)

In transverse section, the petioles are more or less U-shaped, the upper surface distinctly grooved, the lower surface rounded. The cuticle is thin. The epidermal cells are barrel-shaped, parenchymatous, compactly arranged. Below the epidermis are the collenchymatous cells 1-2 layers on both sides. The parenchymatous cells are rounded to polygonal-shaped. The parenchymatous cells surround the large air canal that contains prismatic calcium oxalate crystals.

The vascular bundles of petioles are oval-shaped, collateral and closed type. The vascular tissue consists of main vascular bundles, abaxial bundles and adaxial bundles. The adaxial bundles are divided into center arc and upper arc. Xylem composed of vessels, tracheids, fiber, fiber-tracheids and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma cells. (Figure 16)

Adventitious roots

In surface view, epidermal cells are rectangular and elongated, thin walled, parenchymatous cells. (Figure 17)

In transverse section, the adventitious roots are circular in outline. The epiblema is single layered, barrel-shaped, parenchymatous cells with numerous unicellular root hairs. The exodermis lies below the epiblema layer. The exodermis consists of 3-4 layers of parenchymatous cells, rectangular to polygonal-shaped. The cortex consists of parenchymatous cells, about 10-12 layers, oval to rounded in shape. Endodermis and pericycle are single layered, barrel-shaped, thin walled, parenchymatous cells. Pith lies in the center and composed of parenchymatous cells. Vascular bundles are radial types, polyarch. The xylem is exarch and composed of vessels, tracheids, fibers, fiber-tracheids and xylem parenchyma. Phloem composed of sieve tubes, companion cells and phloem parenchyma. (Figure 18-19)

Tuberous roots

In surface view, epidermal cells are rectangular-shaped, thin walled, parenchymatous cells. It contains numerous yellowish oil cells. (Figure 20)

In transverse section, the epiblema is single-layered, barrel-shaped, thin walled parenchymatous cells. The exodermis consists of 4-6 layers of parenchymatous cells, rectangular-shaped. The cortex contains 18-20 layers of parenchymatous cells, oval to rounded in shape. Endodermis and pericycle consist of single-layered, barrel-shaped parenchymatous cells. Pith lies in the center and composed of parenchymatous cells which are oval to rounded in shape. The parenchymatous cells contain numerous starch grains and yellowish oil cells. Starch grains are oval-shaped and eccentric.

Vascular bundles are radial type, and polyarch. The xylem is exarch composed of vessels, tracheids, fibers, fiber- tracheids and xylem parenchyma. Phloem composed of sieve tube, companion cells and phloem parenchyma. (Figure 21-22)

Rhizomes

In surface view, epidermal cells are polygonal-shaped. The anticlinal walls are straight and smooth. The yellowish oil cells are scattered throughout the section. (Figure 23)

In transverse section, the rhizomes are circular in outline. The epidermal cells are single layered, barrel-shaped. Periderm consists of outer cork and inner cork. The outer cork about 16 layers of parenchymatous cells, rectangular-shaped. The inner cork about 13 layers of parenchymatous cells, rectangular-shaped, tangentially flattened. The cortex is composed of 30-35 layers of thin-walled parenchymatous cells, oval to rounded in shape. Both endodermis and pericycle consists of single layered, barrel-shaped, parenchymatous cells. The stellar region is composed of parenchymatous cells which are rounded to oval shaped. The ground parenchymatous cells contain numerous starch grains and yellowish oil cells. (Figure 24)

Vascular bundles are scattered throughout the cortical and stellar regions. Vascular bundles are collateral and closed type. Xylem composed of vessels, tracheids, fiber tracheids, fibers and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma. (Figures 25 - 26)

Diagnostic characters of powdered aerial shoot and subterranean organs of *Boesenbergia rotunda* (L.) Mansf.

Both powdered aerial shoot and subterranean organs contain oil cells, fibers, fiber-tracheids, vessels (pitted, spiral thickening, reticulate thickening and scalariform thickening). Moreover, fragments of epidermal cells with stomata and calcium oxalate crystals (prism) are also found in aerial shoot while starch grains in subterranean organs. (Figures 27 - 37)

Figure 1 Habit

Figure. 2 Close up view of flower

Figure 3 Flower

Figure 4 Rhizome and Tuberous roots

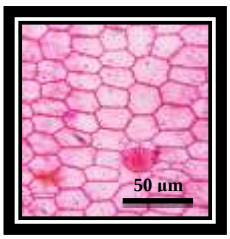


Figure 5 Surface view of upper epidermal cells with tetracytic stoma

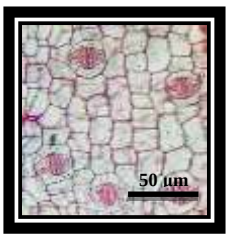


Figure 6 Surface view of lower epidermal cells with tetracytic stomata

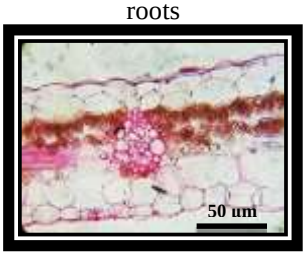


Figure 7 Transverse section of Lamina

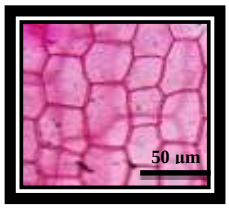


Figure 8. Upper surface of midrib

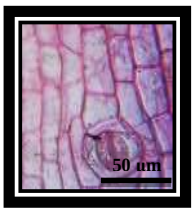
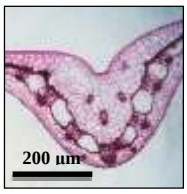
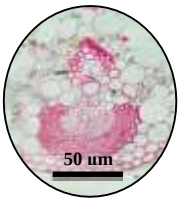


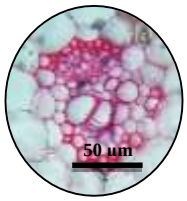
Figure 9 Lower surface of midrib showing tetracytic stoma



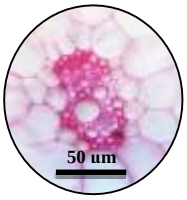
T.S of midrib



main bundle



adaxial bundle



abaxial bundle

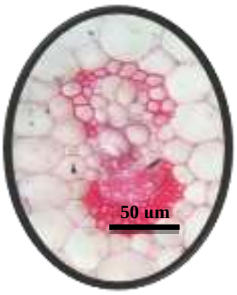
Figure 10 T.S of midrib showing main vaascular bundles, adaxial bundles and abaxial bundles

Leaf sheath (Surface view)

Figure 11 Surface view of upper epidermal cells

Figure 12 Surface view of lower epidermal cells with tetracytic stoma

T.S of leaf sheath



main bundle

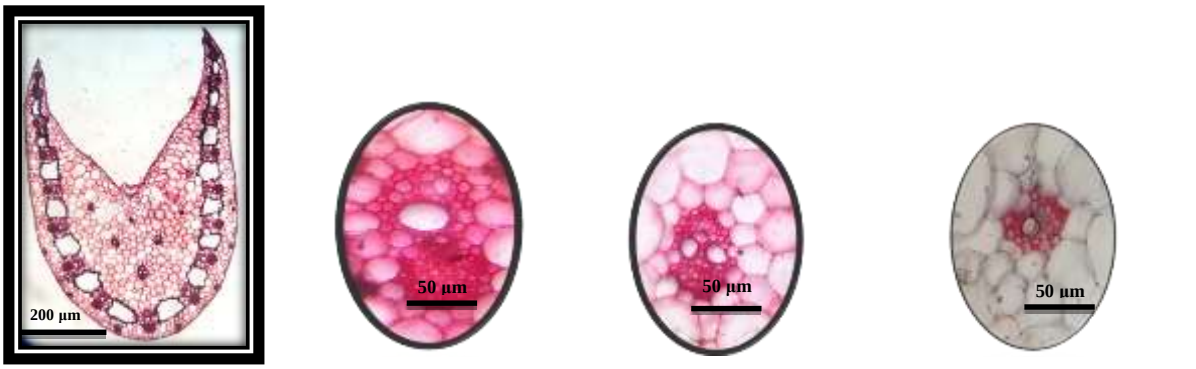
adaxial bundle

Figure 13 T.S of leaf sheath showing main vaascular bundles and adaxial bundles

Petiole (Surface view)

Figure 14 Surface view of upper epidermal cells

Figure 15. Surface view of lower epidermal cells with tetracytic stoma



T.S of petiole | main bundle | adaxial bundle (centre arc) | adaxial bundle (upper arc)

Figure 16 T.S of petiole showing main vaascular bundles and adaxial bundles

Adventitious roots (Surface view)

Figure 17 Surface view of epidermal cells

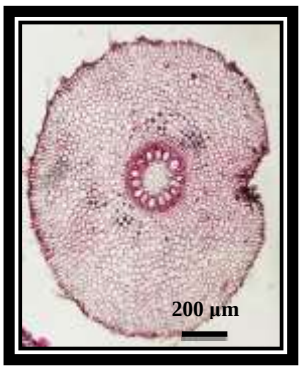


Figure 18 T.S of adventitious root

Figure 19 T.S of adventitious root showing vascular cylinder

Tuberous roots (Surface view)

Figure 20 Surface view of tuberous roots

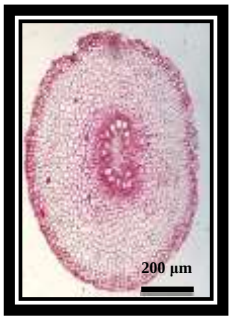


Figure 21 T.S of tuberous roots

Figure 22 T.S of tuberous roots showing vascular

Rhizome (Surface view)

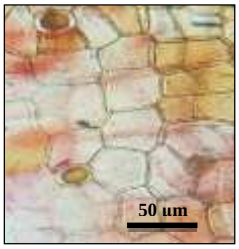


Figure 23 Surface view of lower epidermal cells with oil cells

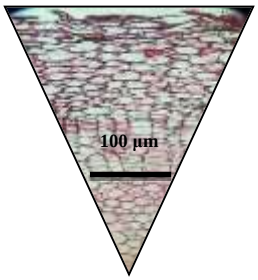


Figure 24 T.S of rhizome showing cork and cortex layers

Figure 25 Close up view of vascular bundle in cortical region

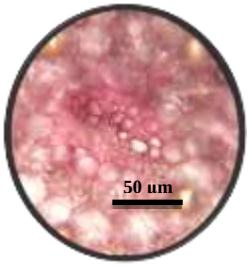


Figure 26.Close up view of vascular bundle in stellar region

Diagnostic Characters of Powdered Aerial Shoots of *Boesenbergia rotunda* (L.) Mansf.

Figure 27 Tracheid

Figure 28 Fibres

Figure 29 Fibre-tracheids

Figure 30. Spiral

Figure 31. Fragments epidermal cells

Figure 32 Pitted vessels

Diagnostic Characters of Powdered Subterranean Organs of *Boesenbergia* rotunda (L.) Mansf.

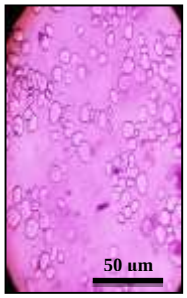


Figure 33 Starch grains

Figure 34 Fibre-tracheids, fibres and fragment of tracheids

Figure 35. Pitted vessels

Figure 36 spiral thickening

Figure 37 Reticulate thickening

Discussion

In this research, the morphological studies on both vegetative and reproductive parts as well as the microscopical examination of the fresh leaves, rhizomes and roots and powdered aerial shoots and subterranean organs were undertaken.

In the morphological study, the plants are aromatic perennial herbs, reddish sheath at the base. This features agreed with the habit of ***Boesenbergia rotunda (L.) Mansf.*** given by Backer, 1968; Dassanayake, 1983; Guzman & Siemonsma, 1999; Wu-Te-lin, 2000).

Rhizomes globose with long finger-like tuberous roots. Rhizomes and tuberous roots are yellow-brown outside and bright yellow inside, aromatic. This finding was in accordance with that of Guzman & Siemonsma, 1999; Wu-Telin, 2000.

Leaves alternate, biseriate, aromatic; leaf sheaths reddish green; ligules triangular; petioles long, channeled; lamina elliptical oblong to broadly lanceolate. This features were in agreement with that given by Backer, 1968; Dassanayake, 1983; Guzman & Siemonsma, 1999; Wu-Telin, 2000.

Inflorescence spike, terminal on leafy shoot, peduncle very short. These characters were in agreement with these given by Dassanayake, 1963; Guzman & Siemonsma, 1999; Wu-Telin, 2000.

Flowers situated in the axil of a bract and a bracteole; bracts and bracteoles oblong-lanceolate; calyx tubular, apex bifid; corolla with white tube, incurved, pink lobes at apex; labellum oblong-obovate, panduriform, apex crenate, undulate plicate, upper half pink, lower half pale pink with red-violet dots within it; staminodes broadly obovate, anther dithecous, pale yellow; gynoecium tri-locular, style filiform. These characters were agreement with those described by Guzman & Siemonsma, 1999.

In surface view of lamina, anticlinal walls of upper and lower epidermal cells are straight. Stomata are of tetracytic type. These features were in accordance with Khatijah et al., 2001.

In transverse section of lamina, the cuticle is thin, epidermal cells are polygonal in shape. These features were agreed with Tomlinson, 1969. The adaxial epidermis is papillate. Hypodermis present on both surfaces, which contain calcium oxalate crystals and oil cells. The mesophyll is differentiated into palisade and spongy layers. The palisade cells are conical-shaped on both sides. The spongy mesophyll cells are rounded or elliptic and may be loosely arranged. These microscopical features of leaves were similar with the findings of Olanrewaju, 1970.

The smallest vascular bundles attached to abaxial surface while the largest attached to both surfaces. Bundle sheath present above and below larger veins but present only below smaller veins. These characters of leaves were agreed with Tomlinson, 1969 and Khatijah et al., 2001.

In transverse section of midrib, adaxial surfaces are slightly curved and grooved. The abaxial surfaces are arced to V-shaped in the middle and basal portion. The vascular tissue consists of vascular bundles arranged in several arces. Air lacunae are present between the bundles. These characters were in accordance with khatijah et al., 2001.

According to in agreements of Olanrewaju 1970, it was found that the axial epidermis of the leaf sheath and petiole is differentiated into costal and intercostals areas. Both the costal and intercostals cells are rectangular but the intercostals cells are more wider than costal cells. In the transverse section of the leaf sheaths and petioles, the cuticle is thin and the epidermal cells are rectangular. The ground tissue is composed mainly of large parenchymatous cells. The vascular bundles have been grouped into main arc I, abaxial arc II, adaxial arc III and IV. A single row of air canals, which vary in size from the center to the margins of leaf sheath and petiole. In transverse section of root, the epiblema is made up of parenchymatous cells. The cortex is composed of

parenchymatous cells. The central pith consists of rounded and thin walled parenchymatous cells. The vascular bundles are radially arranged and polyarch. The xylem is exarch. In the transverse section of rhizome, the periderm is composed of thin-walled cells. The cells of inner layers are arranged in parallel. The cortex is composed of thin-walled parenchymatous cells. The vascular bundles are scattered throughout the cortical and stellar regions. Prismatic calcium oxalate crystals and oil cells are present in lamina, petioles and leaf sheaths. Starch grains are most abundant in rhizomes and tuberous roots. These features were in accordance with Tomlinson, 1969 and Olanrewaju, 1970. Starch grains are simple, spherical or ellipsoidal; hilum eccentric. These features were similar with the findings of Tomlinson, 1969.

In diagnostic characters, oil cells, fibers, fiber-tracheids, vessels (pitted, spiral thickening, reticulate thickening and scalariform thickening) are found in both powdered of aerial shoots and subterranean organs. Moreover, fragments of epidermal cells with stomata and calcium oxalate crystals (prism) are also found in aerial shoot while starch grains in subterranean organs. These characters were agreement with that given by Tomlinson, 1969.

Vegetative period was from June to July. Flowering and fruiting period was July to September. But Surapon et al., 2017 stated that the period of flowering and fruiting is June to September. These differences may depend on the ecological variation of areas.

Conclusion

The morphological and histological studies are ones of the very important parameters in the pharmacognostic study. These parameters are needed for standardization and authentification of medicinal plants with the help of which adulteration and substitution can prevented. Therefore, the morphological and histological characters have been studied for the identification of *Boesenbergia rotunda* (L.) Mansf. in this study.

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ISOLATION AND ANTIBACTERIAL ACTIVITY OF THE TERPENOID COMPOUNDS FROM *CEPHALOSPORIUM* SP.

Htet Htet Zaw[1], Mon Mon Thu[2] and Yee Yee Thu[3]

Abstract

Endophytic fungal strain *Cephalosporium* sp. was isolated from the woods of *Hesperethusa crenulata* (Roxb.) Roem. For extraction, separation, isolation of ten-liter fermentation and antimicrobial activity of the fermented broth on eight test organisms were conducted at Microbiology Lab, Department of Botany, University of Yangon. Antimicrobial activity of the fermented broth indicated highly activity against eight test organisms. After fermentation, the filtrate was extracted on Ambilite XAD 16 resin column with methanol. The methanol extract showed good antimicrobial activity than on *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Micrococcus luteus*, *Salmonella typhus* and *Staphylococcus aureus.* Separation and isolation of the bioactive compounds from the methanol extract were carried out by using silica 34 gel columns with various solvent systems at Microbiology Lab, Department of Botany, University of Yangon. The seven compounds (A to G) were isolated from the methanol extract of 10 L fermentation. Among them, three terpenoid compounds were mentioned in this paper. Among the isolated compounds, the compound A, B and C were terpenoid compounds and they indicated antibacterial activity on five test organisms. Therefore, these compounds are good to inhibit diarrhea and fever on man as well as crown gall and leaf blight diseases on plants.

Keywords: *Cephalosporium* sp., Fermentation studies, Terpenoids

Introduction

Plant endophytic fungi were found in each plant species examined, and there are over one million fungal endophytes existed in the nature (Petrini, 1991). Plant endophytic fungi have been recognized as an important and novel resource of natural bioactive products with potential application in agriculture, medicine and food industry (Strobel, *et al*., 2004). Many scientists have been interested in studying fungal endophytes as potential producers of novel and biologically active compounds (Stierle *et al*., 1993).

In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been successfully discovered from the endophytic fungi. These bioactive compounds could be classified as alkaloids, terpenoids and phenols (Zhang *et al*., 2006). Terpenes play an important role as the signal compounds and growth regulators (Narayan *et al.*, 2017).

The objectives of this study are to extract the bioactive compounds from fermented broth of *Cephalosporium* sp. isolated from the woods of *Hesperethusa crenulata* (Roxb.) Roem., to isolate the bioactive compounds from methanol extract of fermented broth, to study the characterization of the isolated compounds and to evaluate antibacterial activity of the isolated compounds.

Materials and Methods

Fermentation of isolated fungal strain *Cephalosporium* sp.

The small piece (1cm^2) of fungus from the plate culture of *Cephalosporium* sp. was inoculated into 300 mL of conical flask containing 180 mL of sucrose/yeast extract seed medium.

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The flask was incubated at 30°C for two days as seed culture. Two days old seed culture (180 mL) was transferred into ten flasks of 2 L conical flask containing 1 L fermentation medium (15 mL seed culture in each flask). These flasks were incubated on shaker at 100 rpm for a week at room temperature (Strobel and Sullivan, 1999). These fermented broths from 10 flasks were tested for antimicrobial activity on *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Micrococcus luteus*, *Salmonella typhus* and *Staphylococcus aureus.*

Antimicrobial activity by paper disc diffusion assay

Broth culture (50 µL) of test organisms (*Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Micrococcus luteus*, *Salmonella typhus* and *Staphylococcus aureus*) was added to 100 ml assay medium sucrose /yeast medium (SY) and then poured into plates. After solidification, paper discs infused with broth samples were applied on the test plates and incubated at 30°C for 24 hrs. When clear zones (inhibitory zones) showed around the paper discs, they were measured. The paper disc size is 6.0 mm. These inhibitory zones showed the presence of the bioactive compounds that inhibit the growth of the test organisms (Davis and Stout, 1971).

Extraction of the bioactive compounds from fermented broth

After testing antimicrobial activity, 10 L fermented broth was filtered with the filter paper. The mycelia were filtered and eluted with acetone while the filtrate was applied on an Amberlites XAD 16 resin column. The resin column was washed with water, followed by five liters of methanol (Figure 1). The acetone extract and methanol extract were evaporated on water bath at 50-55 °C. These extracts were tested for antimicrobial activities on *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus* (Grabley *et al.*, 1999).

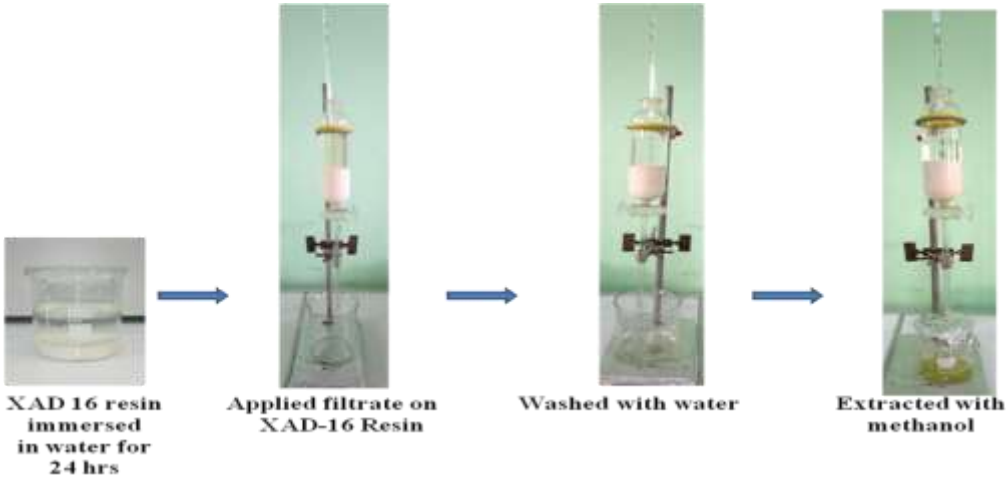


Figure 1 Extraction on Amberlites XAD-16 Resin

Separation, Isolation of terpenoid compounds

This chemical portion was conducted at Microbiology Laboratory, Department of Botany, University of Yangon. The bioactive compounds from fungal strain *Cephalosporium* sp. were separated and isolated by using various solvent systems (PE: DCM (1:1, 1:2,1:3, 1:4, 1:5, 1:7, 1:9, 1:10, (DCM 100%), DCM: MeOH (100%, 97:3, 93:7, 90:10, (EA, 100%), EtOAc: MeOH (95:5, 9:1, 8:2, 6:4), (MeOH 100%)) on 34 silica gel (100g) column. The fractions were collected and the collected fractions were combined according to their behavior on TLC plate.

Silica gel column chromatography

According to TLC result, silica gel column chromatography was carried out. The silica 34 gel (100 g) was dissolved in dicholoromethane thoroughly and then poured into the column. After the level of the silica gel surface in the column was stable, the methanol extract was added through the column. The column was eluted with petroleum ether: dicholomethane (1:1, 1:2, 1:3, 1:4, 1:5, 1:7, 1:9, 1:10, (DCM, 100%), DCM: MeOH (100%, 97:3, 93:7, 90:10, (MeOH 100%) and then nine fractions were collected. The column size was 3.5 cm × 42 cm and flow rate was 2 mL per minute as shown in Figure 2 (Grabley *et al.*, 1999).

The eluting solvent	-	PE:Dicholomethane (1:1, 1:2, 1:3, 1:4, 1:5, 1:7, 1:9, 1:10) DCM 100%, DCM:MeOH (100%, 97:3, 93:7, 9:1) MeOH, 100%
Column Size	-	3.5cm × 42 cm
Flow rate	-	2ml/min

Figure 2 Silica 34 gel column

Characterization of the isolated compounds from *Cephalosporium* sp.

The isolated compounds were characterized by spectroscopic techniques such as UV and FT-IR spectra, and their behaviour on TLC plates. The spectra were undertaken at Universities Research Centre, University of Yangon. The spectral assignments were assigned according to Robert and Francis (2014).

Antimicrobial activity of the isolated compounds from *Cephalosporium* sp.

The three terpenoid compounds were tested their antimicrobial activities on eight pathogenic microorganisms *Agrobacterium tumefaciens, Aspergillus flavus, Candida albicans, Escherichia coli, Malassezia furfur, Salmonella typhi, Staphylococcus aureus* and *Xanthomonas oryzae* by paper disc diffusion assay.

Results

Antimicrobial activity of 10 L fermentation of *Cephalosporium* sp.

In this study, the fermented broths from the ten fermentation flasks showed highly antimicrobial activity against eight test organisms as shown in Table 1.

Table 1 Inhibitory zones (mm) of fermented broths from 10 L fermentation

Fermentation flask	*Asper. flavus*	*Bacillus subtilis*	*Candida albicans*	*E. coli*	*M. furfur*	*Micro. luteus*	*Sal. typhi*	*Staphy. aureus*
Flask 1	19	19	20	19	20	19	19	22
Flask 2	21	19	21	20	24	20	18	23
Flask 3	20	20	20	19	19	18	18	21
Flask 4	22	18	21	20	23	18	20	27
Flask 5	20	18	18	22	22	18	17	23
Flask 6	21	25	23	22	22	17	21	24
Flask 7	13	11	14	18	17	12	12	16
Flask 8	24	26	25	23	27	24	23	29
Flask 9	17	21	19	22	20	18	16	19
Flask 10	16	16	15	20	18	15	14	18

10 -12 mm = weak activity, 13 - 17 mm = high activity, >18 mm = very high activity

Isolation of the bioactive compounds from *Cephalosporium* sp.

The one hundred and eight small fractions were collected from silica gel 34 column with various solvent systems. According to their R_f values and colour spots on TLC plates under UV 254 nm and sprayed with reagent, they were combined into nine large fractions: F1 (1 & 2), F2 (3 & 4), F3 (5-12), F4 (13-41), F5 crystals (42-47), F6 (48-51), F7 (52-68), F8 (69-90) and F9 (91-108) as shown in Figure 3.

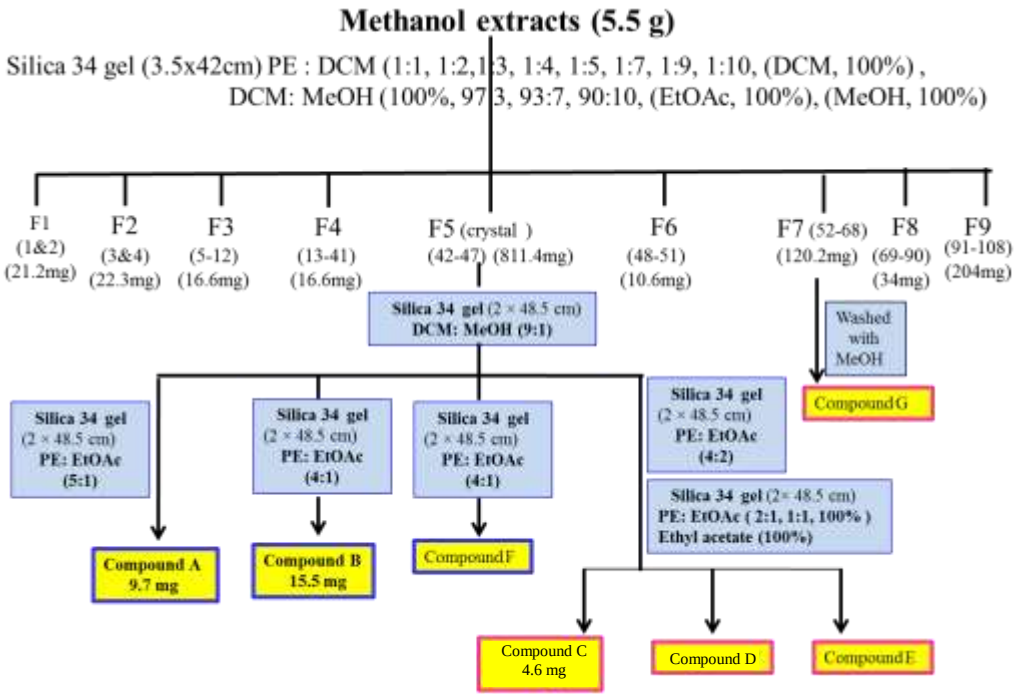
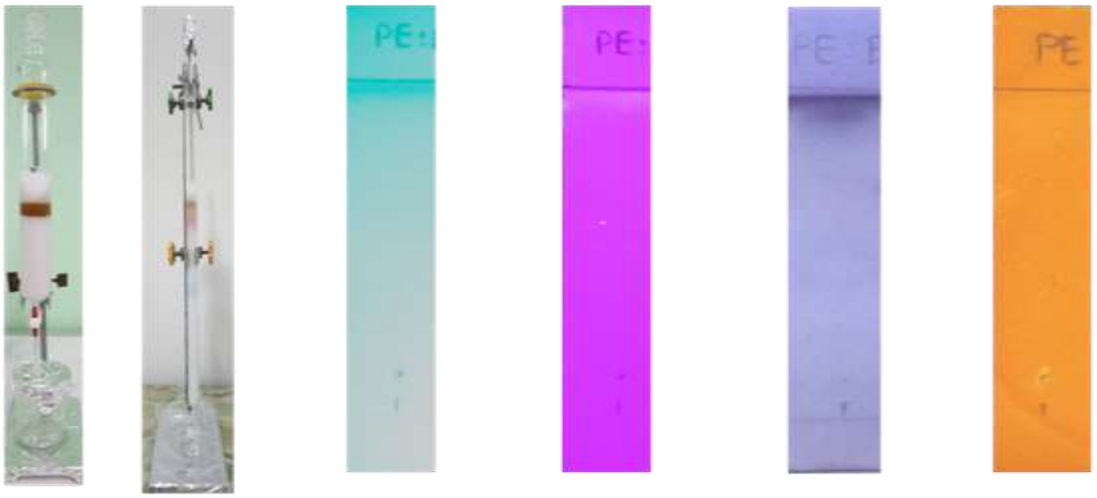


Figure 3 Isolation procedure of bioactive compounds

Physico-chemical properties of the isolated compound A



Silica 34 gel columns **UV 254 nm** **UV 365 nm** **With anisaldehyde** **With Dragendroff**

The eluting solvent = DCM: MeOH (9:1)
= PE: EtOAc (5:1)
Flow rate = 2ml / min
R_f = 0.93 (PE: EtOAc 1:1)
Spot colour = dark blue (Anisaldehyde reagent)

Figure 4 The TLC profile of isolated compound A

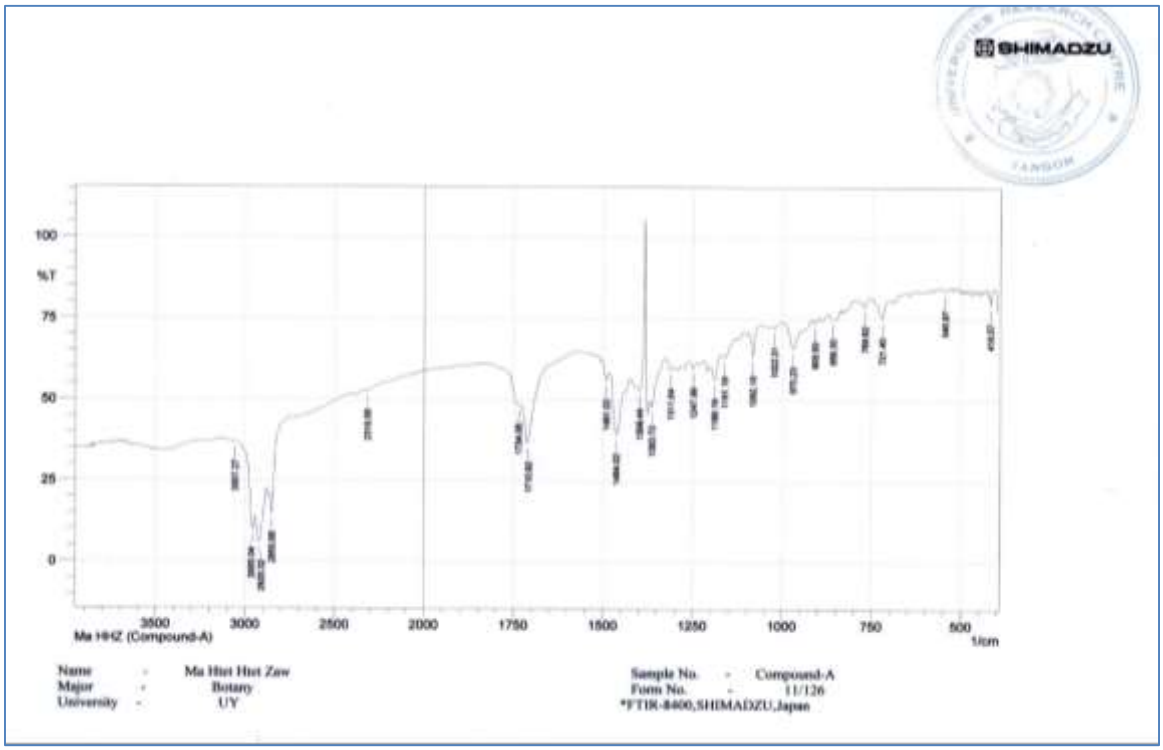


Figure 5 FT-IR spectrum of the isolated compound A

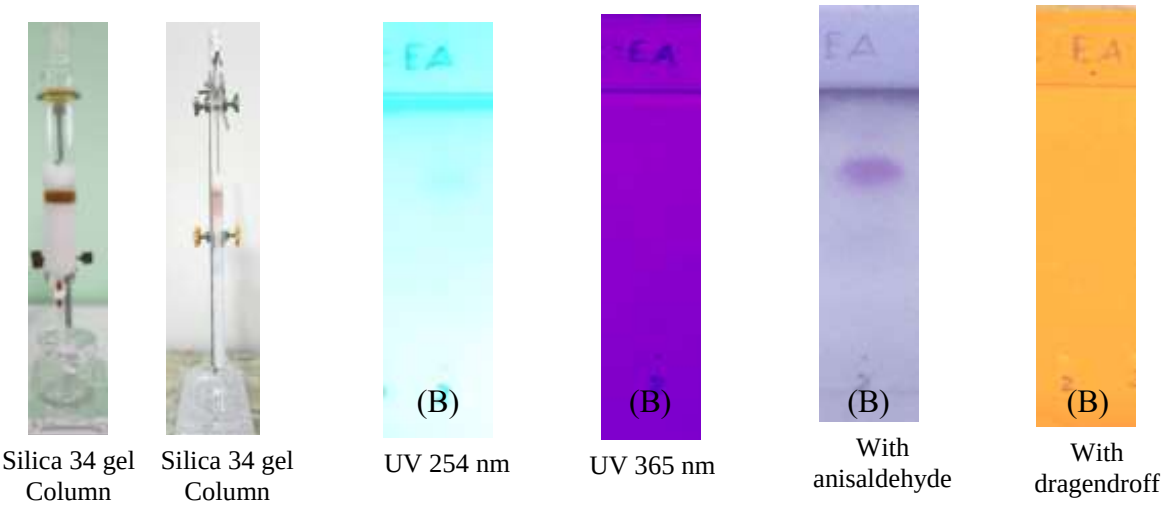
Table 2 FT-IR spectral data assignment of the isolated compound A

Wave number (cm^{-1})	Assignment
3450	O-H stretching vibration of hydroxyl group
2955, 2850	C-H stretching of -CH_3 and -CH_2- group
1710	C=O stretching vibration of carbonyl group
1491,1464, 1363	C-H bending of -CH_3 and -CH_2- group
1464, 970	O-H bending vibration of hydroxyl group
1247, 1181, 1082, 1022	C-O-C stretching vibration (ether)

The compound A was isolated from the fraction 5, it is UV inactive. It has R_f (0.93) (PE: EtOAc, 1:1). It gave a dark pink colour with anisaldehyde reagent firstly. Then, the colour changed into dark blue (Figure 4).

In its IR spectrum (Figure 5), O-H stretching vibration of hydroxyl group was observed at 3450 cm^{-1}. CH_3 and CH_2 groups (C-H stretching) were found at 2955 cm^{-1} and 2850 cm^{-1}. C = O stretching vibration of carbonyl group was shown at 1710 cm^{-1}. CH_3 and CH_2 groups (C-H bending) were observed at 1491 cm^{-1}, 1464 cm^{-1} and 1363 cm^{-1}. O-H bending vibration of hydroxyl group was seen at 1464 cm^{-1} and 970 cm^{-1}. C-O-C stretching vibrations (ether) were found at the wave numbers 1247 cm^{-1}, 1181 cm^{-1}, 1082 cm^{-1} and 1022 cm^{-1}. This substance is good soluble in petroleum ether or dichloromethane. It is a terpenoid compound according to behaviour on TLC plate and IR spectral data.

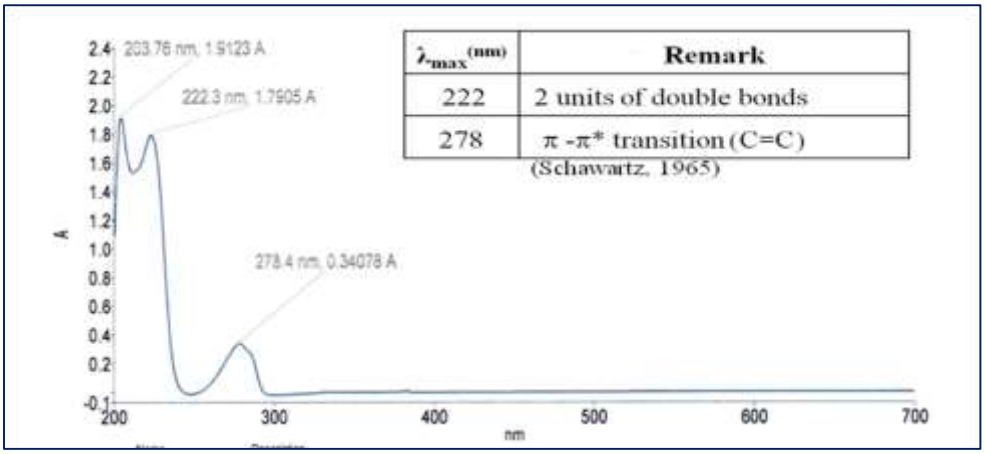
Physico-chemical properties of the isolated compound B



Silica 34 gel Column | Silica 34 gel Column | UV 254 nm | UV 365 nm | With anisaldehyde | With dragendroff

The eluting solvent = DCM: MeOH (9:1)
= PE: EtOAc (4:1)
R_f = 0.73 (PE: EtOAc 1:1)
Spot colour = light colour (under UV 254nm)
= primary colour is dark pink with anisaldehyde reagent but later changed purple colour

Figure 6 The TLC profile of isolated compound B



λ_{max} (nm)	Remark
222	2 units of double bonds
278	π -π* transition (C=C)

(Schawartz, 1965)

Figure 7 UV spectrum of the isolated compound B

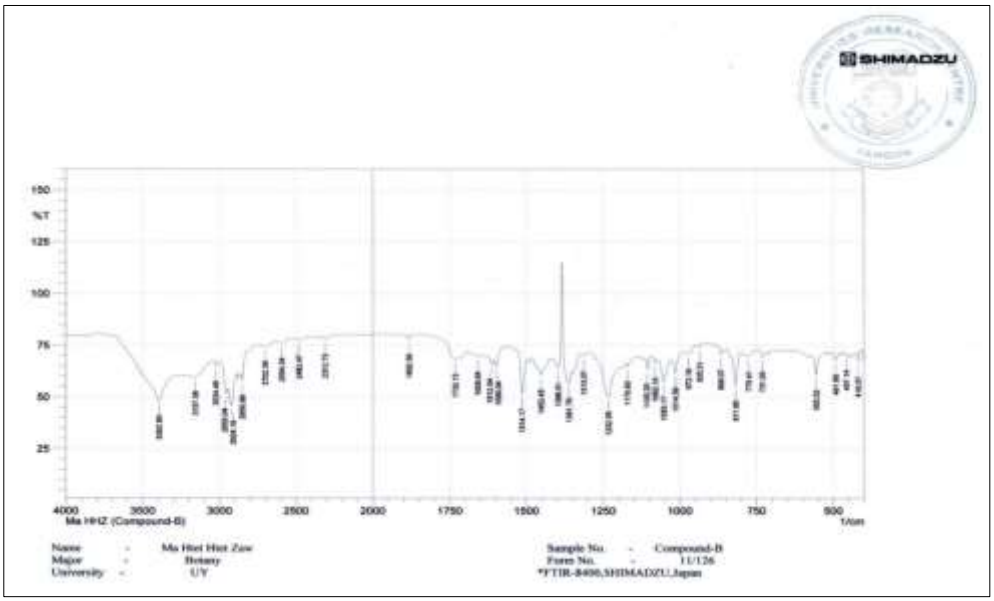


Figure 8 FT-IR spectrum of the isolated compound B

Table 3 FT-IR spectral data assignment of the isolated compound B

Wave number (cm^{-1})	Assignment
3392	O-H stretching vibration in phenolic group
2924, 2850	C- H stretching vibration of -CH_3 and -CH_2 -
1732	C=O stretching vibration
1658, 1514, 1452	C=C stretching vibration (aromatic)
1014, 1053, 1105	C-O stretching vibration of C-O-C
1452, 972	C-OH and O-H bending of aromatic ring

The compound B was isolated from the fraction 5 as a UV active substance under absorbing band at 254 nm. It has R_f 0.73 (PE: EtOAc, 1:1). It gave a dark pink colour with anisaldehyde reagent firstly. Later, the colour changed into purple spot (Figure 6). This substance is good soluble in dichloromethane or methanol. Its UV spectrum showed the two units of double bonds (C=C) at 222 and 278 nm as shown in Figure 7.

In its IR spectrum (Figure 8), O-H stretching vibration of hydroxyl group was observed at 3392 cm^{-1}. C-H stretching vibrations of CH_3 and CH_2 groups were found at 2924 cm^{-1} and 2850 cm^{-1}. C=O stretching vibration was shown at 1732 cm^{-1}. C=C stretching vibrations (aromatic) were found at the wave numbers 1658 cm^{-1}, 1514 cm^{-1} and 1452 cm^{-1}. C-O stretching vibrations of C-O-C were found at 1014 cm^{-1}, 1053 cm^{-1} and 1105 cm^{-1}. C-OH and O-H bending vibrations of aromatic ring were seen at 1452 cm^{-1} and 972 cm^{-1}. It is a terpenoid compound according to behaviour on TLC profile, UV and IR spectral assignments.

Physico-chemical properties of the isolated compound C

Silica 34 gel Column | Silica 34 gel Column | Silica 34 gel Column | With anisaldehyde

The eluting solvent = DCM: MeOH (9:1)
= PE: EtOAc (2:1, 1:1, 100%) Ethyl acetate (100%)
Flow rate = 2ml / min
R_f = 0.36 (PE: EtOAc 1:1)
Spot colour = Inactive
Purple colour (Anisaldehyde reagent)

Figure 9 The TLC profile of isolated compound C

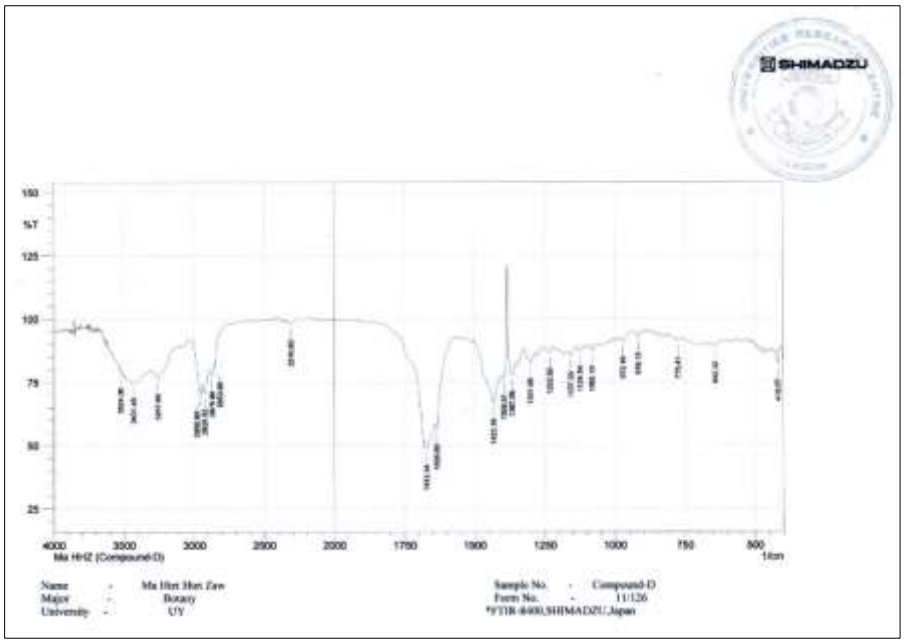


Figure 10 FT-IR spectrum of the isolated compound C

Table 4 FT-IR spectral data assignment of the isolated compound C

Wave number (cm^{-1})	Assignment
3431, 3257	O-H stretching vibration in phenolic group
2958, 2875	C-H stretching of -CH_3, -CH_2- and -CH
1672	C=C stretching vibration (aromatic C=C)
1433, 1396	C-H bending vibration of -CH_3, -CH_2- and -CH
1157	C-O-C stretching vibration of ether

The compound C was also isolated from the fraction 5, and it is UV inactive. It has R_f 0.50 (PE: EtOAc 1:1). It gave a pink colour with anisaldehyde reagent (Figure 9). This substance is good soluble in petroleum ether and ethylacetate.

In its IR spectrum (Figure 10), O-H stretching vibration in phenolic group was observed at 3431 cm^{-1} and 3257 cm^{-1}. The wave numbers 2958 cm^{-1} and 2875 cm^{-1} showed the presence of C-H stretching vibrations for CH_3 and CH_2 group. C=C stretching vibration (aromatic C=C) was shown at 1672 cm^{-1}. C-H bending vibrations of CH_3 and CH_2 and CH were observed at 1433 cm^{-1} and 1396 cm^{-1}. C-O-C stretching vibration of ether was found at the wave number 1157 cm^{-1}. It is a terpenoid compound according to its behaviour on TLC profile and IR spectral assignments.

Antibacterial activities of the terpenoid compounds

The compounds A, B and C showed weakly antibacterial activity on *Agrobacterium tumefaciens, Escherichia coli* and *Salmonella typhi* while the compound A also showed moderately antibacterial activity on *Staphylococcus aureus* and *Xanthomonas oryzae.*

Discussion and Conclusion

Endophytic fungal strain *Cephalosporium* sp. was isolated from the wood of *Hesperethusa crenulata* (Roxb.) Roem., and utilized for extraction, separation, isolation and antimicrobial activity of the bioactive compounds. In antimicrobial activity of 10 L fermentation, all fermentation flasks showed high activity against eight test organisms. In the extraction, the fermented broths were applied on XAD 16 resin column followed by elution with methanol. Mohamed Shaaban *et al*., (2013) also used XAD-16 resin to extract the filtrate using followed by elution with methanol. The methanol extract showed highly activity against eight test organisms.

The three isolated compounds were the terpenoid compounds that showed antimicrobial activity on *Agrobacterium tumefaciens*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Xanthomonas oryzae.* Gerlach *et al*., (2018) reported that the use of sulfuric anisaldehyde reagent greatly improves the detection of terpenes. Miao *et al.,* (2012) stated that harziane diterpenes were isolated from endophytic fungus. Liu *et al.,* (2013) mentioned that new sesterterpenoids were isolated from different endophytic fungi. Yumei *et al*., (2004) reported that the secondary metabolites of *Cephalosporium* sp. showed activity against *M. luteus, B. subtilis, S. aureus, S. typhi, E. coli* and *P. aeruginosa*.

In conclusion, the seven compounds were isolated from the methanol extract of *Cephalosporium* sp. The three from the seven compounds were the terpenoid compounds that possessed antibacterial activity on five test organisms. Therefore, these compounds inhibited diarrhea, fever, and skin infection on man as well as crown gall disease and leaf blight disease on plants.

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We would like to express our gratitude to Chairpersons and Professors from Department of Botany, University of Yangon for their kind permission and suggestion to present this paper on the Conference for Myanmar Academy of Arts and Science (MAAS).

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ISOLATION AND CHARACTERIZATION OF PATHOGENIC FUNGI INFECTED ON *ORYZA SATIVA* L. VAR. PAW SAN BAYKYAR IN MONYWA DISTRICT

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Abstract

The study on the isolation and morphological characterization of pathogenic fungi infected plants of *Oryza sativa* L. (Paw San Baykyar rice variety) was carried out at the Microbiology Laboratory, Department of Botany in Monywa University, Myanmar from June to November, 2018. The disease-infected plant samples were collected from the rice fields in Monywa District. The different cultural media of Low Carbon Agar (LCA), Potato Dextrose Agar (PDA) and Water Glucose Agar (WGA) were used for the isolation of pathogenic fungal strains. The morphological characterization was studied on their colony morphology, macroscopical and microscopical characters. Total of 12 genera (*Alternaria* sp., *Aspergillus* sp., *Cercospora* sp., *Fusarium* spp., *Nigrospora* sp., *Sarocladium* sp., *Curvularia* sp., *Ustilaginoidea* sp., *Penicillium* sp., *Rhizopus* sp., *Cladosporium* sp., *Bipolaris* sp.) and 13 pathogenic fungal strains (HH 01 - HH 13) were isolated and identified from leaves, leaf sheaths, grains and seeds of the disease-infected rice plants. The HH 01 strain as *Alternaria* sp., HH 02 strain as *Aspergillus* sp., HH 03 strain as *Cercospora* sp., HH 04 strain as *Fusarium* sp., HH 05 strain as *Nigrospora* sp., HH 06 strain as *Sarocladium* sp., HH 07 strain as *Curvularia* sp., HH 08 strain as *Fusarium* sp., HH 09 strain as *Ustilaginoidea* sp., HH 10 strain as *Penicillium* sp., HH 11 strain as *Rhizopus* sp., HH 12 strain as *Cladosporium* sp. and HH 13 strain as *Bipolaris* sp. were identified.

Keywords: Pathogenic fungi, *Oryza sativa* L., Paw San Baykyar rice variety, isolation

Introduction

Oryza sativa L. is one of the most important cereal crop of family Poaceae. It is a staple food crop of 60 percent of the world's population. The edible uses of rice include namely, rice flakes, puffed rice, rice wafers and canned rice. It is also used in starch and brewing industries. The byproduct by rice milling that is rice husk and bran are used as a cattle and poultry feed. Rice is one of the diverse crop grown in different agro-climatic conditions (Ramakrishnan 1971). Rice has been the focus in the history of Myanmar economic development. Myanmar' Paw San rice is one of the world's most recognized high quality rice, it was awarded the world's best rice at the Rice Trader's World Rice Conference in 2011 (Myint & Napasintuwong 2016).

Microorganisms play an important role in affecting the quality of seed of which fungi are the largest group. These pathogens are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages (Uma & Wesely 2013). Rice suffers from many diseases caused by fungi, bacteria, viruses, nematodes and other non-parasitic disorders. Fungal disease is considered as the principal disease of rice because of its wide distribution and its destructiveness under favorable conditions for yield loss. The biotic pathogens can infect the crop at any time from seed germination to harvest (Devi & Pushpalatha 2013).

Fungi are a major cause of reduction in the quality of rice due to high moisture and temperature conditions before its harvest (Uma & Wesely 2013). More than 100 species of fungi have been identified on rice seeds so far. However, their severity depends on the time of sampling, location and varieties are different (Monajjem *et al*. 2014).

Rice is affected by as many as 36 seed borne diseases of which 31 were caused by fungi which are mostly namely, *Pyricularia oryzae*, *Alternaria padwickii*, *Helminthosporium* sp.,

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Gibberella fujikuroi, *Gibberella rosa*, *Fusarium cereali*, *Nigrospora* sp., *Epicoccum* sp., *Phyllosticta glumarum*, *Alternaria* sp. and *Helicoceras oryzae*. The most common storage fungi are *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus*, *Chaetomium*, *Dematium*, *Monilia*, *Oidium*, *Streptomyces*, *Syncephalastrum* and *Verticillium* (Sharma & Kapoor 2017).

The aim of present study deals with the isolation and morphological characteristics of pathogenic fungi from infected *Oryza sativa* L. var. Paw San Baykyar from Monywa District. The specific objectives were to isolate the pathogenic fungi which infected on the plants of Paw San Baykyar rice variety, to identify the micro- and macroscopical characters of cells and colony morphology of isolated pathogenic fungal strains, and to record the pathogenic fungi which can be caused diseases on different plant parts of those rice variety under field conditions in Monywa District.

Materials and Methods

Collection of the Infected Plant Parts

The plant samples from the infected plant parts of *Oryza sativa* L. (Paw San Baykyar rice variety) were collected from the rice fields of Monywa District from June to November 2018. Isolation of pathogenic fungi was done as soon as possible after the infected plant samples were brought to the Microbiology Laboratory of Botany Department in Monywa University. Plant pathogenic fungi were isolated by direct isolation method according to Ando (2015), using PDA medium. Low Carbon Agar (LCA) and Water Glucose Agar (WGA) were used for the isolation and identification of pathogenic fungi (Motlagh 2010).

Isolation and Identification of Microorganisms

The disease-infected parts of *Oryza sativa* L. var. Paw San Baykyar were used for the isolation of pathogenic microorganisms. Fungi identification was followed by the methods of Barnett (1955), Larone (1995), Mew & Gonzales (2002), Tanaka (2008), Ladhalakshmi *et al.* (2011), Abass & Mohammed (2014), Kidd *et al.* (2016).

Measuring of Microorganisms

The fungal spores were measured according to the methods of Kokate (2000). These isolated fungal strains were examined under light microscope, XSZ – 107BN and the results were recorded.

Results

Total of 13 pathogenic fungal strains (HH 01 - HH 13) were isolated and identified from the disease-infected parts of *Oryza sativa* L. var. Paw San Baykyar. The macroscopical and microscopical characters of those isolated pathogenic fungal strains were also shown in Table 1 and Figure 1-13.

The isolated pathogenic fungal strains were found in different plant parts of those rice variety with the pathogenic disease-symptoms. The pathogenic fungal strains of HH 01 and HH 02 were found in stackburn symptom infected leaves which caused large oval or circular spots with a pale brown margin. Color of center eventually becomes white and bear dots in leaves (Fig 1 A and Fig 2).

The HH 03, HH 04 and HH 05 strains were found in leaf spot symptom infected leaves which caused short, linear, brown lesions mainly on the leaves (Fig 3 A, Fig 4 and Fig 5). The HH

06, HH 07 and HH 08 strains were found in leaf sheath rot symptom infected leaf sheaths which caused irregular spots, with gray to light brown centers surrounded by distinct dark reddish brown margins (Fig 6 A, Fig 7 and Fig 8). HH 09 strain was found in false smut symptom infected grains which caused greenish spore balls that have velvety appearance. The color of the ball become orange and later yellowish green or greenish black (Fig 9 A). HH 10 and HH 11 strains were found in seed blight symptom infected on grains which caused initially small, oblong, and brown then gradually enlarge and coalesce, becoming whitish with small black dots (Fig 10 A and Fig 11). HH 12 and HH 13 strains were found in black kernel symptom infected seeds which caused black spots, discoloration and empty (Fig 12 A and Fig 13).

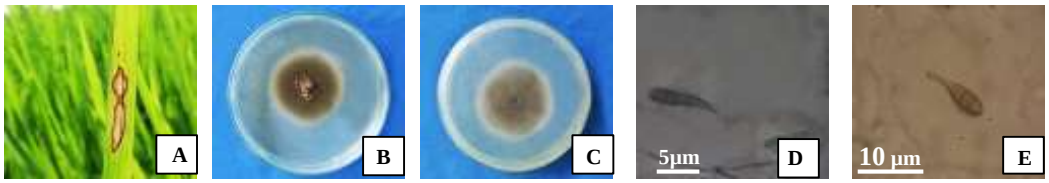

Figure 1 *Alternaria* sp. (HH 01 strain) isolated from stackburn infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. stackburn infected leaves; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. conidia.

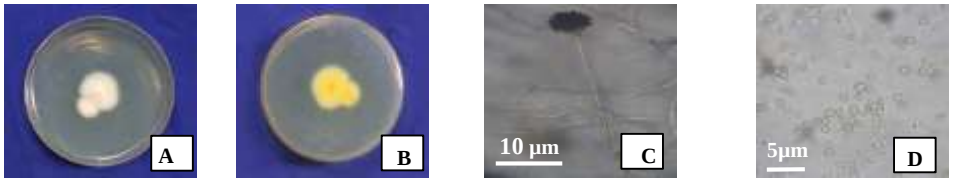

Figure 2 *Aspergillus* sp. (HH 02 strain) isolated from stackburn infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

Figure 3 *Cercospora* sp. (HH 03 strain) isolated from leaf spot infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. leaf spot infected leaves; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

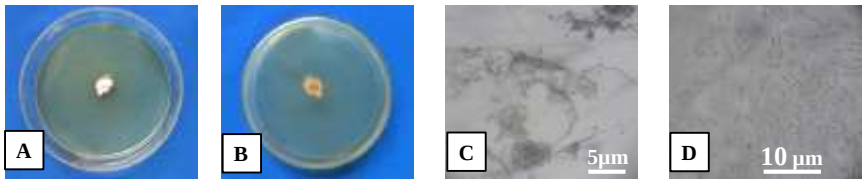

Figure 4 *Fusarium* sp. (HH 04 strain) isolated from leaf spot infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

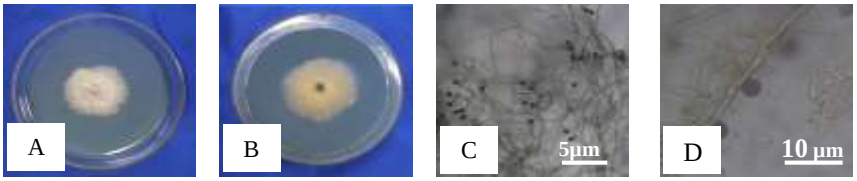

Figure 5 *Nigrospora* sp. (HH 05 strain) isolated from leaf spot infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

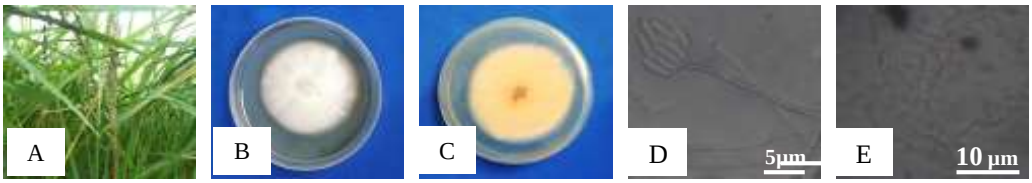

Figure 6 *Sarocladium* sp. (HH 06 strain) isolated from leaf sheath rot infected leaf sheaths of *Oryza sativa* L. var. Paw San Baykyar. A. leaf sheath rot infected leaf sheaths; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

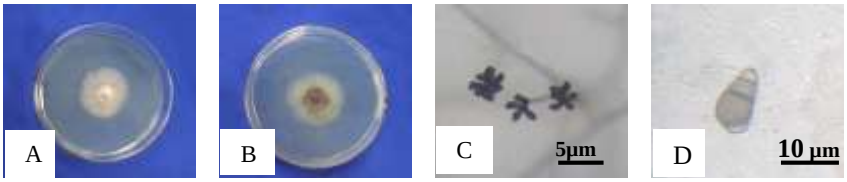

Figure 7 *Curvularia* sp. (HH 07 strain) isolated from leaf sheath rot infeceted leaf sheaths of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

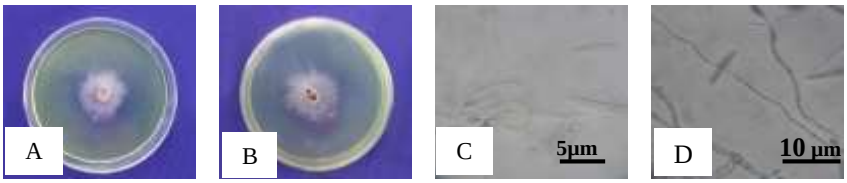

Figure 8 *Fusarium* sp. (HH 08 strain) isolated from leaf sheath rot infected leaf sheaths of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

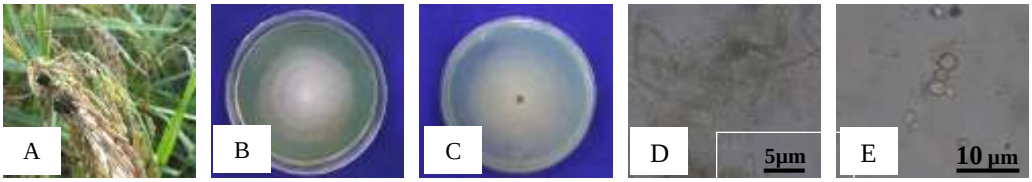

Figure 9 *Ustilaginoidea* sp. (HH 09 strain) isolated from false smut infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. false smut infected seeds; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

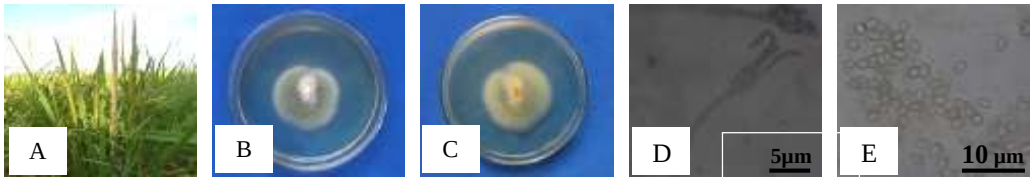

Figure 10 *Penicillium* sp. (HH 10 strain) isolated from seed blight infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. seed blight infected seeds; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

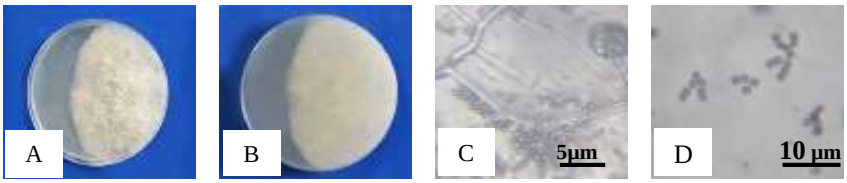

Figure 11 *Rhizopus* sp. (HH 11 strain) isolated from seed blight infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

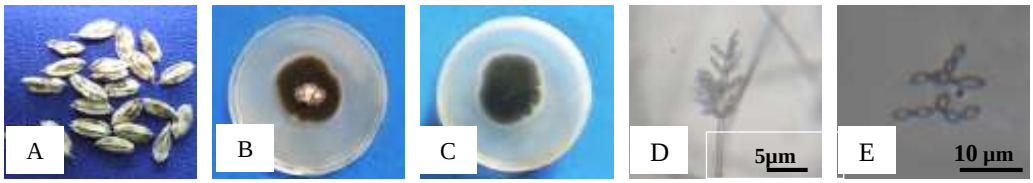

Figure 12 *Cladosporium* sp. (HH 12 strain) isolated from black kernel infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. black kernel infected grains; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

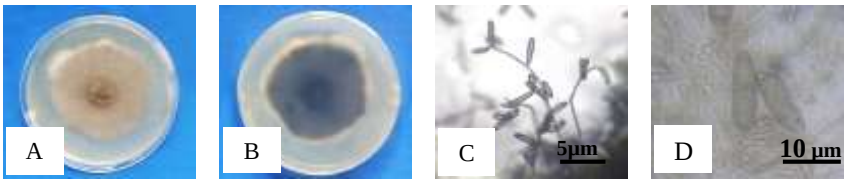

Figure 13 *Bipolaris* sp. (HH 13 strain) isolated from black kernel infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

Table 1 Macroscopical and microscopical characters of isolated pathogenic fungal strains from the disease infected parts of *Oryza sativa* L. var. Paw San Baykyar

Isolated Strains	Pathogenic Fungi	Macroscopical Characters	Microscopical Characters	Diseases
HH 01	*Alternaria* sp.	Greenish black or brown and 5.5 cm. Reverse remained black.	Septate hyphae. Conidiophores were dark and short. Conidia were dark, fusiform to obclavate, thick-walled, 3-5 septate, second cell from the base larger than the rest of the cells and 35.5 - 45.5 µm.	Stackburn
HH 02	*Aspergillus* sp.	Suede-like and cinnamon-buff, white and 2.1 cm. Reverse remained yellow.	Septate hyphae. Conidiophores stipes were usually short, brownish or smooth-walled, terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia were globose to ellipsoidal, slightly yellow, hyaline and 1.5 - 2.5 µm.	Stackburn
HH 03	*Cercospora* sp.	White, with sinuate margins and 2.0 cm. Reverse remained pale red.	Hyphae were swollen. Conidiophores were brown, 3 or more septate. Conidia were hyaline or light olive, 3-10 septate, cylindrical to clavate and 18.1 - 28.0 µm.	Leaf spot
HH 04	*Fusarium* sp.	White and 3.8 cm. Reverse remained white with a dark center.	Septate hyphae. Conidiophores were hyaline, short, simple to multibranched. Conidia were hyaline, fusiform, ovate or clavate, single-celled to 4-celled and 10.5 - 16.0 µm.	Leaf spot
HH05	*Nigrospora sp.*	White to gray and 4.5 cm. Reverse remained gray.	Septate hyphae. Conidiophores were short, simple, inflated below the tip. Conidia were black, spherical, globose or subglobose, single and 4.5 - 8.0 µm.	Leaf spot
HH 06	*Sarocladium* sp.	White and 6.7 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were simple and hyaline. Conidia were grouped in slimy head, hyaline, cylindrical or ellipsoidal and 35.0 - 40.5 µm.	Leaf sheath rot
HH 07	*Curvularia* sp.	Gray and 3.8 cm. Reverse remained black.	Septate hyphae. Conidiophores were dark brown, unbranched or branched typically bent. Conidia were dark brown, boat-shaped, 3-5 celled, end cells lighter; one or two of the central cells enlarged and 12.0 - 18.0 µm.	Leaf sheath rot

Isolated Strains	Pathogenic Fungi	Macroscopical Characters	Microscopical Characters	Diseases
HH 08	*Fusarium* sp.	White and 1.6 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were hyaline, single, lateral. Conidia were hyaline, fusiform, slightly flattened on both end, one or two celled and 4.0 - 8.0 µm.	Leaf sheath rot
HH 09	*Ustilaginoidea* sp.	White and 5.6 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were forming and bearing conidia at the tapering apex. Conidia were yellow to dark color, ovoid and 5.0 - 7.5 µm.	False smut
HH 10	*Penicillium* sp.	Green shade, sometime white and 4.9 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were arising singly, frequently branches, near the apex to form a brush-like, conidia-bearing apparatus, ending in phialides which pinch off conidia in dry chains. Conidia were greenish, ovoid, 1-celled and 5.0 - 8.5 µm.	Seed blight
HH 11	*Rhizopus* sp.	Cottony at first white and then gray or yellowish brown and 6.8 cm. Reverse remained white.	Non septate hyphae. Sporangiophores were simple or branched, arising from stolons opposite rhizoids usually in groups of three or more. Sporangia were greyish black, globose and 2.5 - 5.0 µm.	Seed blight
HH 12	*Cladosporium* sp.	Blackish-brown and 5.0 cm. Reverse remained olivaceous black.	Septate hyphae. Conidiophores were dark, branched variously near the apex or middle portion, clustered or single. Conidia were dark, hilum, 1- or 2-celled, ovoid to cylindrical and 5.0 - 12.0 µm.	Black kernel
HH 13	*Bipolaris* sp.	Grey to dark grey and 6.8 cm. Reverse remained black.	Septate hyphae. Conidiophore were single or in small group, straight to flexuous, pale to mid brown. Conidia were pale to mid golden brown, curved, navicular, fucoid of obclavate, occasionally almost cylindrical, 5-12 distoseptate and 26.0 - 48.0 µm.	Black kernel

Discussion

Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Diseases are considered major constraints in rice production. According to the present study, twelve genera of fungal pathogens concerning with the six kinds of diseases symptoms were observed in the disease-infected parts of *Oryza sativa* L. var. Paw San Baykyar. In the present study, 12 genera of 13 pathogenic fungal strains were found in

the disease-infected plant parts of *Oryza sativa* L. var. Paw San Baykyar. The 13 isolated pathogenic fungal strains (HH 01 - HH 13) were identified.

In this study, HH 01 fungal strain, *Alternaria* sp. found that colonies were greenish black or brown with a light border and 5.5 cm. Reverse remained black. They were septate hyphae. Conidiophores were dark and short. Conidia were dark, fusiform to obclavate, thick-walled, 3-5 septate, second cell from the base larger than the rest of the cells and 35.5 - 45.5 µm. The macroscopical and microscopical characters of *Alternaria* sp. was similar with the statements of Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

The HH 02 fungal strain, *Aspergillus* sp. revealed that colonies were typically suede-like and cinnamon-buff, white and 2.1 cm. Reverse remained yellow. They were septate hyphae. Conidiophore stipes were usually short, brownish or smooth-walled, terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia were globose to ellipsoidal, slightly yellow, hyaline and 1.5 - 2.5 µm. These findings were agreed with Barnett (1955) and Larone (1995).

The HH 03 fungal strain, *Cercospora* sp. found that colonies were white with sinuate margins and 2.0 cm. Reverse remained pale red. They were swollen hyphae. Conidiophores were brown, 3 or more septate. Conidia were hyaline or light olive, 3 to 10 septate, cylindrical to clavate and 18.1 - 28.0 µm. These isolated *Cercospora* sp. was similar with the macroscopical and microscopical characters to the statements of Barnett (1955) and Mew & Gonzales (2002).

In the present study, HH 04 fungal strain, *Fusarium* sp. revealed that colonies were white and 3.8 cm. Reverse remained white with a dark center. They were septate hyphae. Conidiophores were hyaline, short, simple to multi branched. Conidia were hyaline, fusiform, ovate or clavate, 1-4 celled and 10.5 - 16.0 µm. These findings were agreed with Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

HH 05 fungal strain, *Nigrospora* sp. found that colonies were white to gray and 4.5 cm. Reverse remained gray. They were septate hyphae. Conidiophores were short, simple, inflated below the tip. Conidia were black, globose or subglobose, single and 4.5 - 8.0 µm. These findings were agreed with Abass & Mohammed (2014).

In the present study, HH 06 fungal strain, *Sarocladium* sp. revealed that colonies were white and 6.7 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were simple and hyaline. Conidia were grouped in slimy head, hyaline, cylindrical or ellipsoidal and 35.0 - 40.5 µm. HH 07 fungal strain, *Curvularia* sp. found that colonies were gray and 3.8 cm. Reverse remained black. They were septate hyphae. Conidiophores were dark brown, unbranched or branched typically bent. Conidia were dark brown, boat-shaped, 3-5 celled, end cells lighter; one or two of the central cells enlarged and 12.0 - 18.0 µm. These findings were agreed with Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

HH 08 fungal strain, *Fusarium* sp. revealed that colonies were white and 1.6 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were hyaline, single, lateral. Conidia were hyaline, fusiform, slightly flattened on the both ends, one or two celled and 4.0 - 8.0 µm. These findings were agreed with Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

HH 09 fungal strain, *Ustilaginoidea* sp. found that colonies were white and 5.6 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were forming and bearing conidia at the tapering apex. Conidia were ovoid, yellow to dark color and 5.0 - 7.5 µm. These isolated *Ustilaginoidea* sp. was similar with the macroscopical and microscopical characters to the statements of Tanaka (2008) and Ladhalakshmi *et al*. (2011).

In the present study, HH 10 fungal strain, *Penicillium* sp. found that colonies were in shades of green, sometime white and 4.9 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were arising singly, frequently branches, near the apex to form a brush-like, conidia-bearing apparatus, ending in phialides which pinch off conidia in dry chains. Conidia were greenish, ovoid, 1-celled and 5.0 - 8.5 µm. These findings were agreed with Barnett (1955), Larone (1995) and Kidd *et al*. (2016).

In this study, HH 11 fungal strain, *Rhizopus* sp. revealed that colonies were cottony, at first white and then gray or yellowish brown and 6.8 cm. Reverse remained white. They were non septate hyphae. Sporangiophores were simple or branched, arising from stolons opposite rhizoids usually in groups of three or more. Sporangia were globose, greyish black and 2.5 - 5.0 µm. HH 12 fungal strain, *Cladosporium* sp. found that colonies were blackish-brown and 5.0 cm. Reverse remained olivaceous black. They were septate hyphae. Conidiophores were dark, branched variously near the apex or middle portion, clustered or single. Conidia were dark, hilum, 1- or 2-celled, ovoid to cylindrical and 5.0 - 12.0 µm. These findings were agreed with Larone (1995) and Kidd *et al*. (2016).

In the present study, HH 13 fungal strain, *Bipolaris* sp. revealed that colonies were grey to dark grey and 6.8 cm. Reverse remained black. They were septate hyphae. Conidiophore were single or in small group, straight to flexuous, pale to mid brown. Conidia were curved, navicular, fucoid of obclavate, occasionally almost cylindrical, pale to mid golden brown, 5-12 distoseptate and 26.0 - 48.0 µm. These findings were agreed with Larone (1995) and Mew & Gonzale (2002).

The stackburn symptoms included large oval or circular spots with a pale brown margin. Color of center eventually becomes white and bear minute black dots on leaves. Those findings were agreed with Lau and Sheridan (2012) and Asghfaq *et al*. (2017). Lau and Sheridan (2012) also stated that *Alternaria* sp., causal agent of stackburn disease on rice. Asghfaq *et al*. (2017) stated that *Alternaria* sp. was isolated from different rice varieties.

The leaf spot symptoms are short, linear, brown lesions mainly on the leaves. Those findings were in agreement with Elazegui and Islam (2003) who stated that although it may also occur on leaf sheath, pedicels and glume. In this study, *Sarocladium* sp. occurs on rotting leaf sheath enclosing the young panicles. Lesions consist of diffuse reddish brown discoloration in the leaf sheath. These observations are in agreement with Elazegui & Islam (2003) and Rawte (2007).

Ustilaginoidea sp., the fungus transforms individual seeds of the panicles into greenish spore balls that have velvety appearance. The color of the spore balls becomes orange and later yellowish green, or greenish black on grains. These findings were also agreed with the findings of Elazegui & Islam (2003) and Tripathi *et al.* (2011).

In *Bipolaris* sp., brown spot may be manifested as seed blight disease symptom in mature seeds. This fungus may also infected the glumes, causing dark brown to black oval spots, and may also infected the seed, causing a black discoloration. These observations were in agreement with Elazegui & Islam (2003), Tripathi *et al*. (2011) and Lau & Sheridan (2012). In this study, *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. were discoloration in seeds. These findings were agreed with Imolehin (1983).

Conclusion

This study was identified as 12 genera of pathogenic fungi including 13 fungal strains identified from *Oryza sativa* L. var. Paw San Baykyar. Those isolated pathogenic fungi were found in the disease symptoms of leaves, leaf sheaths and seeds of Paw San Baykyar rice variety. Thus, the present study will be provided some information of pathogenic fungi occurring on the Paw San Baykyar in Myanmar. These pathogenic fungi that cause slower growth and loss yield of rice.

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TAXONOMIC STUDY ON TEN SPECIES OF FABACEAE FROM PAUK TOWNSHIP, MAGWAY REGION

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Abstract

Taxonomic study on ten species belonging to the family Fabaceae in Pauk Township was carried out. The flowering specimens were collected and identified from June 2019 to May 2020. Totally 8 genera were resulted. Five species are under the subfamily Caesalpinioideae and the other five species are under Papilionoideae. The caesalpiniaceous species are *Bauhinia acuminata* L., *Bauhinia racemosa* Lam., *Parkinsonia aculeata* L., *Senna auriculata* (L.) Roxb. and *Senna hirsuta* (L.) Irwin & Barneby. The papilionaceous species are *Butea monosperma* (Lam.) Taub., *Crotalaria striata* DC., *Dalbergia oliveri* Gamble ex Prain, *Erythrina microcarpa* Koord. & Valeton and *Millettia pinnata* (L.) Panigrahi. Among the study species, 6 species are trees, 2 species are shrubs and 2 species are herbaceous. The leaf types of 8 species are compound and the other 2 species are simple leaves. The taxonomic descriptions of each species were described with their respective figures. Myanmar name, English name and Flowering period have been mentioned. An artificial key to the genera and species were constructed. It is hoped that the present research will provide the valuable information for further studies and will support in teaching to the students.

Keyword: Taxonomic study, Fabaceae, identified, 8 genera, Artificial key to the genera and species

Introduction

Taxonomy is basically concerned with the classification of organisms (Singh 2010). Taxonomy is a science that includes identification, nomenclature and classification of objects. Nomenclature is concerned with the determination of the correct name of a known plant according to a nomenclatural system. The naming of plants is a subject of international importance. It is a function of taxonomy that is regulated by what are know as the International Rules of Botanical Nomanclature (Lawrence 1951).

The family Fabaceae (Leguminosae) is the third largest family of the flowering plants. The family Fabaceae or Leguminosae is commonly known as the legume, pea or bean. It can be divided into 3-subfamilies. They are Caesalpinioideae, Mimosoideae and Papilionoidaeae (Simpson 2006). The family Mimosaceae, Caesalpiniaceae and Fabaceae were treated under the order Fabales by Cronquist (1981).

Fabaceae consists of about 425 genera, 12000 species distributed worldwide. The more primitive woody genera mostly occur in the hemisphere are in the tropics whereas the more advanced and herbaceous genera are found in temperate regions, especially rich in Mediterrance countries (Anonymous 2008).

The subfamilies Caesalpinioideae can be easily characterized by the flowers; irregular, zygomorphic with five petals which are not differentiated into standard, wings and keel. The stamens are usually ten visibel externally in Caeslpinioideae. In the subfamilies Papilionoideae; flowers are irregular, zygomorphic and is made up of five petals, two wing petals and two petals partially fused together to form a boat-shaped keel. The keel enclose the stamen, which are not visible externally (Haywood 1978).

Papilionoideae commonly known as the legume, pea or bean family are a large and economically important family of flowering plants. It includes trees, shrubs and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume)and their compound

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stipulated leaves. The family is widely distributed and is the third largest land plant family. Papilionoideae is especially important because the seeds and pods are the source of human and animal food (Simpson 2006).

In the present research, Taxonomy on ten species belonging to Fabaceae from Pauk Township, Magway Region was studied. The climate condition of the study area is wet and dry. The temperature is highest in the months of May and June. The temperature is lowest in the months of November and December.

The aims of this research are to study the taxonomy on ten species of Fabaceae and provide the knowledge to other researcher. The objectives are to carry out the morphological characteristics of collected species, to identify and classify them and to record the list of these species.

Materials and Methods

The flowering plant specimens of Fabaceae in Pauk Township were collected from June 2019 to May 2020. Field observation was made by using GPS (Global Positioning System). Locations of the collected plants were also noted. The images of inflorescences and flowers were recorded by taking photograps. Then these specimens were kept into the plastic bags. The morphological characters of collected specimens were recorded by using a dissecting microscope.

The taxonomic identification of collected plants was carried out by referring to Anonymous (2008), Backer (1965), Dassanayake (1980-1996), Hooker (1875) and Nasir & Ali (1977). The genera and species were arranged alphabetically in table. Myanmar names were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003). The valid names of studied species were checked in the website of International Plant Name Index. The taxonomic descriptions were presented with their respective figures. An artificial key to the genera and species were constructed.

Results

Totally 10 species belonging to 8 genera were verified and classified. The arrangement of the genera and species were placed alphabetically in Table 1.

Table 1 List of the collected species from Pauk Township

Group	Order	Family	No.	Scientific name
Eudicot	Fabales	Fabaceae	1.	*Bauhinia acuminata* L.
			2.	*Bauhinia racemosa* Lam.
			3.	*Butea monosperma* (Lam.) Taub.
			4.	*Crotalaria striata* DC.
			5.	*Dalbergia oliveri* Gamble ex Prain
			6.	*Erythrina microcarpa* Koord. & Valeton
			7.	*Millettia pinnata* (L.) Panigrahi
			8.	*Parkinsonia aculeata* L.
			9.	*Senna auriculata* (L.) Roxb.
			10.	*Senna hirsuta* (L.) Irwin & Barneby

1. An artificial key to the genera

1. Corolla caesalpiniaceous ---------- 2
1. Corolla papilionaceous ---------- 4
   2. Leaves simple; flowers bracteolate ---------- *Bauhinia*
   2. Leaves compound; flowers ebracteolate ---------- 3
3. Staminodes absent ---------- *Parkinsonia*
3. Staminodes present ---------- *Senna*
   4. Stamens monadelphous ---------- *Crotalaria*
   4. Stamens diadelphous ---------- 5
5. Leaves 3-foliolate ---------- 6
5. Leaves 5- to many-foliolate ---------- 7
   6. Keels much longer than standards ---------- *Butea*
   6. Keels much shorter than standards ---------- *Erythrina*
7. Leaflets opposite ---------- *Millettia*
7. Leaflets alternate ---------- *Dalbergia*

2. Taxonomic description

1. ***Bauhinia acuminata*** L., Sp. Pl. 1: 376. 1753. (Figure 1)

Myanmar name : Swedaw phyu
English name : Orchid tree
Flowering period : April to July

Perennial erect shrubs, up to 2 m high; stems and branches terete, glabrous. Leaves simple, alternate, bilobed; stipules linear, pubescent; petioles 2.5-3.5 cm long, glabrous; blades ovate, 4.0-7.0 cm by 3.0-5.0 cm, obtuse to subcordate at the base, entire along the margin, deeply cordate at the apex, pubescent beneath. Inflorescences axillary and terminal racemes, few-flowered; peduncles up to 6 cm long, pubescent. Flowers bisexual, zygomorphic, pentamerous, hypogynous, white, 5.5-6.0 cm in diameter; pedicels 1.5-2.0 cm long, pubescent; bract and bracteoles ovate, 0.2 cm long, pubescent, persistent. Calyx splitting spathaceous, 5-lobed; tubes 1.3-2.0 cm long; lobes lanceolate, 0.3-0.5 cm long, green, pubescent. Corolla caesalpinaceous; petals 5, free, white; lobes lanceolate to oblong, 3.0-4.0 cm long, glabrous; claw 0.5-1.0 cm long. Stamens 10, all fertile, inserted; filaments filiform, unequal, 1.5-2.5 cm long, white, glabrous; anthers dithecous, dorsifixed, oblong, 0.3-0.4 cm long, yellow. Carpel 1; ovary superior, linear, about 1 cm long, yellow, unilocular with many ovules on the marginal placentae; style filiform, 0.8-1.2 cm long, glabrous; stigma simple, green; stipe about 1.0 cm long. Pods linear-oblongoid, flat, dehiscent, up to 12 cm long, many-seeded. Seeds compressed, orbicular.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 34.0" and E 94° 24' 37"; elevation 320 m; 8 July 2019; Kay Kay, collection no.1

2. ***Bauhinia racemosa*** Lam., Encycl. 1 (2): 390. 1785. (Figure 2)

Myanmar name : Phalan
English name : Unknown
Flowering period : June to September

Perennial trees, up to 6 m high; stems and branched terete. Leave simple, bilobed, alternate, exstipulate; petioles 1.8-5.0 cm long; blades ovate-orbicular, 4.5-10.5 cm by 3.5-7.5 cm, cordate at the base, entire along the margin, lobed, obtuse at the apex, hairy on both surface. Inflorescence terminal or axillary raceme; many-flowered peduncle 12.0-20.5 cm long, glabrous. Flower bisexual, zygomorphic, pentamerous, hypogynous, pinkish white, 0.5-0.7 cm indiameter; pedicel 0.1-0.3 cm long, pubescent. Calyx spathaceous, 5-lobed; tube cubular, 0.2-0.3 cm long; lobe 0.1-0.2 cm long. Petal 5, free, lobe 4.5-5.5 cm long, glabrous, lanceolate, hairy. Stamen 10, free, exserted, all fertile; filaments filiform; anther dithecous, dorsifixed, oblong, 0.2-0.5 cm long, longitudinal dehiscing. Carpel one; ovary superior, linear, 0.1-0.3 cm long, pubescent, stalked, unilocular with many ovule in the locule on the marginal placentae; style short, 1.0-1.5 cm long; stigma peltate. Pods compressed, linear dehiscent, 5.5-8.5 cm long, many-seeded. Seeds flat.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 38.0" and E 94° 24' 36"; elevation 350 m; 8 July 2019; Kay Kay, collection no.2.

3. ***Butea monosperma*** (Lam.) Taub., Nat. Pflanzenfam. 3 (3): 366, f. 131 M-N. 1894. (Figure 3)

Myanmar names : Pauk

English name : Flame of the forest

Flowering Period : November to March

Perennial deciduous tree, up to 6.5 m high; stems and branches cylindrical, woody, fine hairy when young. Leaves pinnately trifoliate compound, alternate; stipules small, 0.2-0.6 cm long, pubescent; petiole terete, 10.5-19.0 cm long; blades obovate, the terminal leaflet larger than two lateral leaflet, 12.0-23.0 cm by 10.5-15.5 cm, rounded or oblique at the base, entire along the margin, obtuse at the apex glabrous on both surfaces. Inflorescences axillary or terminal fasiculate raceme, many-flowered; peduncle terete, 10.5-43.5 cm long, pubescent. Flowers bisexual, zygomorphic, pentamous, hypogynous, orange, 3.5-5.5 cm in diameter, bract lanceolate, 0.5-0.8 cm long; pedicel terete, 2.0-3.5 cm long, pubescent. Calyx campanulate, 5-lobed; tubes 1.5-3.7 mm long, pubescent. Corolla papilionaceous, vexillariform, 5-lobed, laterally compressed; standard lanceolate, 5.5-8.5 cm long with short claw; wing falcate, 4.5- 6.5 cm long with claw, glabrous. Stamens 10, diadelphous; staminal tube 4.0-7.5 cm long; anther dithecous, basifixed, oblong 2.0-4.0 mm long, longitudinal dehiscing. Carpel 1; ovary superior, 2.0-3.5 cm long, unilocular with 5-10 ovule in the locule on the marginal placentae; style terminal, curved; stigma simple. Pods oblongoid, twisted, pale yellow, 8.5-15.5 cm long, few-seeded. Seeds flat.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 35.0" and E 94° 24' 30"; elevation 700 m; 1 November 2019; Kay Kay, collection no.10.

4. ***Crotalaria striata*** DC., Prodr. 2: 131. 1825. (Figure 4)

Myanmar name : Taw pike san

English name : Smoth crotalaria

Flowering period : June to August

Annual herbs, up to 85 cm high; stems and branches angular, glabrous. Leaves palmately trifoliolate compound, alternate; petioles about 3.5 cm long, glabrous; leaflets ovate, unequal, middle one larger, about 5.0 cm by 2.5 cm, obtuse at the base; entire along the margin, emerginate at apex, glabrous above, pubescent beneath. Inflorescences terminal racemes, many-flowered; peduncles about 10.5 cm long, pubescent. Flowers bisexual, irregular, zygomorphic, pentamerous, hypogynous, about 1.0 cm in diameter, yellow; pedicels about 0.5 cm long, pubescent. Calyx campanulate, 5-lobed; tubes about 0.5 cm long; lobes lanceolate, unequal, 0.1-0.6 cm long, pale

green, slightly pubescent. Corolla papilionaceous, 5-lobed; standards ovate-oblong, about 0.6 cm long, shortly clawed; wings oblong, about 0.7 cm long, shortly clawed; keels beak-shaped, pubescent. Stamens 10, monadelphous; filaments unequal, about 1.5 cm long, pale yellow; anthers dimorphic, dithecous, basifixed and dorsifixed, yellow. Ovary superior, oblong, about 1.0 cm long, hairy, unilocular with many ovules on the marginal placentae; styles curved, about 0.4 cm long, pubescent; stigmas simple. Pods oblong-linear, about 3.5 cm long, pubescent. Seeds obovate, flat, brown, glabrous.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 34.0" and E 94° 24' 36"; elevation 700 m; 5 July 2019; Kay Kay, collection no. 3.

5. ***Dalbergia oliveri*** Gamble ex Prain, J. Asiat. Soc. Bengal. Pt. 2, Nat. Hist. 66: 451. 1897. (Figure 5)

Myanmar name : Tamalan

English name : Burmese rose wood

Flowering period : March to May

Perennial deciduous tree, up to 14.0 m high; stems and branches terete. Leaves unipinnate compound, imparipinnate, alternate; stipules minute, caducous; petioles slender, pubescent, petiole 1.0-2.5 mm long, slender, pubescent; leaflets 5 to 10, oblong, 3.5-5.5 cm long, pale pink when young, pubescent, rounded and unequal at the base, entire along the margin, pubescent on both surfaces. Inflorescences terminal and axillary paniculate raceme; peduncle linear, 10.5-15.5 cm long. Flowers bisexual, zygomorphic, hypogynous, pentamous, purple to pink, 0.8-1.1 cm in diameter; bract caducous. Calyx campanulate; 5 lobed; tube cup-shaped, 0.2-0.3 cm long; lobes 0.1-0.5 cm long, pubescent. Corolla papilionaceous; standards ovate, 0.6-1.5 cm long, clawed; wings oblong, 0.5-0.8 cm long, clawed; glabrous, keels obtuse, 0.4-0.6 cm long, glabrous. Stamens 5+5, diadelphous; filaments 0.2-0.5 cm long; anthers dithecous, dorsifixed, ovoid, 0.1-0.2 cm long, longitudinal dehiscing. Carpel 1; ovary superior, oblong, 0.2-0.4 cm long, pubescent, unilocular with few ovules in the locule on marginal placentae; styles terminal, 0.1-0.3 cm long, glabrous, incurved; stigmas simple. Pods flattened, 1- to 4-seeded with samara. Seeds ovoid, glabrous.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 28.0" and E 94° 24' 39"; elevation 1063 m; 10 May 2020; Kay Kay, collection no.7.

6. ***Erythrina microcarpa*** Koord. & Valeton, Exkurs. Fl. Java 2: 401. 1912. (Figure 6)

Myanmar name : Kathit

English name : Unknown

Flowering period : March to May

Perennial small deciduous trees, up to 5 m high; stems and branches terete, pubescent when young. Leaves pinnately trifoliate compound, alternate; stipules linear, caducous; petioles terete, 5.0- 7.6 cm long, pubescent; rachis 2.5-7.0 cm long; petioles terete, 3.0-7.0 cm long, pubescent; leaflets triangular ovate, terminal leaflet larger than the lateral ones, 3.5-7.0 cm long, truncate at base, entire along the margin, acuminate at the apex, glabrous on both surfaces. Inflorescences terminal and axillary dense raceme, many-flowered; peduncle 5.0-10.0 cm long, pubescent. Flowers bisexual, zygomorphic, pentamerous hypogynous, red, 1.5-3.0 cm in diameter; bracts linear, 1.0-2.0 mm long. Calyx spathaceous; 5-lobed; tube tubular 4.5-6.5 mm long; lobes triangular, 1.0-2.7 mm long, pubescent. Corolla papilionaceous; standards ovate lanceolate, 2.0-3.7 cm long; wings orbicular, 4.5-6.5 mm long, glabrous; keels rhomboid, 1.3-2.7 cm long. Stamens 10, diadelphous; staminal tube 2.0-3.8 cm long, reddish, glabrous; free, filaments filifrom,

1.0-2.0 cm long; anther dithecous, dorsifixed, oblong, 1.0-2.2 mm long, longitudinal dehiscent. Carpel 1; ovary superior, linear, stipitate, 1.5-2.5 cm, unilocular with few ovule in the locule on the marginal placentae; style terminal curved, 1.0-2.0 cm long; stigma simple. Pods linear, stright, black, few-seeded, 5.8-10.5 cm long. Seeds ellipsoid, glabrous.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 32.0" and E 94° 24' 38"; elevation 750 m; 10 May 2020; Kay Kay, collection no. 8.

7. ***Millettia pinnata*** (L.) Panigrahi, Fl. Bilaspur Distr. 1: 210. 1989. (Figure 7)

Cytisus pinnatus L. Sp. Pl. 2: 741. 1753.

Myanmar name : Thinwin pyu

English name : Pongame oiltree

Flowering period : March to May

Perennial deciduous tree, up to 8 m high; stems and branches terete, glabrous. Leaves unipinate compound, imparipinnate, alternate; stipules triangular, 2.0-5.0 mm long, glabrous; petioles slender, 1.5-2.5 cm long; leaflets 5 to 7, opposite, ovate or obovate, 3.0-5.5 cm by 3.5-4.0 cm, rounded and unequal at the base, entire along the margin, glabrous on both surfaces. Inflorescences terminal and axillary raceme, many-flowered; peduncle slender, 2.0-3.0 cm long, glabrous. Flowers bisexual, zygomorphic, pentamerous, hypogynous, pinkish violet, 0.4-0.8 cm in diameter, pedicels 0.5 cm long, pubescent. Calyx cup-shaped; tube 0.3-0.7 cm long. Corolla papillionaceous; standards ovate, 0.4-0.8 cm long; wings oblong, glabrous; keels obtuse, 0.3-0.5 cm long. Stamens 10, diadelphous; filaments linear, 0.5-0.6 cm long; anthers dithecous, versatile, oblong, 0.1-0.2 cm long, longitudinal dehiscing. Carpel 1; ovary superior, oblong, sparsely pubescent, 0.3-0.5 cm long, unilocular with few ovules in the locule on marginal placentae; style terminal; stigma simple. Pods linear, flattened, 5.0-7.5 cm long, 2- to 3-seeded, woody. Seeds linear oblong, glabrous.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 33.0" and E 94° 24' 36"; elevation 1063 m; 10 May 2020; Kay Kay, collection no. 9.

8. ***Parkinsonia aculeata*** L., Sp. Pl. 1: 375. 1753. (Figure 8)

Myanmar name : Mya sein

English name : Jerusalem thorn

Flowering period : August to December

Perennial erect small trees, up to 5 m high; stem and branched terete, pubescent; spines straight, 2 - 3 cm long. Leaves bipinnate compound, imparipinnate, alternate; stipules spinescent; petioles short, about 1 cm long; secondary rachis up to 30 cm long; leaflets 35- to 50-paired; blades obovate-oblong, 0.2-0.4 cm by 0.1-0.2 cm, subrounded at the base, entire along the margin, rounded at the apex, pubescent on both surfaces, usually deciduous. Inflorescences axillary raceme, 10-15-flowered; peduncles 10-15 cm long. Flowers bisexual, zygomorphic, pentamerous, hypogynous, bright yellow, 1.5-2.0 cm in diameter, fragrant; pedicels about 1.5 cm long. Calyx campanulate, 5-lobed, yellowish green; tubes 0.1-0.2 cm long; lobes ovate-oblong, reflexed. Corolla caesalpinaceous; petals 5, limb suborbicular, 1.5-2.0 cm long, clawed, pubescent. Stamens 10, free, all fertile, inserted; filaments filiform, 0.5-1.0 cm long, hairy at the base; anthers dithecous, dorsifixed, oblong, reddish brown. Carpel one; ovary superior, oblong, pubescent, unilocular with many ovules on the marginal placentae; style terminal, reddish, glabrous; stigma

simple. Pods linear, cylindrical, constricted between the seeds, 5.0-13.0 cm long, yellowish brown, indehiscent, few-seeded. Seeds oblong, compressed, glabrous.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 32" and E 94° 24' 38"; elevation 1063 m; 10 August 2019; Kay Kay, collection no. 4.

9. ***Senna auriculata*** (L.) Roxb., Fl. Ind. 2: 349. 1832. (Figure 9)

Cassia auriculata L., Sp. Pl. 379. 1753.

Myanmar name : Peik thingat

English name : Tanner's cassia

Flowering period : August to March

Perennial shrubs, up to 3.0 m high; stems and branches terete, pubescent. Leaves unipinnate compound, paripinnate, alternate; stipules auriculate, about 1.0 cm long, foliaceous, persistent; petioles 1.5-2.5 cm long; racheae 4.0-7.0 cm long, orange-red gland between each pairs of leaflets; leaflets 8- to 12-paired, obovate-oblong to elliptic-oblong, 1.5-2.5 cm by 1.0-1.2 cm, obtuse at the base, entire along the margin, obtuse at the apex, pubescent on both surfaces. Inflorescences axillary or terminal paniculate corymbose raceme, many-flowered; peduncles 5.0-10.0 cm long, glabrous. Flowers bisexual, zygomorphic, pentamerous, hypogynous, bright yellow, about 2.5 cm in diameter; pedicels 1.5-3.0 cm long; bracts linear. Sepals 5, free, ovate, 0.2-0.6 cm long, concave, green. Corolla caesalpinaceous, petals 5, obovate, 1.5-2.0 cm long, shortly clawed, yellow with orange veins, glabrous. Stamens 10, free, 7 fertile, staminodia 3, included; filaments filiform, unequal, 3 longest, 3.0-6.0 mm long; anthers dithecous, basifixed, curved, yellow, opening by apical pores. Carpel 1; ovary superior, linear, about 1.5 cm long, unilocular with many ovules in the locule on the marginal placentae; styles filiform, 1.0-2.0 cm long, curved; stigmas simple. Pods linear-oblongoid, compressed, 8.0-12.0 cm long, brown, few-seeded, septate, dehiscent, wingless. Seeds ovate-oblong, compressed, dark brown.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 33.0" and E 94° 24' 35"; elevation 500 m; 10 August 2019; Kay Kay, collection no. 5.

10. ***Senna hirsuta*** (L.) Irwin & Barneby, Phytologia 44(7): 499. 1979. (Figure 10)

Cassia hirsuta L., Sp. Pl. 378. 1753.

Myanmar name : Kandauk

English name : Wooly wild sensitive

Flowering Period : August to November

Perennial foetid herbs, up to 70 cm high; stem and branches terete, hirsute. Leaves unipinnate compound, paripinnate, alternate; stipules linear, pubescent; petioles 3 - 5 cm long, pubescent, a gland above the pulvinus; racheae 5 - 8 cm long, hirsute; leaflets 3- to 5-paried, opposite; ovate-oblong or elliptic, upper pair of leaflets larger than lower one, 2.5-6.0 cm by 2.5-3.5 cm, rounded at the base, entire along the margin, acuminate at the apex, hirsute on both surfaces. Inflorescences axillary or terminal panicles, few-flowered; peduncles 1.0-2.0 cm long, pubescent. Flowers bisexual, zygomorphic, pentamerous, hypogynous, yellow, about 1.5 cm in diameter; pedicels 1.0-1.5 cm long, pubescent; bracts linear, pubescent. Sepals 5, obovate, concave, unequal, 0.5-0.7 cm long, green, pubescent without. Corolla caesalpiniaceous, petals 5, obovate, yellow, unequal, 0.8-1.5 cm long, shortly clawed, pubescent. Stamens 10, free, 7 fertile; staminodia 3, filaments unequal, 2 longest, 5 medium, 3 shortest, 03-0.8 cm long, yellow; anthers dithecous, curved, unequal, opening by apical pores, pale brown. Carpel 1; ovary superior, linear, pubescent, unilocular with many ovules on the marginal placentae; styles filiform, curved,

about 0.2 cm long, yellow; stigmas capitate, yellow. Pods linear, compressed, up to 12 cm long, hirsute, many-seeded, pubescent. Seeds orbicular, pale brown, glabrous.

Specimens examined: Magway Region, Pauk Township, Say Gyi Mountain area, N 21° 43' 36.0" and E 94° 24' 39"; elevation 1063 m; 10 August 2019; Kay Kay, collection no. 6.

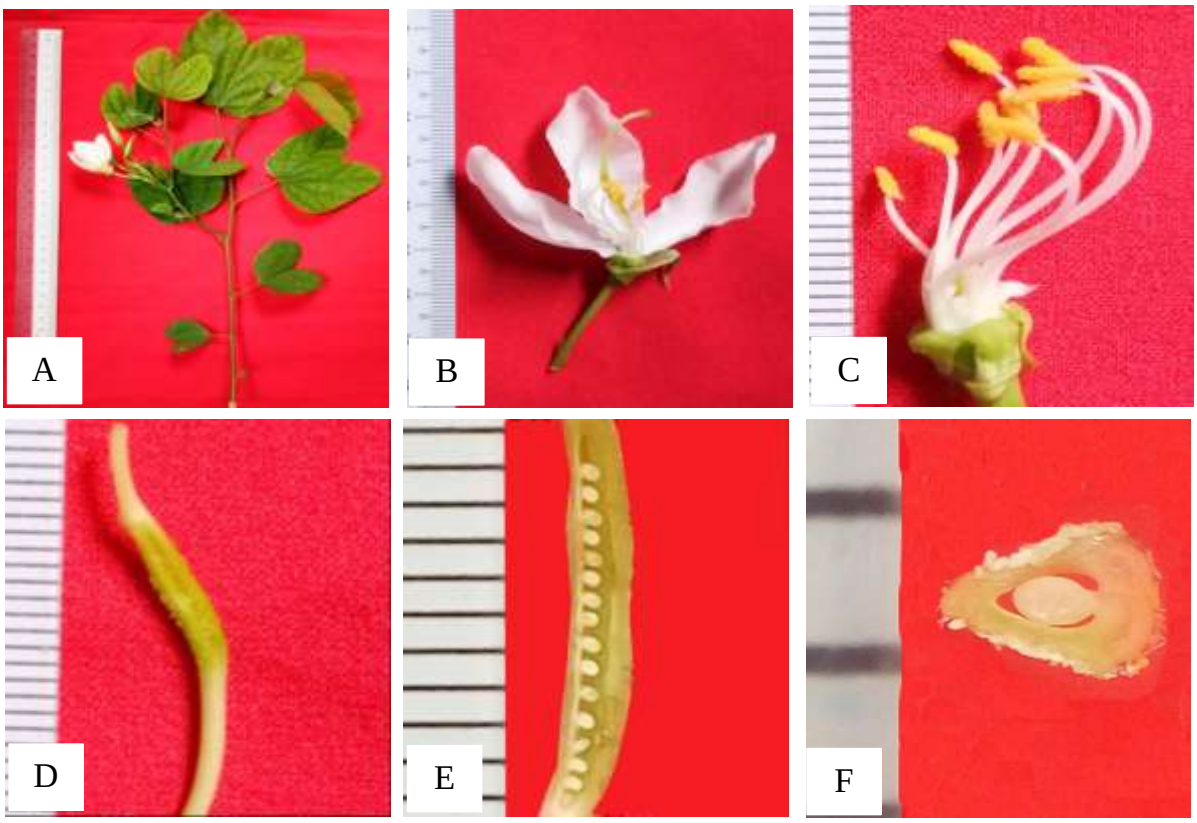
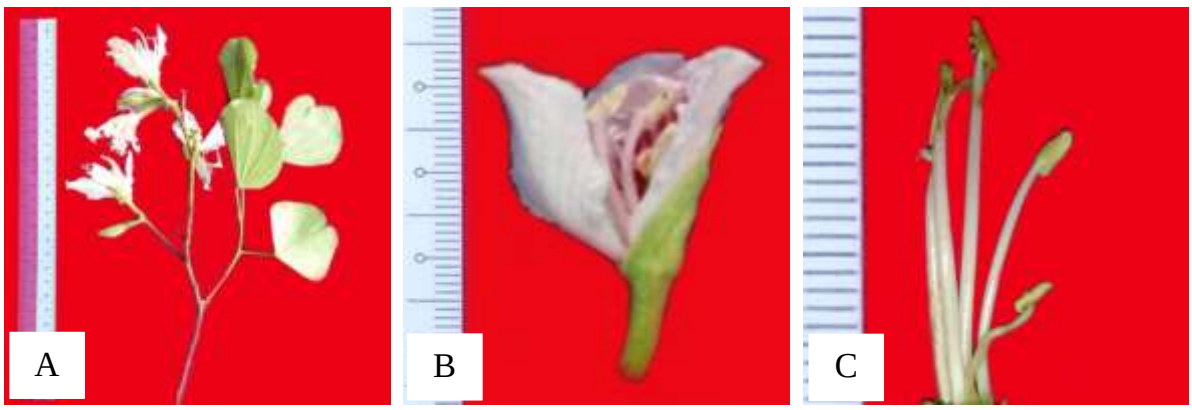


Figure 1 ***Bauhinia acuminata*** L.
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary



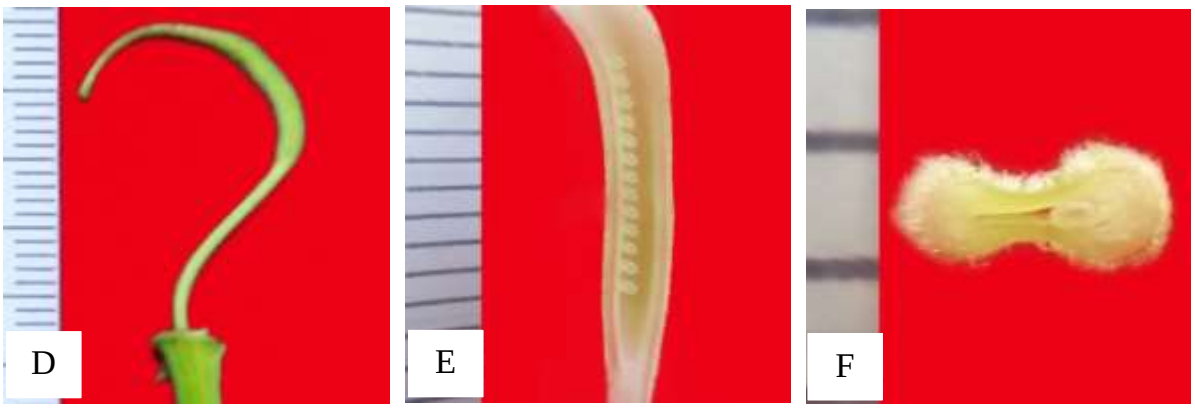


Figure 2 ***Bauhinia racemosa*** Lam.
A. Inflorescence B. Flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

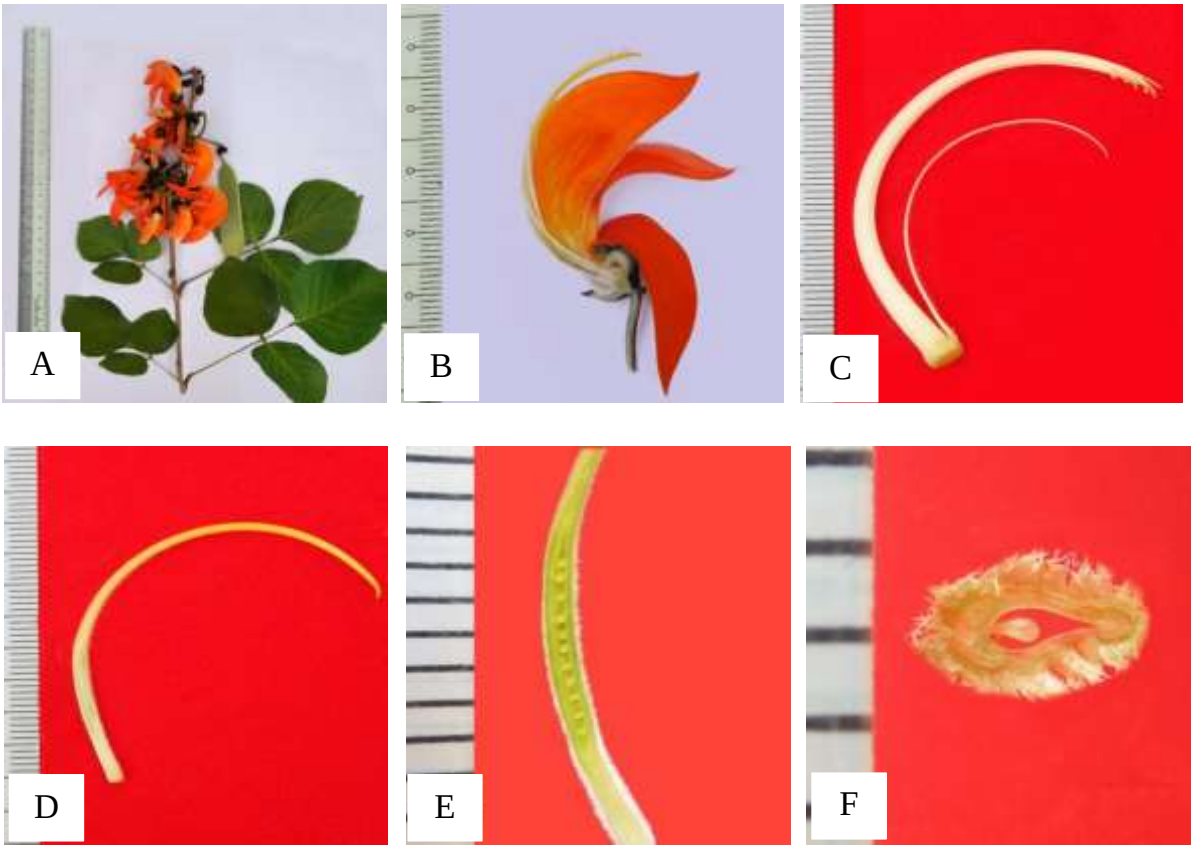


Figure 3. ***Butea monosperma*** (Lam.) Taub.
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

Figure 4 ***Crotalaria striata*** DC.
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

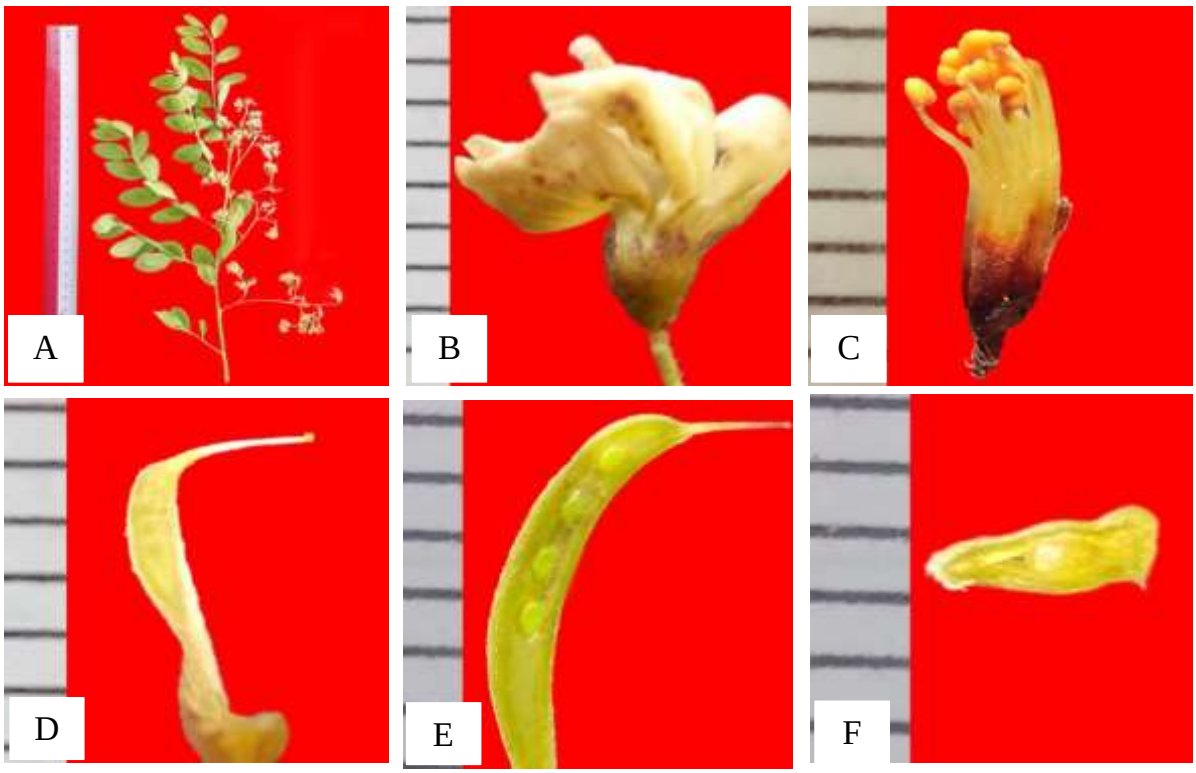


Figure 5 ***Dalbergia oliveri*** Gamble ex Prain
A. Inflorescence B. Flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

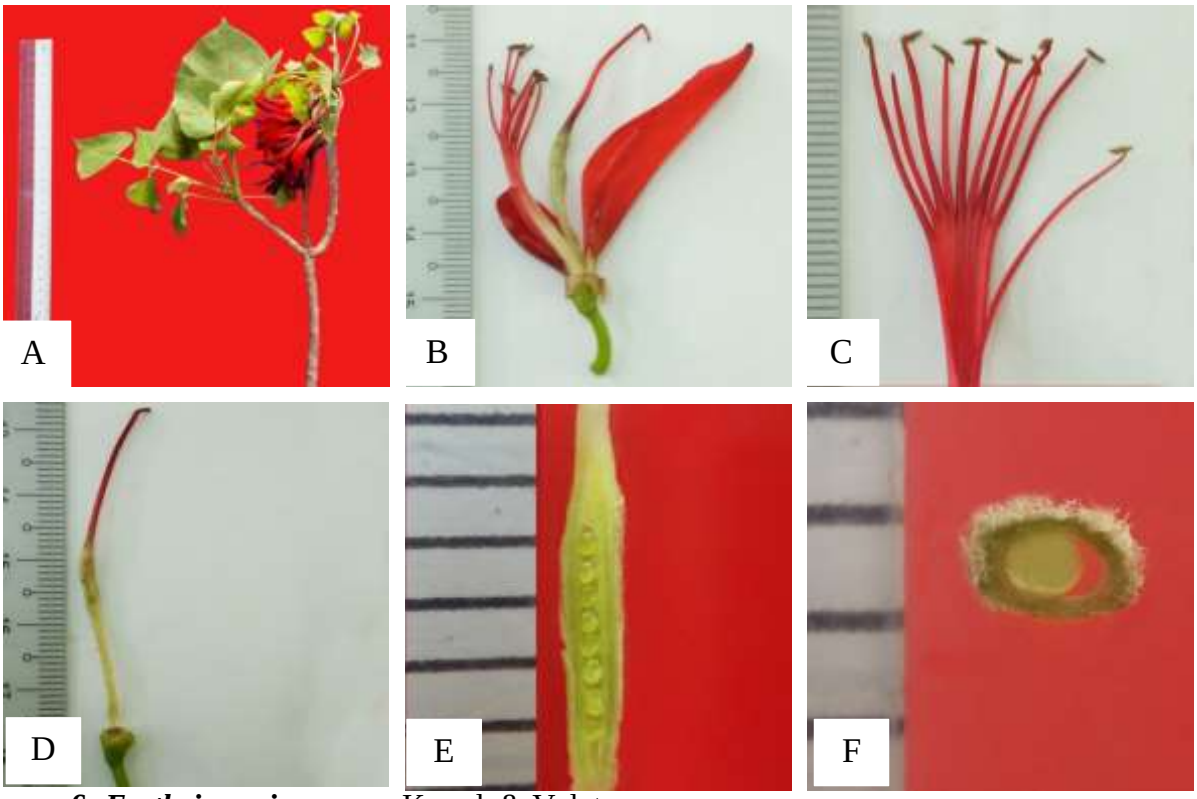


Figure 6 ***Erythrina microcarpa*** Koord. & Valeton
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

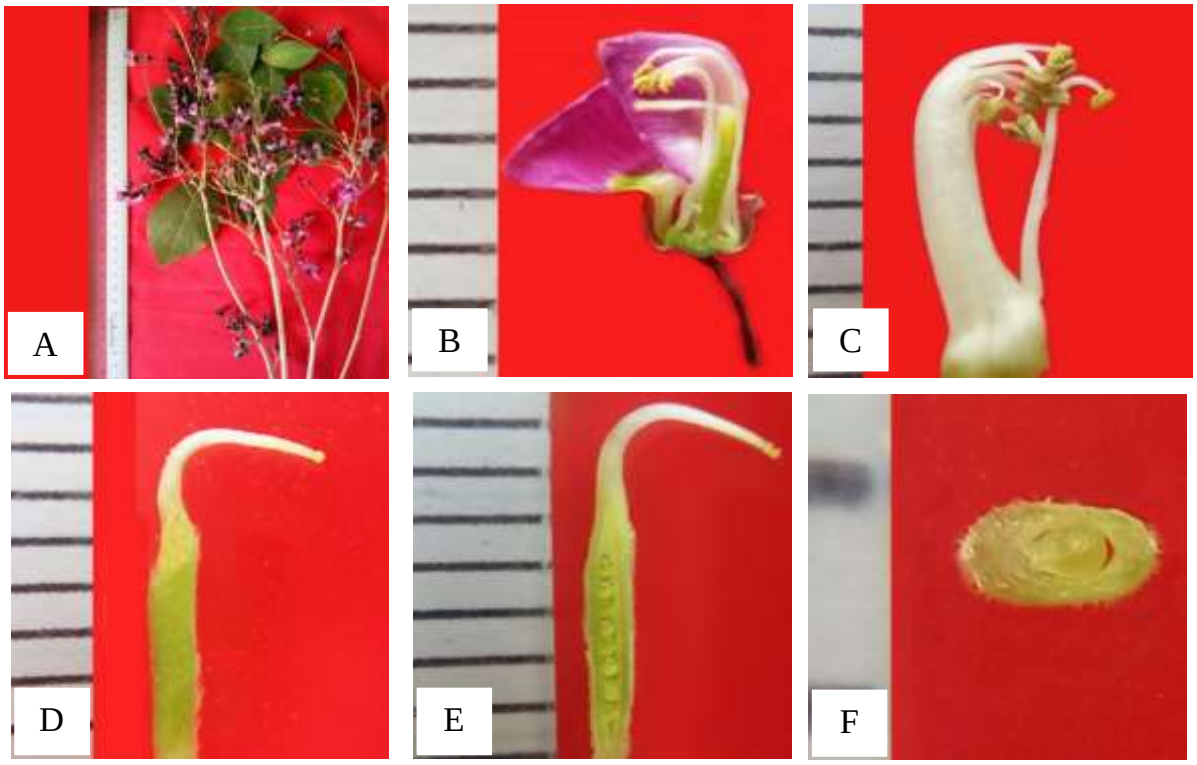


Figure 7 ***Millettia pinnata*** (L.) Panigrahi
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

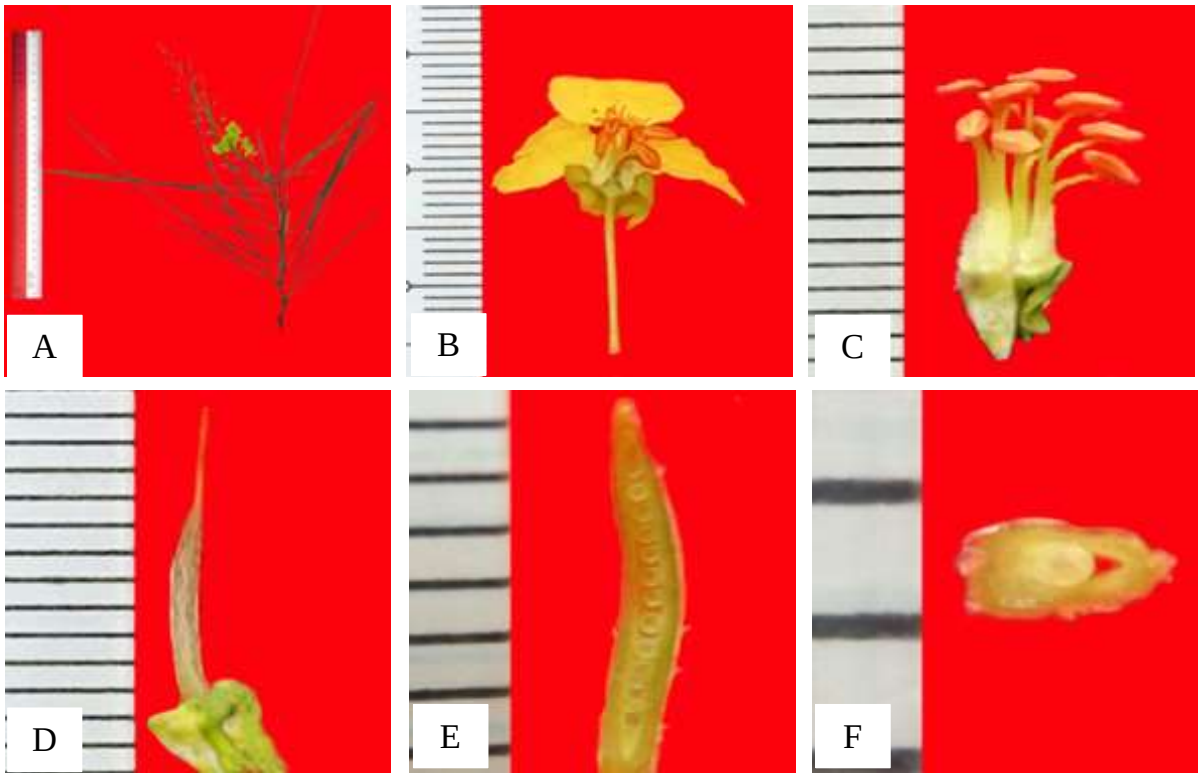


Figure 8 ***Parkinsonia aculeata*** L.
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

Figure 9 ***Senna auriculata*** (L.) Roxb.
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

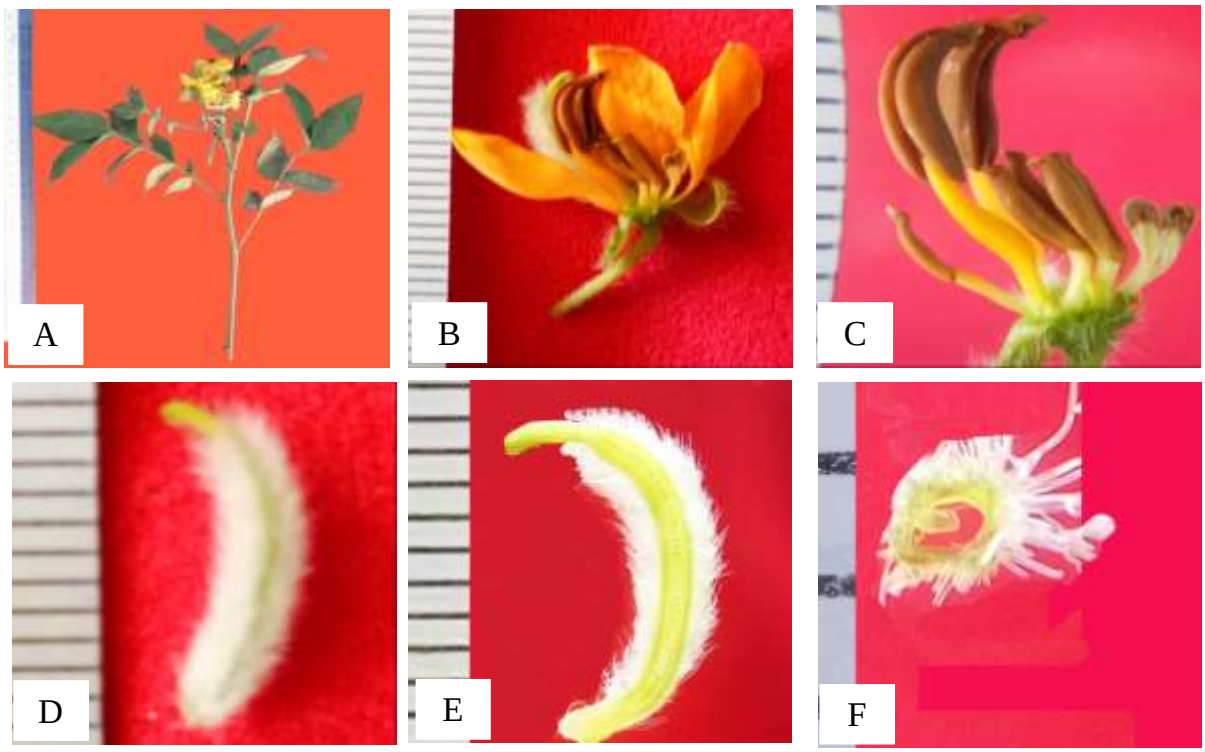


Figure 10 ***Senna hirsuta*** (L.) Irwin & Barneby
A. Inflorescence B. L.S of flower C. Stamens
D. Pistil E. L.S of ovary F. T.S of ovary

An artificial key to the studied species

1. Corolla caesalpiniaceous ---------- 2
1. Corolla papilionaceous ---------- 6
   2. Plants arborescent ----------3
   2. Plants herbaceous or shrubby ---------- 4
3. Plants with spines; leaves compound; flowers bright yellow; anthers dorsifixed ---------- ---------- 8. *Parkinsonia aculeata*
3. Plants without spines; leaves simple; flowers pinkish-white; anthers basifixed ---------- ---------- 2. *Bauhinia racemosa*
   4. Leaves simple; stamens 10, all fertile ---------- 1. *Bauhinia acuminata*
   4. Leaves compound; stamens 10, 7 fertile and 3 staminodes ---------- 5
5. Plants herbaceous, foetid; leaflets 3- to 5-paired; glandular above the pulvinus; stipules linear ---------- 9. *Senna hirsuta*
5. Plants shrubby, not foetid; leaflets 8- to 12-paired; glandular between each pair of leaflets; stipules auriculate ----------10. *Senna auriculata*
   6. Plants herbaceous; stamens monadelphous ---------- 4. *Crotalaria striata*
   6. Plants arborescent; stamens diadelphous ----------7

7. Leaflets trifoliolate ---------- 8
7. Leaflets penta- or more-foliolate----------9
    8. Flowers orange; wings falcate; anthers basifixed ---------- 3. *Butea monosperma*
    8. Flowers red; wings orbicular; anthers dorsifixed ---------- 6. *Erythrina microcarpa*
9. Leaflets pubescent on both surfaces; peduncles 10.5-15.5 cm long; pods with wings ---------- ----------5. *Dalbergia oliveri*
9. Leaflets glabrous on both surfaces; peduncles 2.0-3.0 cm long; pods without wings ---------- ---------- 7. *Millettia pinnata*

Discussion and Conclusion

The taxonomic study on ten species of Fabaceae from Pauk Township were carried out. Totally 8 genera were resulted. Among the resulting species, five species are under the subfamily Caesalpinioideae and the other five species are under the Papilionoideae. The caesalpiniaceous species are *Bauhinia acuminata* L., *Bauhinia racemosa* Lam., *Parkinsonia aculeata* L., *Senna auriculata* (L.) Roxb. and *Senna hirsuta* (L.) Irwin & Barneby. The papilionaceous species are *Butea monosperma* (Lam.) Taub., *Crotalaria striata* DC., *Dalbergia oliveri* Gamble ex Prain, *Erythrina microcarpa* Koord. & Valeton and *Millettia pinnata* (L.) Panigrahi.

The family Mimosaceae, Caesalpiniaceae and Papilionaceae were treated under the order Fabales by Cronquist (1981). A revised and updated classification of the flowering plants at the ranks of orders and families was published by the Angiosperm Phylogeny Group (2009-APG III). The family Mimosaceae, Caesalpiniaceae and Papilionaceae were treated under the Family Fabaceae according to APG III system.

The family Caesalpiniaceae is mainly distributed in tropical and subitropical region and consists of 171 genera and 2200-2300 species (Heywood 2007). Cronquist (1981) stated that this family is widespread in tropical and subtropical regions and it consists about 150 genera and 2200 species. Twenty six genera and 124 species were recorded in the checklist of Myanmar by Kress *et al*. (2003). In the subfamily Caesalpinioideae, three genera and 5 species were verified in the present study and described. The species of *Senna* were abundantly found in the study area.

The family Fabaceae (Papilionaceae) are the third largest family of flowering plants. They were distributed in tropical, subtropical and temperate region. They are mostly herbs, consists of 478 genera and 13,600 to 14,060 species (Heywood 2007). Cronquist (1981) recorded that it consists of about 400 genera and 10000 species and are widespread in temperate and cold tropical region. Kress *et al*. (2003) stated 452 species belonging to 84 genera of Papilionaceae in the checklist of Myanmar. In the present study, 5 species belonging to 5 genera from the subfamily Papilionoideae were presented. This family can be distinguished from other families by its flowers and stamens.

Kress *et al*. (2003) had recorded Pauk as *Butea monosperma* (Lam.) Kuntze in the checklist of Myanmar. Whereas the correct name is *Butea monosperma* (Lam.) Taub. He also stated Thinwin pyu as *Pongamia pinnata* Pierre. It was moved to the genus *Millettia* only recently. The correct name of it was *Millettia pinnata* (L.) Panigrahi. Moreover, Peik thingat had been recorded as *Cassia auriculata* L. It was changed into *Senna auriculata* (L.) Roxb. *Erythrina microcarpa* Koord. & Valeton had not been recorded in the checklist of Myanmar.

The different morphological characters of the study species were observed. According to the resulting data, 6 species are trees, 2 species are shrubs and the rest species are herb. The trees species are *Bauhinia racemosa* Lam., *Butea monosperma* (Lam.) Taub., *Dalbergia oliveri* Gamble

ex Prain, *Erythrina microcarpa* Koord. & Valeton, *Millettia pinnata* (L.) Panigrahi and *Parkinsonia aculeata* L. The shrubby plants are *Bauhinia acuminata* L. and *Senna auriculata* L. Among the collected species, the leaf-type of 8 species is the compound leaves whereas *Bauhinia acuminata* L. and *Senna auriculata* (L.) Roxb. possess simple leaves.

The inflorescence types of all studied species were seen as racemose inflorescence. The flowers characters of all studied species are bisexual, zygomorphic, pentamerous and hypogynous. Most of the flower colours on the studied plants is yellow. The sepal numbers of all study species are 5 and unite to form a tube. The petals of the studied species are not similar. In the flowers of the studied species, various fertile stamens and staminode were found. The fertile stamens 7 can be seen in *Senna auriculata* (L.) Roxb. and *Senna hirsuta* (L.) Irwin & Barneby. The carpel is only one, ovaries are superior and the placentation type is marginal in all studied plants. The shapes of fruits and the seeds are also variable. The fruit type of all studied species is pod. The number of seeds per fruit was found to be few to many.

The most valuable medicinal species were *Bauhinia acuminata* Lam, *Butea monosperma* (Lam.) Taub, *Senna auriculata* (L.) Roxb. and *Senna hirsuta* (L.) Irwin & Barneby. The economically important species are *Butea monosperma* (Lam.) Taub., *Dalbergia oliverri* Gamble ex Prain, *Erythrina microcarpa* Koord. & Valeton and *Millettia pinnata* (L.) Panigrahi. Among the studied species, *Butea monosperma* (Lam.) Taub. and *Erythrina microcarpa* Koord. & Valeton were most abundantly found in the study area. *Crotalaria striata* D.C and *Parkinsonia aculeata* L. were rarely occurred. The other species were widely distributing in the study area.

The present research partically accomplished the information on the members of Fabaceae in Pauk Township. The resulting data including the morphological characteristics are very valuable for identification and classification. It is hoped that the research work of the taxonomic study of ten species of Fabaceae growing in Pauk Township will give the valuable information for other researchers. This research will give the knowledge to students especially in the field of taxonomy. Moreover, this paper will benefit to the botany students.

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MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF *DOLICHANDRONE SPATHACEA* (L.F.) K. SCHUM.

Khin Mar Kyu[1], Aye Pe[2]

Abstract

Myanmar traditional medicine is practically and widely well accepted to many local people. *Dolichandrone spathacea* (L.f.) K. Schum. was utilized effectively for hepatitis and liver cancer. As such morphological and histological studies of this plant were investigated to certain it identification. The specimen was collected from Hta Ma Kan village, Aung Lan Township, Magway Region in july, 2018. The collected plant was identified according to standard procedures. The morphological and histological studies were also carried out by using available literatures at Department of Botany, West Yangon University. *Dolichandrone spathacea* (L.f.) K. Schum., locally known as Tha-kut belongs to the family Bignoniaceae. In Morphological study, this plant was a tree with unipinnately compound leaves, exstipulate. Inflorescences were terminal, corymbose. Flowers were white, fragrant. The fruits were capsule. Seeds were rectangular, membranous winged testa, nonendospermic. In histological study, epidermal cells of both surfaces were polygonal in shape with slightly wavy anticlinal walls. Stomata were found only on lower surface and anisocytic type. Calcium oxalate crystals were present in leaves. Simple unicellular trichomes and glandular peltate trichomes were present on the surfaces of leaves and stems. The powdered samples have been investigated and presented as diagnostic characters for the standardization of powdered drugs.

Keywords: *Dolichandrone spathacea* (L.f.) K. Schum., peltate trichomes

Introduction

Myanmar is well known for its wealth of natural plant resources for there are still many valuable plant materials to be searched. Among these, *Dolichandrone spathacea* (L.f.) K. Schum.is also included. It has enormous traditional uses against various diseases. This plant is deciduous tree, commonly known as Tha-kut. It is widely distributed all over Myanmar. The medicinal plant, *Dolichandrone spathacea* (L.f.) K. Schum. belongs to the family Bignoniaceae. There are five species of genus *Dolichandrone* in Myanmar (Kress *et al.* (2003).

According to the Ashin Nargathiein (1978), this plant is used for bronchitis, asthma and diarrhea caused by spleen disorder. In Philippines, it is used to treat nervous diseases and flatulence (Wiart, 2006). In Indonesia, the leaves are used to treat thrush (Kartikar and Basu, 1975).

Rural people in Amarapura Township, Mandalay region, bark paste is applied to cure the relief of snake bite, scorpion bite and chemical pesticide poisoning. The decoction of leaves and barks are used as a traditional medicine for toothache (PyaePyae Win, 2017). The flowers are eaten as vegetable. In Magway Region, the barks have been utilized effectively by local people for hepatitis and liver cancer. The decoctions of barks were used mostly as an oral medicine. Thus *Dolichandrone spathacea* (L.f.) K. Schum. was investigated in this research to find out the valuable information of this plant.

The aim of this research is to study histological characters of *Dolichandrone spathacea* (L.f.) K. Schum. and the objectives are to verify the morphological characters of *Dolichandronespathacea* (L.f.) K. Schum and to examine the powdered samples of leaves, barks and flowers that can be used for standardization in traditional medicine.

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[2] Professor and Head, Department of Botany, University of Yangon

Materials and Methods

Collection, Classification and Identification of Plants

The plant materials were collected from Hta Ma Kan village, Aung Lan Townships, Magway Region in July, 2017. The collected specimen was identified by using Hooker (1885), Backer and Brink (1965), Kirtikar and Basu (1975), Dassanayake and Fosbery (1981). Myanmar name was referred to Hundley and Chit KoKo (1987) and Kress *et.al.* (2003).

The leaves, barks and flowers were washed with water and then cut into small pieces and air dried at room temperature for three weeks. When constant weight was obtained, the dried samples were pulverized by grinding machine and stored in air tight bottles for further use.

Histological examination of different plant parts and powders of *Dolichandrone spathacea* (L.f.) K. Schum.

The fresh specimens were examined by cutting free hand sections and studied under the microscope. The microchemical tests for the presence of lignin, starch and tannin were made according to the methods and reagents given in Metcalfe and Chalk (1950), Esau (1953), Pandey (1993) and Trease and Evans (1978, 2000) and B.P (1968)

Results

Morphological characters of *Dolichandrone spathacea* (L.f.) K. Schum.

Deciduous trees, 10-20 m in height, stem woody, young shoots puberulous. **Bark** grayish brown to dark brown, fissured in older tree. **Leaves** opposite and decussate, unipinnately compound, imparipinnate, exstipulate; petioles 6.0-8.5 cm long,dark green, puberulous, pulvinate; rachis 17.5-19.0 cm long, green, puberulous; leaflets 1-3 pairs; petiolules 0.8-2.8cm long, green, puberulous; leaflet blades ovate-lanceolate, 9.0-12.5cm x 3.8-6.0 cm, the base oblique obtuse, the margin entire or sometimes wavy, the apex acuminate, dark gren above, pale green beneth, upper surfaces and midveins of lower surface puberulous, fleshy gland on either side of the midrib.**Inflorescences**: terminal, corymbs, peduncles stout, 1-1.3 cm long, grayish green, with distinct scars of shedding flowers, 2 to 6 flowered, fragrant.**Flowers**: ebracts and bracteolate, minute, pale green; pedicels 1.5-2.5 cm long, green, slightly curved, glabrous, complete, bisexual, zygomorphic, penta-merous, hypogynous. **Calyx:** spathaceous, split down one side to the base, oblong with uncinate tip, 5.0-6.0 cm x 2.8-3.2 cm, pale green, two ridges near the middle of outer surface, two grooves inside near the middle, leathery, gland dotted on the outer surface near the apex of calyx, persistent, inferior. **Corolla**: (5), sympetalous, imbricate, infundibuliform, 12.0 -16.5 cm x 7.0-8.0 cm, cylindrical basal tube of 7-8cm long, pale green, tube widened toward the throat to a funnel of 7.0-7.5 cm long, the lobes subequal, 2.0-2.5cm x 2.0-2.5cm, broad and rounded with crenate margin, wart-like gland on the outside of upper part, white, glabrous, inferior. **Androecium:** $4+1^{st}$, epipetalous, didynamous, filaments 4.0-4.5 cm long, inserted, slightly curved, white, slightly yellow at the base, staminode 0.7-1cm long, anthers dithecous, 6-8mm x 1.5-2.0 mm, oblongoid, pale yellow, divergent, introrse, dorsifixed, longitudinal dehiscence, inferior. **Gynoecium**: (2), bicarpellary, syncarpous, ovary linear oblong, about 1.2 cm long, yellowish green, bilocular, placentation axile, many ovules in each locule, style 11-13cm long, slightly curved, greenish yellow, inserted, stigma 2-lobed, ellipsoid, 4-5mm long, white, disc annular, green, superior. **Fruits**: capsule, long-linear, compressed and quadrangular, straight or sickle-shaped, 25-60cm x 1.5-2.3cm, dark green, tuberculate, obscurely ribbed; false septum flat; seeds numerous, rectangular, 12-18 mm x 6-8mm, light brown, wing hyaline (figure 1-8). The results were shown in Figure1-8.

Flowering Period : March to July

Figure 1 Habit

Figure 2 Leaves

Figure 3 Inflorescences

Figure 4 Flower

Figure 5 L.S of flower

Figure 6 T.S of ovary

Figure 7 Fruits

Figure 8 Seeds

Histological characters of leaves, stems and roots of *Dolichandrone spathacea* (L.f.) K.Schum.

Lamina

In surface view, the cuticle is smooth on both surfaces. The epidermal cells of both surfaces are polygonal in shape with with slightly wavy anticlinal wall. The anticlinal walls in lower surface are much wavy than those in upper surface. Anisocytic type of stomata occurred abundantly only on the lower surface. Simpleunicellular trichomes and glandular peltatetrichomes are present on both surfaces. Glandular peltatetrichomes are discoid with entire margin, 8 cells on one plane.

In transverse section, the cuticles are present on both surfaces. Epidermal cells are one layered thick and barrel shaped. The mesophyll tissues are differentiated into palisade and spongy parenchyma. Palisade layer is composed of compactly arranged columnar-shaped cells. They are 1-2 layers thick. The spongy layer is composed of 3-4 layers, closely packed rounded to oval shaped parenchyma cells. Mesophyll cells contain abundant chloroplasts. Calcium oxalate crystals are present in the mesophyll cells. Glandular peltatetrichomes are shortly stalked depressed in the surface and multicellular head with radiating cells. Vascular bundles are closed and collateral type. Each bundle is surrounded by a sheath of large parenchyma cells. Xylem lies towards the upper epidermis and phloem lies towards the lower epidermis (Figure 9-12).

Midrib

In surface view, the epidermal cells are rectangular to polygonal in shape, thin-walled, parenchymatous. Simpleunicellular trichomes are present only on upper surface and glandular peltatetrichomes are present on both surfaces.

In transverse section, the cuticle layer is thin. The upper epidermal cells are barrel-shaped and the lower epidermal cells are oval or rounded in shape. Collenchyma occurs immediately below the epidermis, 3-5 layered, rounded to polygonal in shape. Below the collenchymas, 5-7 layers of rounded or oval parenchymatous cells are present. Sclerenchymatous cells occurred as fiber sheath at the phloem side. Vascular bundles are collateral type. Calcium oxalate crystals are present in the cortical region (Figure 13).

Petiole

In surface view, the epidermal cells of both surfaces are thin-walled and rectangular or polygonal in shape parenchymatous cells. Simpleunicellular trichomes and glandular peltatetrichomes of petiole are similar to those of lamina and midrib.

In transverse section, the petioles are shield shaped in outline and covered with cuticle. Epidermal cells are one layer thick, thin wall, barrel shape. In cortical region, collenchymas cells are foundtowards the peripheral region and parenchyma cells towards the vascular bundle. Collenchymatous cells are 3-5 layers, isodiametric in shape. The parenchymatous cells are 7-9 layers and rounded to oval in shape. Sclerenchymatous patches are present. Vascular bundles are rounded in outline and collateral type. A large main vascular bundle and two small accessory bundles are present. Calcium oxalate crystals are present in the cortical region (Figure 14).

Stem

In surface view, the epidermal cells are thin-walled parenchymatous and rectangular to polygonal in shape. Simple, unicellular trichomes and glandular peltatetrichomes are present.

In transverse section, the stem is oval in outline. Epidermal cells are composed of single layer, barrel-shaped parenchyma and compactly arranged. It bears simple unicellular and glandular peltate trichomes. The cortical region consists of 3-4 layers of collenchymatous cells and 6-7 layers of parenchymatous cells. Collenchymatous cells are isodiametric in shape and parenchymatous cells are rounded to oval in shape. Endodermis is not distinct. Pericycle present as patches and compose of sclerenchymatous cells. Vascular bundles are arranged in the form of a ring, collateral and open type. Cambium cells are 4-6 layers thick, rectangular in shape, radial arrange. Pith composed of thin walled and rounded parenchyma cells (Figure 15-16).

Root

In surface view, the epiblema cells are thin-walled, parenchymatous, rectangular to polygonal in shape and compactly arranged. Root hairs are present.

In transverse section, the root is circular in outline. In young root the outermost layer is made up of single layer of epiblema cells and internal to the epiblema is cortex. It is made up of several layers of thin walled spherical or oval shaped parenchymatous cells. At maturity the epiblema cells become the periderm. The outermost region of root is phellem or cork. The inner region of phellem is phellogen or cork cambium. Innermost region of periderm is phelloderm. Endodermis is not clearly distinguished. Pericycle composed of sclerenchymatous cells as ring. It is discontinuous. Vascular bundles are radial, hexarch and are found within the pericycle at primary stage. The xylem and phloem are arranged in concentric amphicribal ring in mature root (Figure 17-18).

Diagnostic characters of powdered leaves, barks and flowers of *Dolichandrone spathacea* (L.f.) K. Schum.

In powdered leaves, barks and flowers, fragment of mesophyll cells, unicellular and glandular peltate trichomes, calcium oxalate crystal, cork cells, vessels with annular, spiral thickening, papilose and pollen were observed (Figure 19-30).

Table 1 Sensory characters of powdered of *Dolichandrone spathacea* (L.f.) K. Schum.

Sample / Sensory character	Leaves 	Barks 	Flowers 
Colour	Bright green	Brown	Pale brown
Odour	Pungent	Odourless	Pungent
Taste	Slightly bitter	Tasteless	Slightly bitter
Texture	Fibrous	Fibrous	Fibrous

Histological characters of *Dolichandrone spathacea* (L.f.) K. Schum.

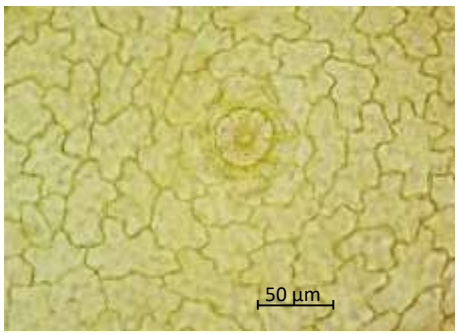

Figure 9 Surface view of upper epidermis

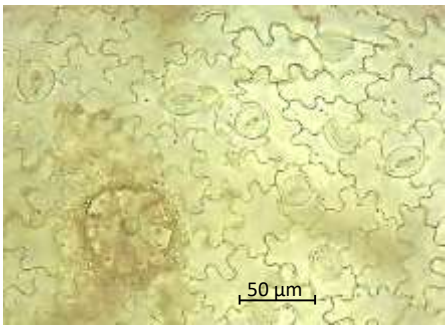

Figure 10 Surface view of lower epidermis with anisocytic stomata

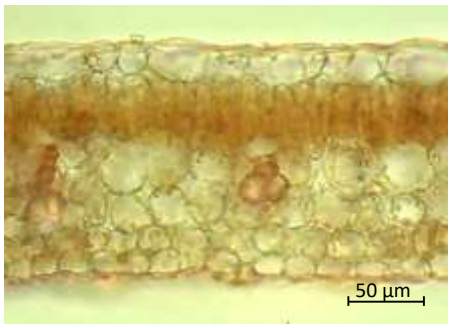

Figure 11 T.S of Laminar

Figure 12 T.S of Peltate trichome

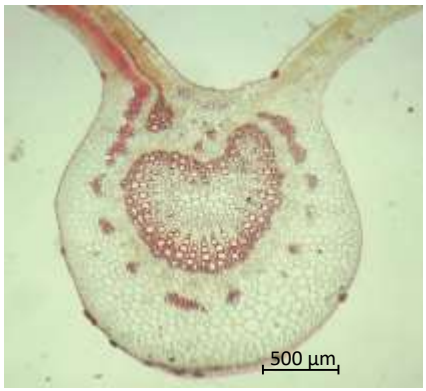

Figure 13 T.S of midrib

Figure 14 T.S of petiole

Figure 15 T.S of young stem

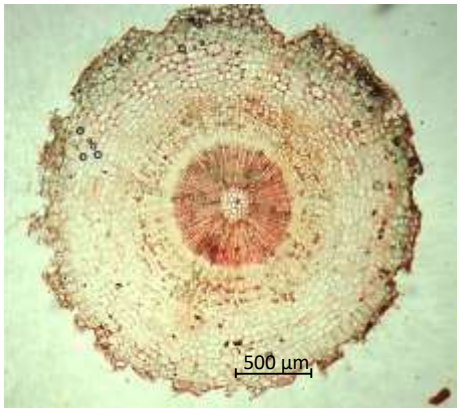

Figure 16 T.S of mature stem

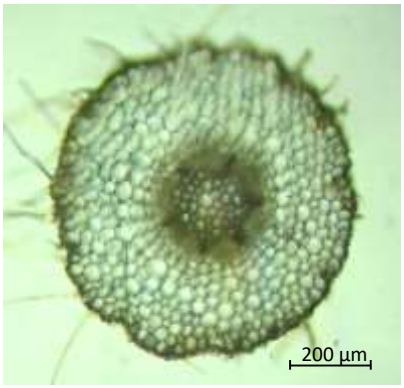

Figure 17 T.S of young root

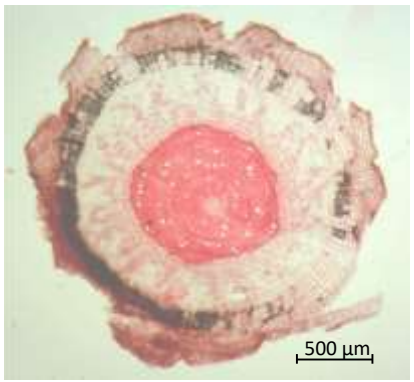

Figure 18 T.S of mature root

Diagnostic characters of Powdered Leaves of *Dolichandrone spathacea* (L.f.)K. Schum.

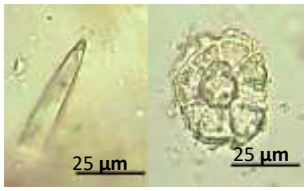

Figure 19 Trichomes

Figure 20 Mesophyll cells

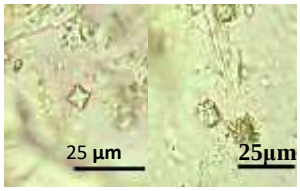

Figure 21 crystals

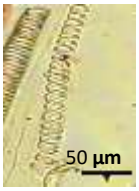

Figure 22 Vessel

Diagnostic characters of Powdered Barks of *Dolichandrone spathacea* (L.f.)K. Schum.

Figure 23 Sclereids

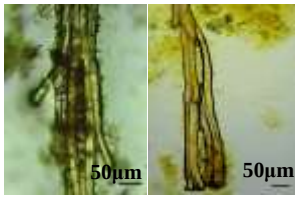

Figure 24 Uniseriate ray

Figure 25 Cork cells

Figure 26 Crystal

Diagnostic characters of Powdered Flowers of *Dolichandrone spathacea* (L.f.)K. Schum.

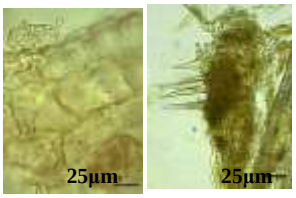

Figure 27 Trichome

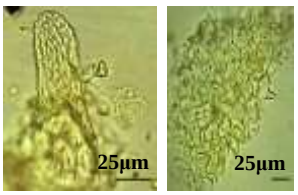

Figure 28 Papilose

Figure 29 Pollen

Figure 30 Vessel

Discussion and Conclusion

In this research, the morphological and histological characters of *Dolichandronespathacea* (L.f.) K. Schum. are presented.

In morphological study, *Dolichandrone spathacea* (L.f.) K. Schum.is deciduous tree. The leaves are unipinnate compound, exstipulate. Inflorescences are terminal corymbs. The flowers are showy, white, and fragrant. The calyxes are spathaceous. The corollas are infundibuliform. The stamens are 4, didynamous with staminode. The pistils are 2 carpelled, syncarpous, placentation axile.The fruits are capsule. Seeds are rectangular with wings. The morphological characters given in this research are in accordance with the characters given by Hooker (1885), Kirtikar and Basu (1975), Dassanayake and Fosbery (1981) and Wiart (2006).

In histological study, epidermal cells of both surfaces are polygonal in shape with slightly wavy anticlinal walls. Stomata are found only on lower surface and anisocytic type. Calcium oxalate crystals are present in leaves. The characters of leaves are in agreement with those given in Metcalfe and Chalk (1950). Cronquist (1981) also revealed that small crystals of calcium oxalate often are present in some of the cell of parenchymatous tissues.

Simple, unicellular trichomes and glandular peltatetrichomes are present on the surfaces of leaves and stem. Glandular peltatetrichomes are the diagnostic characters of this species. Metcalfe and Chalk (1950) described that non-glandular form are simple, unicellular or uniseriate and glandular hairs shortly stalked and scale like in *Dolichandrone*.

In transverse section of petiole, the vascular bundles are one large bundle with small accessory bundles. The petiolule and rachis are characteristically the same in that the microscopical characters of petiole. The anatomical characters of lamina, midrib and petiole observed in this research are similar with Cho ChoNaing (1995).

In stem, vascular bundles are collateral and opened, sclerenchymatous patches present. In roots, vascular bundles are radial and hexarch at primary stage. The xylem and phloem are arranged in concentric amphicribal ring in mature root. The stem and root characters are in agreement with those given in PyaePyae Win (2017). The anatomical characters of leaf and young stem are in agreement with those of the family Bignoniaceae recorded by Metcalfe and Chalk (1950).

The diagnostic characters of powdered leaves, barks and flowers are cork cells, unicellular and glandular peltatetrichomes, calcium oxalate crystal, and vessels with annular, spiral thickening. This combination of sensory and histological characters would assist the identification of powdered drugs of *Dolichandrone spathacea* (L.f.) K. Schum.

In conclusion, the scientific research has helped to promote the development of traditional medicine by revealing the morphological and anatomical characters of *Dolichandronespathacea* (L.f.) K. Schum. Traditionally, the classification of plants is mainly based on morphological and anatomical aspects. Leaf anatomical studies have been proven to be useful for species identification and it has been of great taxonomic significance. Glandular trichomes play a major role in the

characterization of the Bignoniaceae. Characteristics of present study are the valuable evidence for identification on this plant. The sensory characters and diagnostic characters of powdered leaves, barks and flowers would assist the identification and evaluation of powdered drugs of *Dolichandrone spathacea* (L.f.) K. Schum.

Acknowledgements

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EXTRACTION, ISOLATION AND IDENTIFICATION OF PHYTOCONSTITUENTS FROM THE LEAVES OF *CORDIA DICHOTOMA* G.FORST.

Khin Than Oo[1], Swe Swe Aye[2], Khin Chaw Win[2]

Abstract

The present study was the phytochemical analysis of the leaves from *Cordia dichotoma* G. Forst. (Boraginaceae). It was collected from Loilem Township, Southern Shan State. *Cordia* is one of most important genus of family Boraginaceae and involves a wide range of therapeutic uses in traditional medicine. Cool extraction method was followed to prepared the extracts, followed by standard phytochemical methods to identify the phytoconstituents present in it. Thin layer chromatography (TLC) and Column chromatography studies were then done for the confirmation and quantification of phytoconstituents. The defatted methanol extract (8 g) was fractionated by Column Chromatography method. The isolated compounds were identified by TLC, R_f value, melting point, UV and FT IR spectroscopic analysis. It was found that the isolated compound A, steroidal glycoside (R_f value 0.56, yield 0.032 %) was obtained from Fraction I, compound B, lupeol (melting point 215°C, R_f value 0.48, yield 0.005%) from Fraction IV and compound C, quercetin (melting point 321°C, R_f value 0.38, yield 0.014 %) from Fraction V. The presence of steroidal glycoside, lupeol and quercetin are indicative of potential for medicinal use of this plant.

Introduction

Plants are very important source of potentially useful bioactive principles for the development of new chemotherapeutic agents. *Cordia dichotoma* G.Forst., commonly known as Thanatphet or Thanat in Myanmar, belongs to the family Boraginaceae, subfamily Cordioideae The *Cordia* genus comprises more than 300 species, mostly evergreen trees and shrubs distributed widely in the tropical regions.

In Myanmar, *Cordia dichotoma* G. Forst.is especially found in southern Shan State, Kachin State, Kayah State, Mandalay Division, Yangon Division. Leaves, fruit, bark and seed of *Cordia dichotomas* G.Forst. are extensively used in traditional medicine for antimicrobial, anti-inflammatory, anthelmintic, analgesic and diuretic purposes and for treating digestive system, respiratory, urogenital, cardiac, vascular and blood disorders (Matias *et al*., 2015; Kumari *et al*., 2016).

Various secondary metabolites like alkaloids, phenolic compound, tannins, flavonoids, steroids and terpenoids were isolated from the leaves of *Cordia* dichotoma G.Forst.plants. Srivastava (1979) reported that α-amyrins, betulin, lupeol, quercetin, β- sitosterol and quinone are mainly present in leaves and seeds of *Cordia dichotoma* G.Forst. Thus, the aim of this study is to extract and isolate the organic compounds from the leaves of *Cordia dichotoma* G. Forst. and to analyses that isolated compound by modern Spectroscopic Techniques.

[1] PhD Candidate, Department of Botany, University of Mawlamyine
[2] Lecturer, Department of Botany, University of Yangon
[2] Lecturer, Department of Chemistry, University of Yangon

Materials and Methods

Collection and preparation of sample

The sample plant *Cordia dichotoma* G. Forst. was collected from Loilem Township, Southern Shan State during the months of April to July, 2018. The sample leaves were washed with water to eliminate dust and cut into small pieces and air dried in room temperature for several days. After being completely dried, they were pulverized by grinder machine to get powder and stored in airtight containers to prevent it from moisture and air-borne contamination (Fig 1).

Figure 1 Habit of *Cordia dichotama* G. Forst.

Preparation of methanol extract from leaves of *Cordia dichotoma* G. Forst.

The air dried leaves powdered sample of *Cordia dichotama* G. Forst. (200 g) was macerated with 2 liter of Petroleum ether (60-80°C) for 4 weeks and filtered. Defatted marc was macerated with 2.5 liter of methanol for 4 weeks and filtered. This filtrate was concentrated on water bath. Finally, the defatted methanol crude extract (8.95 g) was obtained from dried powdered (200g) of *Cordia dichotoma* G. Forst. (Figure.2).

Isolation of compounds A, B and C from methanolic extract of leaves of *Cordia dichotoma* G. Forst.

Isolation of phytoconstituents by using Column Chromatographic method and identified by R_f value, melting point, UV and FT IR spectroscopic method.

Determination of (R_f) values of isolated compounds (Sherma & Fried, 2005)

$$R_f \text{ (Retardation factor)} = \frac{\text{Distance of chromatographic spot center from the start}}{\text{Distance travelled by the solvent from the start}}$$

Ultraviolet (UV) spectroscopic study of isolated compounds

The ultraviolet spectrums of isolated compounds were determined by UV-1800 Spectrophotometer at Department of Chemistry, University of Yangon. (Figure.7 and 8)

Infra-red (IR) spectroscopic study of isolated compounds

The infra-red spectrums of isolated compounds were determined by using Perkin Elmer Spectrum Two spectrometer at Department of Chemistry, University of Yangon. (Figure.9, 10 and 11)

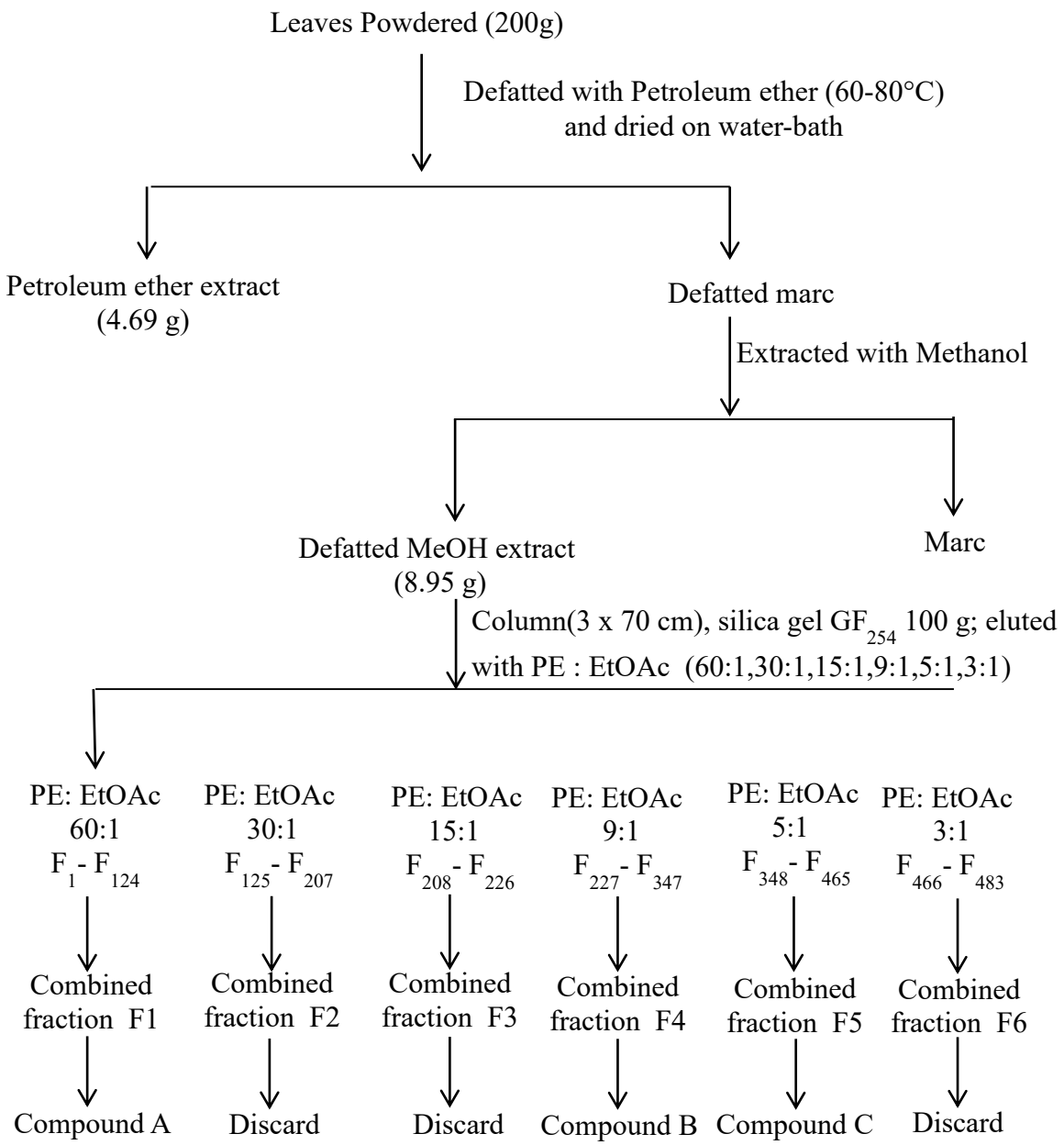


Figure 2 Flow diagram of extraction and isolation of compound A, B and C from the powdered leaves of *Cordia dichotoma* G.Forst.

Results

Isolation, Identification and characterization of phytoconstituents from Cordia dichotoma G.Forst.

The isolation of phytoconstituents from the leaves of *Cordia dichotoma* G.Forst. by using Column chromatography separation method. The three known compounds such as the compound A (light yellow oil), Steroidal glycoside (0.032 %); compound B (colorless needle shape), Lupeol (0.005 %) and compound C (yellow crystal), Quercetin (0.014 %) were isolated. These isolated compounds were identified by R_f value, Thin Layer Chromatography (TLC), physico-chemical characteristics of isolated compounds, UV and FT IR Spectroscopic analysis. The results were shown in Fig (4, 5, 6).

Identification of isolated compound A

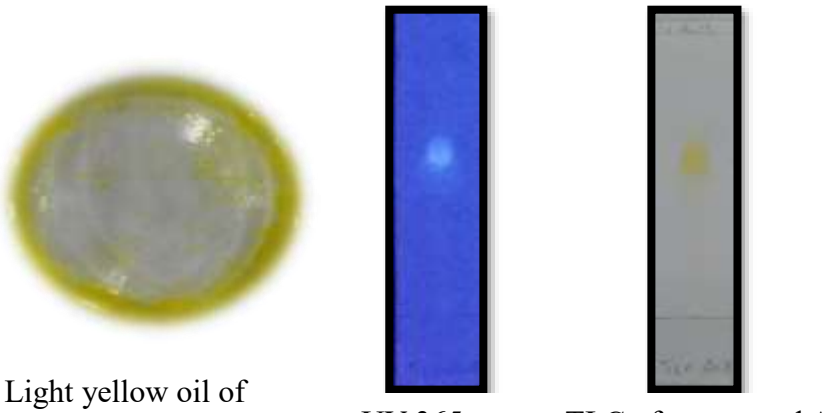
The isolated compound A (oil, 0.032 % yield) was fractioned from fraction F1 (petroleum ether: ethyl acetate, 30:1) was done by using column chromatography method. According the results of physico-chemical characters, Compound A was light yellow color. After Libermann - Burchard test, green colour of compound A was observed. It is UV active (UV 365nm). The single spot was visualized on TLC plate by spraying with 5% H_2SO_4 and vanillin. The compound A may be steroid compound. The R_f value of compound A was 0.56 which was in similar steroidal glycoside (Okwu, & Ohenhen, 2010). Therefore compound A may be steroidal glycoside. The results were shown in Figure 4.

Identification of isolated compound B

The isolated compound B (colorless needle-shape, 0.005 % yield) was fractioned from fraction F4 (petroleum ether: ethyl acetate, 9:1). According the results of physic-chemical characters, Compound B was pink colour by using Libermann-Burchard test. It is UV inactive (UV -254nm & 356nm). The single spot was visualized on TLC plate by spraying with 5% H_2SO_4 and vanillin. The compound B may be terpenoid compound. The R_f value of compound B was 0.48 and melting point 215 °C which was in agreement lupeol (Merck Index, 2001). Therefore, compound B may be lupeol. The results were shown in Figure 5.

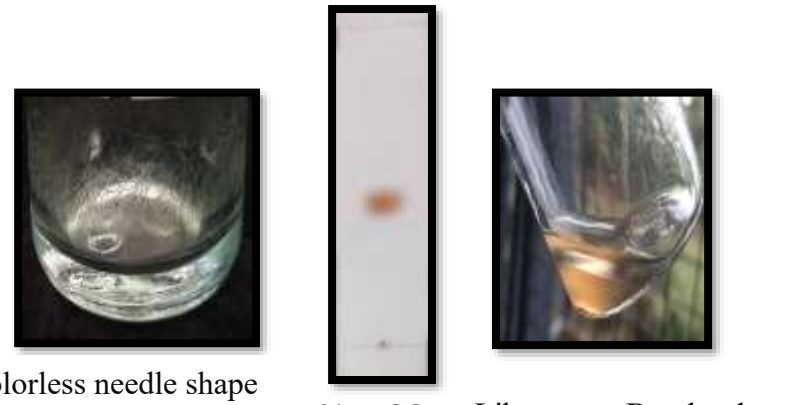
Identification of isolated compound C

The isolated compound C (Yellow crystal shape, 0.014 % yield) was fractioned from fraction F5 by column chromatography with petroleum ether: ethyl acetate (5:1). According the results of physico-chemical characters, Compound C was yellow colour when treated with magnesium ribbon and concentrated hydrochloric acid. So the compound may be flavonoid. The R_f value of compound C was 0.38, melting point 321 C°, which was in agreement with quercetin (Abeer, 2011). Therefore compound C may be quercetin. The results were shown in Figure 6.



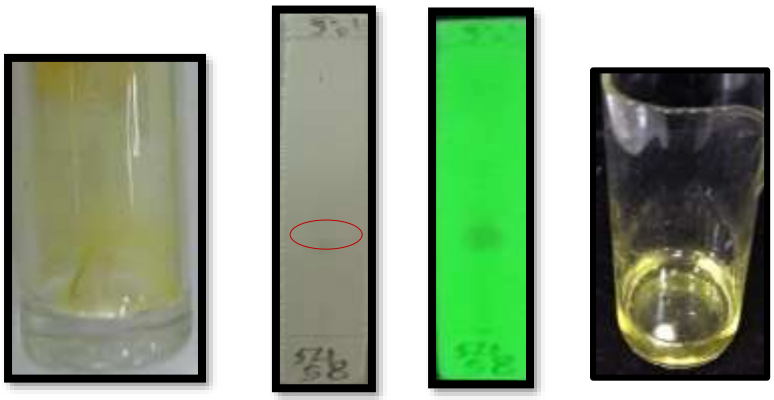
Light yellow oil of compound A | UV-365 nm | TLC of compound A

Figure 4 TLC and crystal shape of isolated compound A



Colorless needle shape crystal of compound B | 5% H_2SO_4 | Libermann-Burchard test

Figure 5 TLC and crystal shape of Isolated Compound (B)

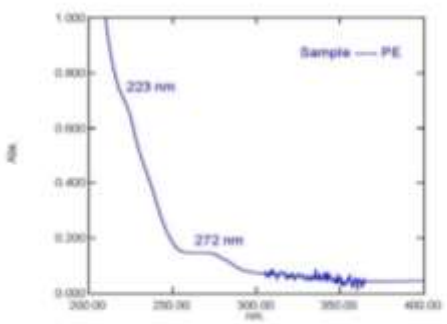


Yellow crystal of compound C | Visible | UV-256 nm | Flavonoid Test

Figure 6 TLC and crystal shape of Isolated Compound (C)

Ultra violet spectroscopic study

The absorption maximum wave length of isolated compound A was found at 223, 272 nm indicating the presence of conjugated double bond. Compound C in methanol observed two absorption maxima at 256 nm (band II) and 373 nm (band I) agreements with flavonoid. By adding of NaOH, band II shifted 285 nm that indicated the presence of 7-OH and band I shifted 421 nm which show the present of 4′ OH. The UV spectra of these compounds were shown in Figure 7 and 8.



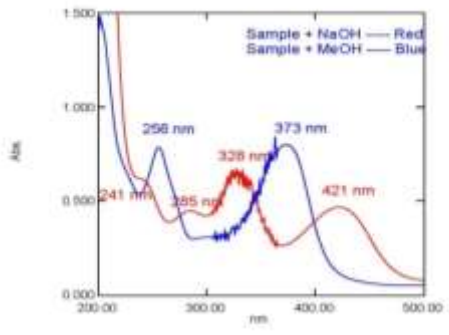


Figure 7 UV spectrum of isolated compound A **Figure 8** UV spectrum of isolated compound C

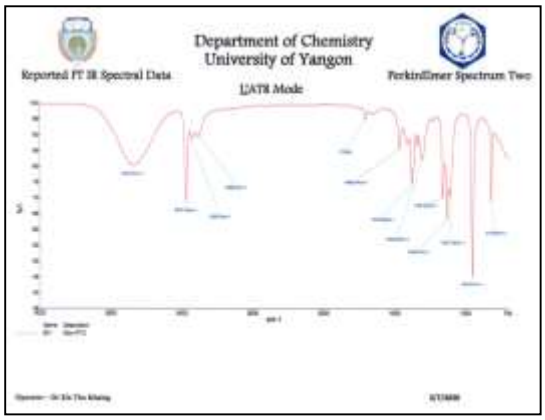


Figure 9 FT IR spectrum of Compound A

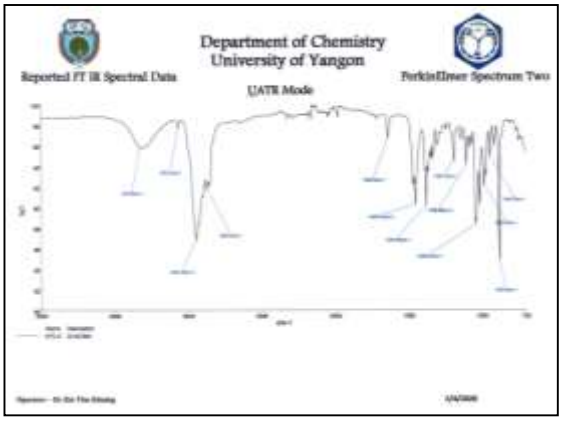


Figure10 FT IR spectrum of Compound B

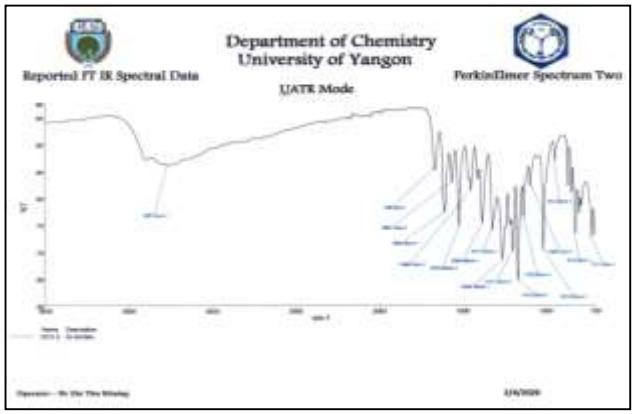


Figure 11 FT IR spectrum of Compound C

Identification of fourier transform infra- red spectrum FT IR of compound A

IR spectrum of isolated compound (A) indicated the presence of OH stretching of alcoholic group (3342 cm^{-1}), C-H stretching of CH_2 group (2970 cm^{-1}), C-H stretching asymmetric of CH_2 and CH_3 group (2970 cm^{-1}), C-H stretching symmetric of CH_2 and CH_3 group (2880 cm^{-1}), C=O stretching of carbonyl group (1708 cm^{-1}), C=C stretching of aromatic ring group (1466 cm^{-1}), C-O stretching of cyclic alcohol group (1378 cm^{-1}), C-O stretching of ether group (1048 cm^{-1}). The FT IR spectrum of the compound (A) was shown in Figure.9. (Okwu & Ohenhen, 2000)

Identification of fourier transform infra- red spectrum FT IR of compound B

IR spectrum of isolated compound (B) indicated the presence of OH stretching of hydroxyl group (3315 cm^{-1}), = C-H stretching of vinylidene group (3070cm^{-1}), C-H stretching asymmetric of CH_2 and CH_3 group (2944 cm^{-1}), C-H stretching symmetric of CH_2 and CH_3 group (2872 cm^{-1}), C=C stretching of alkene group (1636 cm^{-1}), C-H bending of CH_2 and CH_3 group (1452 cm^{-1}), C-H bending of gem dimethyl group (1379 cm^{-1}),), CH-OH stretching of cyclic alcohol (1042 cm^{-1}). The FT IR spectrum of the compound (B) was shown in Figure 10. (Herbone, 1984)

Identification of Fourier transform infra- red spectrum FT IR of compound (C)

IR spectrum of isolated compound (C) indicated the presence of OH stretching of phenolic O-H group (3257 cm^{-1}), C=O stretching of cyclic conjugated system (1667cm^{-1}), C-O stretching of aromatic ring (1608 cm^{-1}), C=O stretching of aromatic ring (1519 cm^{-1}), O-H banding of phenol group (1380 cm^{-1}), C-O-C stretching of ether group (1259 cm^{-1}), C-O stretching of phenol group (1197 cm^{-1}). The FT IR spectrum of the compound (B) was shown in Figure 11. (Bharathi *et al*., 2016)

Discussion and Conclusion

In this research work, extraction, isolation and identification of phytoconstituents from the leaves of *Cordia dichotoma* G.Forst. has been investigated. These organic compounds were obtained from methanol extracts of leaves of *Cordia dichotoma* G. Forst. by Column Chromatographic method.

The isolated compound A with yellow oil (yield - 0.034%) R_f value 0.56. The absorption maximum wave length of isolated compound A was found at 223, 272 nm indicating the presence of conjugated double bond. So, compound A may be steroid compound. According to FT-IR spectrum of compound A, the absorption band at 3342 cm^{-1} indicating the presence of OH-stretching alcohol group, aliphatic CH_2 stretching band appeared at 2970cm^{-1}, the absorption band at1708 cm^{-1} indicating the presence of C=O stretching of carbonyl group, the absorption band at 1048cm^{-1} indicating the presence of C- O stretching of ether group. Therefore, compound "A" may be assigned as steroidal glycoside (Merck index, 2001; Okwa & Ohenhen, 2010).

The melting point of compound B was 215 °C and R_f value was 0.48 and UV inactive. According to physico-chemical tests, isolated compound B was a terpenoid compound. The melting point of compound B is coincident with that of literature lupeol (Herbone, 1984; Merck index, 2001). According to FT IR spectrum of isolated compound B, O-H stretching vibration band appear at 3257 cm^{-1}, the C-H stretching band appeared at 3070 cm^{-1} indicating the presence of vinylidene group, CH stretching band appeared at 2944 cm^{-1} and 2872 cm^{-1} represent the present of CH_2 and CH_3 groups. The absorption band at 1636 cm^{-1} due to C=C stretching indicated the present of double bond. According to the results obtained from FT IR spectral data and melting point, the isolated compound B may be assigned as lupeol(Merck index, 2001).

The isolated compound C was obtained as yellow crystals, melting point 321 °C, R_f value 0.38. The melting point of compound C is similar with that of literature quercetin (Abeer, 2011). The UV spectrum of compound C in methanol observed two absorption maxima at 256 nm (band II) and 373 nm (band I) agreements with flavonoid. By adding of NaOH, band II shifted 285 nm that indicated the presence of 7-OH and band I shifted 421 nm which show the present of 4′ OH (Jain *et al*., 2011) In FT IR analysis, the absorption band occurred at 3257 cm^{-1} due to the OH stretching of phenolic group. The C=O stretching of cyclic conjugated system appeared at 1667cm^{1}. The absorption band at 1608 cm^{-1} and 1519 cm^{-1} were assigned for C=C stretching of aromatic ring. The absorption band at 1380 cm^{-1} appeared due to OH bending of phenolic group and the band at 1259 cm^{-1} associated with C-O-C stretching of ether group and the band at 1197 cm^{-1} was C-O stretching of phenol group. According to melting point, chemical test, UV and FT IR spectral data, the compound C may be assigned as quercetin (Bharathi *et al*., 2016).

It was concluded that, *Cordia dichotoma* G. Forst. is an important therapeutic medicinal plant with varied pharmacological spectrum. The result revealed that *Cordia dichotoma* G. Forst. showed the presence of bioactive compounds (a steroid compound, lupeol and quercetin) which are responsible for varied pharmacological and therapeutic property. The evaluation needs to be carried out on *Cordia dichotoma* G.Forst. in dorder to use the plant in various clinical applications.

Acknowledgements

I would like to express appreciation to Dr. Aung Myat Kyaw Sein, Rector and Dr. San San Aye, Pro-Rector of Mawlamyine University for permission to carry out this research work. I wish to express my special thanks to Dr. Mar Lar Aung, Professor and Head, Department of Botany, Mawlamyine University, Dr Aye Pe Professor and Head, Dr Myint Aung (Professor), Dr Baydar (Professor) and Dr Thandar Aye (Professor), Department of Botany, University of Yangon for their advice and encouragement in this research.

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ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF ISOLATED ANTIFUNGAL COMPOUNDS FROM SOIL BACTERIUM *PSEUDOMONAS* SP.

Moe Moe Win[1], Zar Zar Yin[2] and Yin Yin Myint[3]

Abstract

The focus of the present research, the bacterium *Pseudomonas* sp, isolated from mangrove soil, Shwe thaung yan Township, Ayeyawady Region. It was carried out by paper chromatography with four solvents system. The bacterial culture filtrate was studied by the different ratio of ethyl acetate and fermented broth (1:1, 2:1, 3:1 v/v). The equal ratio (1:1 v/v) ethyl acetate extract showed higher inhibitory effect (19.79 mm) than the other ratio. Crude ethyl acetate extract 2.0 g was obtained from 10 liters of fermented broth with ethyl acetate (1:1 v/v) and subjected to purification was performed using both thin layer chromatography (TLC) method with various solvents system and silica gel column chromatography techniques. By silica gel column chromatographic separation, compound M-1 (colorless needle shape, 1.1 mg, may be sterol), compound M-2 (colorless amorphous powder, 5.6 mg, may be steroid) and compound M-3 (colorless amorphous powder, 5.8 mg, may be steroid) in chloroform: methanol solvent system were isolated. These isolated compounds were characterized by R_f value, physicochemical properties, modern spectroscopic methods such as UV and FT IR. In the further investigation of minimum inhibitory concentrations (MIC), it was observed that MIC value of antifungal compounds M-1 and M-2 were 1.25 µg/ mL and 0.625 µg/mL respectively but M-3 did not exhibit antifungal activity against *Candida albicans* in this experimental concentration range from 20 µg/mL to 0.078 µg/mL. In the present result, indicated that the selected bacterium *Pseudomonas* sp. may be utilized to treat the diseases caused by *Candida albicans.*

Keywords: paper chromatography, silica gel column chromatography, minimum inhibitory concentrations

Introduction

The recent decades are characterized by the novel discoveries of microorganisms capable of producing compounds as a potential source of new antibiotics. Antibiotics are antimicrobial agents produced by microorganisms that inhibit the growth or kill other microorganisms while being harmless to the host cells. Antibiotics are one of the most important commercially exploited secondary metabolites produced by the bacteria and employed in a wide range. Most of the antibiotic producers used today are the soil microbes (Arpigny and Jaeger, 1999). Thin layer chromatography (TLC) is one of the principal separation technique. It can be used in a search from optimum extraction solvents, for identification of known and unknown compounds (Fair *et al*., 2008).

Chromatography is a useful technique for the separation of compounds from a complex mixture, such as a bacterial extract. Base on the physical and chemical properties of compounds and their affinities for certain solid phase materials (e.g., silica), a mixture can be separated into its individual compounds, or at least into mixtures containing fewer compounds with similar characteristics by selecting the appropriate elution solvent or solvent system (Harris, 2003). The most common methods of detection for early stages are: ultraviolet-visible spectroscopy (UV/Vis) that provides information on chromophores present in a compound and FT IR provides information on functional group present in a compound (Henke and Kelleher, 2016).

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The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001). It is well known crude extract isolated from these bacterial metabolites contains complex chemical diversity which is difficult to identify and characterize. Therefore, effort has been made to characterize a bioactive molecule synthesized by isolated bacterial in this study. The aim and objectives of this study were to isolate some organic compounds from the ethyl acetate extract of *Pseudomonas* sp. to characterize the isolated compounds by physicochemical tests and spectroscopic techniques such as UV, FT IR and to determine the Minimum Inhibitory Concentrations (MIC) of bacterial metabolites against *Candida albicans.*

Materials and Methods

Paper chromatography (Tomita, 1988)

The filter paper and four solvents such as 20% NH_4Cl, *n*-butanol saturated with water, *n*-butanol-acetic acid-water (3:1:1) and ethyl acetate saturated with water were used for preliminary characterization of antifungal metabolites. The obtained fermented broth sample was applied on the paper and allowed to dry. The papers were chromatogram in each solvent. Then, bioautography was done to check the antifungal activity of each. Each paper was placed on assay agar plate. After one hour the paper was taken out, and then the plates were incubated for 24-36 hours.

Extraction of antifungal metabolites (Natarajan *et al.*, 2010)

The bacterium was cultivated on nutrient agar medium by inoculating selected bacterium culture in 500 mL conical flask containing 250 mL of the medium. The flask was incubated at room temperature for 2 days. After incubation period, fermentation broth of the bacterium was filtered with filtered paper. The filtrate was extracted with equal ratio of ethyl acetate. Then the mixture was shaken in a separating funnel. The organic layer was separated and collected.

Thin layer chromatographic analysis (Verma *et al.*, 2014)

Thin layer chromatography (TLC) was performed to know the constituent of metabolites of ethyl acetate crude extract from the culture broth of *Pseudomonas* sp. Using pet-ether: ethyl acetate (10:1 and 1:1), chloroform: ethyl acetate, and chloroform: methanol (100:1-1:1) eluting solvent ratios were used. Active culture extract was applied on the TLC plate (1 cm × 6cm). The developing chromatogram was checked under UV lamp (254 nm and 365 nm) and noted down the fluorescence spots. TLC chromatogram was examined with some colour reaction tests such as 5% sulphuric acid, 5% ferric chloride, anisaldehyde/sulphuric acid, vanillin/sulphuric acid and iodine vapour.

Isolation of some organic metabolites by silica gel column chromatography (Simon and Gray, 1998)

According to thin layer chromatographic analysis, the ethyl acetate extract residue of isolated bacterium *Pseudomonas* sp. metabolite was developed to isolate the active compound by silica gel column chromatography with $CHCl_3$: MeOH as eluting solvent. Silica gel (60-200 mesh) (ca. 50g) was dissolved in chloroform and the column was packed by the wet method. EtOAc crude extract (0.58g) was then passed through silica gel column and eluted with chloroform: methanol 90:1-10:1v/v. Fractions of each equal to 2 mL, were collected individually, the compounds present were checked with TLC.

Characterization and identification of isolated antifungal compounds

In an attempt to characterize the isolated antifungal compounds, the following tests were performed:

Determination of solubility of isolated compounds

Each of isolated compounds (0.5 mg) was subjected to 0.5 mL of polar and non-polar solvents such as H_2O, MeOH, EtOAc, $CHCl_3$ and PE in order to known their solubility.

Determination of some chemical properties of isolated compounds

Some coloured reagent such as aq. Potassium permanganate, Iodine vapour, Anisaldehyde/ sulphuric acid, 5% Sulphuric acid, 5% Ferric chloride, Vanillin/sulphuric acid and 2,4 Dinitrophenylhydrazine (DNP) were used to study their behavior on TLC.

Study under UV-visible spectroscopy

For the identification of isolated compounds, ultra violet absorption spectra were also recorded and examined. A Shimadzu UV-1800 UV- visible spectrophotometer at Department of Chemistry, Pathein University were used.

Study under FT IR spectroscopy

The FT IR spectra of isolated compounds were sample recorded by spectrum II spectrophotometer (Perkinelmer) FT IR Fourier Transform Infrared at Department of Chemistry, Pathein University.

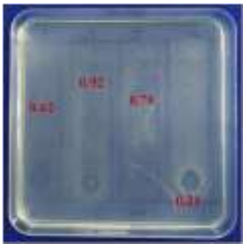
Minimum Inhibitory Concentration (MIC) of isolated compounds

Minimum Inhibitory Concentration (MIC) was carried out by two fold serial dilution method (Andrew, 2001). The using experimental concentrations were ranging from 20.0 µg/mL, 10.0 µg/mL, 5.0 µg/mL, 2. 5 µg/mL 1.25 µg/mL, 0.625 µg/mL, 0.312 µg/mL, 0.156 µg/mL and 0.078 µg/mL respectively. The test organism was *Candida albicans.* After incubation for 24 hours, the MIC were determined by selecting the lowest concentration of metabolite which caused complete inhibition of test growth.

Results

Paper chromatography

In this study, four kinds of solvents 20% NH_4Cl, *n*-butanol saturated with water, *n*-butanol acetic acid-water (3:1:1), ethyl acetate saturated with water were used. Ethyl acetate was more extractable the antifungal metabolites than other solvents. The chromatography bioautographic assay was shown in Figure 1.



1. 20% NH_4Cl
2. *n*-butanol saturated with water
3. *n*-butanol-acetic acid – water (3:1:1)
4. ethyl acetate saturated with water

Figure 1 Bioautographic assay of paper chromatography

Antifungal activity of metabolites in *Pseudomonas* sp. extracted with different volume of EtOAc

From the results of paper chromatography, using ethyl acetate extract (1:1 v/v) resulted in inhibition zone was 17.79 mm, followed by 16.83 mm and 16.60 mm in ethyl acetate extract with fermentation broth (2:1 v/v) and (3:1 v/v) respectively. These results were showed in Table 1 and Figure 2.

Table 1 Antifungal activity of *Pseudomonas* sp. extracted with different ratio of EtOAc to FB on *Candida albicans*

Different ratio of solvent: fermented broth(v/v)	Inhibition diameter Zone (mm)
1:1	19.79
2:1	16.83
3:1	16.60

Well size =8mm

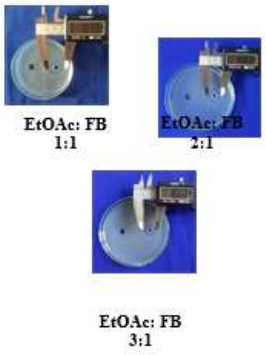


Figure 2 Antifungal activity of *Pseudomonas sp.* extracted with different ratio EtOAc to FB on *Candida albican*

Extraction of antifungal metabolites

10 liters of selected bacterium *Pseudomonas* sp. were fermented in suitable synthetic fermentation medium (48 hrs, 20% size of inoculum, temperature 35º C, pH 7, 2 days fermentation period, under shaking culture) and extracted with equal ratio of ethyl acetate to fermented broth (1:1 v/v) to yield 2.0 g was obtained from the culture filtrate.

Thin layer chromatographic analysis

Thin layer chromatography (TLC) was performed on ethyl acetate crude extracted by employing various solvent system. The extract showed well- separated spots on TLC by using $CHCl_3$:MeOH solvent systems under UV 254 nm and 365 nm and some color reagent tests. Therefore, the solvent system $CHCl_3$:MeOH was chosen to isolate pure compounds by silica gel column chromatography.

Isolation of some organic metabolites by silica gel column chromatography

Gradient elution was performed successively with increasing polarity. According to the procedure in Figure 4, compound M-1 (colorless needle shape 1.1 mg) and compound M-2 (colorless amorphous powder, 5.6 mg) and compound M-3 (colorless amorphous powder, 5.8 mg) were obtained from the respective fractions F-I, F III and F-IV. The remaining fractions F-II and F-V were observed as mixtures and no antifungal activity was recorded. These isolated compounds M-1, M-2 and M-3 have significant activity on *Candida albicans* with inhibitory zone 19.66 mm, 18.06 mm and 16.43 mm respectively. Thin layer chromatogram of compounds (M-1, M-2 and M-3) and their antifungal activity were presented in Figures 3 and 11.

(a)

Compound M-1
Solvent System : $CHCl_3$
R_f value : 0.61 ($CHCl_3$: MeOH, 120 : 1)
Spraying reagent : 5% H_2SO_4, heat
Amount : 1.1 mg

(b)

Compound M-2
Solvent System : $CHCl_3$:MeOH
R_f value : 0.43 ($CHCl_3$:MeOH 20:1)
Spraying reagent : 5% H_2SO_4, heat
Amount : 5.6 mg

(c)

Compound M-3
Solvent System : $CHCl_3$:MeOH
R_f value : 0.26 ($CHCl_3$:MeOH 20:1)
Spraying reagent : 5% H_2SO_4, heat
Amount : 5.8 mg

Figure 3 Thin layer chromatogram of isolated compounds (a) compound M-1, (b) compound M-2 and (c) compound M-3

Procedure for selection of stationary phase

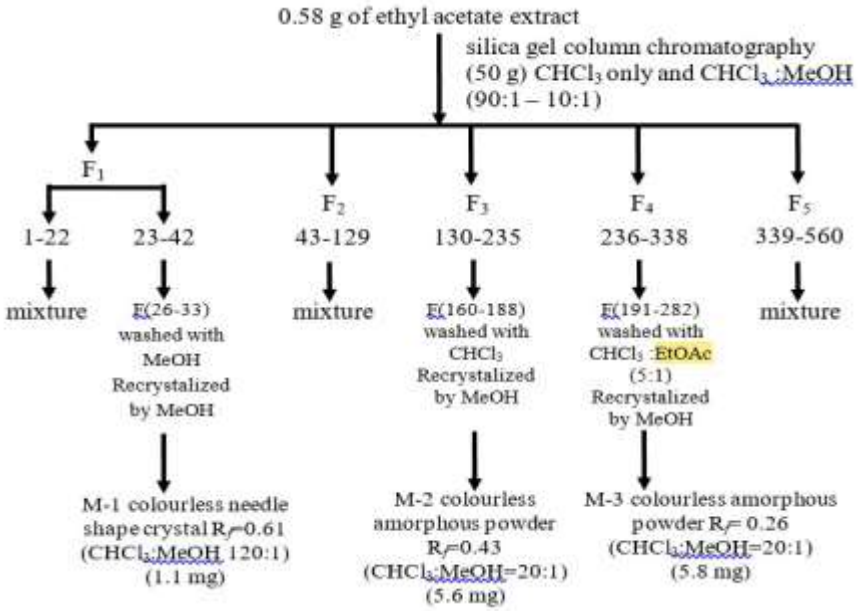


Figure 4 Flow diagram of separation of compounds from ethyl acetate extract of selected bacterium *Pseudomonas* sp. by column chromatography with $CHCl_3$:MeOH

Characterization of isolated antifungal compounds

The isolated compounds were characterized by physicochemical tests, solubility tests, modern spectroscopic techniques such as UV and FT IR. These resultant data were given as follow;

Table 2 Chemical Reagent Tests of Isolated Compounds M-1, M-2 and M-3

Reagent	Observation on Isolated Compounds			Remark		
	M-1	**M-2**	**M-3**	**M-1**	**M-2**	**M-3**
Anisaldehyde/ sulphuric acid, Δ	violet	purple	purple	steroid	steroid	steroid
Vanillin/ sulphuric acid, Δ	chared	chared	chared	steroid	steroid	steroid
5% $FeCl_3$	no brown colour	no brown colour	no brown colour	no	no	no
I_2vapour	yellow colour change purple to colourles	yellow colour immediately change purple to colourless	yellow colour immediately change purple to colourless	phenolic double bond double bond	phenolic double bond double bond	phenolic double bond double bond
aq.$KMnO_4$						
2, 4- Dinitrophenyl hydrazine	no ppt.	yellow ppt.	yellow ppt.	no carbonyl group	carbonyl group	carbonyl group

ppt. = precipitate (Δ) = heat

Identification of isolated compounds from ethyl acetate extract of bacterium *Pseudomonas* sp.

Compound M-1

It was soluble in EtOAc, MeOH, PE and $CHCl_3$ but insoluble in H_2O. The R_f value of compound M-1 was found to be 0.61 in $CHCl_3$ only solvent system, it gave yellow spot on TLC chromatogram with iodine vapour, violet spot with anisaldehyde/sulphuric acid followed by heating. According to the UV absorption spectral data of compound M-1, the maximum wavelength in methanol is 231 nm. This wavelength indicated to be the presence of π bond in compound M-1. The FT IR spectrum of compound M-1 is illustrated in Figure 5, 6 and the corresponding data assignments are interpreted in Table 3,4. According to the results of the physicochemical properties, R_f value, UV and FT IR spectral data, isolated compound M-1 may be sterol.

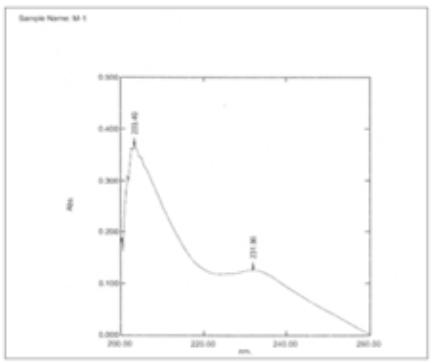


Figure 5 UV spectrum of isolated compound M-1

Table 3. UV Spectral Data of Isolated Compound M-1

Observed λ_{max} (nm) in methanol	* Remark
231	double bond

*(Kasal *et al.*, 2010)

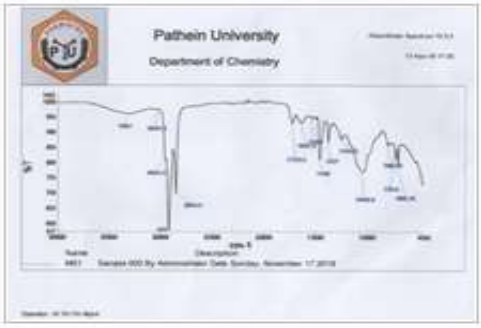


Figure 6 FT IR spectrum of isolated compound M-1

Table 4 FT IR Spectral Data of Isolated Compound M-1

Wave number (cm^{-1})		Band Assignment
Observed	*Literature	
3307	3625-3200	Streching O-H in alcohol
3024	3050-3000	Streching C-H for -HC=C-
2921	2970-2850	Streching C-H in CH_2 and CH_3
1632	1635-1605	Streching C=C for alkane
1459	1475-1448	Bending C-H in CH_3
1377	1390-1370	Bending C-H in CH_2
1048	1060-1000	Streching C-O in alcohol

(*Kasal *et al.*, 2010)

Compound M-2

It was soluble in EtOAc, MeOH and $CHCl_3$ but insoluble in PE, and H_2O. The R_f value of compound M-2 was found to be 0.43 in $CHCl_3$:MeOH (20:1 v/v) solvent system and it gave yellow spot on TLC chromatogram with iodine vapour, purple spot with anisaldehyde/sulphuric acid followed by heating, yellow color with 2, 4 DNP. According to the UV absorption spectral data of compound M-2, the maximum wavelength in methanol are 227 nm, 276 nm and 284 nm. This wavelength indicated to be the presence of π bond and atom with non bonded electrons. According to the results of the physicochemical properties, R_f value, UV and FT IR spectral data, isolated compound M-2 may be steroid Table 5, 6 and Figure 7, 8.

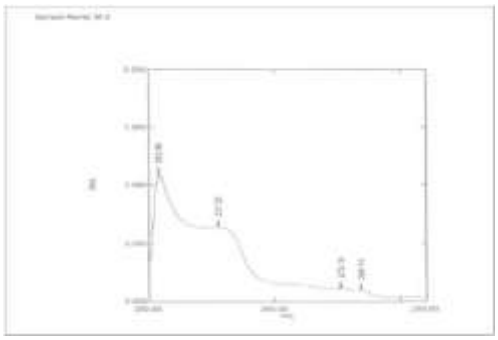


Table 5 UV Spectral Data of Isolated Compound M-2

λ_{max} in methanol (nm)	* Remark
227 276, 284	double bond atoms contained non bonded electrons

(*Kasal *et al.*, 2010)

Figure 7 UV spectrum of isolated compound M-2

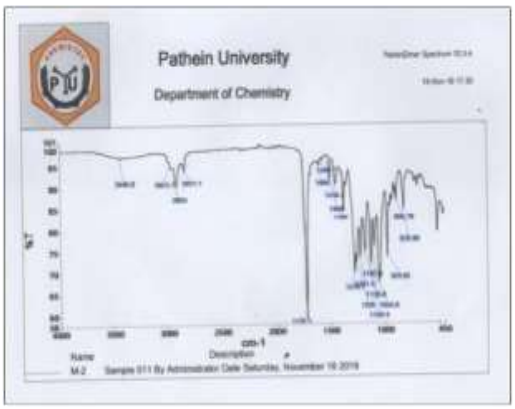


Figure 8 FT IR spectrum of isolated compound M-2

Table 6 FT IR Spectral Data of Isolated Compound M-2

Wave number (cm^{-1})		Band Assignment
Observed	*Literature	
2972-2851	2970-2850	Streching C-H in CH_2 and CH_3
1720	1800-1650	Streching C=O in carbonyl
1632-1605	1635-1605	Streching C=C in α, β unsaturated
1456	1475-1445	Bending C-H for methylene
1380	1390-1370	Bending C-H for methyl
1064	1060-1000	Streching C-O for alcohol
978, 896, 826	970-700	Streching of plane bending C-H in

(*Kasal *et al.*, 2010)

Compound M-3

It was soluble in EtOAc, MeOH, and $CHCl_3$ but insoluble in PE, and H_2O. The R_f value of compound M-3 was found to be 0.26 in $CHCl_3$:MeOH(20:1 v/v) solvent system and it gave yellow spot on TLC chromatogram with iodine vapour, purple spot with Anisaldehyde/sulphuric acid followed by heating, yellow color with 2, 4 DNP. According to the UV asorption spectral data of compound M-3, the maximum wavelength in methanol are 228 nm, 277 nm and 284 nm. This wavelength indicated to be the presence of π bond and atom with non bonded electrons. According to the results of the physicochemical properties, R_f value, UV and FT IR spectral data, isolated compound M-3 may be steroid.

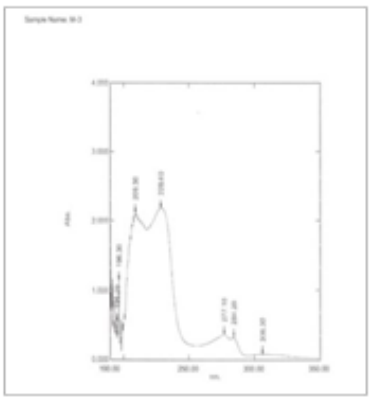


Figure 9 UV spectrum of isolated compound M-3

Table 7 UV Spectral Data of Isolated Compound M-3

λ_{max} in methanol (nm)	* Remark
228	double bond
277, 284	atom contained non bonded electrons

(*Kasal *et al.*, 2010)

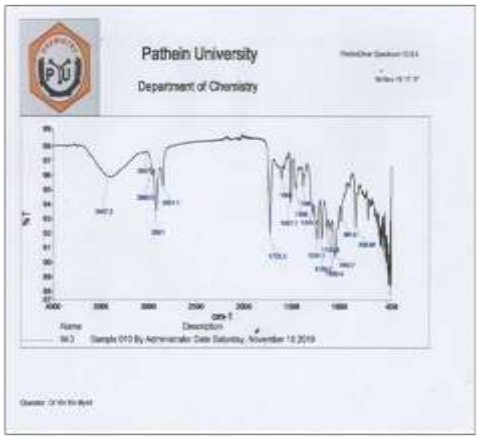


Figure 10 FT IR spectrum of isolated compound M-3

Table 8 FT IR Spectral Data of Isolated Compound M-3

Wave number (cm^{-1})		Band Assignment
Observe	***Literature**	
3407-2851	3550-3200	Streching O-H in alcohol
2921	2970-2850	Streching C-H in CH_2 and CH_3
2851	2970-2850	Streching C-H in CH_2 and CH_3
1723	1720-1700	Streching C=O in carbonyl
1608	1635-1605	Streching C=C in , β
1450	1457-1445	Bending C-H for methylene
1380	1390-1370	Bending C-H for methyl
1042	1260-1000	Streching C-O for OH
826	970-700	Bending C-H in CH_3

* (Kasal *et al.*, 2010)

Antifungal activity of isolated compounds

Bioassay for determination of isolated compounds were undertaken by agar well diffusion method. From the results, isolated compound M-1 showed antifungal activity (19.66 mm), compound M-2 exhibited (18.06 mm) and compound M-3 showed (16.43 mm) on *Candida albicans*

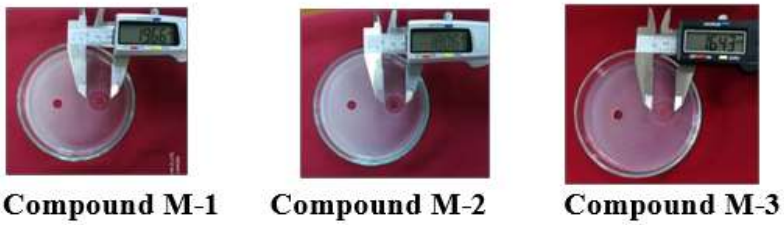


Figure 11 Antifungal activity of isolated compounds on *Candida albicans* by agar well diffusion method

Minimum Inhibitory Concentration (MIC) of isolated compounds

MICs were read in µg/mL after overnight incubation. It was observed that MIC value of compound M-1 was 1.25 µg/mL and for compound M-2 was 0.625 µg/mL but M-3 did not exhibit antifungal activity against *Candida albicans* in this experimental concentration range of 20 µg/mL to 0.078 µg/mL

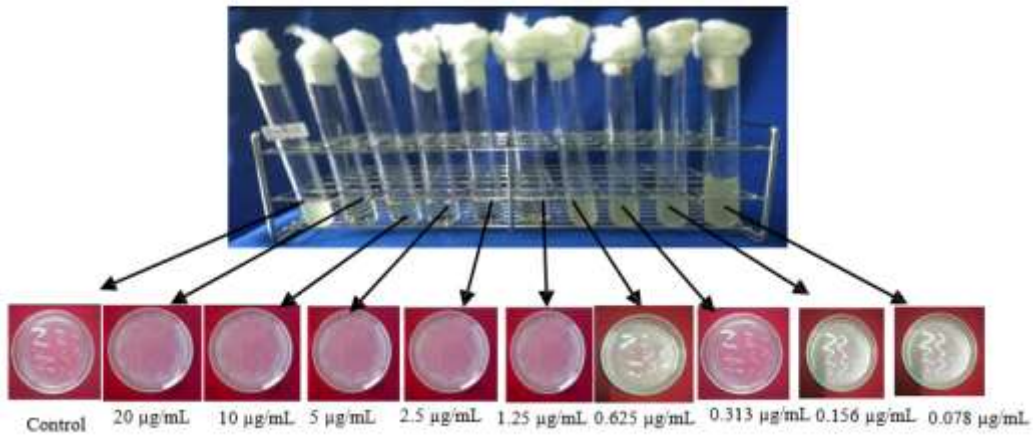


Figure 12 Minimum inhibitory concentration of secondary metabolites from compound M-1 on *Candida albicans*

Agar well size = 8 mm

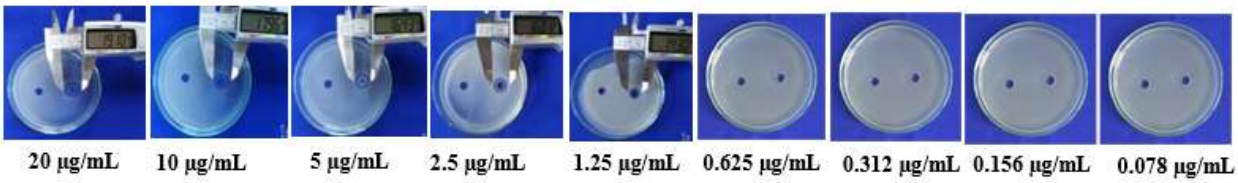


Figure 13 Minimum inhibitory concentration of secondary metabolites from compound M-1 on *Candida albicans* (agar well diffusion method

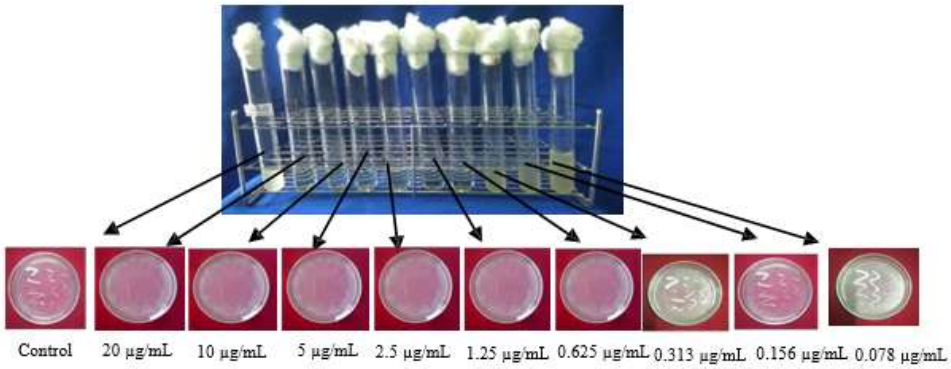


Figure 14 Minimum inhibitory concentration of secondary metabolites from compound M-2 on *Candida albicans*

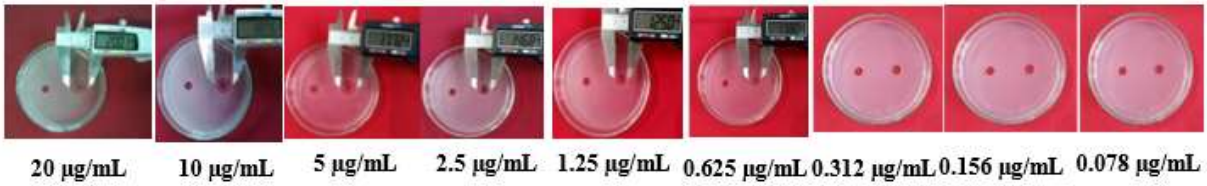


Figure 15 Minimum inhibitory concentrations of secondary metabolites from compound M-2 on *Candida albicans* (agar well diffusion method)

Discussion and Conclusion

The production of secondary metabolites from Pseudomonas species has been the most economical and biotechnological sources for the discovery of new bioactive compounds. In the investigation of paper chromatography, four kinds of different solvents were applied to observe the optimum extraction ability of secondary metabolites. Ethyl acetate was more extractable the antifungal metabolites than other solvents. On studying antifungal activity of bacterium extracted with different ratio (1:1, 2:1, 3:1 v/v) of EtOAc. The equal ratio of ethyl acetate extract to fermented broth showed the highest activity (19.79 mm). Jain and Pundir, 2011 reported that fermentation broth and ethyl acetate solvent (1:1 v/v) was applied and the maximum antimicrobial metabolite was obtained by using this ratio.

In this study, for containing the organic constituents in crude extract was examined by various solvent systems with TLC method. The crude extract showed well separated spots on TLC by using chloroform: methanol (1:1 v/v) solvent system. According to the TLC result, ethyl acetate crude extract (0.58 g) was subjected with silica gel column chromatography and eluted with starting from the optimized solvent systems used was chloroform: methanol at ratio 90:1, 70:1, 50:1, 30:1 and 10:1v/v. The isolated fractions were collected by same characteristic of TLC chromatogram which ultimately resulted in major five fractions after examined UV lamp (254 nm and 365 nm) as well as some color reaction tests. Compounds M-1, M-2 and M-3 were obtained from the respective fractions F-I, F-III, and F-IV and remaining fractions F-II and F-V were observed as mixture. The fraction (F-I) $f_{26\text{-}33}$; isolated compound M-1 with R_f value 0.61 showed antifungal activity 19.66 mm. Inhibitory zones 18.06 mm was observed in fraction (F-III) $f_{160\text{-}188}$; isolated compound M-2 with R_f value 0.43 and fraction (F-IV) $f_{191\text{-}282}$; isolated compound M-3 with R_f value 0.26 showed 16.43 mm inhibitory zone. The purified active compounds obtained were subjected to various examinations such as some chemical reagent tests, ultraviolet (UV) and FT IR (Fourier Transform Infrared). In order to these data, the isolated

compound, M1, M2 and M3 sterol and steroid respectively. Balandrin *et al*., 1998 described that steroids are a group of cholesterol derived lipophilic, low-molecular weight compounds found in derived from a variety of different marine, terrestrial, and synthetic sources. Steroid family includes the sterols, bile acids a number of hormones (both global and adrenal cortex hormones) and some hydrocarbons. In a study of Minimum Inhibitory Concentrations (MIC) of isolated compounds, the antifungal metabolites affected on the growth of *Candida albicans* at least MIC of 1.25 µg/mL for compound M-1 and 0.625 µg/mL for compound M-2 and compound M-3 did not exhibit antifungal activity against *Candida albicans* in this experimental range from 20 µg/mL to 0.078 µg/mL. A lower MIC is an indication of a better antimicrobial activity (Andrews, 2001).

The present study indicates that soil bacterium *Pseudomonas* sp. were isolated from mangrove soil for an antifungal compound against antibiotic resistant human pathogenic fungi *Candida albicans*. Purification and structure elucidation of active compound and investigation its molecular mechanisms can be a promising approach for further antimicrobial drug development programs.

Acknowledgements

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ISOLATION AND ANTIMICROBIAL ACTIVITIES OF ENDOPHYTIC FUNGUS, NF-01 FROM *CROTON ROXBURGHIANUS* N.P.BALAKR.

Nway Darli Ko[1], Mya Htet Htet Aung[2] and Khine Swe Nyunt[3]

Abstract

A total of 20 endophytic fungi were isolated from three different plants, *Croton roxburghianus* N.P.Balakr (Thet-yin-gyi) , *Tadehagi triquetrum* (L.) H. Ohashi. (Lauk-thay), *Cassia siamea* L. (Mezali) collected from Pathein Township. In the investigation of antimicrobial activities of 20 endophytic fungi with ten kinds of test organism, *Agrobacterium tumefaciens, Aspergillus paraciticus, Bacillus subtilis, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas fluorescens, Saccharomyces cerevisiae, Salmonella typhimurium* and *Staphylococcus aureus* were used for the test throughout the research studied. In this study, endophytic fungi NF-01(27.85mm) and NF-07(26.67mm) showed highly activity against *Bacillus subtilis.* Among them, fungus NF-01 isolated from *Croton roxburguianus* n.p.balakr. was screened for further investigation based on the results of maximum inhibition against *Bacillus subtilis*. In the investigation of carbon and nitrogen sources utilization, the excellent growth of NF-01 fungus was found on carbon sources such as glucose and glycerol; nitrogen sources were yeast extract and peanut cake.

Keywords: endophytic fungi, antimicrobial activity and *Bacillus subtilis.*

Introduction

Endophytic microorganisms are survived inside the living tissues of plants without causing any harmful effects or damages and have symbiotic relationships with host plants (Specian *et al*., 2012). These endophytes have an ability to produce a variety of secondary metabolites (Sandhu *et al*., 2014). Nevertheless, increasing levels of antibiotic resistance in both nonpathogenic and pathogenic bacteria has spurred the search for new antibiotics to manage diseases (Petrini, 1991).

Since the population has been increased, this was not possible to afford plant-based medicine. Due to the increasing demand of medicine and destruction of medicinal plants, a huge work carried out in the field of endophytes for producing bioactive compounds that can be used in the treatment of diseases (Onifade, 2007). Endophytes are the synthesizers inside plants that produce bioactive compounds with low toxicity toward higher plants (Owen and Hundley, 2004). Endophytes provide an extensive variety of bioactive secondary metabolites with unique structure, synthesized via various metabolic pathways i.e. polyketide, isoprenoid, amino acid derivatives (Tan and Zou, 2001).

Materials and Methods

Collection of plant samples

The plant samples were collected at different places in Pathein Area. These plant specimens were identified according to the available references and internet websites information.

[1] PhD Candidate, Microbiology, Pathein University
[2] Lecturer, Department of Botany, Monywa University
[3] Assistant Lecturer, Department of Botany, University of Yangon

Table1 Plant used for isolation of endophytes

No.	Scientific Name	Family	Myanmar Name	Location
1	*Croton roxburghianus* N.P.Balakr.	Euphorbiaceae	Thet-yin-gyi	Yadanar street, Pathein Uni, Campus.
2	*Tadehagi triquetrum* (L.) H. Ohashi.	Fabaceae	Lauk-thay	Yadanar street, Pathein Uni, Campus.
3	*Cassia siamea* L.	Caesalpiniaceae	Mezali	Near BDC, Pathein Uni, Campus.

Isolation procedure of endophytes from plants (Tomita, 1998)

The plants were washed in running tap water for 15 mins. The plant leaves were cut into about 1cm pieces. Sterilize the surface of plant part by soaking it in 75% ethanol for 2mins. These parts were dried on sterilized paper and then they were placed on agar plates containing medium. The plates were incubated for 3days to 1 week at room temperature.

Preliminary Study for Antimicrobial Activities by Paper Disc Diffusion Assay (NITE, 2004)

The isolated fungi were grown at 25ºC for 5 days on Potato Glucose Agar medium. These isolated fungi were inoculated into seed medium and incubated at 25ºC for 3 days (Tomita, 1998)**.** Then, 10mL of seed culture were transferred into the fermentation medium. The fermentation was carried out for 10 days. The fermented broth (20µL) was used to examine the antimicrobial activity against test organisms by paper disc diffusion assay. Paper disc having eight-millimeter diameter (Advantee, Toyo Roshi Kaisha Co., Ltd., Japan) were utilized for antimicrobial assays. The assay medium was used for the antimicrobial activity test. One percent of test organisms was added to assay medium, then poured into plates. After solidification, paper disc impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated 24-36 hours at 25ºC.The appearance of clear zone (inhibitory zones) around the test disc indicates the presence of antimicrobial activity. The test organisms used in paper disc diffusion assay were as followed (Table 2).

Table 2 Test organisms and diseases used in antimicrobial activities

Test organisms	Code number	Diseases
Agrobacterium tumefaciens	NITE 09678	Plant disease, Crown gall disease and tumors.
Aspergillus paraciticus	IFO 5123	Fruits disease.
Bacillus subtilis	IFO 90571	Fruits and seeds disease.
Candida albicans	NITE 09542	Candidosis.
Escherichia coli	AHU 5436	Cholera, diarrhoea and vomiting, urinary tract infections.
Micrococcus luteus	NITE 83297	Skin disease.
Pseudomonas fluorescens	IFO 94307	Rice disease and pulmonary disease.
Saccharomyces cerevisiae	NITE 52847	Food spoilage, empyema, pneumonia, liver abscess, asthma and diarrhea.
Salmonella typhi	AHU 7943	Typhoid fever and food poisoning.
Staphylococcus aureus	AHU 8465	Skin disease, food poison, wound infection, burns, abscesses, blood stream infection, staphylococcal pneumonia.

In the investigation of carbon and nitrogen sources utilization, carbon sources such as glucose, sucrose, glycerol, soluble starch, oat and tapioca powder were employed whereas nitrogen sources such as peptone, yeast extract, malt extract, meat extract, peanut cake and sesame cake. The cultures for NF-01 were undertaken on plates containing these carbon and nitrogen sources for 6 days at 25ºC.

Effects of Carbon and Nitrogen Utilization

Carbon sources (1.0%) with basal medium		Nitrogen sources (1.0%) with basal medium	
Yeast extract	0.6%	Glucose	1.5%
Corn powder	0.6%	Glycerol	1.5%
K_2HPO_4	0.002%	K_2HPO_4	0.002%
$MgSO_4$	0.002%	$MgSO_4$	0.002%
$CaCO_3$	0.002%	$CaCO_3$	0.002%
pH	6.0	pH	6.0
DW	100mL	DW	100mL

Results

Isolation of endophytic fungal strains

Twenty fungal strains were isolated from the leaves of *Croton roxburghianus* N.P.Balakr, *Tadehagi triquetrum* (L.) H.Ohashi ,*Cassia siamea* L. In the investigation of antimicrobial activities of these endophytic fungi (NF-01 to NF- 10) showed antimicrobial activities. Among them, NF-01 and NF-07 (isolated from the plant leaves of *Croton roxburghianus* N.P.Balakr (Thet-yin-gyi)) were highly activity against *Bacillus subtilis* than the other fungi. Therefore, these strains were selected for further investigation of fermentation.

Table 3 Isolation of endophytic fungal strains

Strain	Source
NF-01 to 08	Leaves of *Croton roxburghianus* N.P.Balakr.(Thet-yin-ghi)
NF-09 to 14	Leaves of *Tadehagi triquetrum* (L.) H. Ohashi (Lauk-thay)
NF-14 to 20	Leaves of *Cassia siamea* L. (Mezali)

Table 4 Antimicrobial activities of isolated fungi

Test organisms / Endophytes	*Agrobacterium tumefaciens*	*Aspergillus paraciticus*	*Bacillus subtilis*	*Candida albicans*	*Escherichia coli*
NF-01	-	12.61mm	27.89mm	15.67mm	-
NF-02	-	10.81mm	24.46mm	12.22mm	-
NF-03	-	11.48mm	23.07mm	12.07mm	-
NF-04	-	14.24mm	23.07mm	12.80mm	-
NF-05	-	13.02mm	24.55mm	14.68mm	-
NF-06	-	-	21.52mm	11.98mm	-
NF-07	-	12.61mm	26.35mm	12.74mm	-
NF-08	-	-	20.64mm	11.36mm	-
NF-09	-	14.09mm	24.15mm	13.98mm	-
NF-10	-	-	25.37mm	11.98mm	-

Table 5 Antimicrobial activities of isolated fungi

Test organisms / Endophytes	*Micrococcus luteus*	*Saccharomyces cerevisiae*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Pseudomonas fluorescence*
NF-01	26.64mm	-	-	19.61mm	-
NF-02	25.66mm	-	21.53mm	20.46mm	-
NF-03	19.50mm	-	17.54mm	22.79mm	-
Nf-04	20.17mm	-	-	23.63mm	-
NF-05	23.36mm	-	19.38mm	21.50mm	-
NF-06	21.95mm	-	17.91mm	19.14mm	-
NF-07	18.92mm	-	17.94mm	21.67mm	-
NF-08	20.58mm	-	17.93mm	21.21mm	-
NF-09	22.52mm	-	19.87mm	22.47mm	-
NF-10	23.74mm	-	-	18.99mm	-

Table 6 Antimicrobial Activities of isolated fungi

Test organisms / Endophytes	*Agrobacterium tumefaciens*	*Aspergillus paraciticus*	*Bacillus subtilis*	*Candida albicans*	*Escherichia coli*
NF-11	13.61mm	13.52mm	12.81mm	-	-
NF-12	10.11mm	12.83mm	10.61mm	-	-
NF-13	14.28mm	14.05mm	11.34mm	-	-
NF-14	12.44mm	16.35mm	14.04mm	-	-
NF-15	12.02mm	16.89mm	13.42mm	-	-
NF-16	10.56mm	19.08mm	-	-	-
NF-17	16.61mm	18.42mm	12.71mm	-	-
NF-18	16.08mm	15.44mm	-	-	-
NF-19	14.09mm	13..21mm	14.22mm	-	-
NF-20	17.09mm	12.21mm	-	-	-

Table 7 Antimicrobial activities of isolated fungi

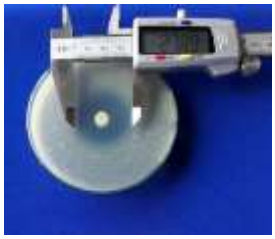
Test organisms / Endophytes	*Micrococcus luteus*	*Saccharomyces cerevisiae*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Pseudomonas fluorescence*
NF-11	20.43mm	22.43mm	19.55mm	-	-
NF-12	-	19.43mm	19.77mm	-	-
NF-13	15.74mm	23.44mm	22.09mm	-	-
NF-14	16.73mm	21.61mm	22.75mm	-	-
NF-15	18.39mm	19.71mm	19.75mm	-	-
NF-16	12.71mm	21.31mm	23.53mm	-	-
NF-17	20.94mm	18.69mm	21.31mm	-	-
NF-18	19.39mm	21.44mm	19.39mm	-	-
NF-19	18.77mm	17.09mm	-	-	-
NF-20	-	-	21.75mm	-	-

Figure 1 Antimicrobial activity of isolated fungi (NF-01 to 10) on *Bacillus subtilis.*

Table 8 Antimicrobial activity against *Bacillus subtilis*

Fermentation Period / Endophytes	2days	3days	4days	5days	6days	7days	8days	9days	10days
NF-01	-	21.51 mm	27.89 mm	27.66 mm	26.78 mm	23.72 mm	20.42 mm	-	-
NF-07	-	23.55 mm	26.35 mm	24.77 mm	22.49 mm	20.5 2mm	18.09 mm	-	-

Based on the results of antimicrobial activity test, it was found that 20 strains showed activities on six test organisms, among them, NF-01 and NF-07 showed highly activity against *Bacillus subtilis.*

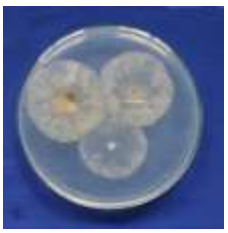


NF-01(27.89 mm)

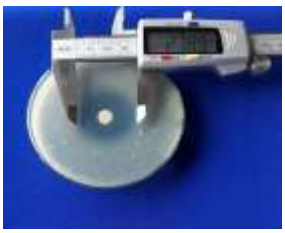
NF-07(26.35 mm)

Figure 2 Antimicrobial activity against *Bacillus subtilis*

Front view



Reverse view



Antimicrobial activity on *Bacillus subtilis*

Photomicrograph X 40

Figure 3 Morphology, Antimicrobial activity and Photomicrograph of selected fungus NF-01 against on *Bacillus subtilis*

Investigation of Carbon and Nitrogen Sources Utilization

In the study for the growth with carbon and nitrogen sources utilization, the excellent growth of NF-01 fungus was found on carbon sources such as glycerol, glucose and nitrogen sources such as yeast extract and peanut cake gave excellent growth. It was found that the good growth of NF-01 on carbon sources were sucrose and tapioca powder, nitrogen sources were peptone and meat extract. Carbon source were oat and soluble starch and nitrogen sources were malt extract and sesame cake gave poor growth. These results are shown in Table 9 and 11, Figure 4 and 6.

Table 9 Morphological Characters of NF-01 on Various Carbon Sources

Carbon Source	Growth	Colour
Glycerol	Excellent	White
Sucrose	Good	White
Glucose	Excellent	White
Oat	Poor	White
Tapioca powder	Good	White
Soluble starch	Poor	White

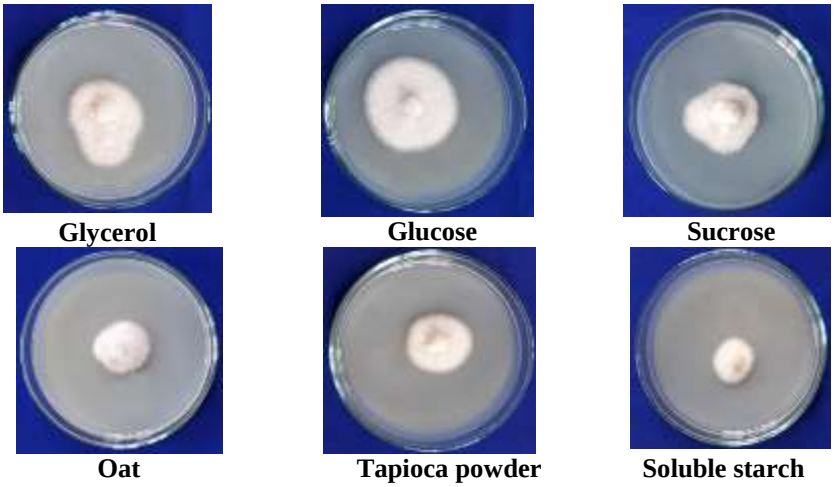


Figure 4 Morphological characters of NF-01 on various carbon sources

Table10 Effects of Different Carbon Sources Utilization against on *Bacillus subtilis*

Day / Sources	1 day	2 days	3 days	4 days	5 days	6 days
Glycerol	21.55 mm	24.60 mm	28.33 mm	34.21 mm	35.67 mm	**38.93 mm**
Sucrose	19.88 mm	23.53 mm	36.23 mm	36.88 mm	37.90 mm	**37.94 mm**
Glucose	**22.36 mm**	**23.60 mm**	**24.52 mm**	**38.23 mm**	**38.27 mm**	**42.52 mm**
Oat	20.17 mm	20.19 mm	21.07 mm	32.68 mm	34.65 mm	**35.11 mm**
Tapioca powder	19.74 mm	18.94 mm	23.16 mm	32.16 mm	33.36 mm	**34.04 mm**
Soluble starch	23.13 mm	25.16 mm	27.59 mm	29.72 mm	33.59 mm	**36.32 mm**

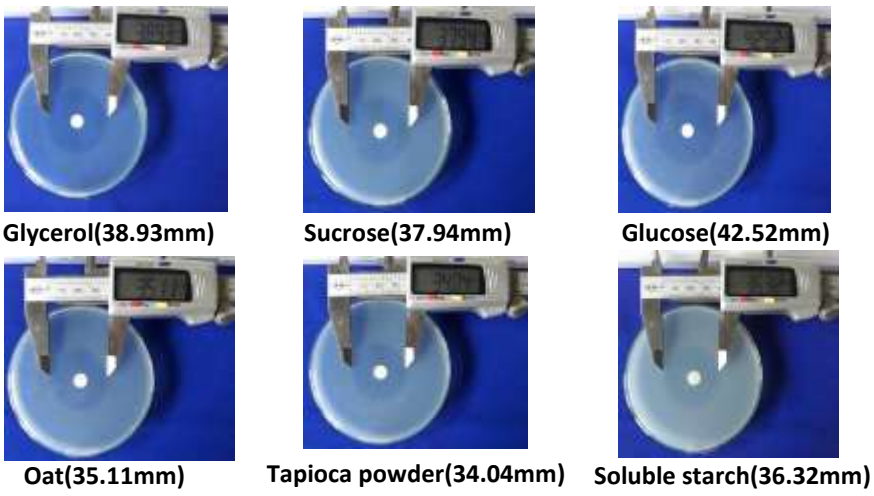


Figure 5 Carbon sources activities of fungus NF-01

Table 11 Morphological Characters of NF-01 on Various Nitrogen Sources

Nitrogen Source	Growth	Colour
Sesame cake	Poor	White
peptone	Good	White
Meat extract	Good	White
Malt extract	Poor	White
Peanut cake	Excellent	White
Yeast	Excellent	White

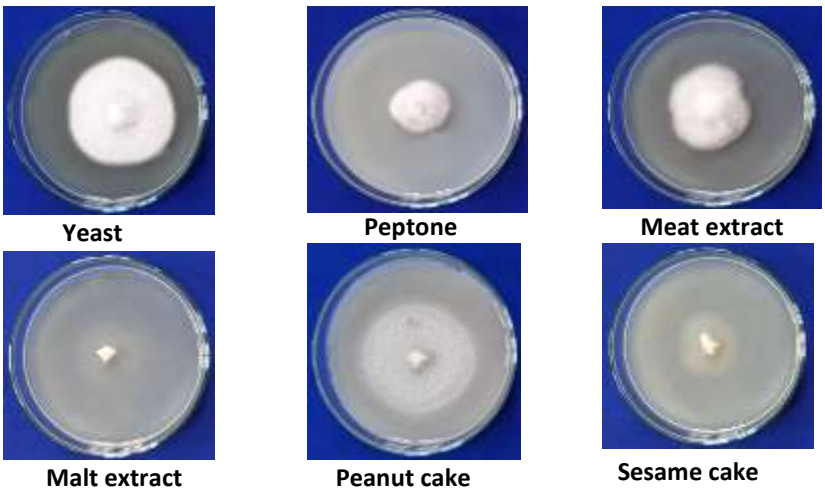
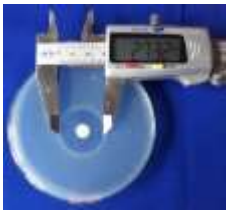


Figure 6 Morphological characters of NF-01 on various nitrogen sources

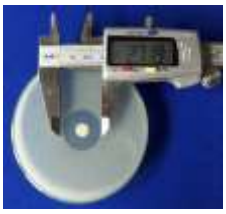
Table 12 Effects of Different Nitrogen Sources Utilization against on *Baciullus subtilis*

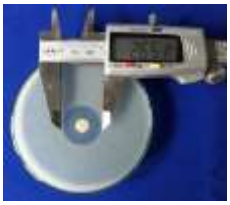
Day / Sources	1 day	2 days	3 days	4 days	5 days	6 days
Sesame cake	**29.31 mm**	27.32 mm	26.17 mm	26.32 mm	25.33 mm	24.03 mm
Peptone	**27.32 mm**	27.03 mm	24.79 mm	24.29 mm	22.48 mm	21.79 mm
Meat extract	**26.75 mm**	25.30 mm	24.07 mm	23.54 mm	23.36 mm	22.46 mm
Malt extract	**27.19 mm**	26.44 mm	25.36 mm	24.36 mm	23.48 mm	23.29 mm
Peanut cake	**26.51 mm**	25.73 mm	25.48 mm	25.25 mm	23.11 mm	21.08 mm
Yeast extract	**30.23 mm**	**29.14 mm**	**28.82 mm**	**26.73 mm**	**24.65 mm**	**22.65 mm**


sesamecake(29.31mm)


Peptone(27.32mm)


Meat


Malt extract(27.19mm)


Peanut


Yeast extract(30.23mm)

Figure 7 Nitrogen sources activities of strain NF-01

Discussion and Conclusion

During the study of the isolation of endophytic fungi from three different plants collected from Pathein Area. The isolation of endophytic fungi, 20 fungi were isolated. In the investigation of antimicrobial activities of these endophytic fungi (NF-01 to NF- 20) showed antimicrobial activities. Among them, NF-01 and NF-07 (isolated from the plant leaves of *Croton roxburghianus* N.P. Balakr (Thet-yin-gyi)) showed highly activity against *Bacillus subtilis.* Therefore, these strains were selected for further investigation of fermentation.

In conclusion, the isolation of endophytic fungi from three different plant leaves samples and screening them for antimicrobial activity by *Bacillus subtilis.* Among them, these active strains NF-01 showed highly activity than NF-07. Therefore NF-01 was selected for carbon and nitrogen sources utilization. In the investigation of carbon and nitrogen sources utilization, the excellent growth of NF-01 fungus was found on carbon sources such as glucose and glycerol; nitrogen sources were yeast extract and peanut cake.

The excellent growth of NF-01 fungus was found on carbon sources activity such as glucose (42.52mm) in 6 days fermentation period. In nitrogen sources activity yeast extract was (30.23mm) in 1 day fermentation period. Among them, these active strain NF-01 was selected further investigation of optimal fermentation conditions.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Si Si Hla Bu, Rector, Pathein University for providing me an opportunity to do this work. I would like to record my deep thank to Professor Dr Wah Wah Lwin, Head of Botany Department, Pathein University for her suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Mya Htet Htet Aung, Lecturer, Department of Botany, Monywa University, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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POLLEN MORPHOLOGY OF SOME SPECIES OF BIGNONIACEAE IN LASHIO AREA

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Abstract

The pollen grains of 15 species from 14 genera of Bignoniaceae in Lashio area were investigated by using light microscope. The species studies were included in genera *Campis*, *Dolichandrone*, *Haplophragma*, *Jacaranda*, *Mansoa*, *Mayodendron*, *Millingtonia*, *Oroxylum*, *Podranea*, *Pyrostegia*, *Radermachera*, *Spathodea*, *Tabebuia* and *Tecoma*. Pollens collected from mature flowers were analyzed by acetolysis method of Erdtman 1960. The pollen grains of the species studied were monads with radially symmetrical; apolar or isopolar; tricolpate in 7 species, tetracolpate in only one species, tricolporoidate with ill-defined endoaperture in 4 species, tricolporate with lolongate endoaperture in 2 species and polycolpate in only one species; the apertures position showed zonocolpate in most species but pantocolpate in *Mansoa alliacea*; mostly subprolate to prolate except from those of *Tecoma stans* and *Radermachera yunnanensis*; circular, rounded triangular to triangular amb; exine sculpture psilate in one species, microreticulate in 3 species and reticulate in 11 species; homo- or heterobronchate lumen, with simpli or duplibaculate muri. The resulting pollen morphological diversity may offer some taxonomic potential and interrelationships among the studied species.

Keywords: Pollen, Morphology, Bignoniaceae, Species, Lashio.

Introduction

The study area, Lashio, is situated in Northern Shan State of Myanmar. It lies between 22º 39' 53" and 23º 04' 27" N latitude and 97º 30' 10" and 97º 47' 40" E longitude. Generally, the elevation of this part is roundly 855m above sea level. The total area of Lashio is 4832 sqkm. It is divided into 12 quarters. It is bounded by Kawonlon and Hopan townships on the east, Nanmatau Township on the west, Tantyann and Mairal townships on the south and Theini township on the North. Lashio area is rich in the variety of flora due to its tropical mountainous climate. The natural vegetation is evergreen and dry deciduous forest in the study area.

Bignoniaceae Juss. is one of the dicotyledonous families of flowering plant. Lohmann (2004) stated that the Bignoniaceae composed of 120 genera and about 800 species in worldwide distribution; of these, *Tabebuia*, *Jacaranda*, *Arrabidaea*, *Anemopaegma* and *Adenocalymma* are the largest and comprise almost half of the species in the family. The rest genera are small and monotypic. Hundley & Chit Ko Ko (1987) recorded 39 species in 21 genera and Kress *et al.* (2003) listed that 40 species and 22 genera of Bignoniaceae were distributed in Myanmar.

The study of pollen and spores is called palynology. The term palynology was first coined by Hyde and Willian (1945). In 1952, Erdtman has worked on the publication of the pollen morphology and plant taxonomy included 327 families of monocot and dicot. Pollen characters are very distinctive, easily recognizable and identifiable to the family, genus or even species level. Furthermore, sporopollinin included in pollen is very durable and does not decay. Therefore, pollen remains as durable natural marker in environment (Ugbabe *et al.*, 2007). Therefore, pollen morphology has great significance in the taxonomy of angiosperms. Nowadays an attempt has been made to classify the plants by using the pollen characters.

The pollen morphology of the Bignoniaceae was studied by many authors: Erdtman (1952), Bove (1993), Ugbabe *et al*. (2007, 2013), Saensouk & Saensouk (2011) and Souza *et al.* (2019). Erdtman (1952) recorded about 25 species and 20 genera of Bignoniaceae. According to Kress *et al.* (2003), about half of total species of Bignoniaceae were distributed in Lashio area. The pollen

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morphology of Bignoniaceae from Lashio had not been recorded by other authors. Therefore, the present study attempts to describe the pollen morphology of Bignoniaceae from the Lashio area.

The aim of this study was to record the characters of the pollen of Bignoniaceae in Lashio area. The objectives were to identify the species of Bignoniaceae and to provide palynological information which may contribute to the understanding and identification of the family Bignoniaceae.

Materials and Methods

The plant materials of the fifteen species of Bignoniaceae were obtained from collection of Lashio area, Northern Shan State, from December 2018 to November, 2019. Polliniferous samples were harvested from the anther of fresh flower specimens. The collected materials were preserved in a glass vial with glacial acetic acid. The pollen grains were prepared for light microscopy by acetolysis method described by Erdtman (1960). The pollen morphology was characterized by using the following parameter.

Qualitative characters: dispersal unit, symmetry, type and position of aperture, shape of endoaperture, pollen shape in equatorial and polar view and exine ornamentation including muri and lumen in reticulate sculpture. Quantitative characters: number of apertures, length and width of aperture, size of pollen grain, measurement of polar axis and equatorial diameter, length and width of muri and exine thickness.

The shape of pollen grains was determined by the polar axis/equatorial diameter ratio in accordance with the classification proposed by Erdtman (1952). A measurement of equatorial diameter and polar axis of ten pollen samples were taken by using ocular stage division and the measuring unit converted into milimicron (μm). The pollen size was categorized on the basis of classification by Erdtman (1952). The length and width of colpi, pore, muri and exine thickness were also measured. Microphotographs of the prepared sample were made with camera Cannon A 3500 IS under 40x and using 10x eye piece.

The pollen morphology was presented alphabetic order of scientific name. Myanmar name and English name were also checked by Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003). The primitive and advanced character states were defined by method of Luo *et al.* (2015). Morphology of the collected specimens were identified with the help of literatures and flora like Flora of Java (Backer, 1965), Flora of Ceylon (Dassanyake, 1981) and Flora of China (Wu & Raven, 1998). The descriptive terms of perisynclopate, colporoidate and microreticulate were followed by Bove (1993). The terminologies were used in accordance with Erdtman (1952), Paldat (2005) and Hesse (2009).

Results

In the present study, pollen morphology of 15 species belonging to 14 genera of Bignoniaceae had been investigated. The collected species were presented in Table 1 and Figure 1. The results of the pollen grain morphological studies are represented in Table 2, 3 and Figure 2. The palynological descriptions were arranged according to the following pollen characters: dispersal unit, polarity, shape and size of pollen, type of aperture and exine sculpture. The resulting pollen characters may serve as taxonomic markers to distinguish the taxa of Bignoniaceae.

Polarity, dispersal unit, size and shape

In the present study, isopolar pollen grains were found in most of the species and the apolar grain was found in *Mansoa alliacea*. In the current study the dispersal unit of all studied species is

monads. The size of pollen grain was measured in equatorial view including equatorial diameter and polar axis. The size of pollen grains varies between 23.5 - 95.0µm polar axis and 17.5 - 75.0 µm equatorial diameter. The largest one was observed in *Spathodea campanulata* and the smallest one was found in *Campis grandiflora*.

The shape of pollen grain can sometime be useful in identification of species. It may vary within one genus and one family. The shape is defined by the ratio between the length of polar axis and the equatorial diameter. In this research, the pollen grains are mostly subprolate to prolate. The subprolate shape was found in *Millingtonia hortensis, Podranea ricasoliana, Pyrostegia venusta* and *Spathodea campanulata,* and prolate in *Campis grandiflora*, *Dolichandrone spathacea*, *Jacaranda mimosifolia*, *Mayodendron igneum*, *Tabebuia aurea* and *Tecoma capensis*. However other shape like suboblate and spheroidal are also occurred. AMB is circular in most species but triangular in *Jacaranda mimosifolia* and *Tecoma capensis*, rounded triangular in *Campis grandiflora, Dolichedrone spathacea* and *Tabebuia aurea*.

Number and type of apertures

Tricolpate in most species but polycolpate in *Manosa alliacea*. The apertures found in *Manosa alliacea* were distributed around the pollen grain so it is called pantocolpate. Zonocolpate, the aperture equally distributed along the equatorial plane were observed in the remaining 14 species. According to an aperture type, five types can be found including tricolpate, tetracolpate, polycolpate, tricolporoidate and tricolporate. The aperture joined toward the pore (syncolpate) were observed in *Haplophragma adenophyllum* and *Jacaranda mimosifolia*, parasyncolpate (aperture join at the pole remaining triangular space) in *Millintonia hortensis* and *Tecoma stans* and perisyncolpate in *Manosa alliacea*. The colpi were longicolpate in all studied species. *Jacaranda mimosifolia* featured the colpus constricted in psilate. In this study, 11 species possess reticulate sculpture, 3 species in microreticulate and only one species in psilate. The muri are simplibaculate in most species but duplibaculate in *Pyrostegia venusta*. The lumen is homobranchate in *Haplophragma adenophylla* and *Millintonia hortensis*, and heterobronchate in the rest species. The muri are smaller than the polar region in *Oroxylum indicum*. Sexine thickness is the same as nexine thickness in 11 species, thinner in 2 species and thicker in 2 species.

Table 1 List of collected species

No	Scientific name	Local name	Common name	Flowering period	Uses
1	*Campis grandiflora* (Thunb.) K. Schum.	Egayit Nwe	Chinese Trumpet Vine	May to Aug	Ornamental
2	*Dolichandrone spathacea* (L.f.) Seem.	Thakut	Mangrove Trumpet Tree	March to June	Flower vegetable
3	*Haplophragma adenophyllum* (Wall.) P. Dop	Kyaung Sha	Katsagon	Oct to Dec	Flower vegetable
4	*Jacaranda mimosifolia* D. Don	Seinban Apya	Blue Jacaranda	June to Aug	Ornamental
5	*Mansoa alliacea* (Lam.) A.H.Gentry.	Thahtay Warda	Garlic Vine	June to Oct	Ornamental
6	*Mayodendron igneum* Kurz	Egayit	Orange Tree Jasmine	Oct to Dec	Ornamental
7	*Millingtonia hortensis* L. f.	Egayit	Tree Jasmine	Nov to Dec	Medicinal
8	*Oroxylum indicum* (L.) Benth. ex Kurz	Kyaung Sha	Indian Trumpet Flower	Oct to Dec	Flower & fruit vegetable

No	Scientific name	Local name	Common name	Flowering period	Uses
9	*Podranea ricasoliana* (Tanf.) Sprague	Egayit	Pink Trumpet Vine	Oct to Jan	Ornamental
10	*Pyrostegia venusta* Miers	Thaw Ka Nwe	Flame Vine	Jan to March	Ornamental
11	*Radermachera yunnanensis* C.Y.Wu	Yae Mhwe Pan	Kunming Tree Jasmine	May to Aug	Ornamental
12	*Spathodea campanulata* P.Beauv.	Ar Fri Ka Kyu Lit	African Tulip Tree	October to December	Ornamental
13	*Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex S.Moore	Ta Bay Bu Ah	Tree of Gold	Feb to April	Ornamental
14	*Tecoma capensis* (Thunb.) Lindl.	Egayit	Cape Honeysuckle	Jan to March	Ornamental
15	*Tecoma stans* (L.) Juss. ex Kunth	Sein Ta Kyu	Yellow Trumpet Bush	Sep to Dec	Ornamental

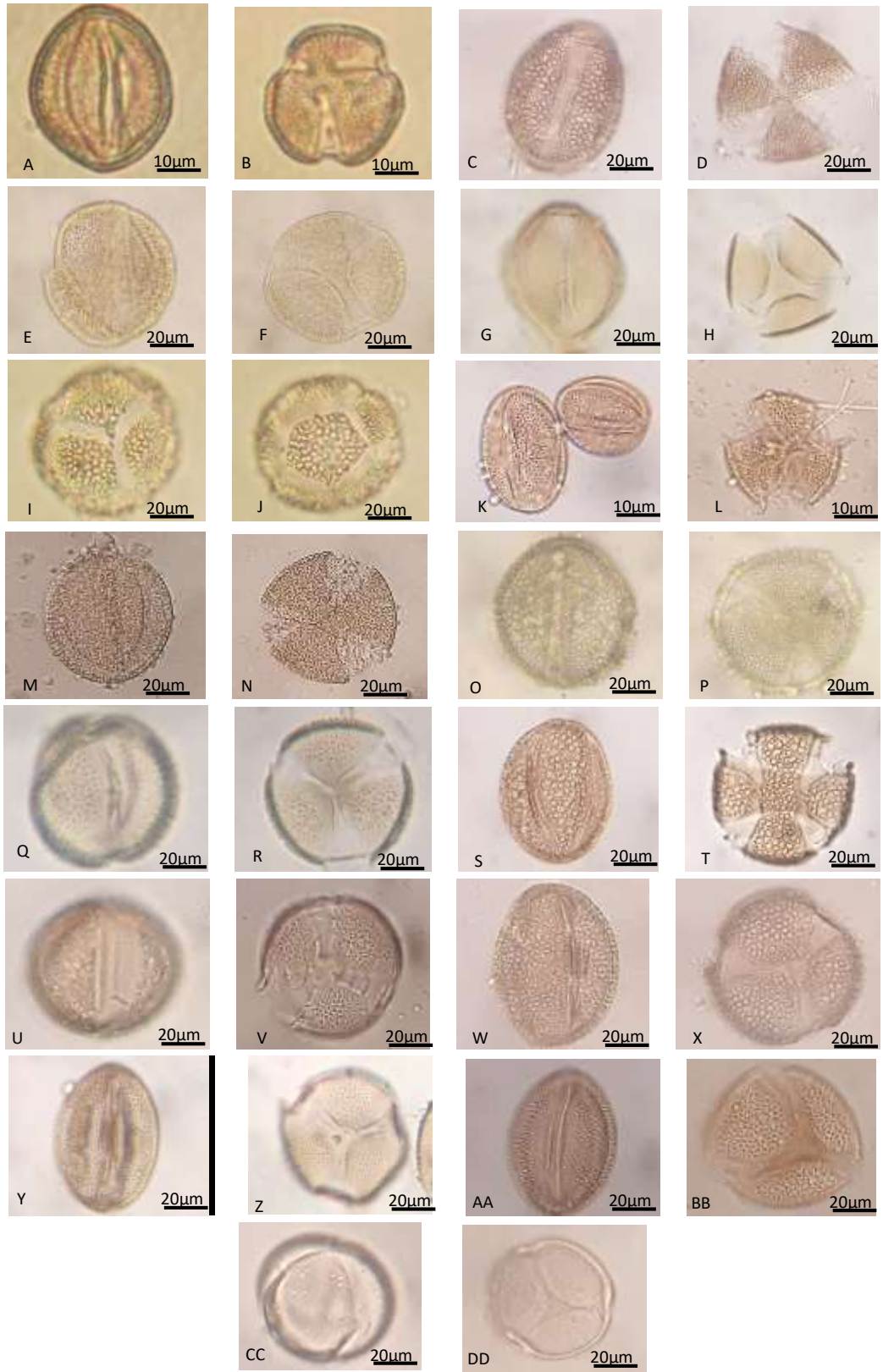
Figure 1 Flowering branches of collected species **A**. *Campis grandiflora* (Thunb.) K.Schum. **B**. *Dolichandrone spathacea* (L.f.) Seem.**C**. *Haplophragma adenophyllum* (Wall.) P. Dop **D**. *Jacaranda mimosifolia* D.Don **E**. *Mansoa alliacea* (Lam.) A.H.Gentry. **F**. *Mayodendron igneum* Kurz**G**. *Millingtonia hortensis* L. f. **H**. *Oroxylum indicum* (L.) Benth. ex Kurz **I**. *Podranea ricasoliana* (Tanf.) Sprague **J**. *Pyrostegia venusta* Miers **K**. *Radermachera yunnanensis* C.Y.Wu **L**. *Spathodea campanulata* P.Beauv. **M**. *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S.Moore **N**.*Tecoma capensis* (Thunb.) Lindl. **O**.*Tecoma stans* (L.) Juss. ex Kunth

Table 2 Qualitative pollen characters of investigated taxa

No	Scientific Name	Aperture type	Aperture position	Shape class	AMB	Exine sculpture
1.	*Campis grandiflora*	colporoidate	zonocolpate	prolate	rounded triangular	reticulate
2.	*Dolichandrone spathacea*	colpate	zonocolpate	prolate	rounded triangular	reticulate
3.	*Haplophragma adenophyllum*	syncolpate	zonocolpate	prolate spheroidal	circular	microreticulate
4.	*Jacaranda mimosifolia*	colpate	zonocolpate	prolate	triangular	psilate
5.	*Mansoa alliacea*	perisyncolpate	pantocolpate	spheroidal	circular	reticulate
6.	*Mayodendron igneum*	colpate	zonocolpate	prolate	circular	reticulate
7.	*Millingtonia hortensis*	parasyncolpate	zonocolpate	subprolate	circular	microreticulate
8.	*Oroxylum indicum*	colpate	zonocolpate	Prolate spheroidal	circular	reticulate
9.	*Podranea ricasoliana*	colporoidate	zonocolpate	subprolate	circular	reticulate
10.	*Pyrostegia venusta*	colpate	zonocolpate	subprolate	circular	reticulate
11.	*Radermachera yunnanensis*	colpate	zonocolpate	suboblate	circular	reticulate
12.	*Spathodea campanulata*	colporoidate	zonocolpate	subprolate	rounded triangular	reticulate
13.	*Tabebuia aurea*	colporoidate	zonocolpate	prolate	rounded triangular	reticulate
14.	*Tecoma capensis*	syncolpate	zonocolpate	prolate	triangular	reticulate
15.	*Tecoma stans*	parasyncolpate	zonocolpate	suboblate	circular	microreticulate

Table 3 Quantitative pollen characters of investigated taxa

No	Scientific Name	Aperture number	Polar axis(µm)	Equatorial diameter (µm)	P/E ratio	Colpi length(µm)	Colpi width(µm)	Exine thickness (µm)
1.	*Campis grandiflora*	3	23.5 - 25.0	17.5 - 20.0	1.34	15.0 - 17.5	3.75 - 5.00	2.50 - 3.75
2.	*Dolichandrone spathacea*	3	60.0 - 62.5	42.5 - 45.5	1.42	42.5 - 45.5	5.0 - 7.5	2.50-3.75
3.	*Haplophragma adenophyllum*	3	55.0 - 57.5	50.5 - 52.5	1.10	-	7.5 - 10.0	1.25-2.50
4.	*Jacaranda mimosifolia*	3	57.5 - 60.0	42.5 - 45.0	1.34	-	-	1.25-2.50
5.	*Mansoa alliacea*	15	42.5 - 47.5	-	1.00	12.5-17.5	2.5 - 7.5	3.75 - 5.00
6.	*Mayodendron igneum*	3	40.0 - 45.0	25.0 - 30.0	1.60	35.0 – 37.5	5.0 - 6.5	1.25 - 2.5
7.	*Millingtonia hortensis*	3	45.0 - 47.5	40.0 - 42.5	1.30	-	2.50 - 3.75	1.25 - 2.5
8.	*Oroxylum indicum*	3	72.5 - 75.0	67.5 - 70.0	1.07	67.5 - 70.0	5.0 - 10.0	2.5-3.75
9.	*Podranea ricasoliana*	3	40.0 - 42.5	32.5 - 37.5	1.23	32.5 - 37.5	5.00 - 6.25	1.25 - 2.50
10.	*Pyrostegia venusta*	3 - 4	70.0 - 72.5	67.5 - 70.0	1.27	65.0 - 62.5	5.0 - 7.5	2.5-3.75
11.	*Radermachera yunnanensis*	3	27.5 - 30.0	30.0 - 32.5	0.91	27.5 - 30.0	3.75 - 5.00	1.25 - 2.50
12.	*Spathodea campanulata*	3	90.0 - 95.0	70.0 - 75.0	1.28	70.0 – 75.0	7.50 - 8.75	2.5-5.0
13.	*Tabebuia aurea*	3	47.5 - 50.0	32.5 - 35.0	1.48	45.0 - 47.5	2.50 - 3.25	1.25-2.0
14.	*Tecoma capensis*	3	42.5 - 47.5	30.0 - 35.5	1.41	-	5.00 - 6.25	1.25-2.50
15.	*Tecoma stans*	3	32.5 - 35.0	37.5 - 40.0	0.87	-	5.0 - 7.5	1.25-2.50



Figurer 2 Equatorial view and Polar view of pollen for each species **A.,B**. *Campis grandiflora* (Thunb.) K.Schum. **C.,D**. *Dolichandrone spathacea* (L.f.) Seem. **E.,F**. *Haplophragma adenophyllum*(Wall.) P. Dop **G.,H**. *Jacaranda mimosifolia* D.Don **I.,J**. *Mansoa alliacea* (Lam.) A.H.Gentry. **K.,L**. *Mayodendron igneum* Kurz **M.,N**. *Millingtonia hortensis* L. f. **O.,P**. *Oroxylum indicum* (L.) Benth. ex Kurz **Q.,R**. *Podranea ricasoliana* (Tanf.) Sprague **S.,T**. *Pyrostegia venusta* Miers **U.,V**. *Radermachera yunnanensis* C.Y.Wu **W.,X**. *Spathodea campanulata* P.Beauv. **Y.,Z**. *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S.Moore **AA., BB**. *Tecoma capensis*(Thunb.) Lindl. **CC.,DD**. *Tecoma stans* (L.) Juss. ex Kunth

Discussion and Conclusion

The current study was done by analyzing the pollen morphology of some species of Bignoniaceae which are known for their ornamental and medicinal uses. In this study, *Mayodendron igneum* and *Millingtonia hortensis* were cultivated as ornamental and medicinal purposes; *Dolichandrome spathacea*, *Haplophragma adenophyllum* and *Oroxylum indicum* as medicinal plants for their floral and fruit vegetable and the rest species as ornamental plant for their large and showy attractive flowers.

Nowadays, the pollen characters are considered together with morphological feature of the plants. Therefore, they become useful complementary tool in solving the problem of taxonomy and phylogeny. The present study has shown the differences and similarities in the pollen morphology of some species of Bignoniaceae.

Generally, in Bignoniaceae, the pollens are monads, radially symmetrical, isopolar and apolar, tricolpate, tetracolpate, tricolporate, tricolporoidate and polycolpate and psilate, microreticulate to reticulate. This result is in line with the work of Erdtman (1952), Gentry (1980), Bove (1993), Saensouk & Saensouk (2011) and Ugbabe *et al.* (2007, 2013). The pollens of the species analyzed in this study are tricolpate, oblate shape and reticulate sculpture which is typical of the family studied. This confirms that the species investigated are members of Bignoniaceae. Gentry (1980) assumed that the tricolpate and reticulate pollen is ancestral status.

Tsymbalyuk (2014) reported that the pollen of the genus *Campis* was tri – tetracolpate and reticulate sculpture while the present study reveals tricloporoidate and reticulate sculpture in *Campis grandiflora.* It was noted that the pollen morphology of *Dolichandrome spathacea* was not reported by any authors in family Bignoniaceae. The current study described them as tricolpate, prolate and reticulate sculpture. According to Saensouk & Saensouk (2011), the pollen of *Haplophragma adenophylla* is tricolpate and prolate; in the present study, it is tricolpate, syncolpate and prolate spheroidal. In the studies carried out by Bove (1993), the pollen of *Jacaranda* was examined as tricolporoidate, oblate spheroidal to prolate and psilate sculpture. The results presented by this author were different from the trisyncolpate aperture type of the current study.

The perisyncolpate aperture type and reticulate sculpture found in *Mansoa alliacea* by Bove (1993) was the same one found by the present study. The pollen of *Mayodendron igneum* was tricolpate and prolate. This finding is in accordance with those of Saensouk & Saensouk (2011). They also described the pollen of *Millintonia hortensis* as tricolpate, prolate and microreticulate. The current study coincides with the results found by these authors. The pollen of *Oroxylum indicum* is tricolpate, prolate spheroidal and reticulate. This finding is agreement with those of Ugbabe *et al.* (2007).

The pollen of *Podranea ricasoliana* was studied by Trigo (1991). The results of those are confirmed to the present study of tricolporoidate pollen with reticulate sculpture. The current study described the aperture of *Pyrostegia venusta* as tri - tetracolpate and exine sculpture as reticulate. These characters are confirmed to the results of Bove (1993). The pollen characters of *Ridermachera yunnanensis* corroborate those of Wei *et al.* (2001) in relation to the pollen characters being tricolpate. Ugbabe *et al.* (2013) found the pollen of *Spathodoea campanulata* to be tricolporate and subprolate; in the current result, it is tricolporoidate and prolate.

Bove (1993) analyzed that the pollen of *Tabebuia* was tircolporoidate, prolate and reticulate; Ugbabe *et al.* (2007) described it as tricolporate and prolate-spheroidal; the results of this study were confirmed to those of the first author. The pollen of *Tecoma capensis* was described as having tricolpate and prolate by Saensouk & Saensouk (2011) similar to the pollen grain

described in this paper. The tricolporate aperture type found in *Tecoma stans* by Ugbabe *et al.* (2007, 2013). The results of the present research were similar to the findings of this author.

In the study of pollen morphology, it was categorized on the basis of dispersal unit, polarity, symmetry, shape, size, aperture types and exine sculpture. However, from an evolutionary point of view, aperture type and exine sculpture are the most important pollen character. The pollen of Bignoniaceae shows both primitive and advance characters. These characters were defined by the method of character states used by Luo *et al.* (2015).

The dispersal unit of studied species was monad which was considered by Luo *et al*. (2015) as primitive character while Dajoz 1991 as advances one. The apolar as in *Mansoa alliacea* was primitive whereas isopolar of the rest species was advanced. The symmetry of pollen was radial in this paper. According to Luo *et al.* (2015), bilateral symmetry proceed into radial symmetry.

The shape of pollen was examined under equatorial orientation. It was mostly prolate in present species. Luo *et al.* (2015) defined that the prolate was advanced, spheroidal as in *Mansoa alliacea* was intermediate and suboblate as in *Tecoma stans* and *Radermachera yunnanensis* was primitive. In the present investigation, the pollen grain size was classified based on polar axis by method of Erdtman (1952). In this paper, 5 species of medium and 10 species of large size were recorded. Luo *et al.* (2015) analyzed that the medium size pollen was more primitive than small one.

Luo *et al.* (2015) stated that the porate derived from colpate but syncolpate were the most advance one. In an evolutionary point of view, the morphology of angiosperm pollen has an increasing number of apertures; the more apertures a pollen grain has, the more quickly its germinate (Dajoz, 1991). In the present study the number of aperture were mostly three in number but many in *Mansoa alliacea*. Luo *et al.* (2015) classified inaperturate as primitive, three apertures as mediate and more than three as advanced condition.

According to Luo *et al.* (2015), zonocolpate like *Campis grandiflora* was more primitive than pantocolpate as in *Mansoa alliacea*. Exine sculpture was mostly reticulate pattern but rarely observed psilate in *Jacaranda mimosifolia*. Luo *et al.* (2015) analyzed psilate evolved into reticulate. According to the above results, the presence of primitive status namely aperture type, number, position, and the advance character namely polarity, symmetry, shape and sculpture indicates the Bignoniaceae lie intermediate evolutionary trends.

In angiosperms, the most important pollen characters at the higher taxonomic levels involve the number, position and structure of aperture, exine sculpture and in some cases size and shape (Bose, 2012). From a palynological perspective, the family Bignoniaceae is known as heterogenous or eurypalynous. The results show that the size, shape and exine sculpture have little diagnostic value, while the number, position and type of the aperture have higher taxonomic value. It can be concluded that the pollens of the species investigated was found to have taxonomic value and also supports the identification of species. These resulting pollen morphological characters may offer some taxonomic tools and interrelationships among the species studied.

Acknowledgements

We are deeply indebted to Dr Khin San San Win, Pro-Rector, Mohnyin University for her permission to present this paper. We also thank to Dr Hlaing Hlaing Myint, Professor and Head of Department of Botany, Mohnyin Degree College for her encouragement to carry out this research.

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ISOLATION OF SOIL FUNGI FROM THREE VILLAGES OF KATHA TOWNSHIP AND THEIR ANTIMICROBIAL ACTIVITIES

Tin May Htwe[1], Zar Zar Yin[2]

Abstract

In the present research, soil samples were collected from three different places of Katha Township, Sagaing Region, during July 2019 and isolated them by the serial dilution method. The media used for the isolation includes Blakeslee's Malt Extract Agar (BMEA) medium and Potato Dextrose Agar (PDA) medium, incubated for 3-7 days at room temperature. Pure colonies were preserved into slant culture containing PDA medium. Twenty fungal strains were obtained. The surface colours of all isolated fungi are white, black, blue, brown, cream, green, dark green, pale yellow, pink, yellow and greenish yellow and their reserve colours are brown, cream, pale yellow, pink, red and yellow. In the colony morphology, the isolated fungi are medium and large in size. The margin of isolated fungi are entire, undulate, filamentous and the elevation of isolated fungi are flat, umbonate and raised. In the form, isolated fungi are irregular, circular and filamentous. Furthermore, the antimicrobial activity of all fungal strains were tested by agar well diffusion method on eight test organisms. Among them, four fungal strains showed the antimicrobial activity on all test organisms. Especially, TM- 14 and 16 showed the highest antimicrobial activity. These findings suggested that the soil fungi may be utilized for screening of the antimicrobial substances and to treat the diseases caused by pathogenic microorganisms.

Keywords: Soil Fungi, Colony Morphology, Antimicrobial Activity

Introduction

Soil is considered as one of the most suitable environments for microbial growth (Cavalcanti *et al.*, 2006). Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Anisworth, 1995). Fungi are one of the dominant groups present in soil, which strongly influence ecosystem structure and function. Thus they play a key role in many ecological services (Rajendra, 2016).

Antimicrobial agents play the most important role in the treatment of bacterial infections (Hacioglu. N., 2011) and wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic production microbes (Oskey, 2004).

Several fungal species produces bioactive compounds, secondary metabolites and chemical matels having pharmaceutical importance. There are about 23000 known secondary metabolites, 42% of which are produced by actinobacteria, 42% by fungi (eg. *Penicillium* spp.) and 16% by other bacteria. Antibiotics can be classified according to their made of actions (Lambert, 1977). Antibiotic are classified as broad-spectrum antibiotics when they have the ability to affect a wide range of gram-positive and gram-negative bacteria while antibiotics that only effective towards certain group of bacteria are known as narrow-spectrum antibiotics (Lambert, 1977).

Therefore, the aim of this research work is to produce antimicrobial compounds by isolated fungi from three different places of soil in Katha Township. To achieve this aim, the physicochemical properties of soil from Katha Township were analyzed. Then, fungi were isolated from different soil samples of Katha Township and Secondly, the different forms of colony morphology were studied and recorded them. After that the preliminary antimicrobial activities of isolated fungi were studied through eight test organisms.

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Materials and Methods

Collection of soil samples

The soil samples were collected from three different places in various locations of Katha Township, during July, 2019. These samples were taken from different places (up to 15 cm depth) and put into sterilized polyethene bags after removing the surface soil for the isolation of fungi which were brought to the laboratory of Biotechnology and Development Center of Pathein University.

Table 1 Collected soil samples from three different places at Katha Township

No	Place	Location	
1	Kyan Taw	24.194588 N	96.325729 E
2	Between Kyan Taw and Lan Gwa	24.349149 N	96.196676 E
3	Pa Lway Shwe	24.214439 N	96.359594 E

Physicochemical analysis of Soil Samples

The collected soil samples were characterized by its physicochemical properties. Physicochemical parameters include organic carbon, nitrogen, pH, moisture content and temperature etc. The temperature and colour of soil samples were recorded. The physicochemical parameters of the soil samples were analyzed at Department of Agricultural Research, Yezin, Myanmar (Table 3).

Serial Dilution Method (Dubey, 2002)

One gram of soil sample was put into a conical flask containing 99 mL of distilled water. The flask was shaken for about 30 minutes in order to make the soil particles free from each other. This solution was then serial diluted from 10^{-3} to 10^{-7} dilution in separated test tubes and 1 mL each of the above dilution was separately transferred into sterile petri dishes under aspetic condition.The sterilized medium in the conical flask was cooled down to about 45°C and separately poured into each of the petri dish containing the respective soil dilutions. The inoculated plates were shaken in a clockwise and anti-clockwise direction for about 5 minutes in order to make uniform distribution of the fungi inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at 27°C-30°C for 3-7 days. The isolated pure fungi had been preserved in slant culture containing PDA medium for further experimentations.

Agar Well Method (Collins, 1965)

Isolated strains were tested by agar well method for the preliminary antimicrobial activities. The wells (8 mm in diameter) were made by Cork borer in the autoclaved basal antimicrobial test medium. Wells impregnated within 3-6 days old culture fermented broth (20 μL) were incubated at room temperature for 24-28 hours. After 24-28 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones had been observed as potent activity as shown by representative strain. The clear zones which are surrounding the wells indicated the presence of antimicrobial activities which inhabit the growth of the test organism selectively.

Table 2 Eight kinds of Test Organisms used for Antimicrobial Activity (NITE and PRD)

Test No.	Test Organisms	Infection
1	*Escherichia coli* AHU 5436	Diarrhoea, pneumonia, abdominal pain
2	*Bacillus subtilis* IFO 90571	Fever
3	*Bacillus pumilus* IFO 90571	Fever
4	*Candida albicans* NTTE 09542	Candidasis, skin disease
5	*Pseudomonas fluorescens* IFO 94307	Septicemia
6	*Staphylococcus aureus* AHU 8465	Boil and Food poisoning
7	*Agrobacterium tumefaciens* NITE 09678	Crown gall disease
8	*Malassezia furfur* UY	Dandruff, Seborrhoeic dermatitis

NITE = National Institute of Technology Evaluation, Japan
PRD = Pharmaceutical Research Department, Yangon, Myanmar

Results

In the present research work, soil samples were collected and its physicochemical properties were studied. Fungal diversity of any soil depend on a large number of factors of the soil such as pH, organic content, moisture and soil texture. The results of the physicochemical properties of soil samples showed that soil environments between Kyan Taw and Lan Gwa, Pa Lway Shwe were Sandy Loam while the sample from Kyan Taw was Sandy Clay Loam.

The pH values of the soil samples showed that moderately acidic and neutral between 5.1 to 7.18. The temperature of soil environments of Katha Township during this investigation (the rainy season) showed that the soil environment of Katha Township at temperature range between 30°C to 34°C with great variation in present moisture content (4.6-19.3 %), organic carbon (0.26-0.96%), organic nitrogen (41-87 mg/kg) and potassium (50-383 mg/kg). These results were shown in Table 3.

Table 3 Physicochemical Properties of soil samples collected from three different places of Katha Township

Sample No.	Place	Soil Color	Text-ure	pH	T (ºC)	Moisture (%)	Organic Carbon (%)	Organic Nitrogen (mg/kg)	Organic Potassium (mg/kg)
1	Kyan Taw	Brown	SCL	5.1	32	5.7	0.26	41	50
2	Between Kyan Taw & Lan Gwa	Brown	SCL	5.35	30	19.3	052	71	78
3	Pa Lway Shwe	Brown	SL	5.31	33	18.0	0.9	81	79

*CL = clay loam, SCL = sandy clay loam, SL = sandy loam
Soil temperature = between 30 and 35°C

In the isolation of soil fungi, 20 fungal isolates were obtained, 11 strains from Kyan Taw, 4 strains from between Kyan Taw and Lan Gwa, 5 strains from Pa Lway Shwe, These fungi were cultured on potato dextrose agar (PDA) and Blaskeslee's Malt Extract Agar (BMEA) and each ten strains were isolated from these two media.

Table 4 Isolation of Soil Fungi on Two Different Media

sample No.	Place	PDA	BMEA	Total
1	Kyan Taw	TM-1, 2, 3, 4, 5	TM-6, 7, 8, 9, 10, 11	11
2	Between Kyan Taw and Lan Gwa	TM-12, 13	TM-14, 15	4
3	Pa Lway Shwe	TM-16, 17, 18	TM-19, 20	5
	Total	10	10	20

In the colony morphology, isolated strains were medium and large in size, entire in margin, raised, flat, convex in elevation and form in circular and irregular. Their colony morphology, microphotograph and their antimicrobial activities were also performed.

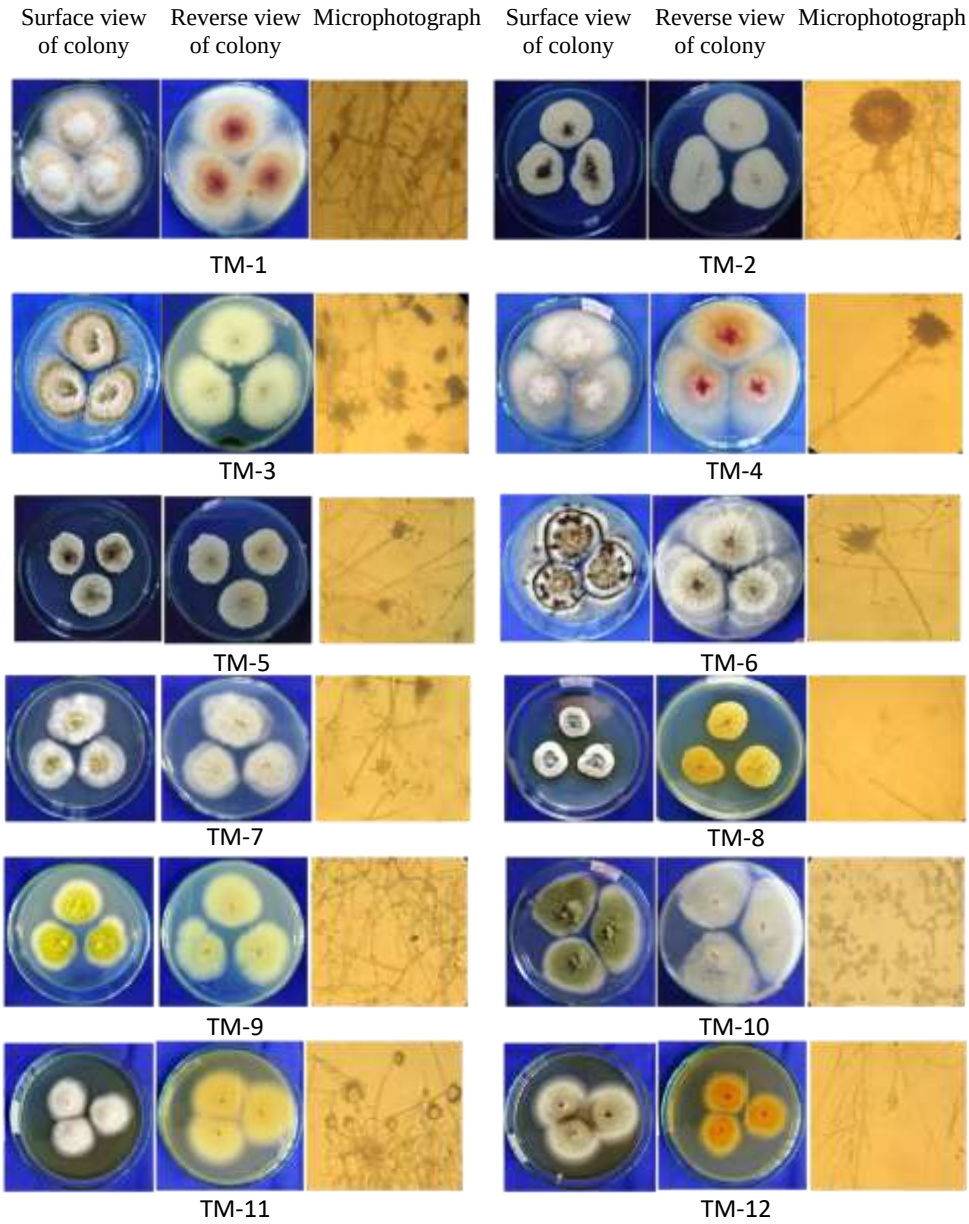


Figure 1 Morphology and their microscopical characters of isolated fungi TM-1 to TM-12

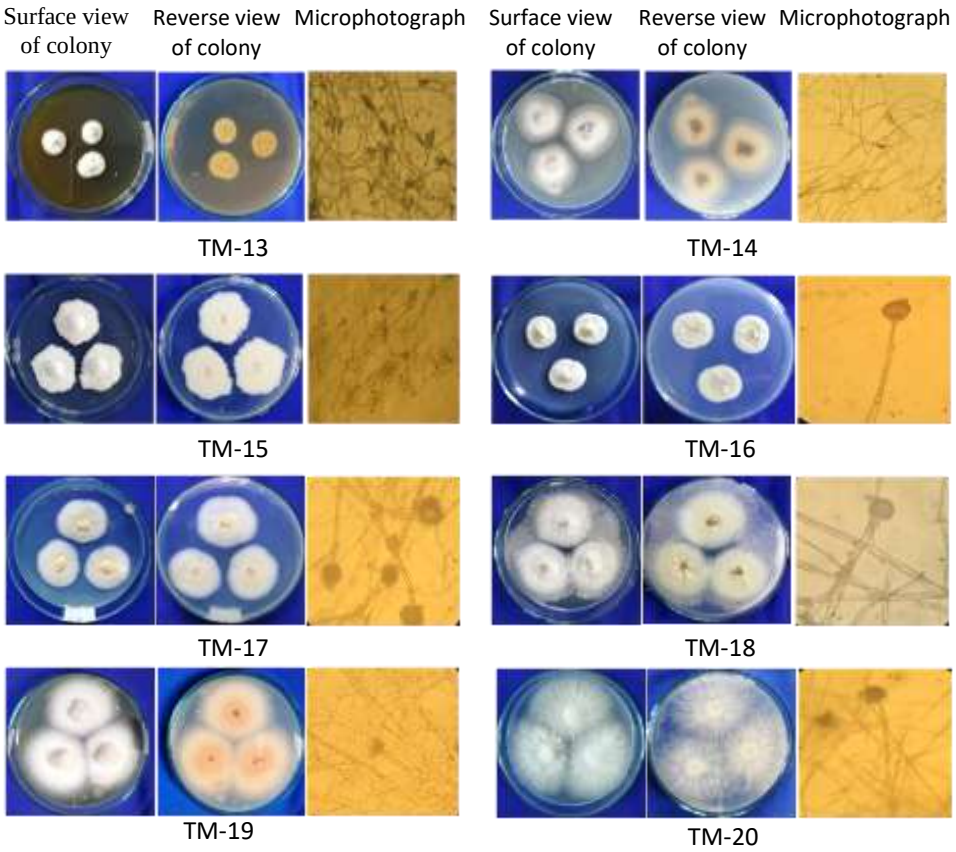


Figure 2 Morphology and their microscopical characters of isolated fungi TM-13 to TM-20

In the screening of antimicrobial activity, all strains were tested on eight test organisms. Among them, four strains showed different levels of antimicrobial activities and were selected for further study.

Table 5 Antibacterial Activity of Isolated Fungal strains against *Escherichia coli*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	-	-	19.09	+
2	TM-14	18.88	22.32	**31.59**	20.22
3	TM-16	-	-	**24.60**	+
4	TM-20	+	+	+	18.21

Table 6 Antibacterial Activity of Isolated Fungal strains against *Bacillus subtilis*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	17.38	20.76	**26.49**	22. 98
2	TM-14	20.85	21.58	22.94	19.44
3	TM-16	+	18.95	20.34	19.53
4	TM-20	+	+	**25.10**	21.2

Table 7 Antibacterial Activity of Isolated Fungal strains against *Bacillus pumilus*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	16.59	**26.22**	26.62	15.22
2	TM-14	18.23	20.44	24.30	17.18
3	TM-16	-	21.26	22.19	18.21
4	TM-20	+	**25.24**	17.10	+

Table 8 Antifungal Activity of Isolated Fungal strains against *Candida albicans*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	+	+	22.54	+
2	TM-14	+	22.64	23.66	18.15
3	TM-16	21.32	**33.68**	20.12	+
4	TM-20	-	28.00	**24.38**	+

Table 9 Antibacterial Activity of Isolated Fungal strains against *Pseudomonas fluorescence*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	-	+	+	18.29
2	TM-14	-	+	17.45	**29.74**
3	TM-16	-	29.35	**32.59**	19.19
4	TM-20	-	+	29.35	17.27

(+) present (-) no activity Agar well = 8 mm

Table 10 Antibacterial Activity of Isolated Fungal strains against *Staphylococcus aureus*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	-	+	21.36	-
2	TM-14	-	-	18.45	+
3	TM-16	18.00	**22.70**	19.33	+
4	TM-20	-	+	**20.25**	19.52

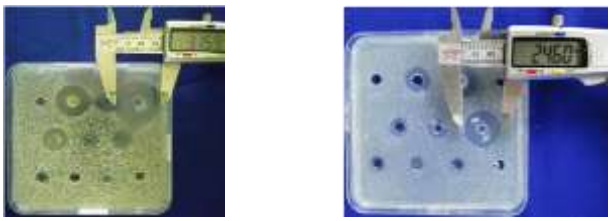
Table 11 Antibacterial Activity of Isolated Fungal strains against *Agrobacterium tumefaciens*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4		+	24.14	**26.19**
2	TM-14	-	17.59	18.33	+
3	TM-16	-	+	16.57	+
4	TM-20	-	**25.23**	22.59	19.19

(+) present (-) no activity Agar well = 8 mm

Table 12 Antifungal Activity of Isolated Fungal strains against *Malassezia furfur*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	+	18.21	19.55	+
2	TM-14	+	17.08	16.45	+
3	TM-16	14.87	**24.00**	**27.67**	18.04
4	TM-20	+	20.33	25.57	18.23.

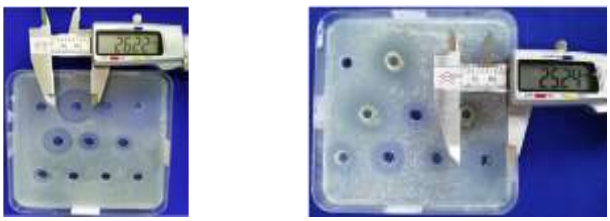


TM-14 TM-16

Figure 3 Antibacterial Activity of Isolated Fungal strains against *Escherichia coli*

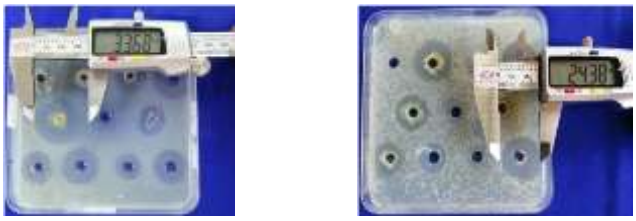
TM-4 TM-20

Figure 4 Antibacterial Activity of Isolated Fungal strains against *Bacillus subtilis*



TM-4 TM-20

Figure 5 Antibacterial Activity of Isolated Fungal strains against *Bacillus pumilus*



TM-16 TM-20

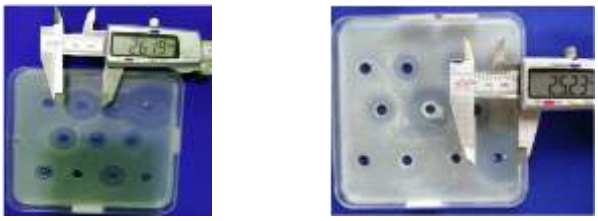
Figure 6 Antifungal Activity of Isolated Fungal strains against *Candida albicans*

TM-14 TM-16

Figure 7 Antibacterial Activity of Isolated Fungal strains against *Pseudomonas fluorescence*

TM-16 TM-20

Figure 8 Antibacterial Activity of Isolated Fungal strains against *Staphylococcus aureus*



TM-4 TM-20

Figure 9 Antibacterial Activity of Isolated Fungal strains against *Agrobacterium tumefaciens*

TM-16 TM-16

Figure 10 Antifungal Activity of Isolated Fungal strains against *Malassezia furfur*

Discussion and Conclusion

In the present study, the colour of soil samples is brown with variation in pH (5.11-7.18). During the investigation (rainy season) showed that the soil environment of Katha Township at temperature ranging between 30°C to 35°C with great variation in present moisture content (4.6-19.3 %), organic carbon (0.26-0.96 %), organic nitrogen (41-87 mg/kg) and potassium (50 -79 mg/kg). Total number of colonies obtained in Kyan Taw is eleven with pH 5.11 (moisture 5.7 %). The results showed that low pH and optimum moisture content favour for the growth of fungi. Normal soil contains a large number of microbes and substantial quantities of microbial biomass.

It is also known that the bacteria thrive well in natural and alkaline soils, whereas fungi show the best activity under acidic conditions. A total of 20 fungi were isolated from three different soil samples and cultured on BMEA and PDA medium. The isolated fungi were designated as TM-1 to TM-20. The surface colours of all isolated fungi are white, black, blue, brown, cream, green, dark green, pale yellow, pink, yellow and greenish yellow and their reserve colour are brown, cream, pale yellow, pink, red and yellow.

Among all of the strains, the surface color of TM-1 has changed from red to orange and the reverse colour of TM-6 has changed from gray to cream on slant culture after six days. The reverse color of TM-9 has occured yellow pigment in slant culture after seven days on PDA medium. A wide range of media is used for growing fungi, as a result media have affected on colony morphology and their colour. All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activities Among them, 4 strains showed that the different levels of antimicrobial activities.

TM-14 exhibited the antibacterial activity (31.59 mm) on *Escherichia coli* at 5^{th} day, (22.94 mm) on *Bacillus subtilis* at 5^{th} day, (24.30 mm) on *Bacillus pumilus* at 5^{th} day and (23.66 mm) on *Candida albicans* at 6^{th} day. TM-16 showed the highest antimicrobial activities (33.68 mm) on *Candidas albicans* at 4^{th} day, (27.67 mm) *on Malassezia furfur* at 5^{th} day and then (32.59 mm) on *Pseudomonas fluorescens* at 5^{th} day and (22.70 mm) on *Staphylococcus aureus* at 4^{th} day. Especially TM-16 showed moderate antimicrobial activity against most of the test organism.

It can be concluded that the present research is to isolate the fungi from different soil samples and to study the antimicrobial activities of isolated fungi on eight test organisms. This study will be focused on the fermentation conditions of selected fungus and extraction of antimicrobial compounds.

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ISOLATION AND CHARACTERIZATION OF SELECTED SOIL BACTERIUM SY-7 FROM MYINT THA TOWNSHIP, MANDALAY REGION

Shwe Yee Win[1], Zar Zar Yin[2]

Abstract

The present paper was focused on the isolation and characterization of selected soil bacterium. Soil samples were collected from three different area of Yoar Thit village of Myint Tha Township, Mandalay Region and cultured on FLO medium and Nutrient Agar Medium. The total of ten bacterial colonies were obtained and designated as SY-1 to SY-10. Antimicrobial activities of all strains were carried out by agar well diffusion assay on 5 test organisms. Among them, SY-7 showed the highest antifungal activity (32.26 mm) on *Candida albicans*. Therefore, SY-7 was selected and identified by morphological, microscopical and biochemical characteristics. In the morphological characterization, SY-7 was medium in size, rod-shaped, margin entire, spore present and cream colour. According to the results of biochemical characters, SY-7 was characterized as the genus *Bacillus.* This research can provide screening the antimicrobial activity and identification of soil bacteria by using the different biochemical characters.

Keywords: Soil Bacteria, Antimicrobial activity, Biochemical characterization

Introduction

Soil microorganisms, such as bacteria, and fungi, control ecosystem functioning through decomposition and nutrient cycling and may serve as indicators of land-use changes and ecosystem health (Balser *et al.,* 2010). Microbes are very small living organisms, so small that most of them are invisible (Subba, 1999).

Soil bacteria are source of higher members of bioactive natural products with biological activity which are extensively used as pharmaceutical and agrochemicals. Infectious diseases are a clear cut threat to the physical health and economic well-being of the world irrespective of site of residence (Young, 2007). Most of the Antibiotics in currents use for the treatment of various infections diseases are microbial products. Antibiotics resistance against infections diseases has increased in recent years (Tawish etc, 2012).

Bacilli are rod-shaped, Gram-positive, speculating aerobes as facultative anaerobes. Most bacilli are saprophytes. Each bacterium creates only one spore, which is resistant to heat, cold radiation, desiccation and disinfectants.

The most commonly used biochemical tests involve the observation of whether as not a growth of the bacterium in liquid nutrient medium will ferment particular sugar such as glucose, lactose or mannitol.

Bacillus spp, produces many kinds of antibiotics which share a full range of such antimicrobial activities as bacterian, pamalin and Gramcidin (Todar, 2005).

In the present study, soil bacterium *Bacillus* was isolated from three different areas of Yoar Thit village of Myint Tha Township. However, nobody has carried out the antimicrobial activity and identification of soil bacteria from above places. Therefore, antimicrobial activity and identification of *Bacillus* sp was mainly studied in this research.

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Materials and Methods

Study area and collection of soil samples

Soil samples were collected from three different stations of Yoar Thit Village, Myint Tha Township, Mandalay Region. Soil samples were collected from 0-3 inches, 1-6 inches and under 6 inches deep from each of these stations using a sterile spatula. The experiments were carried out at the Microbiology Laboratory of Biotechnology and Development Center of Pathein University.

Isolation of Bacteria from the Soil Samples (Altas, 1993)

The soil Bacteria were isolated by serial dilution method and media such as FLO Medium and Nutrient Agar Medium.

Medium used for Isolation of Bacteria

FLO Medium (Altas, 1993)	
Casein	10.0 g
Peptone	10.0 g
K_2HPO_4	1.5 g
$MgSO_4$. $7H_2O$	1.5 g
Agar	15.0 g
Distilled Water	1000 mL
pH	5.0

Nutrient Agar Medium (Altas, 1993)	
Peptone	5.0 g
NaCl	5.0 g
Yeast extract	2.0 g
Agar	15.0 g
Beef extract	1.0 g
Distilled Water	1000 mL
pH	5.0

Medium used for Antimicrobial Activity

Seed and Fermentation Medium Nutrient, Broth Medium (Dubey and Mahesh Wari 2007)	
Peptone	5.0 g
Beef extract	3.0 g
Sodium chloride	5.0 g
Yeast extract	10.0 g
Distilled water	1000 mL
pH	6.8-7.2

Assay Medium Glucose Yeast Peptone (GYP) Medium (Atlas, 1993)	
Glucose	10 g
Yeast extract	3 g
Peptone	2 g
Agar	16 g
Distilled water	1000 mL
pH	6.5

After autoclaving, Nystatin (1.5 mL) was added to the medium.

Serial Dilution Method of Soil Samples (Collins, 1965)

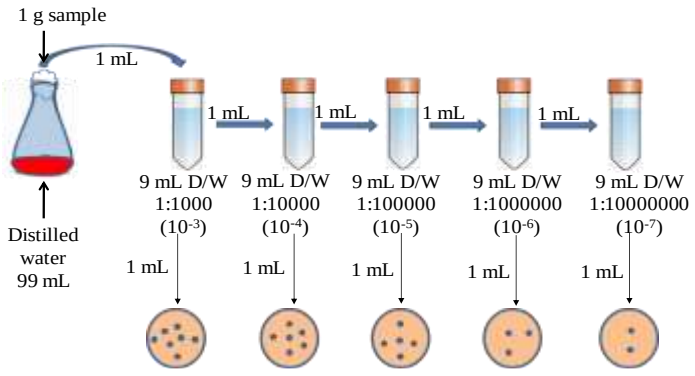

Figure 1 Serial dilution method for soil samples (Collins, 1965)

Screening of Antimicrobial Activity by Agar Well Method (Collins, 1965)

This method was used for the antimicrobial activity by seven test organisms. The assay medium (glucose – 1.0 g, yeast extract – 0.3 g, peptone – 0.2 g, agar – 1.6 g) was utilized for these bacteria. Isolated strains were subjected with antimicrobial activity by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclave basal antimicrobial test medium.

Well impregnated with 1-5 days old culture fermented broth (0.1 mL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Clear zone surrounding the test wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Table 1 Test organisms used in antimicrobial activities (NITE, 2004)

Sr No.	Test organisms	Infection
1.	*Agrobacterium tumefaciens* NITE 09678	Plant disease
2.	*Bacillus subtilis* IFO 90571	Fever
3.	*Bacillus pumilus* IFO 90571	Wound and burn infection
4.	*Candida albicans* NITE 09542	Candidosis
5.	*Escherichia coli* AHU 5436	Cholera, Diarrhea and vomiting, urinary tract infections

NITE = National Institute of Technology Evaluation
PRD = Pharmaceutical Research Development (Ministry of Industry)

Identification of Selected Bacterium

The identification of isolated bacterial strains were carried out by using their colony morphology, gram staining methods (Dubey and Maheshwari, 2002), and biochemical tests which include the motility test (Tittsler and Sandholzer, 1936), methyl red test (Aneja, 1996), sugar fermentation test (sucrose, lactose, maltose) (Atlas, 1993), nitrate reduction test (Dickey and Kelman, 1988), starch hydrolysis test (Aneja, 1996), catalase test (Dickey and Kelman, 1988), oxidase test (Dickey and Kelman, 1988), oxygen requirement (aerobic/anaerobic) (Prescott, 2002), citrate utilization test (Atlas, 1993), Voges-Proskauer VP test (Cruickshank, 1963), Urea test (Woodland, 2004), respectively.

Identification of selected bacterium SY-7

KB013-1KT, HiBacillus™ Identification Kit

Result interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Malonate	—	Malonate utilization	Bluish green	Dark Blue	Bluish green
2	Voges Proskauer's	1-2 drops of Barritt reagent A and 1-2 drops of Barritt reagent B	Detects acetoin production	Colourless/ Light yellow	Pinkish red	Colourless/ Light copper
3	Citrate	—	Citrate utilization	Light Green	Dark Blue	Light Green
4	ONPG	—	Detects Beta galactosidase	Colourless	Yellow	Colourless
5	Nitrate Reduction	1-2 drops sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Naphylamine	Detects Nitrate reduction	Colourless / Light yellow	Pinkish Red	Colourless
6	Catalase	3% H_2O_2 solution	Detects Catalase activity	Colourless	Effervescence coming out from the loop	No Effervescence seen
7	Arginine	—	Arginine utilization	Olive Green to Light Purple	Purple / Dark Purple	No Change in Colour or yellow
8	Sucrose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Glucose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Arabinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Trehalose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

Results

The total of 10 bacterial strains (SY-1 to 10) were isolated from the Yoar Thit Village, Myint Thar Township.

Table 2 Isolated Bacteria from Soil Samples

Soil Sample No.	FLO Medium	Nutrient Agar Medium
S - 1	SY 1 – 2 = 2	SY 3-4 = 2
S - 2	SY 5 – 6 = 2	SY 7 = 1
S - 3	SY 8 = 1	SY 9-10 = 2

Table 3 Colony and Cell Morphology of Isolated Bacteria

Isolated Bacteria	Shape	Size of Colony	Margin	Color	Elevation and Form	Cell Mor-phology	Gram Staining
SY - 1	Circular	Medium	Lobate	White	Flat	Rod	-
SY - 2	Circular	Medium	Entire	White	Flat	Rod	-
SY - 3	Filamentous	Medium	Rhizoid	White	Raise	Rod	-
SY - 4	Circular	Small	Entire	Cream	Flat	Rod	-
SY - 5	Circular	Small	Entire	Cream	Flat	Rod	-
SY – 6	Irregular	Large	Entire	Cream	Flat	Rod	-
SY – 7	Circular	Medium	Entire	Cream	Flat	Rod	+
SY – 8	Circular	Medium	Undulate	Cream	Flat	Rod	-
SY – 9	Circular	Large	Entire	Cream	Flat	Rod	-
SY - 10	Circular	Small	Entire	Cream	Flat	Rod	+

Small < 2mm diameter/ Medium between 2mm and 5mm diameter
Large > 5mm diameter + = Gram positive - = Gram negative

Morphology Microscopical characters Morphology Microscopical characters


SY-1


SY-1 (×40)


SY-2


SY-2 (×40)


SY-3


SY-3 (×40)


SY-4


SY-4 (×40)

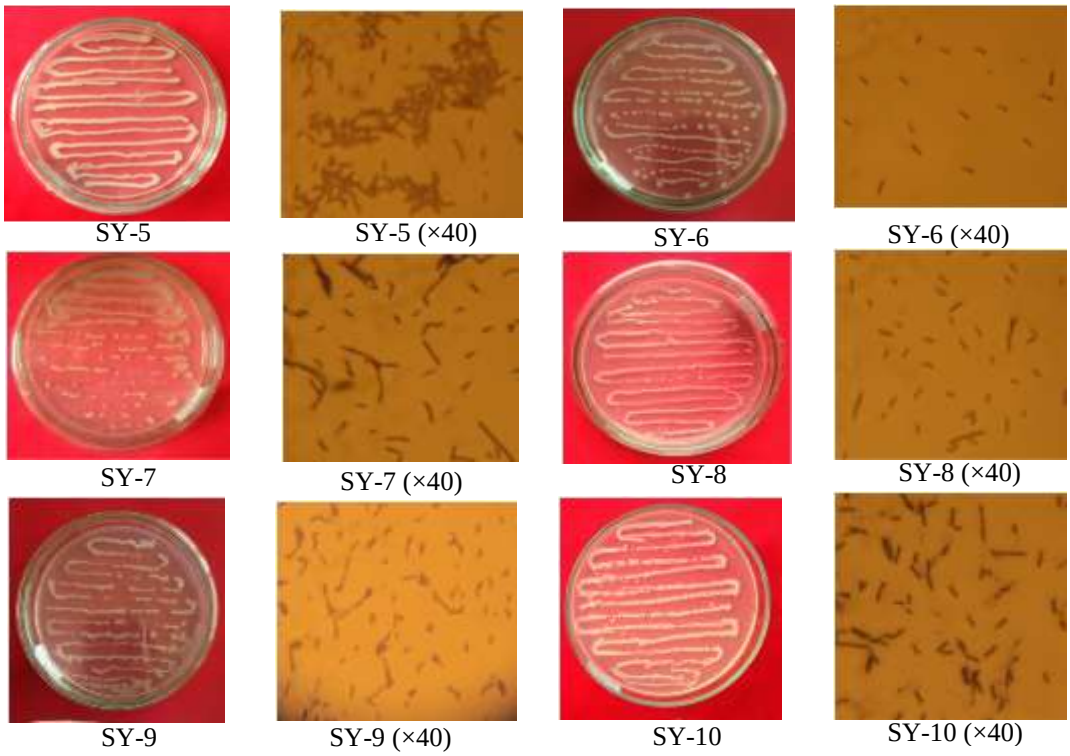

Figure 2 Cultural Character and Cell morphology of Isolated Bacteria SY-1 to SY-10

Isolated bacteria and their Antimicrobial Activity

Seven isolates (SY-1, SY-2, SY-4, SY-6, SY-7, SY-8 and SY-9) had antimicrobial activity and SY-7 showed the highest antifungal activity (32.26) against on *Cadida albicans* followed by 30.44 mm on *Bacillus subtilis.* Other bacteria (SY-3, 5 and 10) could not produce antimicrobial metabolites.

Table 4 Antimicrobial Activity of Isolated Bacteria

No.	Isolated bacteria	Test Organisms and Antimicrobial Activity (mm)				
		Agrobacterium tumefaciens	***Bacillus pumilus***	***Bacillus subtilis***	***Candida albicans***	***E. coli***
1	SY - 1	21.41	23.31	15.61	15.76	16.99
2	SY - 2	19.79	19.89	21.22	23.66	19.46
3	SY - 3	-	-	-	-	-
4	SY - 4	18.77	19.17	18.22	21.86	18.82
5	SY - 5	-	-	-	-	-
6	SY - 6	16.11	17.86	19.21	14.03	16.33
7	**SY-7**	**26.20**	**29.91**	**30.44**	**32.26**	**20.88**
8	SY-8	14.21	16.33	17.18	16.77	14.11
9	SY-9	17.62	17.64	18.11	26.88	16.01
10	SY-10	-	-	-	-	-

The antimicrobial activity of these strains were tested by using five different test organisms. Seven strains showed the activity on *A. tumefaciens, B. pumilus, B. subtilis, C. albicans* and *E. coli*.

A. tumefaciens *B. pumilus* *B. subtilis* *C. albicans* *E. coli*

Figure 3 Antimicrobial Activity of Ten Isolated Bacteria

Therefore, SY-7 was selected and identified by colony morphology, Gram-staining and Biochemical characteristics. In the colony and cell morphology, SY-7 was medium in size, entire margin, cream colour, rod shaped and spore present.

(A) **(B)** **(C) X 100** **(D) X 100**

Figure 4 (A) Colony character (B) Colony morphology (C) Gram staining (D) Spore staining of selected bacterium SY-7

Table 5 Carbohydrate Fermentation of Selected Strain SY-7

Sugar sources	Responces Yellow colour	Acid production	Gas production
Sucrose	Change in medium	+	-
Glucose	Change in medium	+	-
Maltose	Change in medium	+	-
Xylose	Change in medium	+	-
Fructose	Change in medium	+	-

\+ = acid and gas was produced - = acid and gas was not produced

The positive results of SY-7 were able to ferment the glucose, sucrose, maltose, xylose and fructose that is responsible for sugar fermentation and produced acid.

C T (A) C T (B) C T (C) C T (D) C T (E)

Figure 5 Carbohydrate Fermentation Test of Selected Bacterium (SY-7) A. Sucrose, B. Glucose, C. Maltose, D. Xylose, E. Fructose (All positive)

Table 6 Biochemical Characteristics of Selected Strain SY-7

No	Biochemical tests	Responses	Results
1	Urea hydrolysis test	No change in colours	-
2	Nitrate reduction test	Change in orange colour	+
3	Methyl red test	No change colour	-
4	Voges proskaucer test	Change in orange colour	+
5	Citrate utilization test	Medium change from green to blue	+
6	H_2S production test	Change black	+
7	Catalase test	Release free oxygen gas bubble	+
8	Oxidase test	No change color	-
9	Gelatin hydrolysis	No clear zone around the colony	-
10	Potato plug	Growth in streak line	+
11	starch hydrolysis		
	(i) Soluble starch	Clear zone around in streak line	+
	(ii) Tapioca powder	Clear zone around in streak line	+
	(iii) Sticky rice powder	Clear zone around in streak line	+
	(iv) Wheat powder	Clear zone around in streak line	+
	(v) Rice	Clear zone around in streak line	+
12	Caesin hydrolysis	Clear zone around the colony	+
13	Esterase activity	No colour change in medium	-
14	Salt tolerance test		
	(i) 1% NaCl	High growth	+
	(ii) 2% NaCl	High growth	+
	(iii) 3% NaCl	Moduate growth	+
	(iv) 4% NaCl	Moduate growth	+
	(v) 5% NaCl	Poor growth	-
	(vi) 6% NaCl	Poor growth	-
15	Triple Sugar Iron	Change in pink colour	+
16	Motility	Motile	+
17	Aerobic/Anaerobic Test	Facultative anaerobic	
18	PPA (Phenylalanine)	no change in colour	-

\+ = Gram positive - = Gram negative

In the biochemical characteristics properties, SY-7 was found to be positive in catalase reaction, motility, aerobic and anaerobic and hydrogen sulfide production. Oxidase test was negative.

(a) Catalase test (positive) (b) Oxidase test (negative) (c) Hydrogen sulfide test (positive) (d) Motility test (positive)

Figure 6 Biochemical Characteristics of Selected Bacterium (SY-7)

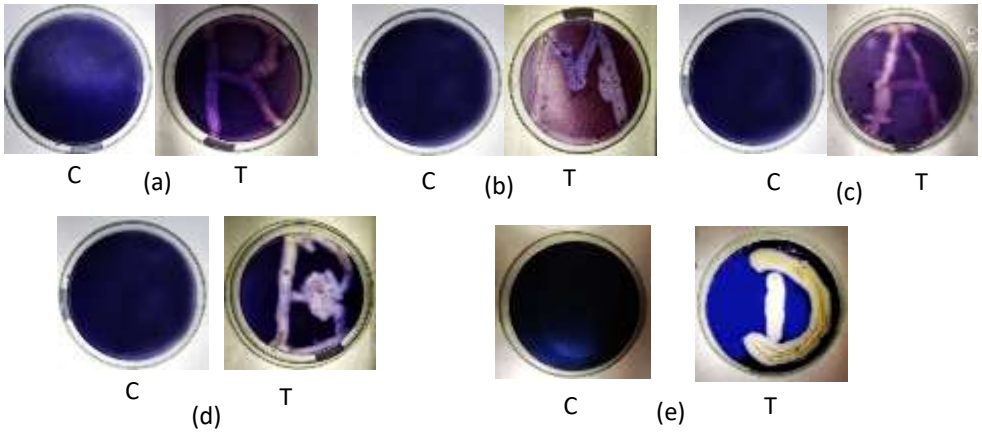

Figure 7 Starch Hydrolysis Test of Selected Bacterium SY-7 (a) Rice (positive), (b) Sticky rice (positive), (c) Wheat (positive), (d) Starch (positive), (e) Tapioca powder (positive)

SY-7 can grow well in 1% and 4% NaCl at room temperature. SY-7 was found to be positive in voges-proskauer (VP) and citrate utilization and negative in methyl red.

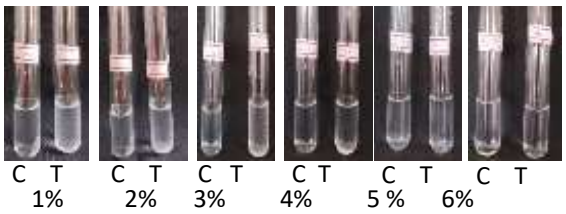

Figure 8 NaCl Tolerance Test of Selected Bacterium SY-7 (1%, 2% highest growth),(3%, 4% moderate growth), (5%, 6% poor growth)

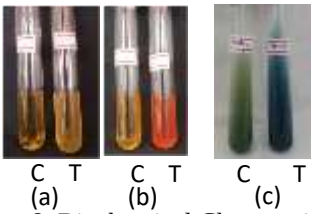

Figure 9. Biochemical Characteristics of Selected Bacterium SY-7
(a) Methyl red (negative),
(b) Voges Proskucer (positive),
(c) Citrate utilization (positive)

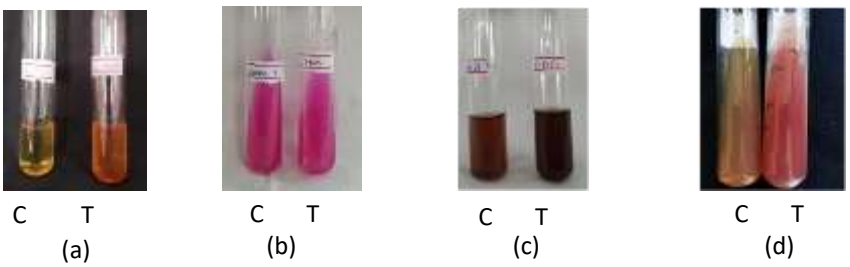

Figure 10 Biochemical Characteristics of Selected Bacterium SY-7 (a) Nitrate reduction (positive), (b) Urea hydrolysis (negative), (c) Phyenylalanine (PPA) (negative), (d) Triple Sugar Iron (TSI) (positive)

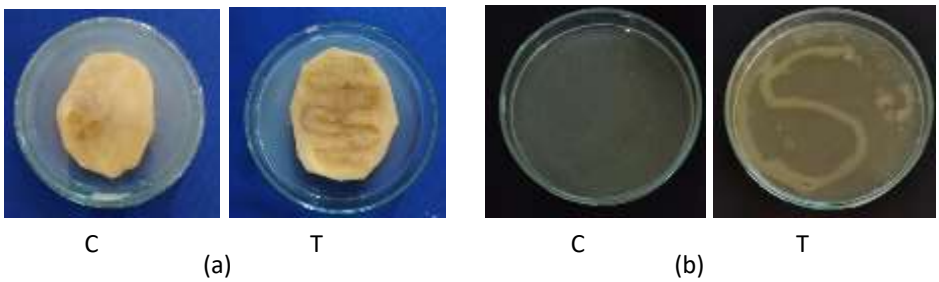

Figure 11 Potato Plug Test and Esterase Activity of Selected Bacterium SY-7 (a) Potato plug test (positive) (b) Esterase activity (negative)

Figure 12 Gelatin (negative) and Casein hydrolysis (positive) Test of Selected Bacterium SY-7

Table 7 Identification of selected bacterium SY-7 (KB013-1KT, HiBacillus™ dentification Kit)

No.	Test	Original Color of the medium	Result
1.	Malonate	Bluish green	Dark blue (+)
2.	Voges Proskauer's	Colourless/Light yellow	Pinkish red (+)
3.	Citrate	Light Green	Dark blue (+)
4.	ONPG	Colourless	Yellow (+)
5.	Nitrate Reduction	Colourless/Light yellow	Pinkish red (+)
6.	Catalase	Colourless	Effervescence coming out from the loop (+)
7.	Arginine	Olive Green to Light Purple	Purple/Dark purple (-)
8.	Sucrose	Pinkish Red/Red	Yellow (+)
9.	Mannitol	Pinkish Red/Red	Yellow (+)
10.	Glucose	Pinkish Red/Red	Yellow (+)
11.	Arabinose	Pinkish Red/Red	Yellow (+)
12.	Trehalose	Pinkish Red/Red	Yellow (+)

SY-7 was identified by KB013-1KT, HiBacillus™ Identification Kit and the results were the same with those of manual biochemical tests.

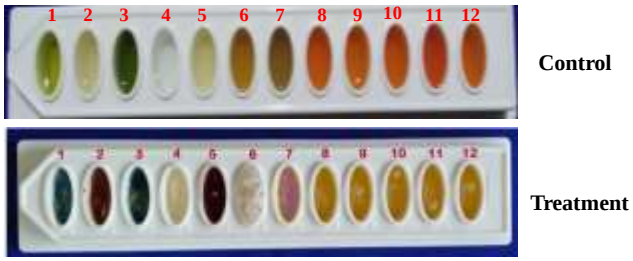

Figure 13 Identification of selected bacterium SY-7 (KB013-1KT, HiBacillus™ Identification Kit)

Discussion and Conclusion

Soil is a primary source of microorganisms. Soil bacteria and fungi have played a significant and an important role in antibiotic discovery. (Mashoria *et al*., 2014)

In the course of the isolation of bacteria, three different samples were collected from Myit Tha Township, Mandalay region and 10 bacterial strains were obtained from these soil samples.

Two different media were employed in the investigation of the isolation of bacteria. Five strains were obtained from FLO medium and other five strains from Nutrient Agar medium.

Cell morphology of isolated strains was studied by Gram staining, colony characters and shape of cell. Among them, all strains were rod.

Hoorman, 2011 described that most of the bacteria belong to three main shapes: rod (rod shaped bacteria are called bacilli), sphere (sphere shaped bacteria are called cocci), and spiral (spiral shaped bacteria are called spirilla) and slender branching filaments called actinomycetes.

Antimicrobial activity of isolated bacterial strains were tested by agar well diffusion method on five test organisms and these strains showed different level of antimicrobial activities.

Among them, SY-7 exhibited the highest antimicrobial activity (32.26 mm) on *Candida albicans* followed by (30.44 mm) on *Bacillus subtilis* and (29.91 mm) on *Bacillus pumilus* respectively.

Shapiro, 2002 described that research on antimicrobial compounds as a new class of drugs has increased in the recent past as they exhibit both narrow and broad spectrum inhibitory activities against Gram-positive and Gram-negative bacteria.

In the characterization of SY-7, the results of colony character and biochemical characterization were similar to the previous research of Buchanan, 1974 and Vargar *et al.*, 2004.

Vargar *et al.*, 2004 reported that *Bacilli* are described as aerobic or facultative anareobic, gram positive, rod-shaped, flagellated motile bacteria, catalase positive belong to the division Frimicutes with a wide ecological diversity mostly saprophytic they are commonly found in soil, dust, milk, plant surface, a few are animal or insect parasites or pathogen.

Moreover, SY-7 was identified by KB013-1KT, HiBacillus^{TM} Identification Kit and the results were the same with those of manual biochemical tests.

Therefore, the selected bacterium SY-7 was characterized as the genus *Bacillus* spp.

Further study will be studied the purification and identification of isolated compounds and minimum inhibitory concentration (MIC).

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ISOLATION AND CHARACTERIZATION OF ANTIBACTERIAL COMPOUNDS ISOLATED FROM ENDOPHYTIC ACTINOMYCETES (TG-16)

Theingi Aung[1], Zar Zar Yin[2] and Nant Si Si Htay[3]

Abstract

The research was concerned with isolation, purification and partial characterization of antibacterial compounds of isolated endophytic Actinomycetes TG-16 from the stem of *Tadehagi triquetrum* (L.)H.Ohashi. These experiments were conducted by solvent extraction, thin layer chromatography, silica gel column chromatography, UV and FT IR spectroscopic analyses. The plant sample was collected from Pathein University Campus. The fermented broth of isolated strain TG-16 was carried out by paper chromatography using with four solvents for the extraction of antibacterial metabolites. the Actinomycetes culture filtrate was studied by the different ratio of two solvents. According to this result, the active metabolite, 15 liter was extracted by using *n*-butanol to fermented broth (1:1v/v). The analysis of separation, purification, and partial characterization of isolated compounds indicated that compound A (aromatic derivatives, 15 mg, colorless crystal) and compound B (steroid derivatives, 14 mg, white amorphous solid). Further evaluation of minimum inhibitory concentration of compounds showed the value of 3.125 µg/mL in compound B while compound A did not exhibit antibacterial activity against *E. coli*. This study revealed the presence of bioactive compounds in isolated strain TG-16, which may be a promising resource for the discovery of bioactive metabolites against multidrug resistance of *E. coli.*

Keywords: paper chromatography, silica gel column chromatography, minimum inhibitory cocentration

Introduction

Endophytic Actinomycetes can produce novel antibiotic compounds and other secondary metabolites and this has resulted in the isolation of metabolites from Actinomycetes. Only a few resent studies have heighted the bioactive importance of endophytic actinomycetes, including biocontrol of fungal plant pathogens, production of antimalarial and antimicrobial agents, production of anticancer compounds and production of enzymes (EI- Shatoury *et al*., 2006). Dilution methods are used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents and are reference methods for antimicrobial susceptibility testing (Owuama, 2017). Among microbes producing bioactive secondary metabolites, actinomycetes produced highest number of bioactive metabolites (Prashith Kekuda, 2016). The aim and objectives of this study was to be extraction, isolation and purification of two compounds from endophytic streptomyces, TG-16 and to observe partial characterization and minimum inhibitory concentractions (MIC) of these compounds.

Materials and Methods

Paper Chromatography (Tomita, 1988)

The extraction of antibacterial metabolites of selected strain TG-16 was done by using paper chromatography. The filter paper and four solvents; 20% NH_4Cl, *n*-butanol saturated with water, *n*-butanol-acetic acid -water (3:1:1) and ethyl acetate saturated with water, were used for preliminary characterization of antibacterial metabolites. The obtained fermented broth samples (100 µL) were applied on the paper and allowed to dry. The papers were chromatographed in each

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solvent. Each paper was placed on assay agar plate. Then, bioautography was done to check the antibacterial activity of each.

Preparation of *n*-BuOH extract fromTG-16 fermented broth (Natara *et al*., 2010

The actively growing culture of TG-16 was inoculated with broth of ISP-2 medium and incubated at room temperature for 5 days. It was them centrifuged at 100 rpm for 15 min. After incubation period and the supernatant was passed through a filter paper to get a spore free filtrate. For extraction of antibacterial compounds containing in fermented broth, the filtrate was then treated with an equal volume ratio of *n*-BuOH to fermented broth. Then, the mixture was shaken in a separating funnel. The organic layer was separated and collected. The solvent was removed in vaccum using a rotary vaccum evaporator.

Thin layer chromatographic analysis (Verma *et al*., 2014)

Thin layer chromatography (TLC) was performed on *n*-BuOH crude extract from the culture broth of the isolated strain TG-16. For this, bioactive culture extracts were applied to the TLC plate (Merck silica gel plate 60 GF 254, 0.2 mm) using chloroform : methanol (30:1 v/v), chloroform : ethyl acetate (5:1, 1:1 v/v), chloroform only, hexane : chloroform (1:1 v/v), hexane : ethyl acetate (10:1,5:1, 3:1, 1:1 v/v) and hexane : *n*-BuOH (60:1, 50:1, 40:1, 30:1,20:1, 10:1,5:1, 3:1, 2:1, 1:1 v/v). The spots on the plates were developed and observed under UV light at 254 nm and 365 nm and also observed by color developing chemical reagents such as I_2 vapour, 5 %H_2SO_4 and 5% $FeCl_3$ and their R_f values were recorded.

Isolation of organic metabolites by silica gel column chromatography (Simon and Gray, 1998)

According to thin layer chromatographic analysis, the *n*-butanol extract residue of selected Actinomycetes metabolite was developed to isolate the active compound by silica gel column chromatography with *n*-hexane:*n*-BuOH as eluting solvent. Silica gel (60-120 mesh) (ca.50g) was dissolved in *n*-hexane:*n*-BuOH 60:1 v/v and the column was packed by wet method. Gradient elution was performed successively with increasing polarity (*n*-hexane:*n*-BuOH, 60:1,50:1, 40:1, 30:1, 20:1 10:1, 5:1, 2:1,and 1:1 v/v). Fractions of 2 mL each were collected individually and the compounds present were checked on TLC. The fractions with same spots were mixed and the solvent was evaporated on rotary evaporator.

Characterization of Isolated Antibacterial Compounds

In an attempted to characterize the isolated antibacterial compounds, the following tests were performed;

Determination of solubility of isolated compounds

A 0.5 mg each of isolated compounds was subjected to 0.5 ml of polar and non-polar solvents such as H_2O, MeOH, EtOH, EtOAc, $CHCl_3$, *n*-BuOH and Hexane in order to known their solubility.

Determination of some chemical properties of isolated compounds

The isolated compounds were subjected to TLC analysis and then treated with some coloured reagents such as Liebermann-Burchard reagent, 5%H_2SO_4, I_2 vapour, 5% $FeCl_3$ and 5 % Anisalehyde sulphuric acid and noted their behavior on TLC.

Study on UV-visible spectroscopy

For the identification of isolated compounds, ultra violet absorption spectra were also recorded and examined. A Shimadzu UV-18000 UV-visible spectrophotometer at Customer Support & Laboratory (AMTT).

Study on FT IR spectrometry

The FT IR spectra of the isolated compounds A and B were recorded by using Spectrum II (Perkin elmer) spectrophotometer at Chemistry Department, Pathein University.

Antimicrobial activity of isolated compounds

Isolated compounds A and B were checked for their activity against *E.coli* by agar well diffusion method. The isolated compounds A and B (100 µg each) were separately dissolved in 1 mL of methanol and their antibacterial activity were performed by agar well diffusion method.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by tow fold serial dilution method (Andrew, 2001). The using concentrations were ranging from 50µg/mL to 0.195µg/mL with control solution. The minimum inhibitory concentration of compound A and B were determined by streak culture method and agar well diffusion method. The selected test organism was *Escherichia coli.* After incubation for 24 hours, checked the MIC values of isolated compounds.

Results

Paper Chromatography

In this study, according to R_f values, *n*-butanol was the most extractable solvent for the antibacterial metabolites (R_f : 0.79) followed by ethyl acetate solvent (R_f : 0.76), ethyl acetate-acetic acid-water 3:1:1 (R_f : 0.74) and finally NH_4Cl the lowest R_f :0.21). The chromatography bioautographic assay was shown in Figure 1

1. 20% NH_4Cl
2. *n*-butanol saturated with water
3. ethyl acetate- acetic acid-water (3:1:1v/v)
4. ethyl acetate saturated with water

Figure 1 Paper chromatography bioautographic assay

Comparison of antibacterial activity of TG-16 extracted with different ratios of *n*-BuOH and EtOAc to fermented broth against *E. coli*

In this study, the *n*-BuOH esxtracts obtained by *n*- BuOH to (fermented broth) FB in the ratios of (1:1, 2:1, 3:1 v/v) showed antibacterial activety with inhibition zone diameter of 27.01 mm, 26.02 mm and 25.00 mm, respectively. Similarly, EtOAc extracts obtained by EtOAc to FB in their ratio of 1:1, 2:1, 3:1 v/v showed antibacterial activity against *E.coli* with inhibition zone diameter 22.47 mm, 20.80 mm, 20.72 mm, respectively. According to the results, the *n*-BuOH extract (1:1v/v) of TG-16 displayed the highest antibacterial activity against *E.coli*. with

inhibition zone diameter of 27.01 mm. Therefore, antibacterial metabolites were extracted with equal volume of *n*-BuOH to fermented broth.

Table 1 Comparison of Antibacterial Activity Aginst *E.coli* of TG- 16 Extracted with Different Ratios of Solvent to Fermented Broth

Different ratio of solvent to fermented broth (v/v)	Inhibition zone diameter (mm)	
	***n*-BuOH extract**	**EtOAc extract**
1:1	27.01	22.47
2:1	26.02	20.80
3:1	25.00	20.72

Agar well size = 8mm

Extraction of antibacterial metabolite

15 liters of selected strain TG-16 were fermented in suitable synthetic fermentation medium (120 h, 20 % size of inoculums, at room temperature, pH 8, 6day fermentation period, under shaking culture) and extracted with equal ratio of *n*-butanol to fermented broth (1:1 v/v) to yield 4.0g was obtained from the culture filtrate.

Thin layer chromatographic analysis

According to the thin layer chromatography (TLC) was performed on *n*-BuOH crude extract by employing various solvent systems, the extract showed well- separated spots on TLC by using *n*-hexane : *n*-BuOH solvent system under UV 365nm and some color reagent tests. So, the solvent system (*n*-hexane: *n*-BuOH) was selected to isolate pure compounds by silica gel column chromatography.

Isolation of organic metabolites from *n*-BuOH extract of fermented broth of TG-16 by silica gel column chromatography

In this study, successive fractions obtained were combined on the basic of their behavior on TLC. Finally, fifteen main fractions (F1 to F15) were collected. Antibacterial activity of each fraction was examined in bioassays to determine the fraction containing the active compound by agar well diffusion method. Fractions F-1-2, F-4 and F-15were found to be inactive against *E.coli*, F-5, F-6 and F-9 were significantly showed antibacterial activity against *E.coli* while remaining fractions have a few effect on it. From the resulting fractions, compounds A and B were obtained from the respective fractions F-6 and F-9 and the remaining fractions were Found as mixture (Figure 3).

Figure 2 The antibacterial activity of collected fractions F-1 toF-15 from *n*-BuOH extract

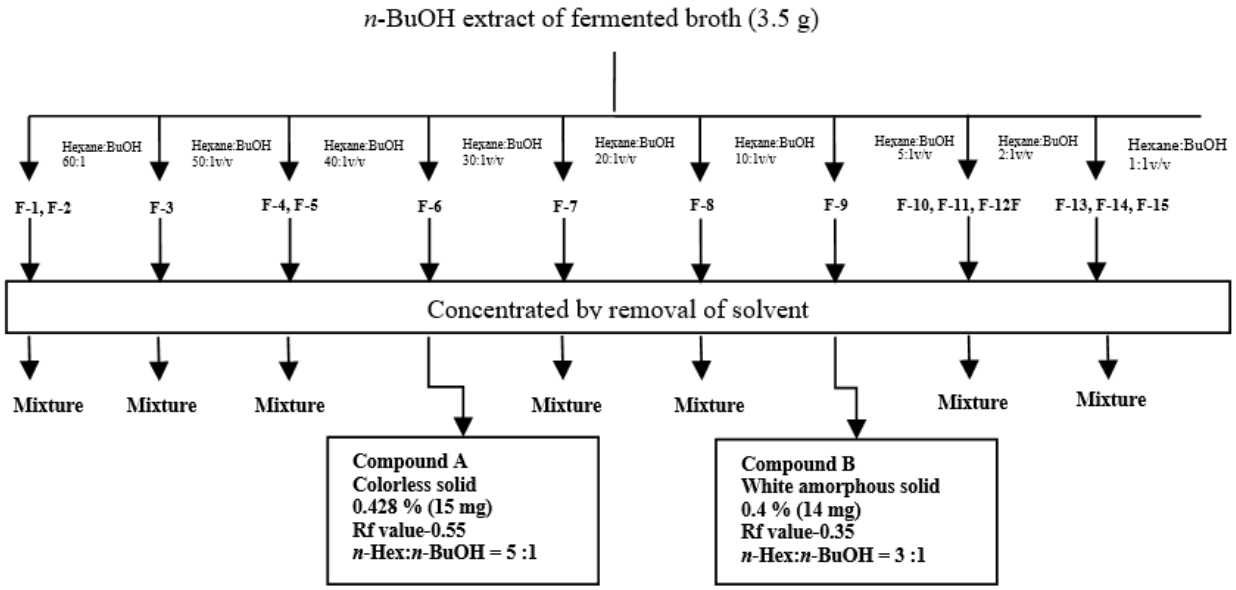


Figure 3 Isolation of organic metabolites from n-BuOH crude extract, culture broth of isolated strain TG-16 by column chromatography with *n*-Hexane : *n*-BuOH solvent system

Compound A
R_f value : 0.55
Solvent system : *n*-Hexane : *n*BuOH (5:1 v/v)
UV 365 nm : Fluorescence

Compound B
R_f value :0.35
Solvent system : *n*-Hexane:*n*-BuOH (3:1 v/v)
Spraying agent : 5 % Anisaldehy sulphuric acid

Figure 4 Isolated compound A and B and their thin layer chromatogram

Characterization and classification of isolated compounds

The isolated compounds A and B were characterized by physical properties such as R_f value, solubilities and by some chemical reagent tests and modern spectroscopic techniques such as UV and FT IR.

Compound A

From the above result, fraction F-6 was evaporated and washed with *n*-Hexane and then with *n*-Hexane : *n*-BuOH (60:1 v/v) and then purified by recrystallization from MeOH, to give 0.428 % (15 mg) of compound A as colorless solid. It was soluble in EtOH, Hexane, BuOH, $CHCl_3$, MeOH but insoluble in H_2O.The R_f value of compound A was found to be 0.55, solvent system of *n*-Hexane:*n*-BuOH (5:1v/v) and it fluorescence under UV 365 nm, it gave yellow spot on TLC with iodine vapor but not responds in spraying with 5% H_2SO_4, anisaldehyde sulphuric acid, Libermann Burchard and $FeCl_3$ (Table 2). In the UV spectrum, maximum absorption bands at λ_{max} of 204 nm and 283 nm indicating the present of conjugated double bond system (Figure 5 and Table 3).

The IR spectrum of the compound included a diagnostic peak at 3339 cm^{-1} which is indicative of the -OH group. However, the peak at 3068 cm^{-1} was assigned to aromatic -CH stretching. The peak appearing at 2921cm^{-1}, 2855 cm^{-1} was assigned to C-H stretching vibration of $-CH_3$– and $-CH_2$. The peak absorption band appearing at 1713 cm^{-1} was assigned to C-O stretching vibration of ketone, the absorption bands at 1640 cm^{-1} was assigned to C=C stretching vibration of olefinic group, the absorption bands at 1534cm^{-1} and 1513cm^{-1} were due to C= C stretching

vibration of aromatic ring. The absorption bands at 1458cm^{-1} and 1376 cm^{-1} were due to bending -CH_3 and -CH_2. The peak appearing at 1040cm^{-1}was assigned to C-O group of alcohol and the peak at 910cm^{-1}wasdue to C-H bending vibration of olefinic group. The absorption bands at 749cm^{-1}and 719cm^{-1} were due to C-H bending vibration of monosubstituted aromatic compound (Figure 6 and Table 4). According to the results of UV, FT IR and some chemical coloration tests, compound A may be classified as an aromatic derivative.

Table 2 Some Chemical Properties of Isolated Compound A and B

Experiment	Observation (compounds)	
	A	B
UV (MeOH /nm)	Active	Inactive
5% H_2SO_4	Colorless	Pink
I_2 vapor	Yellow	Yellow
5% $FeCl_3$	Colorless	Colorless
Anisaldehyde Sulphuric acid	Colorless	Violet
Libermann Burchard	Colorless	Greenish blue

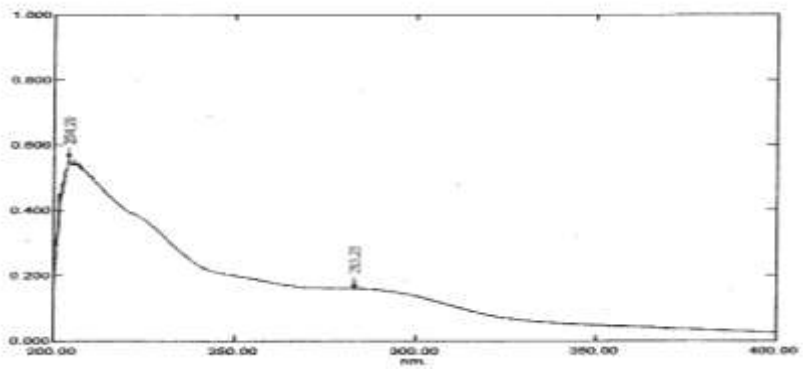


Figure 5 Ultraviolet absorbance of the compound A

Table 3 UV Spectral Data of Isolated Compound A

Solvent used	Observed (nm)	Remark
MeOH	204,283	Conjugated double bond

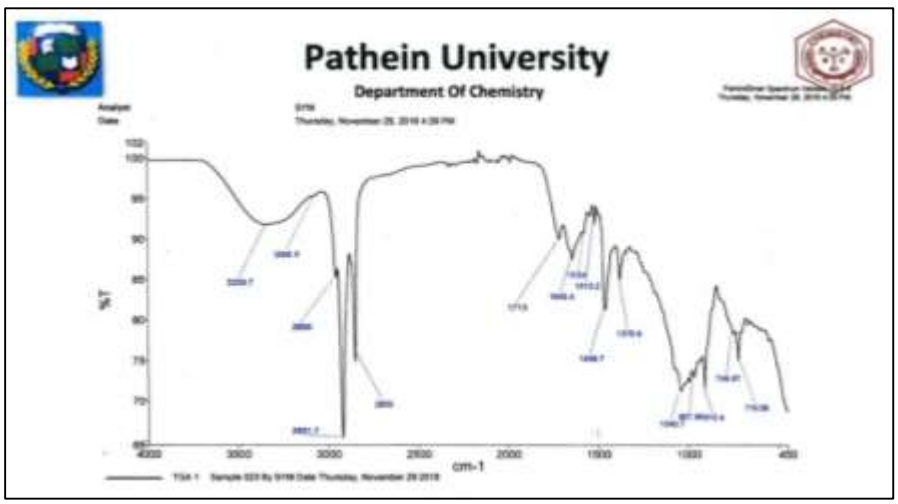


Figure 6 FTIR spectrum of the compound A

Table 4 FTIR Spectral Data of Isolated Compound A

Wave number (cm^{-1})	Literature value*(cm^{-1})	Band assignment
3339	3400-3200	υ_{O-H} of alcohol
3068	3100-3000	$\upsilon_{=C-H}$ of aromatic ring
2921, 2855	3000-2850	υ_{C-H} (asym & sym) of CH_3 and CH_2
1713	1730-1715	$\upsilon_{C=O}$ of ketone
1640,1534,1513	1600-1400	$\upsilon_{C=C}$ of aromatic ring
1458,1376	1470-1450	δ_{C-H} of (asym & sym) of $CH_3$and CH_2
	1390-1370	
1040	1300-1000	υ_{C-O} of alcohol
910	900-680	δ_{C-H} of olifinic group
749, 719	900- 675	$\delta_{(oop)C-C}$ of aromatic ring

*Silverstein *et al*., 2005

Compound B

Compound B isolated from fraction F-9, it washed with *n*-Hexane and then with *n*-Hexane : *n*-BuOH (30:1 v/v) and then purified by recrystallization from MeOH, to give 0.4 % (14 mg) of compound B as white amorphous solid. It was soluble in *n*-Hexane, EtOH, *n*-BuOH, $CHCl_3$, MeOH but insoluble in H_2O and it was inactive under UV (365 & 254 nm).The R_f value of compound B was 0.35 in the solvent system of *n*-Hex to *n*-BuOH (3:1v/v). It gave a yellow with iodine vapor, pink coloration while spraying 5% H_2SO_4 followed by heating and developed into violet coloring while spraying anisaldehyde sulphuric acid followed by heating (Table 2). It gave greenish blue coloration with Liebermann Bruchard reagent. The IR spectrum of the compound included a diagnostic peak at 3315 cm^{-1} which is indicative of the -OH group of alcohol. The peak appearing at 2921cm^{-1}, and 2873 cm^{-1}were assigned to asymmetric and symmetric -CH stretching vibration of -CH_3–and–-CH_2 of groups. The peak at 1640 cm^{-1} was assigned to C=C stretching vibration of olefinic group, and the absorption bands at 1413cm^{-1}, 1376 cm^{-1}were due to -CH bending vibration of -CH_3 and -CH_2 groups, the C=O stretching vibration of alcohol was observed in 1007cm^{-1} (Figure 7 and Table 5). From the results of FTIR and some chemical coloration tests, compound B may be classified as a steroid derivative.

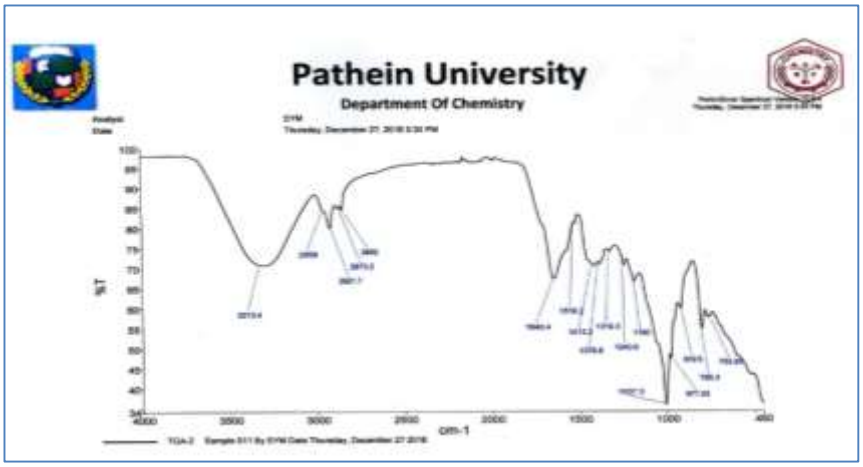


Figure 7 FTIR spectrum of the compound B

Table 5 FT IR Spectral Data of Isolated Compound B

Wave number (cm^{-1})	Literature value* (cm^{-1})	Band assignment
3315	3400-3200	υ_{O-H} of alcohol
2921,2873	3100-2850	υ_{C-H} asym & sym of CH_3 and CH_2
1640	1680-1600	$\upsilon_{C=C}$ of olefinic group
1413-1376	1465 -1375	δ_{C-H} of CH_3 and CH_2
1007	1300-1000	υ_{C-O} of alcohol

*Silverstein *et al*., 2005

Antibacterial activity of isolated compounds

Bioassay for determination of isolated compounds were undertaken by agar well diffusion method. From the results, isolated compound A exhibited antibacterial activity against *E.coli* with inhibition zone diameter :16.82 mm and compound B showed antibacterial activity with inhibition zone diameter: 22.98 mm. The photograph illustrating the inhibition zones provited by the isolated compounds against *E.coli* are presented in Figure 8.

Compound A **Compound B**

Figure 8 Antibacterial activity of isolated compound A and B

Concentration (MIC) of isolated compounds

In the study of Minimum Inhibitory Concentration (MIC) of isolated compounds, it was observed that MIC value of compound B was 3.125 µg/mL but isolated compound A did not exhibit antibacterial activity against *E.coli* in this experimental concntration range from 50 µg/mL to 0.195 µg/mL.

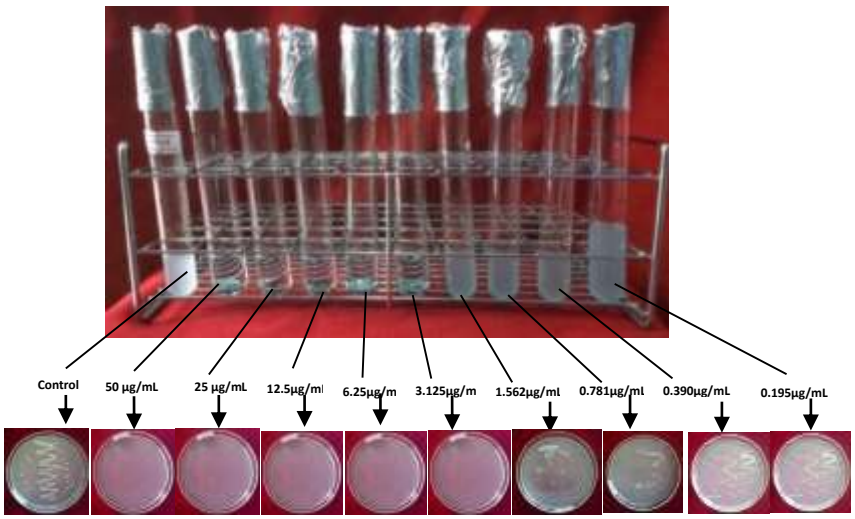
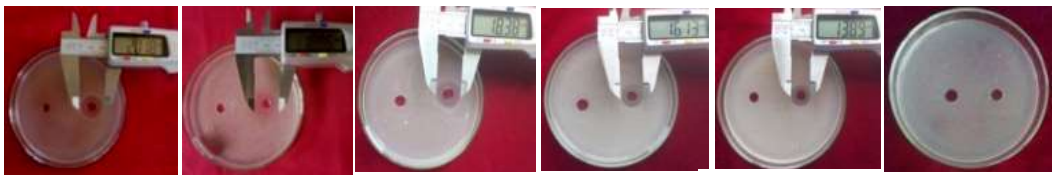


Figure 9 Minimum inhibitory concentration of secondary metabolites from compound B against *E. coli* (Streak culture method)



50 µg/mL 25 µg/mL 12.5µg/mL 6.25µg/mL 3.125 µg/mI 1.562µg/mL

Figure 10 Minimum inhibitory concentration of secondary metabolites from compound B against *E. coli* (agar well diffusion method)

Discussion and Conclusion

Investigation on the extraction of antibacterial metabolites of TG-16, the active metabolites were the best extracted with *n*-BuOH to fermented broth, 1:1 v/v. The present result was described by Sekiguchi, *et al*., 2007. In the study of separation of active compounds by thin layer chromatography, the crude extract showed well separated spots on TLC by using *n*-Hexane: *n*-BuOH solvent system. According to the result of silica gel column chromatographic analysis, all the collected fractions was analyzed by TLC and the fractions with similar behavior on TLC were combined, which ultimately resulted in major fifteen fractions. In the study of bioassay for measuring the antibacterial activities of collected fractions 1-15, fraction F_1, F_2, F_4, and F_{15} were found to be inactive on *E.coli* and F_5, F_6 and F_9 were to have the highest activity.

From column chromatographic separation of fermented broth TG-16, compound A and B were obtained from the respective fractions F6 and F9 and remaining fractions were found as mixture. In the UV spectrum of compound A, it was seen that a maximum absorption bands at λ_{max} 204 nm and 283 nm were due to conjugated double bond system. From the results of FT IR spectral data of compounds A, the absorption band at 3339.7, which indicates OH- groups, peaks at 1640 cm^{-1} (C=C groups), it shows the presence of aromatic ring. In addition, absorption band at 2921cm^{-1}, it exhibits the presence of CH- groups and peak at 1040cm^{-1} was assigns to C-O groups of alcohol. The UV and FT IR spectra data were agreement with Oskay, 2011 Augustine *et al*., 2005 and Dhanasekaran *et al*., 2008. From the results of UV, FT IR and some chemical coloration tests, compound A may be characterized as an aromatic derivative.

The compound B was inactive under (365 nm and 254 nm). The result of FT IR spectral data of compound B indicated that bands at 3420 cm^{-1} indicated the presence of OH groups of alcohol, the peak appearing 1640 cm^{-1} was assigned to C=C of olefinic group. These characters were aggrement with Jenifer *et al*., 2013 from the results of some chemical reagent tests on TLC and FT IR spectral data, compound B may be classified as a steroid derivative. The results were expressed as the minimum inhibitory concentration of the compound B against *E coli* were 3.125 µg/mL. Isolated compound A did not exhibit antibacterial activity against *E.coli* in this experimental concentration range of 50µg/mL to 0.195µg/mL. Antimicrobial resistance in *E.coli* has been reported worldwide and increasing rates of resistance among *E.coli* is a growing concern in both developed and developing contries (Kibret and Abera, 2011). This research indicated that the selected strainTG-16 produced antibacterial compounds, which are found effective against multi drug resistant bacteria, *E.coli.*

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PROBIOTIC EFFECT OF *LACTOBACILLUS* SP. (H-1) ISOLATED FROM SAUERKRAUT AND USED IN CHICKEN FEED

Yin Kyay Khin[1] and Htein Htein Lin[2]

Abstract

In this study, the *Lactobacillus* sp. (H-1) isolated from sauerkraut that was used as probiotic in chicken feed. There were 7 dietary treatments for investigation of probiotic activity on broiler chicken growth. The experiments were conducted during May 2019 to November 2019 in the Fermentation Department (Pharmaceutical Research Department, Ministry of Industry - 1, Yangon Region) and chicken farm in Ngwe Nantthar village, Hlegu Township. The chicks in the group D which were fed with 0.015% dried isolated bacteria (with nitrogen source) provide the optimal body weight gain than other group but the chicks in group E which were fed with 0.005% dried isolated bacteria (without nitrogen source) showed lowest body weight gain among 7 groups. Similarly, the chicks in the group D also provided feed consumption, reduce mortality rate compared to those of control group. It can be concluded that the best results were found in group D (0.015% dried bacteria of *Lactobacillus* sp.) fed to the chickens.

Keyword: *Lactobacillus*, nitrogen source, mortality rate.

Introduction

Antibiotics are used to fight bacterial infections. However, a selective pressure gave rise to bacteria resistant to antibiotics. This leaves scientists worried about the danger to human and animal health. Some strategies can be initiated to reduce the use of antibiotics in chicken farms. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health.

Among these, the most popular are probiotics, prebiotics, enzymes, phytogenic feed additives and etc. (Mehdi *et al.,* 2018). Probiotics are live microbial feed supplements that have a beneficial effect on the health and well-being of the host (Bovill *et al.,* 2001). A positive impact of probiotics supplementation in poultry has been well reported on production performance, feed intake, weight gain and feed conversion efficiency, immune responses, and body's resistance to infectious diseases and help lowering of chick mortality (Bansal *et al.,* 2011, Hatab *et al.,* 2016).

Rich medium and suitable conditions are the key environmental parameters required for good bacterial growth (Manzoor *et al.,* 2017). The nitrogen sources was necessary for the growth and product formation in microbial cultivation (Zammaretti *et al.,* 2005). Yeast extract is widely used for the cultivation of lactobacilli because it is an abundant source of nitrogen, the vitamin B group, purine, and pyrimidine (Yeo *et al.,* 2018). The aim of this study is to investigate the effects of *Lactobacillus* sp. (H-1) as probiotic in chicken feed, to study the effect of nitrogen source (yeast extract) on the biomass production and growth performance of broiler and to observe the effects of probiotic supplementation on the body weight gain, feed consumption, feed conversion rate and mortality rate.

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Materials and Methods

Microorganisms

Bacterial strain of *Lactobacillus* sp. (H-1) was isolated from sauerkraut and was incubated in the laboratory of Pharmaceutical Research Department (PRD), Ministry of Industry - 1, Yangon Region.

Culture maintenance

Strain was maintained on tomato juice agar medium slant.

Preparation of inoculum

Bacterial cells were grown in 500 ml conical flask containing 100 ml of tomato juice broth medium. Culture was incubated at 37°C in incubator.

Tomato juice agar medium (Sigma-Aldrich, 2013)

Composition per liter

Tomato juice	20.00 g
Yeast extract	10.00 g
Dextrose	10.00 g
Dipotassium phosphate	0.50 g
Monopotassium phosphate	0.50 g
Magnesium sulphate	0.20 g
Manganese sulphate	0.01 g
Ferrous sulphate	0.01 g
Sodium chloride	0.01 g
Agar	20.00 g

Fermentation (Dubey and Maheshwari, 2002)

Cells were grown in 5000 ml flasks containing 4000 ml medium. The flasks were inoculated using 10 % seed culture and then placed in the incubator for 3 days. At the end of fermentation stage, the bacteria were present as a suspension of cells in the medium. The bacteria was separated by centrifugation at 2000 rpm for 20 min. After centrifugation, the supernatant was discarded and the sediments were collected. Then, for dry weight determination these sediments were filtered by using filter paper. The filtrate was dried in oven at 60°C for 10 hours in order to get the constant weight.

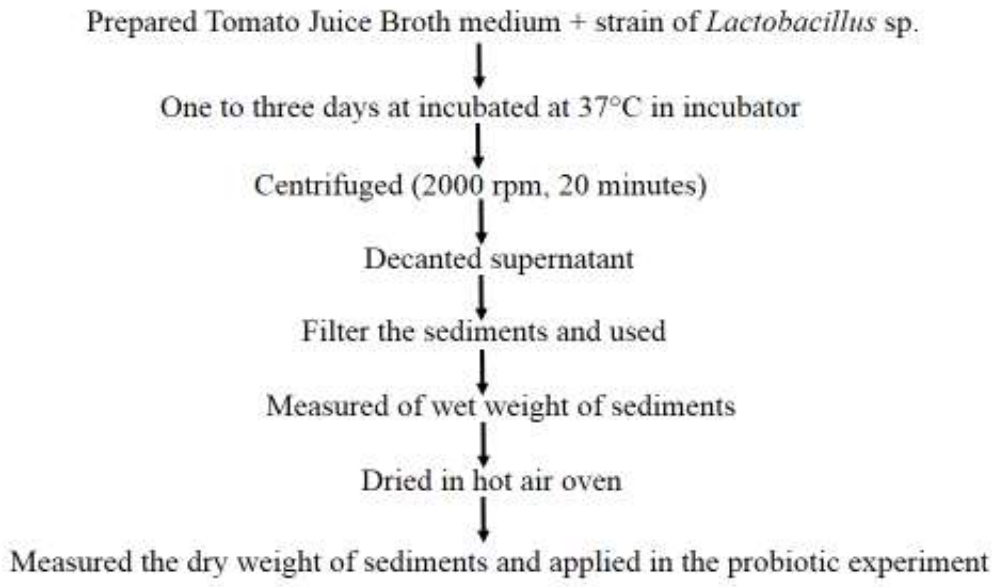

Figure 1 Flow chart for determining percentage (%) dry cell mass of *Lactobacillus* sp.

Preparation as probiotics

After drying, the dried cells was grounded to powder. The resulting powder was used as probiotics and mixed with the normal food for chicken.

Selection of chick for probiotics test

Three hundred and fifty healthy meat broiler was chosen at random. The tested birds were collected immediately after hatching from the broiler trading, Sunjin Myanmar Co., Ltd. They were transported to Ngwe Nantthar village, Hlegu Township where the research work was undertaken.

Application of *Lactobacillus* sp. (H-1) strain as probiotic in chicken feed (Pourakbari *et al.*, 2016)

The experimental broilers were divided into seven groups. Group A was fed on basal diet as control. Group B was fed with basal diet plus 0.005% of feed of *Lactobacillus* sp. (H-1) strain. Group C was fed with basal diet plus 0.010% of *Lactobacillus* sp. (H-1) strain. Group D was fed with basal diet plus 0.015% of *Lactobacillus* sp. (H-1) strain. Similarly, group E was fed with basal diet plus 0.005%, group F was fed with basal diet plus 0.010% and group G was also fed with basal diet plus 0.015% of *Lactobacillus* sp. (H-1) strain. Groups B, C, D contained nitrogen source but groups E, F, G did not contain nitrogen source in *Lactobacillus* dried cell powder.

Rearing and feeding of chick

The chicken house was divided into seven units. The units were enclosed by wire mesh netting. An experiment with 7 treatments (0%, 0.005%, 0.010% and 0.015%) including with/without nitrogen source and each included 50 chicks in the experiment. The experimental diets fed at three different breeding (starter; 0 - 10 days, grower; 11 - 28 days and finisher; 29 - 42 days). These basic constituents of the feed contained soybean meal, animal protein, broken rice, rice bran, corn, wheat bran, cassava, amino acids, minerals, vitamins, etc. The feeds were produced by a commercial mill (Green Feed) in Shwe Pyi Thar Township. Body weight and feed consumption were measured weekly. Death bird was recorded daily.

Calculation of feed utilization (Heuser, 1995)

Feed utilization (feed conversion rate or FCR) was calculated as the ratio between weight of feed consumed and body weight gained.

$$\text{Feed conversion rate} = \frac{\text{Weight of feed consumed}}{\text{Body weight gained}}$$

Vaccination program

The tested chicks were vaccinated according to the following schedule program based on Than Shwe and Fatt (2000). Environmental temperature for the first week of broiler was 32°C and gradually decreased according to the age, 25°C until the end of the experiment.

Table 1 Vaccination schedule

Vaccines	Age of chicken (day)	Method of vaccination
Infectious Bronchitis and Newcastle Disease (1st time)	3	Eye drop
Infectious Bursal Disease (1st time)	7	Mouth drop
Infectious Bronchitis and Newcastle Disease (2nd time)	14	Mouth drop
Infectious Bursal Disease (2nd time)	21	Mouth drop

Chicken feed


Weighting chick broiler chicken


One day old broiler chicken


Farm of broiler


Two week old broiler chicken


Three week old broiler chicken

Four week old broiler chicken

Five week old broiler chicken

Six week old broiler chicken

Figure 2 Development stages of broiler chicken during two to six weeks in the experiment I

Results

Determination of dry mass

Lactobacillus sp. (H-1) isolated from the sample

The optimal condition of *Lactobacillus* sp. (H-1) isolated from the sample was 3 days of culture, 10% size of inoculum and medium of pH-6 in 3 days of fermentation periods. The results showed that the maximum dried cell weight were obtained 1.38400 g per liter (with nitrogen source) and 0.57917 g per liter (without nitrogen source). The yield of dried mass were an average of 1.0 g per liter (with nitrogen source) and 0.5 g per liter (without nitrogen source). The result of bacterial dry mass were presented in Tables 2 and 3.

Table 2 Dried mass and wet weight of *Lactobacillus* sp. (H-1 with nitrogen source) at optimal condition (3 days of 10 % inoculum)

Batch number in liter	Wet weight in gram	Dry weight in gram	Dry mass%
1	3.29720	1.11490	33.81
2	3.18802	1.08125	33.92
3	4.14500	**1.38400**	33.39
4	3.00018	1.00208	33.40
5	2.95860	0.98863	33.42
6	3.15229	1.05220	33.38
7	2.69095	**0.92850**	34.50
8	2.69895	0.92952	34.44
9	3.42531	1.16625	34.05
10	3.42521	1.14525	33.44
Total	31.98171	10.79258	337.75
Average	3.198171	1.079258	33.775

Table 3 Dried mass and wet weight of *Lactobacillus* sp. (H-1 without nitrogen source) at optimal condition (3 days of 10 % inoculum)

Batch number in liter	Wet weight in gram	Dry weight in gram	Dry mass%
1	1.54237	0.50674	32.85
2	1.73745	**0.57917**	33.33
3	1.55904	0.51125	32.79
4	1.35193	0.46032	34.05
5	1.35109	**0.45990**	34.04
6	1.35278	0.46073	34.06
7	1.67201	0.56629	33.87
8	1.52990	0.50568	33.05
9	1.51603	0.50256	33.15
10	1.65702	0.55242	33.34
Total	15.26962	5.10506	334.53
Average	1.526962	0.510506	33.453

Experiment I

Application of *Lactobacillus* sp. (H-1) strain isolated from sauerkraut as probiotic in chicken feed

Data presented in Tables showed the effect of different levels of dried bacteria *Lactobacillus* sp. (H-1) on body weight gain, feed consumption, feed conversion rate and mortality rate in all treatments. At the end of experiment, mean of body weight (2996.9 g, 3037.3 g, 3074.8 g, 3108.6 g, 2991.7 g, 3005.4 g, 3029.0 g), mean of feed consumption (1050.0 g, 1089.6 g, 1096.8 g, 1123.6 g, 1049.4 g, 1085.4 g, 1087.6 g), mean of feed conversion rate (1.39 %, 1.51 %, 1.41 %, 1.45 %, 1.47 %, 1.44 %, 1.42 %), and mortality rate (4 %, 2 %, 0 %, 0 %, 4 %, 4 %, 2 %) were observed in the group A, B, C, D, E, F and G respectively.

Body weight gain was increased in all group during 0-21 days and 35-42 days. However, body weight gain was not increased during 22-34 days. In this experiment, feed consumption of groups B, C, D, F and G were increased than control (group A) during 2-6 weeks but group E was decreased than control. However, groups E, F and G were lower than groups B, C and D in the result of feed consumption at this experiment. In the 2nd week, the feed conversion ratio (FCR) is not different from that of control (1.57 g for control and groups B, C, D and, 1.66 g, 1.65 g, 1.64 g for groups E, F, G respectively). FCR is lower than that of control in (group E in 3rd week, groups E and F in 4th week, groups B, C, D, E and G in 5th week). In the 6th week, FCR of control (group A) is lower than other groups. The motility rate was much higher in control (group A) and groups E, F 4%, followed by groups B and G which were 2% and finally groups C and D was 0%. In the present study, group D (0.015 % probiotic) showed the best result in the body weight gain, feed consumption and mortality rate. The results were presented in **Tables 4-8** and **Figures 3, 4**.

Table 4 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in two week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	659.6	586.6	512.4	1.57	0
B	680.4	607.2	512.4	1.57	0
C	691.0	617.4	512.4	1.57	0
D	700.8	627.0	512.4	1.57	0
E	656.4	582.8	512.4	1.66	0
F	661.6	588.8	512.4	1.65	0
G	672.0	597.2	512.4	1.64	0

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 5 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in three week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	1215.6	556.0	722.8	1.30	0
B	1242.0	561.6	750.4	1.34	0
C	1269.2	578.2	768.0	1.33	0
D	1287.4	586.6	782.2	1.33	0
E	1212.6	556.2	717.0	1.29	0
F	1219.8	558.2	747.3	1.37	1
G	1228.8	558.6	755.1	1.39	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 6 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in four week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	1744.9	529.3	940.8	1.86	1
B	1771.8	529.8	984.0	1.86	0
C	1802.2	533.0	1022.2	1.92	0
D	1820.6	533.2	1057.2	1.98	0
E	1731.4	518.8	925.0	1.78	0
F	1745.7	529.8	971.8	1.83	1
G	1759.6	530.8	988.6	1.86	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 7 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in five week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	2239.2	494.3	1060.4	2.32	2
B	2269.6	497.8	1057.0	2.12	0
C	2297.8	498.6	1110.0	2.24	0
D	2333.2	512.6	1176.0	2.29	0
E	2232.9	501.5	1039.6	2.23	1
F	2249.2	503.5	1093.3	2.34	2
G	2263.7	504.1	1095.9	2.17	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 8 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in six week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	2996.9	757.7	1050.0	1.39	2
B	3037.3	767.7	1089.6	1.51	1
C	3074.8	775.1	1096.8	1.41	0
D	3108.6	775.4	1123.6	1.45	0
E	2991.7	758.8	1049.4	1.47	2
F	3005.4	766.2	1085.4	1.44	2
G	3029.0	765.3	1087.6	1.42	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

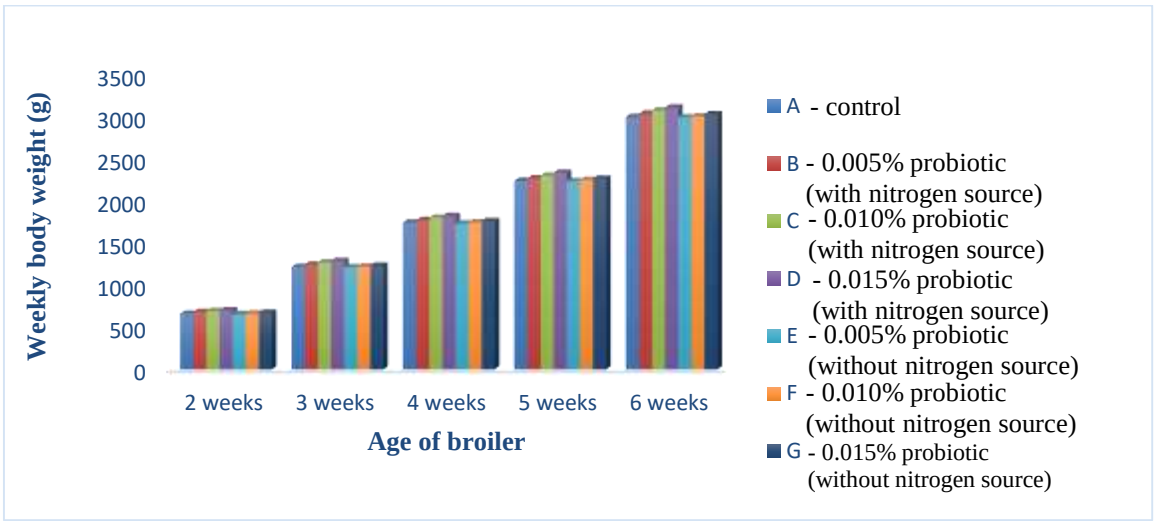

Figure 3 Comparison of weekly body weight in the experiment I

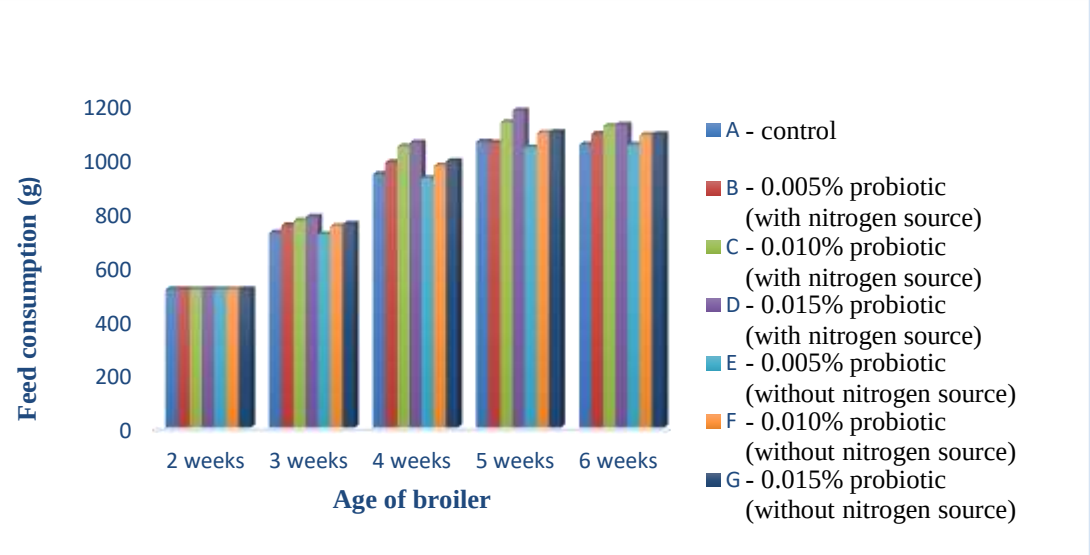

Figure 4 Comparison of weekly feed consumption in the experiment I

Discussion and Conclusion

The optimal condition of *Lactobacillus* sp. (H-1) isolated from the sample was 3 days age of culture, 10% size of inoculum and medium of pH-6 in 3 days of fermentation periods. The results showed that the maximum dried cell weight were obtained 1.38400 g per liter (with nitrogen source) and 0.57917 g per liter (without nitrogen source). The yield of dried mass were an average of 1.00 g per liter (with nitrogen source) and 0.50 g per liter (without nitrogen source).

Hwang *et al.*, 2015 observed that dried cell weight of *Lactobacillus acidophilus* was 1.06 g L^{-1} and 1.61×10^{9} CFU/ml. Rosa *et al.*, 2013 reported that maximum cell yield was achieved a pH value of 6.45 and 1.04 g L^{-1} of inoculum. The data of present research were found to be in agreement with above authors. Salma *et al.*, 2017 observed that *Lactobacillus helveticus* was found optimized at temperature of 40ºC and pH 6.25 which yielded 3.25 g L^{-1} dry cell biomass in MRS medium. According to this literatures, the results of present study were somewhat different.

Dietary probiotic significantly enhanced the feed intake and weight gain in starter phase (0-21 days) only was reported by Cengiz *et al.*, 2015. Body weight gain was increased in all group during 0-21 days and 35-42 days. However, body weight gain was not increased during 22-34 days. This results was also similar to the result of Cengiz *et al.*, 2015. In this experiment, feed consumption of groups B, C, D, F and G were more increased than control (group A) during 2-6 weeks but group E was decreased than control.

However, groups E, F and G were lower than groups B, C and D in the result of feed consumption at this experiment. In the 2nd week, the feed conversion ratio (FCR) is not different from that of control (1.57 g for control and groups B, C, D and, 1.66 g, 1.65 g, 1.64 g for groups E, F, G respectively). FCR is lower than that of control in (group E in 3rd week, groups E and F in 4th week, groups B, C, D, E and G in 5th week). In the 6th week, FCR of control (group A) is lower than other groups. The motility rate was much higher in control (group A) and groups E, F 4%, followed by groups B and G were 2% and finally groups C and D was 0%.

Khin Thu Zar Min, 2011 reported that feed conversion rate was not different between control and treatment groups. Samad *et al.,* 2011 found that feed conversion rate was lower for birds supplemented with probiotics than in control bird but no significant differences were reported between treatment groups. Afsharmanesh and Sadaghi, 2014 stated that some probiotics had no effect on feed intake and feed conversion ratio during the starter phase while feed intake increased during the grower-finisher phase. Therefore, these reported data were similar with this experiment.

Cengiz *et al.,* 2015 stated that the feed intake was reduced, whereas the feed conversion was improved significantly when birds were fed DFM at 0-7 days of age. According to Cengiz *et al*, 2015, the results of feed consumption and feed conversion ratio were somewhat different. In this experiment, body weight gain of groups B, C, D (with nitrogen source) were higher than groups E, F, G (without nitrogen source). Yeo *et al.,* 2018 reported that the yeast extract concentration (ranging between 20 and 30 g/l) enhanced the biomass production and growth rate of *L. helveticus*. Therefore, this reported data of Yeo *et al.,* 2018 was nearly the same with this experiment.

In the present study, probiotic supplementation in broiler feed was effective in improving body weight gain, feed consumption and reduce mortality rate. Results of the present study show that the treatment 0.005 %, 0.010 % and 0.015 % of dried bacteria used as probiotic had higher body weight gain and feed efficiency compared with the control group. The obtained results confirmed the previous finding of Pourakbari *et al.,* 2016. They reported that two hundred one-day-old male chickens were allocated to one of five treatments: control, and the same basal diet supplemented with 0.005%, 0.010%, 0.015% and 0.020% of probiotics. They described that probiotics in feed at 0.010% or higher levels of supplementation improved body weight gain and feed conversion rate compared with the control.

In this study, group D (0.015 % probiotic) showed the best result in the body weight gain, feed consumption and reduce mortality rate. The present result was nearly the same with those reported by Pourakbari *et al*., 2016. The present study reveals that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of growth and reducing the mortality. Therefore, it may be assumed that the application of probiotic in the feeding method of poultry would be safer and effective.

Acknowledgements

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WATER QUALITY AND THIRTY-FIVE SPECIES OF BLUE-GREEN ALGAE FOUND IN TWIN TAUNG LAKE

Zin Mar Soe[1], May Win Soe[2] and Khin San Yi[3]

Abstract

The present study deals with the seasonal variation of algae and water analysis of Twin Taung Lake, Budalin Township, Monywa District, Sagaing Region. Algae and water samples were collected from October, 2018 to September, 2019. Physico-chemical parameters were analyzed in the laboratory of Department of Quality Control, June Pharmaceutical and Food Industry in Sagaing. Statistical analysis was carried out using R Studio software. Totally 35 species of Cyanophyceae were classified and recorded. Among them, *Chroococcus*, *Microcystis*, *Anabaenopsis* occurred throughout the year. Maximum values of temperature, sodium bicarbonate, total hardness and nitrate were found in summer; sodium carbonate, total alkalinity and total chloride were in rainy season and the maximum pH and phosphate occurred in winter. Highest value of salinity was in January and April. Maximum density of *Chroococcus* and *Anabaenopsis* was observed in summer. The growth of *Chroococcus* was positively correlated with salinity and negatively correlated with total alkalinity and phosphate. Growth of *Anabaenopsis* was positively correlated with pH, salinity, total hardness and nitrate. Growth of *Microcystis* was observed in winter and it was significantly correlated with season and positively correlated with pH and total hardness. It may be due to fluctuations of physico-chemical parameters of water in three seasons.

Keywords: Blue-green algae, monthly variation, physico-chemical parameters, Twin Taung Lake.

Introduction

Freshwater algae, also known as phytoplankton, are found in a large range of habitats and vary in shape and color (Anand *et al.* 2011). A great majority of them are truly aquatic and grows in ponds, lakes, puddles, etc. Besides occurring in aquatic habitats, algae are found abundantly on tree, trunks rocks and in association with other plants and animals (Gupta & Pamposh 2014). Algae are frequently occurred in polluted and unpolluted water and due to this point they are generally regarded as indicators to determine the quality of water because water is extremely important and necessary for life (Rajurkar & Dalal 2014).

Algae are playing a vital role in this world and it is the predominant primary producer in any aquatic ecosystems. It is very significant ecologically because they are involving in symbiosis with bacteria in different ecosystems. It supplies food and oxygen for many species in the aquatic environment and it's vitally crucial to maintain CO_2 of carbon cycle via photosynthesis to balance the CO_2 concentration in atmosphere (Ramaraj *et al.* 2010).

Cyanobacteria are a large and morphologically diverse group, which can survive in all kinds of water, with some species living in freshwater while others thrive in brackish water or the marine environment (Malakar & Kalita 2012). Cyanobacteria are an ancient group of prokaryotic microorganisms showing the general features of Gram-negative bacteria. They can be observed in almost all environments, including freshwater, seawater, non-acidic hot springs and deserts. Some cyanobacteria also have the ability to fix atmospheric nitrogen, yet relatively little is known about ecology of natural populations and the diversity of nitrogen-fixing cyanobacteria (Boonkerd *et al.* 2002).

The division Cyanophyta includes about 150 genera and 2000 species. They are found in the most various habitats in freshwater and in the sea, on damp soil and even in such extreme and

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inhospitable places as glaciers, deserts and hot springs. Most, however, thrive in freshwater, blue-green algae are frequently to be found in the phytoplankton of still or slowly flowing freshwaters (Hoek *et al.*1995).

Dynamics in the phytoplankton biomass are the results of the complex interaction of physical, chemical and biological processes. The availability of nutrients influences the range of the phytoplankton. From the past few decades, there is much interest to study various factors influencing the development of phytoplankton in correlation to the physico-chemical characteristics (Sharma & Tiwari 2018).

The FDA (Food Drug Administration) recognized *Spirulina* by the issuance of a GRAS (Generally Recognized as Safe) certificate in 1981. *Spirulina* is legally marketed as food or food supplement without hazard to human health. *Spirulina* is one of the most studied microalgae around the world, due to its high nutritional value and the presence of active biomolecules. *Spirulina* inhabit in natural environment characterized by brackish, alkaline and natroned waters (high concentrated in carbonates and bicarbonates) of the intertropical zone. It grows in warm conditions (28 – 40 ºC), with a high light intensity (Vernès *et al.* 2019). In Myanmar, natural *Spirulina* is produced from the natural lakes of Twin Taung, Twin Ma, Taung Pyauk and Yae Kharr in Sagaing Region. Twin Taung Lake is located in Budalin Township, Twin Ma and Taung Pyauk Lakes are located in Kani Township and Yae Kharr is located in Sagaing Township. After 2014, the blooming of natural *Spirulina* is disappeared at all in Twin Taung Lake. After falling commercial production of *Spirulina* from Twin Taung Lake, there was not any research to know the diversity of algae growing in Twin Taung Lake by previous workers in Myanmar.

Therefore, it was interested to study on algal flora of Twin Taung Lake. The aims of this study were to know which algae occur in which month of a year, to determine which algae grow all the year round and to access the physico-chemical parameters of water in Twin Taung Lake. The objectives of this study were to study the seasonal variation of algae and to evaluate the water quality of Twin Taung Lake.

Materials and Methods

1. Study Area

Twin Taung Lake is situated at Monywa District in Sagaing Region. It is about 9.66 km from east west of Budalin Township. The surface area of lake is 89.4 hactares; 1040 m from east to west, 1150 m from north to south and the depth of lake is about 51.21 m at the deepest place. It is situated between North Latitudes 22° 20' 56.38" and 22° 22' 44.22", between East longitude 95° 0' 24.28" and 95° 2' 12.66", 82 m of elevation as shown in Figure 1.

2. Sampling Sites

Eleven sampling sites were chosen as shown in Figure 2. Among these, the fresh water from Chindwin River is leaking into the lake at Moemalal (site 10) and Yaenatgyi (site 11).

3. Collection and Classification of the Algal Specimens

Algae specimens were collected monthly during October 2018 to September 2019, by using plankton net. The collected specimens were identified up to specific level based on their morphological characters by referring on Desikachary (1959), Prescott (1962), Komárek & Anagnostidis (2005), John *et al*. (2011) and Komárek (2013).

4. Counting the Number of the Cells and Relative Abundance Percent of Algae

The number of algae belonging to different genera were determined and counted under the microscope using a haemacytometer and are calculated with the following formula used by Lavens and Sorgeloos (1996) and then calculated by relative abundance (%).

Number of cell mL^{-1} = $(n_1 + n_2) / (2 + 80) + 80 + 10^3 + d$

= $(n_1 + n_2) / 2 + 10^3 + d$

n_1 = number of cells counted in upper rafter

n_2 = number of cells counted in lower rafter

d = dilution factor

For greater accuracy make 3 duplicate counts

The relative abundance (%) of a particular algae type was calculated by employing the following formula:

$$\text{Relative abundance (\%)} = \frac{Y}{X} \times 100$$

Where,

X = total number of samples collected

Y = number of samples from which a particular algae type was isolated

5. Collection of Water Samples and Analysis of Water Quality

Water samples of all sampling sites were collected monthly from the different layers-: surface, 1.52 m and 3.05 m intervals of the water column by using water sampler. Physico-chemical parameters (i.e. salinity, hardness, alkalinity, sodium carbonate, sodium bicarbonate, chloride, nitrate and phosphate) of water samples were analysed at laboratory of Department of Quality Control, June Pharmaceutical and Food Industry of Sagaing. Temperature and pH of water were measured by thermometer and pH meter in the field.

6. Statistical analysis

Multiple regressive analyses were performed to determine the relationship of algae with physico-chemical parameters. Statistical analysis was carried out using R Studio software.

Figure 1 Location Map of Budalin Township, Monywa District, Saging Region

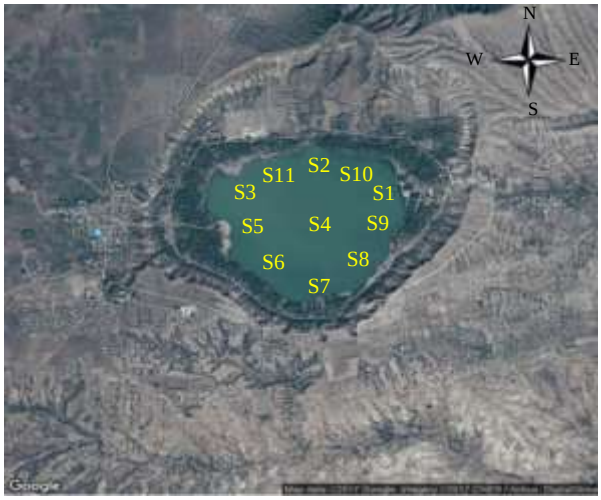

Figure 2 Map showing the collection sites

S1 (sampling site 1) = North-east part of lake, S2 = North part of lake,
S3 = North-west part of lake, S4 = Middle part of lake, S5 = West part of lake, S6 = South-west part of lake, S7 = South part of lake, S8 = South-east part of lake, S9 = East part of lake, S10 = Moemalal and S11 = Yaenatgyi.

Results

Totally 35 species belonging to 11 genera, 9 families and 5 orders of the class Cyanophyceae was classified, described, and recorded. The list of algae species was as shown in Table 1.

1. Monthly variation of algae in all sampling sites

Although microalgae belonging to class Cyanophyceae occurred throughout the year the species of *Chroococcus, Microcystis* and *Anabaenopsis* were observed monthly (Figure 3). The highest growth of *Chroococcus* was in April and the lowest in December. The highest growth of *Microcystis* was found in November and lowest in August. The maximum growth of *Anabaenopsis* occurred in early summer month of February and lowest in April.

According to statistical analysis, the growth of *Chroococcus* was positively correlated with salinity and negatively correlated with total alkalinity and phosphate (Table 2). The growth of *Microcystis* was observed in winter and positively correlated with pH and total hardness (Table 3). The growth of *Anabaenopsis* was positively correlated with pH, salinity, total hardness and nitrate (Table 4).

Table1 List of 35 species of blue-green algae observed in Twin Taung Lake

No.	Species Names	No.	Species Names
1.	*Aphanocapsa grevillei* (Berkeley) Rabenhorst	20.	*Phormidium chalybeum* (Merterns ex Gomont) Anagnostidis & Komarek
2.	*A. rivularis* (Carmichael) Rabenhorst	21.	*P. tenue* Gomont
3.	*Chroococcus globosus* (Elenkin) Hindak	22.	*Arthrospira argentina* (Frenguelli) Guarrera & Kuhvemann
4.	*C. minimus* (Keissler) Lemmermann	23.	*A. massartii* Kuffareth
5.	*C. tenax* (Kirchner) Hieronymus	24.	*A. platensis* Gomont
6.	*C. turgidus* (Kutzing) Nageli	25.	*Spirulina laxissima* forma *major* Desikachary
7.	*C. turgidus* var. *maximus* Nygaard	26.	*S. maior* Kutzing ex Gomont
8.	*Merismopedia minima* G. Beck	27.	*S. subsalsa* Oersted ex Gomont
9.	*M. punctata* Meyen	28.	*Borzia periklei* Anagnostidis in Anagnostidis & Komarek
10.	*Microcystis aeruginosa* (Kutzing) Kutzing	29.	*Anabaena minispora* M. Watanabe
11.	*M. flos-aquae* (Wittrock) Kirchner	30.	*Anabaenopsis arnoldii* Aptekar
12.	*M. marginata* (Meneghini) Kutzing	31.	*A. arnoldii* var. *indica* Ramanathan
13.	*M. protocystis* W. B.Crow	32.	*A. circularis* var. *javanica* Woloszynska
14.	*M. viridis* (A. Braun) Lemmermann	33.	*A. magna* Evans
15.	*Oscillatoria acuminate* Gomont	34.	*A. milleri* Woronichin
16.	*O. acuta* Bruhl *et* Biswas, Geitler	35.	*A. tanganyikae* (G. S. West) Woloszynska & V. V. Miller
17.	*O. euboeica* Anagnostidis		
18.	*O. meslinii* Fremy		
19.	*O. princeps* Vancher ex Gomont		

2. Physico-chemical parameters of water samples in all sampling sites

The result on the variation of physico-chemical parameters at monthly intervals as shown in Figure 4 – 8. The range of temperature was observed from 28.4 ºC to 35.2 ºC. The highest was in April and the lowest in December. The value of pH ranged from 9.6 to 9.9 and the highest value was in December and the lowest in March, April, July, August and September. The highest salinity value was 4‰ and it was in January and April (late winter and summer). The value of sodium carbonate (Na_2CO_3) was ranging from 878 – 1143 mg/L, the maximum value was June and the minimum value was in October. The value of sodium bicarbonate ($NaHCO_3$) was ranging from 1600 – 1932 mg/L, the maximum value was April and the minimum value was in December. The highest value of total alkalinity was 2984 mg/L in June and the lowest value 2614 mg/L was in December. The value of total chloride was highest in September and it was 302 mg/L and the lowest value 135 mg/L in October was gradually increased up to May with the value of 194 mg/L. The peak result of total hardness was 275 mg/L in February and the lowest one was 226 mg/L in January. The highest nitrate level was detectable in March 33.6 mg/L and revealed a wide variation and the lowest value was 11.1 mg/L in November. The highest phosphate level was measured in December and it was 1.57 mg/L and did not show much variation in other months during the study period.

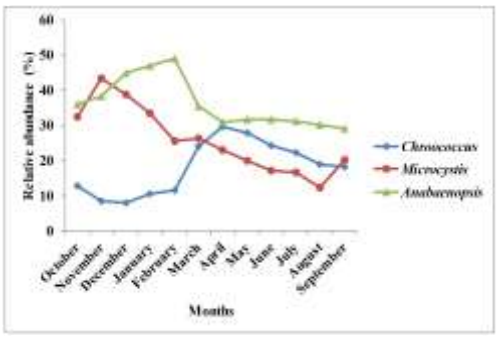

Figure 3 Monthly variation of the population of genus *Chroococcus*, *Microcystis* and *Anabaenopsis* as in one unit

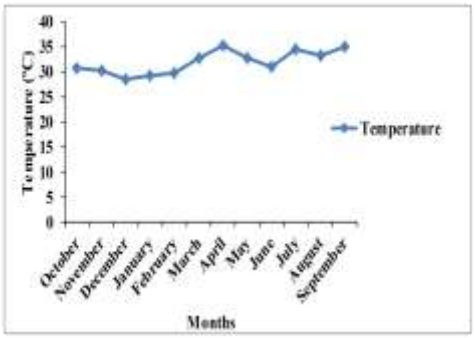

Figure 4 Monthly variation temperature (ºC) of water in Twin Taung Lake

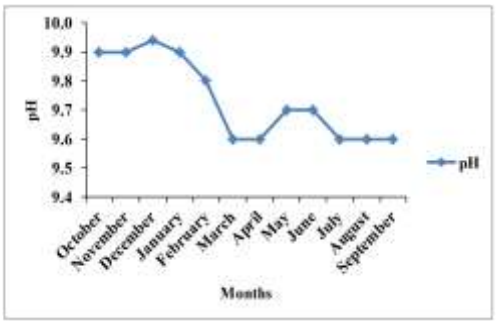

Figure 5 Monthly variation pH of water in Twin Taung Lake

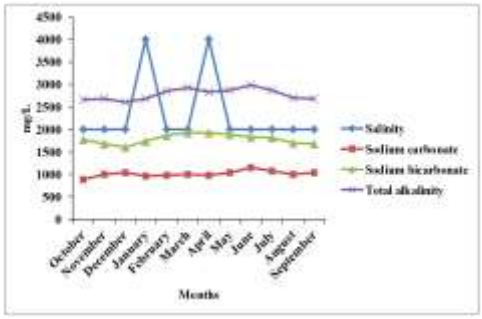

Figure 6 Monthly variation of salinity, sodium carbonate, sodium bicarbonate and total alkalinity of water in Twin Taung Lake

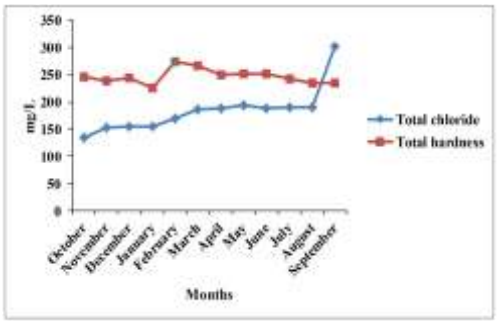

Figure 7 Monthly variation of total chloride and total hardness of water in Twin Taung Lake

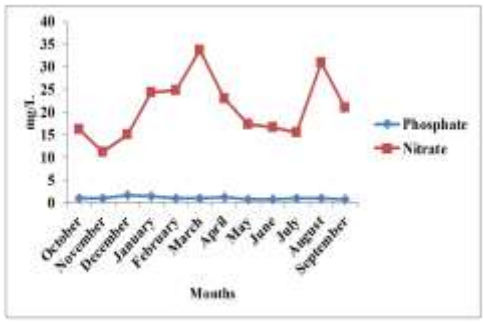

Figure 8 Monthly variation of phosphate and nitrate of water in Twin Taung Lake

Table 3 Regression analysis result of genus *Chroococcus* and environmental variables

Coefficient:	Estimate	Std. Error	t value
(Intercept)	47.16295	36.43983	1.294
Temperature	-0.01724	0.04683	-0.368
pH	-4.93420	3.68214	-1.34
Salinity	0.67014	0.33659	1.991*
Na_2CO_3	0.00334	0.00333	1.003
$NaHCO_3$	0.00311	0.00294	1.058
Alkalinity	-0.00416	0.00195	-2.13*
Chloride	-0.00563	0.00633	-0.889
Hardness	0.03954	0.02727	1.450
NO_3	-0.00925	0.02082	-0.444
PO_4	-1.13773	0.35014	-3.249**
factor (Season) Summer	0.39643	0.70456	0.563
factor (Season) Winter	-0.23136	1.3045	-0.177

*Significance at 0.05 level
**Significance at 0.01 level

Table 4 Regression analysis result of genus *Microcystis* and environmental variables

Coefficient:	Estimate	Std. Error	t value
(Intercept)	-1.22E+02	4.71E+01	-2.584*
Temperature	8.47E-02	6.05E-02	1.399
pH	9.91E+00	4.76E+00	2.083*
Salinity	1.22E-01	4.35E-01	0.28
Na_2CO_3	3.09E-03	4.30E-03	0.719
$NaHCO_3$	2.16E-03	3.80E-03	0.569
Alkalinity	-2.50E-04	2.52E-03	-0.099
Chloride	6.99E-03	8.18E-03	0.854
Hardness	7.62E-02	3.52E-02	2.163 *
NO_3	1.42E-02	2.69E-02	0.529
PO_4	-2.09E-01	4.52E-01	-0.461
factor (Season) Summer	2.13E+00	9.10E-01	2.336*
factor (Season) Winter	7.22E+00	1.69E+00	4.283***

*Significance at 0.05 level
***Significance at 0.001 level

Table 5 Regression analysis result of genus *Anabaenopsis* and environmental variables

Coefficient:	Estimate	Std. Error	t value
(Intercept)	-3.75E+02	5.76E+01	-6.499 ***
Temperature	1.12E-01	7.41E-02	1.517
pH	3.61E+01	5.82E+00	6.201 ***
Salinity	1.47E+00	5.32E-01	2.757 **
Na_2CO_3	-3.52E-03	5.26E-03	-0.669
$NaHCO_3$	-5.00E-03	4.65E-03	-1.075
Alkalinity	6.41E-04	3.09E-03	0.208
Chloride	4.03E-03	1.00E-02	0.403
Hardness	1.38E-01	4.31E-02	3.196 **
NO_3	1.10E-01	3.29E-02	3.327 **
PO_4	-3.50E-02	5.54E-01	-0.063
factor (Season) Summer	2.77E-01	1.11E+00	0.248
factor (Season) Winter	-2.18E+00	2.06E+00	-1.057

**Significance at 0.01 level
***Significance at 0.001 level

Discussion and Conclusion

Totally 35 species of Cyanophyceae collected from Twin Taung Lake during 2018 – 2019 were recorded in the present study. In lake Twin Taung, the maximum population of Cyanophyceae occurred in winter and early summer season and the minimum population was found in rainy season. This finding agreed with Agale *et al.* (2013).

During the study period, the relative abundance (%) of 3 genera-: *Chroococcus, Microcystis* and *Anabaenopsis* of Cyanophyceae were more dominant than the others and they were observed throughout the year. Among them, *Anabaenopsis* was observed as the highest relative abundance (%) and followed by *Microcystis* and *Chroococcus*.

In the present study, the highest growth of *Chroococcus* was observed in April (summer) and the lowest was observed in December (winter). In April, the highest value of temperature and sodium bicarbonate occurred and the lowest value was observed in December. Similar observation was in agreements with Sharma & Tiwari (2018).

The highest growth of *Microcystis* was observed in November (winter) and the lowest content of nitrate was observed in this month. The high growth of this genus occurred in high pH (9.9) of lake water this area. This finding agreed with Imai *et al*. (2009).

During the study period, the maximum value of *Anabaenopsis* occurred in early summer month of February and the highest content of total hardness was observed in this month. In late summer the growth of this genus was decreased up to September. This observation was agreed with Aguilera *et al.* (2016).

During October 2018 to September 2019, the lowest temperature was 28.4 ºC in December and the highest was in April (35.2ºC). pH of lake water was observed in the range of 9.6 to 9.9 indicating alkalinity throughout the period of this study. Generally, high level of pH of water promotes the growth of algae. In the present study, higher values of pH were recorded in winter and lower during rainy season. This finding agreed with Suresh (2013) who stated that the higher values of pH were recorded in winter and lower during the monsoon.

The highest value of salinity was 4‰ in January (late winter) and April (summer), 2‰ in other months and not constant. These findings were similar to Singh (2015). The value of sodium carbonate (Na_2CO_3) was ranging from 878 – 1143 mg/L, the maximum value was in June and the minimum was in October. The value of sodium bicarbonate ($NaHCO_3$) was ranging from 1600 – 1932 mg/L, the maximum value was in April and the minimum was in December. These observations were similar to Sahni & Yadav (2012).

Total alkalinity value was between 2614 mg/L and 2984 mg/L. The highest concentration of total alkalinity was recorded in rainy and summer. Low concentration of total alkalinity was recorded in winter. This finding agreed with Dorche *et al.* (2018) and Jyotsna *et al*. (2014). The value of chloride was 302 mg/L highest in September, the lowest was 135 mg/L in October and it was gradually increased up to September. The growth of algae observed throughout of the year was not in high growth in September. Perrotte (2008) stated that the chloride salts, when used cause the decrease in population of thousands of organisms, especially algae.

The highest value of total hardness was observed in early summer (February) and the lowest was in late winter. This finding agreed with Sahni & Yadav (2012) and Agale *et al.* (2013). The highest nitrate level (33.6 mg/L) was detectable in March and the lowest one was 11.1 mg/L in November revealed a wide variation. The highest phosphate level was measured in December and it was 1.57 mg/L and did not show much variation in other months during the study period. These findings agreed with Sahni & Yadav (2012).

It may be concluded that as in the seasonal variation, maximum algal density of *Chroococcus* and *Anabaenopsis* was recorded in summer due to high temperature and rich in nutrients in water and high the rate of photosynthesis in summer months. The low density of phytoplankton in rainy may be due to heavy flood water inflow and they were resumed again due to dilution. And then this may be due to the fluctuation of physico-chemical parameters of the water body in Lake Twin Taung.

According to statistical analysis, the growth of *Chroococcus* was positively correlated with salinity and negatively correlated with total alkalinity and phosphate. The growth of *Microcystis* was observed in winter and it was significantly correlated with the season and positively correlated with pH and total hardness. The growth of *Anabaenopsis* was positively correlated with pH, salinity, total hardness and nitrate.

This research is beneficial for country. After 2014, the production rate of natural *Spirulina* was decreased and incomes of national government fall down due to disappearance of beneficial algae, therefore it will be done for further study from the phycological and liminological point of view.

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EFFECT OF THE WATER REQUIREMENTS ON *SOLANUM LYCOPERSICUM* L. DURING RAINING SEASON

Zin Moe Moe[1], Ohn Mau[2], Tingn Tin Maw[3]

Abstract

This experiment was conducted to evaluate the effect of amount of water on the vegetative and reproductive growth of tomato and to assess the suitable irrigation to improve the economic efficiency of tomato production. Present investigation was carried out during April to July, 2019 at Oakshitpin Village, Padaung Township, Bago Region to observe the effects of variable water of tomato crop. The four treatments of water requirements were used. This experiment was treated with T_1 (0.05-0.27 L), T_2 (0.21 - 2.07 L), T_3 (0.36 - 2.22 L) and T_4 (0.50-2.37 L). Each treatment had five replications set up in Randomized Completely Block Design (RCRD). Tomato was evaluated in farmer's field with farmer's participation under plastic house condition for yield potential and other yield characters using the effect of amount of water during rainy season. The germination rate of *Solanum lycopersicum* L. was 75.60 %. Vegetative growth such as plant height (17.21 cm), number of leaves per plant (17.97), leaf length (12.22 cm) and single leaf area (78.95 cm^2) were the best in T_2 (0.21 - 2.07 L). Reproductive growth as earliest first flowering days (36 DAT), number of clusters per plant (7), number of fruits per cluster (6.9), single fruit diameter (5.01 cm), fruits weight per plant (282.10 g) and fruits yield (4798.74 kg ha^{-1}) were highest in T_2 (0.21 - 2.07 L). T_2 (0.21 - 2.07 L) could be recommended for commercial production under plastic house condition during rainy season.

Keywords: water requirements, tomato, RCBD

Introduction

Tomato is originated from Western South America and it was introduced into European gardens in the early sixteenth century. Tomato is a member of the Solanaceae family or night shade family (Wien, 1997). Growing tomatoes in greenhouses allows producers to grow plants at a time when it would be impossible to grow outside because of the weather. Tomatoes prefer well drained soil because they are sensitive to water logging. Water should be given in proper amount and accurate time application. Therefore, water management is a key to avoid plant moisture stress during the crop growth stages (Priyanka *et al*., 2015).

Tomato seedling of 7.5 to 10 cm in height is ready for transplanting or 4-5 weeks old or when it has attained 5-6 leaves and irrigates well before and during transplanting. Growth of all plants can be divided into three stages with regard to watering practice: vegetative, flowering, and fruiting (Hansen *et al.*, 1982).

In general, most of the crops show that timely irrigations (watering) are more important than total number of irrigations (Allen *et al.*, 1998). In the growing period of tomato, daily mean air temperature varied between 18.5 and 27.7°C with overall mean air temperature was 22.8°C and the daily minimum and maximum temperatures were 8.5 and 33.3°C, respectively. During the study period, a meager 3.6 mm rainfall was received (Kumar *et al*., 2015). Tomato can be produced both as rainy season crops in the highlands and as cool season vegetables in lowland areas (Yu Yu Tun and Aung Phyo, 2019).

Tomato can be consumed as raw or as an ingredient in many dishes, sauces, salads, and drinks. Factors influencing the considerable increase in tomato consumption include consumer awareness of benefits such as preventing cancer and chronic diseases. This beneficial effect is due

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to the action of antioxidant compounds, which reduce oxidative damage in the body. Tomatoes are not as sweet due to its lower sugar content then other edible fruits. Tomatoes are low in calories and a good source of vitamins A and C, the flavor and texture (Ray *et al*., 2016).

The sustainable use of water in agriculture has become a major concern. The adoption of strategies for saving irrigation water and maintaining acceptable yields may contribute to the preservation of this ever more restricted resource. Irrigation water quality can affect soil fertility and irrigation system performance as well as crop yield and soil physical conditions. Therefore, knowledge of irrigation water quality is critical in understanding the management changes that are necessary for long-term productivity. Tomato is one of the most important vegetable crops and is one of the most demanding in terms of water use (Mahmud *et al*., 2012). Insufficient water at any growth stage will reduce yield and fruit quality. Tomato grows well in moist but not soggy soil, and well-timed furrow or drip irrigation is effective (Ramathani *et al*., 2018).

The aims of the study were to introduce fruit production of tomato in rainy season and the objectives were to study the growing of tomato in the plastic house, to record the water requirements of amount, accurate time application on growth stages and to evaluate growth and yield of tomato under different water supply conditions.

Materials and Methods

Experimental Site

The experiment of water requirements for *Solanum lycopersicum* L. was conducted Oakshitpin Village, Padaung Township, Bago Region, during April to July, 2019.

Planting material used

Tomato seeds were used as planting material for this research. These tomato seeds (var: kyar chay) were purchased from the Marlarmyaing Agricultural shop.

Germination test

The seeds of tomato, *Solanum lycopersicum* L. (Kyar Chay), were germinated in the prepared soil (2 soil: 1 sand). The numbers of germinating seeds were daily recorded. The germination rate was calculated using the method of Soupe (2009).

$$\text{Germination rate (\%)} = \frac{\text{Total number of germinated plants}}{\text{Total number of sown seeds}} \text{ x } 100$$

Analysis of soil sample

The soil samples were collected from the experimental field in the depth of 30 cm. The collected soil samples were analyzed in the soil laboratory, Land Use Division, Department of Agriculture, Yangon Region.

Soil Preparation

Firstly the soil of the experimental area was cleaned the wastes. Then the soil from the field was mixed with paddy rice char in the ratio of 5:1. The prepared soil was sprayed water with thoroughly mix. The soil mix was covered with plastic sheet for one week. Twenty four kilogram of soil mix was put into a polyethylene bag; it was contained nine parts of soil and one part of cow dung which was the basal fertilizer treatment.

Cultural management practices

Five weeks after germination, the seedlings with the high or 5-7 cm containing 2-3 leaves were transplanted into the prepared soil medium of polyethylene bag. After transplanting the individual tomato plant was watered with the same amount of water to protect from the transplanting shock. The inorganic compound fertilizer (NPK 15-15-15) was applied at the vegetative growth stages (1WAT) and at early developmental stages (6 WAT). Water treatment was started one week after transplanting. In the treatment T_1 (0.05 – 0.27 L), T_2 (0.21 - 2.07 L), T_3 (0.36 - 2.22 L) and T_4 (0.50 – 2.37 L) were given per day to the assigned plants. The control plants were watered as the amount of water used by (0.05 – 0.27 L) the local farmers. The spraying of tawnid pesticide and weeding were carried out when necessary (Table 1).

Table 1 Weekly water treatments on growing of *Solanum lycopersicum* L. in the plastic house

Treatments	Weekly water treatment (Liter)							
	Vegetative stage				Early developmental stage			Late developmental stage
	1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8-10 WAT
T_1	0.05	0.08	0.10	0.13	0.15	0.20	0.24	0.27
T_2	0.21	0.36	0.47	0.62	0.83	1.33	1.86	2.07
T_3	0.36	0.50	0.62	0.77	0.98	1.48	2.01	2.22
T_4	0.50	0.65	0.77	0.92	1.12	1.63	2.16	2.37

WAT = weeks after transplanting, Lewis, 2014

Experimental layout

There were four treatments, each with five replications were out in Randomized Complete Block Design (RCBD) which was inside the plastic house. L x W x H (65268000 cm^3) the spacing between the plants and row were 60 cm x 60 cm. The total experimental area was 683100 cm^2 (Figure 1).

Meteorological data

The meteorological data such as temperature, rainfall and humidity were recorded from Meteorological Department, Pyay Township, Bago Region.

Data collection

The vegetative growth such as plant height, petiole length, number of leaves per plant, single leaf width, single leaf length and single leaf area in this experiment and reproductive growth such as first flowering days, fruits per cluster, clusters per plant, single fruit diameter, fruits weight per plant and fruit yield were collected in this experiment using IRRISTAT software.

Single leaf area

The single leaf area (cm^2) was calculated at flowering using leaf length and leaf width measurements following the formulae as follows;

$$\text{Leaf area} = K \times L \times W,$$

K = constant coefficient, L = leaf length, W = leaf width (Bertin, 1993)

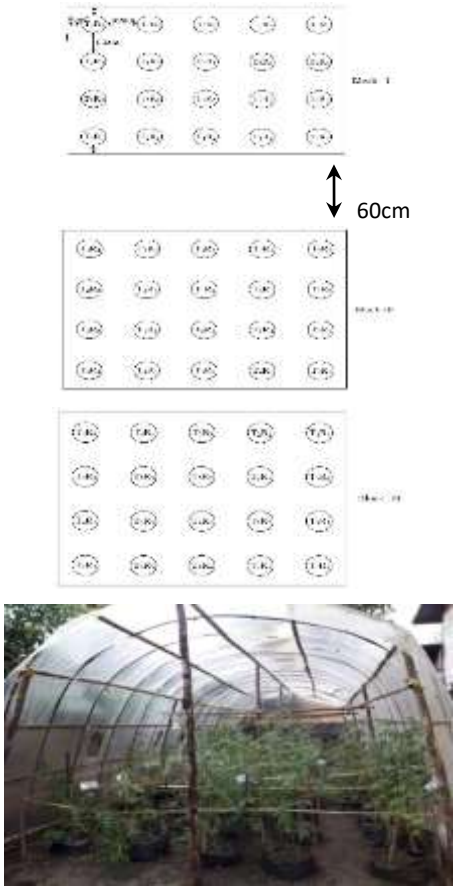

Figure 1 Growing of tomato in RCBD with four treatments and five replications

Results

Soil analysis

The pre planting results of soil laboratory test indicated that the soil in the study site is Oakshitpin Village, Padaung Township, Bago Region, texturally sandy loam, low in nitrogen content. The sample analysis further indicated that the experimental soils have high in potassium and K_2O, very high in phosphorus and neutral in pH (Table 2).

Table 2 Physical and chemical characteristics of experimental area

Parameters	Analyzed results	Rating
pH (soil : water, 1:2.5)	6.59	Neutral
Total N %	0.16	Low
Exchangable K (meq/100 gm)	0.47	High
Available Nutrients, P, ppm (Bray)	50.71	Very high
Available Nutrients, K_2O (mg/100gm)	21.91	High
Moisture (%)	1.39	-
Texture	Sandy loam	-
Sand (%)	67.12	-
Silt (%)	17.00	-
Clay (%)	15.88	-

meq = milliequilivalent, ppm = parts per million, Bray = Bray method

Measurement of temperature, rainfall and humidity

Everyday weather data was recorded from Department of Meteorology and Hydrology, Pyay Township, Bago Region (Table 3).

Table 3 Temperature and rainfall data of Pyay Township in May, June, July 2019

Date	Mean temp (°C)	Mean Rain fall	Mean humidity
May, 2019	31.92	2.00	65.43
June, 2019	29.56	8.73	80.30
July, 2019	29.91	9.10	85.19
Total	30.46	6.61	230.92

Germination test of *Solanum lycopersicon* L.

Two hundred tomato seeds cv. kyar chay were tested in the tray. Among 40 seeds in each plot, plot 1 had 31 germinated plants; plot 2 had numbers of germinated plant 30, plot 3, 33 germinated plant and plot 4, 27 germinated plants, respectively. Therefore germination rate is 75.63 % and the result showed that the germination rate was 75.63 % and thus tomato seeds cv. kyar chay were chosen as planting materials (Table 4).

Table 4 Germination rate of *Solanum lycopersicum* L.

Plot	No. of sown seeds	Germinated	Germination %
1	40	31	77.50
2	40	30	75.00
3	40	32	80.00
4	40	28	70.00
Total	160	121	**75.63**

Vegetative Growth

Plant height

The result of the plant height response to different watering levels on growth stages showed that T_2 (0.21 L – 2.07 L) had the longest height 17.21 cm followed by T_4 (0.50 L – 2.37 L) 17.07 cm, T_1 (0.05 L- 0.27 L) had 16.32 cm and T_3 (0.36 L – 2.22 L) 16.17 cm respectively (Table 5 and Figure 2).

Petiole length

The result of the petiole length response to different watering levels on growth stages showed that T_3 (0.36 L – 2.22 L) had the longest length 5.30 cm followed by T_2 (0.21 L – 2.07 L) 5.06 cm, T_4 (0.50 L – 2.37 L) 4.90 cm and T_1 (0.05 L- 0.27 L) had 4.80 cm respectively (Table 5 and Figure 2).

Number of leaves per plant

The results of the number of leaves per plant response to different watering levels on growth stages revealed that T_2 (0.21 L – 2.07 L) had the most numbers of leaves 17.97. The second and third highest leaf number were observed in T_1 (0.05 L- 0.27 L) 17.24 and T_3 (0.36 L – 2.22 L) 16.42 and the fourth was T_4 (0.50 L – 2.37 L) 15.92 (Table 5).

Leaf length

The results of the leaf length among the treatments showed that T_2 (0.21 L – 2.07 L) had highest leaf length 12.22 cm. It was followed by T_1 (0.05 L- 0.27 L) 10.93 cm, T_3 (0.36 L – 2.22 L) 10.91 cm and T_4 (0.50 L – 2.37 L) had least leaf length of 9.97 cm respectively (Table 5 and Figure 2).

Leaf width

Single leaf width among the treatments gave that T_4 (0.50 L – 2.37 L) was highest leaf width 9.62 cm. It was followed by T_3 (0.36 L – 2.22 L) 8.99 cm, T_1 (0.05 L- 0.27 L) 8.89 cm and T_2 (0.21 L – 2.07 L) had least leaf width of 8.80 cm respectively (Table 5 and Figure 2).

Single leaf area

The single leaf area among the treatments showed that T_2 (0.21 L – 2.07 L) had the highest single leaf area 78.95 cm^2. It was followed by T_3 (0.36 L – 2.22 L) 73.53 cm^2, then T_4 (0.50 L – 2.37 L) 71.76 cm^2 respectively. T_1 (0.05 L- 0.27 L) was the least result 71.53 cm^2 among treatments (Table 5).

The summarized results of vegetative growth expressed the effect of different watering levels on growth stages that the highest plant height was 17.21 cm, T_2 (0.21 L – 2.07 L), the longest petiole length, 5.30 cm, T_3 (0.36 L – 2.22 L), the most number of leaves per plant 17.97, T_2 (0.21 L – 2.07 L), the maximum leaf length 12.22 cm, T_2 (0.21 L – 2.07 L), the broadest leaf width 9.62 cm T_4 (0.50 L – 2.37 L) and the largest single leaf area 78.95 cm^2 T_2 (0.21 L – 2.07 L) respectively (Table 5).

Table 5 Summarized data of vegetative growth of *Solanum lycopersicum* L. responsed to different water treatments

Water treatments	Plant height (cm)	Petiole length (cm)	Number of leaves per plant	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
T_1 (0.05 L- 0.27 L)	16.32	4.80	17.24	10.93	8.89	71.53
T_2 (0.21 L – 2.07 L)	**17.21**	5.06	**17.97**	**12.22**	8.80	**78.95**
T_3 (0.36 L – 2.22 L)	16.17	**5.30**	16.42	10.91	8.99	73.53
T_4 (0.50 L – 2.37	17.07	4.90	15.92	9.97	9.62	71.76

(a) Plant height (b) Petiole length (c) Leaf length (d) Leaf width

Figure 2 Vegetative growth of *Solanum lycopersicum* L.

Reproductive Growth

First flowering days

The mean number of the earliest first flowering days is 36 (DAT) days after transplanting in T_2 (0.21 L – 2.07 L) followed by T_3 (0.36 L – 2.22 L) and T_4 (0.50 L – 2.37 L) 39 DAT and then 40 DAT in T_1 (0.05 L- 0.27 L) respectively. The statistical results of these experiments were significant (Table 6 and Figure 3).

Table 6 First flowering days of *Solanum lycopersicum* L. responsed to different water treatments

Water treatments	First flowering days (DAT)
T_1 (0.05 L- 0.27 L)	40
T_2 (0.21 L – 2.07 L)	**36**
T_3 (0.36 L – 2.22 L)	39
T_4 (0.50 L – 2.37 L)	39
F test	*
CV%	0.36
5%LSD	1.6

Number of clusters per plant

Compared the mean value of number of clusters per plant among the treatments showed that T_2 (0.21 L – 2.07 L) had the highest number 7, followed by T_3 (0.36 L – 2.22 L) 5.2, T_4 (0.50 L – 2.37 L) 5 and finally T_1 (0.05 L- 0.27 L) 4.5 respectively. The statistical results of these numbers of clusters per plant were significant (Table 7).

Table 7 Number of clusters per plant of *Solanum lycopersicum L.* responsed to different water treatments

Water treatments	Number of clusters plant^{-1}
T_1 (0.05 L- 0.27 L)	4.5
T_2 (0.21 L – 2.07 L)	7
T_3 (0.36 L – 2.22 L)	5.2
T_4 (0.50 L – 2.37 L)	5
F test	*
CV%	2.0
5%LSD	0.70

Number of fruits per cluster

The fruits per cluster of tomato plant had the highest 6.9 T_2 (0.21 L – 2.07 L) followed by 5.4 (T_3 0.36 L– 2.22 L), 5 T_4 (0.50 L – 2.37 L) and 3.5 T_1 (0.05 L- 0.27 L) respectively. According to the statistical analysis showed that all data were significant (Table 8).

Table 8 Number of fruits per cluster of *Solanum lycopersicum L.* responsed to different water treatments

Water treatments	Number of fruits cluster^{-1}
T_1 (0.05 L- 0.27 L)	3.5
T_2 (0.21 L – 2.07 L)	6.9
T_3 (0.36 L – 2.22 L)	5.4
T_4 (0.50 L – 2.37 L)	5
F test	*
CV%	2.5
5%LSD	0.20

Single fruit diameter and Fruits weight per plant

The results of the single fruit diameter response to different watering levels on growth stages showed that largest single fruit diameter had T_2 (0.21 L – 2.07 L) 5.01cm, followed by T_3 (0.36 L – 2.22 L) 4.56 cm and then T_1 (0.05 L- 0.27 L) 4.21 cm. The least diameter was T_4 (0.50 L – 2.37 L) 4.10 cm. According to statistical analysis, all recorded data of single fruit diameter were significant (Table 9 and Figure 3).

The result of the fruits weight per plant among treatment observed that T_2 (0.21 L – 2.07 L) had the largest weight 282.10 g, followed by T_3 (0.36 L – 2.22 L) 213.3 g, then T_4 (0.50 L – 2.37 L) 203.6 g and the least weight was 151.40 g in T_1 (0.05 L- 0.27 L) respectively. According to the statistical analysis, four treatments were significant (Table 9 and Figure 3).

Table 9 Single fruit diameter per plant and Fruits weight per plant of *Solanum lycopersicum L.* responsed to different water treatments

Water treatments	Single fruit diameter	Fruits weight plant^{-1} (g)
T_1 (0.05 L- 0.27 L)	4.21	151.40
T_2 (0.21 L – 2.07 L)	5.01	282.10
T_3 (0.36 L – 2.22 L)	4.56	213.3
T_4 (0.50 L – 2.37 L)	4.10	203.6
F test	*	*
CV%	3.6	14.70
5%LSD	0.28	3.22

Fruits yield

The fruits yield of T_2 (0.21 L – 2.07 L) had the highest 4798.74 kg ha^{-1} and T_3 (0.36 L – 2.22 L) 3628.40 kg ha^{-1}, followed by T_4 (0.50 L – 2.37 L) 3463.40 kg ha^{-1} and then, T_1 (0.05 L- 0.27 L) 2575.43 kg ha^{-1} respectively. The statistical analysis showed that the four treatments were significant (Table 10)

The summarized results of reproductive growth stated the effects of different watering levels on growth stages that the earliest first flowering days 36 DAT, the maximum number of cluster per plant 7, the highest number of fruits per cluster 6.9, the largest single fruit diameter 5.01 cm, the biggest fruits weight per plant 282.10 g and the fruits yield 4798.74 kg ha^{-1} respectively were observed in T_2 (0.21 L – 2.07 L) (Table 11 and Figure 3).

Table 10 Fruits yield of *Solanum lycopersicum* L. responsed to different water treatments

Water treatments	Fruits yield (kg ha^{-1})
T_1 (0.05 L- 0.27 L)	2575.43
T_2 (0.21 L – 2.07 L)	4798.74
T_3 (0.36 L – 2.22 L)	3628.40
T_4 (0.50 L – 2.37 L)	3463.40
F test	*
CV%	0.46
5%LSD	11.7

Table 11 Summarized data of reproductive growth of *Solanum lycopersicum* L. responsed to different water treatments

Treatments	First flowering days (DAT)	Number of clusters plant^{-1}	Number of fruits cluster^{-1}	Single fruit diameter (cm)	Fruits weight per plant (g)	Fruits yield (kg ha^{-1})
T_1 (0.05 L- 0.27 L)	40	4.5	3.5	4.21	151.40	2575.43
T_2 (0.21 L – 2.07 L)	36	7	6.9	5.01	282.10	4798.74
T_3 (0.36 L – 2.22 L)	39	5.2	5.4	4.56	213.3	3628.40
T_4 (0.50 L – 2.37 L)	39	5	5.0	4.10	203.6	3463.40
F test	*	*	*	*	*	
CV %	0.36	2.0	2.5	3.6	14.70	
5 % LSD	1.6	0.70	0.20	0.28	3.22	

(a) Flower with tomato plant (b) Single fruit diameter (c) Fruits weight per plant

Figure 3 Reproductive growth of *Solanum lycopersicum* L.

Table 12 Evaluation on different water treatments and yield in the experiment

Treatments	Growth stages water treatment (Liter)			Total amount of water	Total amount of water per hectare	Fruits yield per plant (g)	Fruits Yield (kg ha^{-1})
	Vegetative stage	Early developmental stage	Late developmental stage				
	1-4 WAT	5-7 WAT	8-10 WAT				
T_1 (0.05 L- 0.27	2.52	4.13	5.67	12.32	1746.44	151.4	2575.4
T_2 (0.21 L – 2.07	11.62	28.14	43.47	83.23	11798.4	282.1	4798.7
T_3 (0.36 L – 2.22	15.75	31.29	46.62	93.66	13276.9	213.3	3628.4
T_4 (0.50 L – 2.37	19.88	34.37	49.77	104.02	14745.5	203.6	3463.4

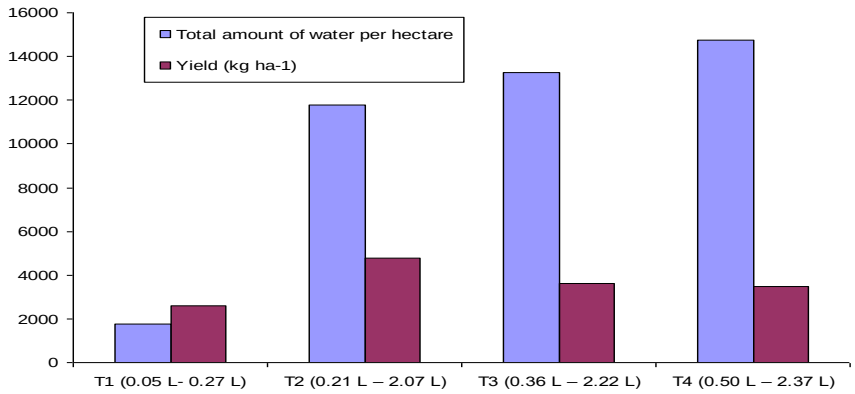

Figure 3 Evaluation of water requirements and yield

Discussion and Conclusion

This research was conducted during April-July, 2019 at Oakshitpin Village, Padaung Township, Bago Region. The growing of *Solanum lycopersicum* L. (kyar chay) using different watering levels on plant growth stages was studied. Properties of the soil prior to experimentation are shown in Table (1). The soil was sandy loam in texture, low in N %, and high in exchangeable K, very high in available P exchangeable Ca and the exchangeable K and high in available nutrients K_2O. The present investigation used NPK fertilizer (15-15-15) for the increasing yield of tomato. Edossa *et al*. (2013) reported that it is well documented that application of N promotes vegetative growth and fruit yield of tomato, and later application in the growing stages favors fruit development. Similarly, application of phosphorus is an important nutrient for tomato plant growth and development, a deficiency of P leads to reduced growth and reduced yields. Tomatoes have the greatest demand for phosphorus at the early stages of development. According to climatic data at experiments site, temperature data was 28-33°C during vegetative growth, 26-33°C during reproductive growth and average temperature 29.05°C, total rainfall 556 mm and mean humidity 62.59, in the whole growing season (Table 3). Yu Yu Tun and Aung Phyo (2019) observed that in Myanmar, while lowland tomatoes can be easily produced in the winter, yields are much lower in the summer and in the rainy season. Relatively cool temperatures and drier conditions in Southern Shan State are very favorable for tomato production throughout the year. In Southern Shan State, highland tomatoes are mainly grown on and around Inle Lake. The main production areas for lowland tomatoes are Pyinmana (Nay Pyi Taw Region), Magway Region (several areas), Monywa (Sagaing Region), Dike Oo and Binnar (Bago East Region) and Watpoke (Bago West Region). Harel *et al*. (2014) mentioned that the relationship between mean daily temperatures and the reproductive stage of tomato plants, found that at daily mean temperatures of 29°C, fruit number, and percentage fruit set and fruit weight per plant decreases in comparison with those at 25°C. Optimum relative humidity in glasshouse crops range from 60-80%. In this research paper, the seedlings transplanted into the field cultivation (polyethylene bags) during 5-7 cm and 2-3 leaves (5 WAS, weeks after sowing). Then these transplanted plants were watered on different rates at one week after transplanting. Shankara *et al*. (2005) reported that the seedlings transplant to the field 3 to 6 weeks after sowing. A week before transplanting, seedlings should be hardened by reducing the application of water, but 12-14 hours before they are taken out of the seedbed they should be thoroughly watered again to avoid excessive damage to the roots. Seedlings of 15-25 cm tall with 3-5 true leaves are most suitable for transplanting. In this experiment, germination and early growth with initial leaves (35 DAS), vegetative stage (28 days, 1-4 WAT), early developmental stage (21 days, 5-7 WAT) and late developmental stage (21 days, 8-10 WAT) were observed. Shamshiri *et al*. (2018) revealed that the five growth stages of tomato as germination and early growth with initial leaves (between 25 and 35 days), vegetative period (20 to 25 days), and flowering (20 to 30 days), early fruiting (20 to 30 days), and mature fruiting (15 to 20 days). In this paper, tomato-vegetative growth as petiole length was the best results in T_3 (0.36 L – 2.22 L) and leaf width, T_4 (0.50 L – 2.37 L). Moreover, plant height, number of leaves per plant, single leaf length and leaf area investigated in T_2 (0.21 L – 2.07 L) (Table 5). This research paper investigated that the differences among the treatments on days to first flowering from transplanting, number of clusters plant^{-1}, and number of fruits cluster^{-1}, single fruit diameter, single fruit weight, and fruits weight plant^{-1} and fruits yield was significant. T_2 (0.21 L – 2.07 L) was the best number of cluster, The highest number of fruits per cluster, the biggest single fruit diameter, the largest fruits weight per plant and the best fruits yield (Table 11). Tomato is grown in the plastic house during rainy season. T_2 (0.21 L – 2.07 L) was the more resultant yield than other treatments. Shova *et al*. (2018) reported that deficient watering may require for tomato crop production in rainy season in this experiment. The low cost plastic tunnels can be used to protect the crops from excessive rainfall and provide the favorable environment for the production of better quality crops over the period of time. This paper observed that the water requirements of vegetative stage

(28 days, 1-4 WAT) was T_1 (0.05 L- 0.27 L) 2.52 L, T_2 (0.21 L – 2.07 L) 11.62 L, T_3 (0.36 L – 2.22 L) 15.75 L and T_4 (0.50 L – 2.37 L) 19.88 L. The water requirements of early developmental stage supplied T_1 (0.05 L- 0.27 L) 4.13 L, T_2 (0.21 L – 2.07 L) 28.14 L, T_3 (0.36 L – 2.22 L) 93.66 L and T_4 (0.50 L – 2.37 L) 34.37 L. The water requirements of late developmental stage supplied T_1 (0.05 L- 0.27 L) 5.67 L, T_2 (0.21 L – 2.07 L) 43.47 L, T_3 (0.36 L – 2.22 L) 46.62 L and T_4 (0.50 L – 2.37 L) 49.77 L. Total water requirements growing on tomato was T_1 (0.05 L- 0.27 L) 12.32 L (1746.44 L ha^{-1}), T_2 (0.21 L – 2.07 L) 83.23 L (11798.40 L ha^{-1}), T_3 (0.36 L – 2.22 L) 83.66 L (13276.92 L ha^{-1}) and T_4 (0.50 L – 2.37 L) 104.02 L (14745.51 L ha^{-1}). Then Tomato yield investigated that T_1 (0.05 L- 0.27 L) was 2575.43 kg ha^{-1}, T_2 (0.21 L – 2.07 L) 4798.74 kg ha^{-1}, T3 (0.36 L – 2.22 L) 3628.40 kg ha^{-1} and T_4 (0.50 L – 2.37 L) 3463.40 kg ha^{-1}. T_2 (0.21 L – 2.07 L) among them was the best yield. These results studied that T_1 (0.05 L- 0.27 L) using local farmer watering was the lowest yield and T_2 (0.21 L – 2.07 L), the best yield (Table 12). The volume of water requirement is length x breadth x height. The volume of 1 m^3 converts 1000 liters. The volume of water 1 mm converts to 0.001 m^3 (or) 1liter (https:// www. Mackillopgroup. com.au/wp-content/uploads/ 2019/03/Irrigation-Glove-Box- Guides.pdf). Yang *et al*. (2017) revealed that the whole growing season was divided into three stages:, the watering applied 3.567 mm (3.567 L) in stage one, vegetative stage, 11.195 mm (11.195 L) in stage two, and 6.139 mm (6.139 L) stage three, flowering and fruit development stage with interval three days (except rainy day) and the whole season 25.501 mm (25.501 L). Moreover, the maximum fruit per plant was 1056.0 g $plant^{-1}$. Water requirements of tomato plant are 10,000 m^3 ha^{-1} (10,000 L ha^{-1}) in greenhouses. Tomato plants are fairly resistant to moderate drought. However, proper management is essential to assure high yield and quality. Water requirements will differ at various growth stages. The requirement increases from germination until beginning of fruit setting, reaching a peak during fruit development and then decreasing during ripening (https://www.haifa roup.com/files/Guides/ tomato/ Tomato.pdf). These results show clearly that the crop water requirement varies from region to region. The results indicated that daily irrigated treatments resulted in better crop growth characteristics and the best yield. The maximum watering treatment gave the highest yield while the minimum treatments gave the least. Tomato yield varied from 4.44 kg m^{-2} (44400.00 kg ha^{-1}) to 3.26 kg m^{-2} (32599.10 kg ha^{-1}) for daily irrigated treatments (Luvai *et al*., 2014). In this paper, tomato grew inside the plastic tunnel (L 840 cm x W 300 cm x H 259 cm) because this plant grew during offseason (rainy season). Tomato production during rainy season in open field condition is very difficult mainly due to serious disease attack. Rainy season tomato production under low cost plastic shelter by avoiding direct contact of rain with tomato foliage avoids favorable condition for disease development. This practice is therefore helpful to produce tomatoes without the use of fungicides contributing towards ensuring continuous production and constant supply of fresh tomatoes throughout the year. Furthermore by improving the microclimatic condition such as raising the temperature under the shelter, favorable environment for the production of high tomato yield with superior quality will be created (Getahun, 2019). It can be concluded that tomato production inside the plastic house during rainy season has become very profitable where there is market access. Adoption of plastic house technology can improve yield and productivity of tomato crop in off season cultivation. Since tomato is very expensive during rainy season, farmers could fetch good price besides its high initial investment cost. For that reason the farmer can know and get the appropriate amount of water during offseason (rainy season) and extend this method to the other growers for dissemination for boosting the national economy.

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TAXONOMIC STUDY ON SOME WILD ORCHIDS OF KENNEDY MOUNTAIN IN TEDIM TOWNSHIP, CHIN STATE

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Abstract

Chin state is mostly covering with subtropical evergreen forests and distribution with many wild orchids. The members of Orchidaceae from Kennedy Mountain, Tedim Township, Falam District in Chin State, the North-western part of Myanmar had been collected and studies. Its area is about 2410 square kilometer. The orchids were collected in all seasons within the December, 2019 to June, 2020. Altogether, 35 species of Orchidaceae in study area were collected, identified and classified. Of these, 6 genera and 10 species including 9 species epiphyte and 1 species lithophyte. All collected species are fully described with necessary photographs and figures. Moreover, on artificial key to the species of the plants, their local name, English name and flowering periods are also described of the 2 species of sub-family Vandoideae and 8 species of sub-family Epidendroideae were included. The system of Seidenfaden (1992) was adopted as the classification system in the present research.

Keywords: Wild Orchids, Morphological character

Introduction

Chin State is composed of three Districts and nine Townships. Falam district consists of Falam, Tedim and Tunzang Townships. The Chin State which lies in the west of Myanmar is situated on a mountain range far away from Myanmar proper. The study area is situated between 23° 19′ 03″ N latitude and 93° 45′ 42″ E longitude. The total land area of Tedim Township is 2410 km sq. Tedim Township lies on 1728 meter above sea level.

Kennedy Mountain is a 2703 meter (8868 ft) peak in the Tedim Township of the Chin State of Myanmar that dominates the Tedim Road. It is one of the world's ultra-prominent peaks, as it rises 4951 feet more than 1509 m above all other peaks nearby. It is the second highest mountain in Chin State. Instead, a rough road leads to the summit from Sozang village, about 15 miles from Tedim on the road back to Kalaymyo, which huge the base of the mountain.

Orchids are lacking, also in the most extreme desert environments though they may be found in oasis in sheltered desert canyons, and in cactus thorn shrub or thorn forest. Orchid plants may be abundant in drier forest, but such communities have relatively few orchid species are rarely found. Orchids are one of the most striking, elegant, glamorous flowers to be found in nature and it is very interested.

They are widely distributed throughout the country. Since 50% of the rain forest covering the world had been destroyed by human activities, the orchid population is at risk of extinction due to their habitat destruction. Therefore, it is essential to record the orchids of Kennedy Mountain, Tedim Township in Northern Chin State of Myanmar.

The aim and objectives of this research are to conduct and record the Orchidaceae flora of Kennedy Mountain in Tedim Township, Northern Chin State, to investigate the various species of orchids in study area, their distribution and morphological characteristics, to understand the value of natural orchid species from Kennedy Mountain, Tedim Township, to get the value information

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of species from the study area, and to give a partial fulfillment of the floristic information of Kennedy Mountain in Tedim Township, Chin State in Myanmar.

Material and Methods

Orchidaceae species are collected from Kennedy Mountain, Tedim Township, Falam District in Chin State, from December 2019 to June 2020, were preserved and use for identification and description. All the collected species are noted by photographs while flowering. The diagnostic characters are recorded in detail. Field notes are made of precise locations and its plant characters. Identification of an unknown specimen is carried out by referring to Flora of British India (1894), Flora of Ceylon (1918) Flora of Java (1963; 1968), Flora of Malaya (1964), the orchids of Indochina (1992). The first step is solving the generic name. Again the species names of the collected plants are resolved. The final verification is made by examination of the herbarium species from old records. The index for normenclatural data refered in Index kewensis by which the names and synonyms of plant up to the rank of species being confirmed all of the normenclatural studies were finalized by referring to the web site International Plant Name Index, (http:// www ipni. org).

All the collected specimens have been identified and descried their characters, then constructed the artificial key to the genera and also to the species. Most of the plant specimens have been air dried and pressed.

Results

The present study deals with (10) species of Orchidaceae. As a result of collection (10) species belong to (6) genera of 2 sub-families. They are collected from Kennedy Mountain, Tedim Township, Chin State. The list of collected species are shown in Table.1. All the resulting species are treated systematically. An artificial key to the species are constructed and stated.

Table 1 List of Collected Species

Sub-Families	Tribes	Sub-Tribes	Genus	Species
Epidendroideae	Arethuseae	***Coelogyneae***	1.***Coelogyne***	1.***Coelogyne viscosa*** Rchb.f.
			2. ***Pleione***	2.***Pleione praecox*** Lindl.
	Epidendreae	***Dendrobieae***	3.***Bulbophylum***	***3.Bulbophylum pictum*** Parish & Rchb. f.
			4.***Dendrobium***	4.***Dendrobium bellatulum*** Rolfe.
				5.***Dendrobium cariniferum*** Rchb.f.
				6.***Dendrobium peguanum*** Lindl.
				7.***Dendrobium pendulum*** Roxb.
				8.***Dendrobium sutepense*** Rolfe ex Downie
Vandoideae	Cymbidieae	Sarwntheae	**5.*Pelatantheria***	9.***Pelatantheria insectifera*** Rchb.f.
			6.*Vanda*	10. ***Vanda coerulea*** Grift ex. Lindl.

1. Key to the Subfamilies

1 .Pollinia soft, waxy, without stipes----------1.**Epidendroideae**

1. Pollinia cartilaginous or bony, with stipes----------2.**Vandoideae**

Subfamilies Epidendroideae

Key to the Tribes

1 Pollinia with caudicles, superposed; plant with pseudobulbs of one internodes------ ----------**Coelogyneae**

1. Pollinia without caudicles, no superposed; plant with pseudobulbs of severed internodes--------**Epidendreae**

Tribe

Key to the Subtribe

1. Inflorescence terminal, lateral, or on a leafless scape; pollinia equal, 1-seriate, connected by not appendage----------**Dendrobieae**

1. Inflorescence terminal; pollinia subequal, 2-seriate, connected by appendage----------**Coelogyneae**

Subtribe Coelogyneae

Key to the genera

1. Epiphytes; leaves linear-lanceolate; flowers white to orangish – white ----------***Coelogyne viscosa***

1. Lithophytes; leaves elliptic-oblanceolate; flowers pinkish purple----------***Pleione praecox***

Coelogyne viscosa Reieghb. f. in Allgemein. Berline Gartenz. 1856.

Local Name - Ngwe-Hni-Phyu-Myo-Kywe

Flowering periods from December to January.

Sympodial epiphytes. Roots clinging cylindrical, white to brownish white, glabrous. Pseudobulbs one-jointed, narrowly ovoid to fusiform, yellow to yellowish-green glabrous. Leaves 2, at apex pseudobulbs; sessile, blade lanceolate to linear lanceolate, green. Inflorescence basal racemes, erect, 1-3 flowered; pedunclar bracts, ovate to ovate-lanceolate, peduncle, slender, orangnish-yellow, glabrous. Flower 4.5-5.0cm across at anthesis, white to orangnish-white; floral bracts caducious; pedicels, cylindrical, orangnish-white, glabrous; dorsal sepal, elliptic, white; lateral sepal, linear-lanceolate, white; lateral petal, oblanceolate, coriaceous, lip 3 lobes, oblong, pale-yellow streaks, spur not distinct; column, flat, orangnish-white, anthercap, ovoid, pale-orange; pollinia 4, waxy, oblanceolate, yellow, visidium, ovate, yellow, glabrous; stigmatic surface, ovoid, orangnish-white; ovary, oblanceolate, orangnish-white, glabrous.

Specimen Examined: Chin State; Tedim Township; Kennedy; January 29. 2020; 23° 07′ 29.8″ N, 94° 01′ 40.8″E; Ah Nge Htwe and groups; collection No.7.

2. *Pleione praecox* Lind., Coll. Bot. 37; in wall. Cat.1965.

Local Name - Phar-la-tet-thitkhwa

Flowering periods from November to December.

Sympodial lithophytes. Roots fibrous, cylindrical, white. Pseudobulbs, turbinate, dark green with purplish brown mottled. Leaves 2, top of the pseudobulbs, elliptic to elliptic-oblanceolate, plicate, green. Inflorescences basal racemes, erect, 1-2 flowered; peduncular bracts, ovate-lanceolate, brownish-green blotch; peduncle, slender, green. Flower 5.0-6.0cm across at anthesis, pinkish purple flower, lip white with purple markings; floral bracts, oblong-lanceolate, brownish-white; pedicels, slender, yellownish-green; dorsal sepals, oblong-lanceolate, pinkish purple; lateral sepal, slightly oblique to obvate-lanceolate; lateral petal, linear lanceolate, pinkish purple; lip distinctly 3-lobe, sidelobe, oblong, pale purple, midlobe, margins deeply fimbriate, tips emarginated, white purple marking and yellow streaks; spur saccate, tips obtuse, white; column long flat, white; anthercap 2-loculed, ovoid, white, pollinia 4, waxy, 2 pairs of 4, clavate, yellow, stigmatic surfaces, quardrangular, pale purple, ovary, trigonous, yellownish-green.

Specimen examined: Chin State; Tedim Township; Kennely Mountain; December 14.2019; 23° 09′ 29.8″N, 94° 01′ 40.8″ E; Ah Nge Htwe and groups; collection No.1.

Subtribe Dendrobieae

Key to the genera

1. Caudicles and viscidium absent----------2
1 Caudicles and viscidium present----------***Bulbophyllum pictum***
   2. Anthercap oblong or orbicular----------3
   2. Anthercap ovate----------5
3. Spur significant; pseudobulbs without swollen internodes----------4
3. Spur insignificant;pseudobulbs with swollen internodes----------***Dendrobium pendulium***
   4. Leaves oblong; peduncular bract ovate; lateral petal elliptic----------***Dendrobium cariniferium***
   4. Leaves linear-oblong; peduncular bract ovate-lanceolate; lateral petal oblanceolate----------***Dendrobium peguanum***
5. Peduncular bract pubescent; dorsal sepal ovate-lanceolate; spur saccate----------***Dendrobium bellatulum***
5. Peduncular bract glabrous; dorsal sepal oblong; spur funnel-shaped----------***Dendrobium sutepense***

3. *Bulbophyllum pictum* E.C Parish & Rchb. f. Soc. London 30: 150. 1874.

Local Name - Unknown

Flowering periods from January to February.

Sympodial epiphytes. Pseudobulbs one-jointed, erect, ovate-lanceolate, green. Leaves 1, at apex of pseudobulbs, elliptic-lanceolate, margins entire, tips acute, green. Inflorescence lateral from the rhizomatose part, 1- flowered; peduncular bract, ovate, brown; peduncle slender, brown. Flower 1.0-1.5cm across at anthesis, sepals dirty yellow with many purple dots, petals dark purple, lip proximal part yellow with red dots, distal part reddish-purple; floral bract, ovate-lanceolate,

brown; pedicels, slender, green with red dots, glabrous; dorsal sepal, ovate-lanceolate, dirty yellow with many purple dots; lateral sepal, broadly ovate, dirty yellow with many purple dots; lateral petal, ovate, creamy-white with many purple dots, lip, auricles, proximal part yellow with red dots, glabrous; column stout, white, glabrous; column, slender, white; anthercap, obovoid, anther with a horn at apex, reddish-purple, glabrous; pollinia 4, waxy, obovoid, yellow, glabrous; caudicle absent; visidium minute, stigmatic surface, ovoid, white, glabrous; ovary, oblong ,green, glabrous.

Specimen Examined: Chin State, Tedim Township; Kennedy Mountain; February 7,2020; 23° 19′ 03″ N, 93° 45′ 42″E; Ah Nge Hwe and groups; collection No.10.

4. *Dendrobium bellatulum* L. Soc. 36: 10, 1903.

Local Name - Unknown

Flowering periods from January to March.

Sympodial epiphytes. Roots clinging, cylindrical, white. Pseudobulbs 3-6 jointed, erect, fleshy, cylindrical to fusiform, green. Leaves alternate and distichous, leafless at anthesis, ovate-oblong, green. Inflorescences terminal racemes, erect, 1-2 flowered; peduncular bracts, ovate, margins entire, tips acute, brown, pubescent; peduncle, cylindrical, pale green. Flower 4.5-5.0cm across at anthesis, creamy-white flower with bright orange lip; floral bracts, ovate, margins entire, tips acute, brown, pubescent; pedicels, slender, pale green, glabrous; dorsal sepal, ovate-lanceolate, creamy-white, glabrous; lateral sepals, oblanceolate, creamy-white, glabrous; lateral petal, oblong-lanceolate, creamy-white, glabrous; lip, oblong, tips by-fixed, lip with fleshy glossy medium band, on each side by laminate keel with crose denate edges; spur, saccate, white, glabrous; column stout, yellownish-white, glabrous; anthercap, ovoid, yellow, glabrous; pollinia 4, waxy, cohering in 2 pairs, ovoid, yellow; stigmatic surfaces, elliptic-ovate, white; ovary, ovate, pale-green.

Specimen Examined: Chin State; Tedim Township; Kennedy; January 25.2020, 23° 07′ 29.8″ N, 94° 01′40.8″ E; Ah Nge Htwe and groups; collection No.6.

5. *Dendrobium cariniferum* Rchb. f. in Gard. Chron. 611. 1869.

Myanmar Name - Mahar-deiwi

Flowering periods from December to January.

Sympodial epiphytes. Roots clinging, cylindrical, white, glabrous. Pseudobulbs 3-6 jointed, erect, terete, pale green, pubescent with black. Leaves alternate and distichous, oblong, upper the dark green and beneath the pale green, glabrous. Infloresscence axillary and terminal racemes, erect and drooping, 2-3 flowed; peduncular bracts 2, ovate, pubesecent with black; peduncle short, terete, green,glabrous. Flower 1.5-3.5cm across at anthesis, white flower sepal and petals creamy-white with yellow tips, midlobe of lip creamy yellow, sidelobe darker yellow, throat reddish orange and ciliated; floral bracts ovate-lanceolate, brownish-white, glabrous; pedicels, slender, greenish-white, glabrous; dorsal sepal, ovate, creamy-white, glabrous; lateral sepal, ovate-lanceolate, creamy white, glabrous; lateral petal, elliptical, creamy white, glabrous; lip distinctly 3-lobes, sidelobes, ovate, creamy white with darker yellow with streaks, glabrous; midlobe, oblong-reniform, creamy-white with dark yellow stain, glabrous; spur, funnel-shaped, tips tubular, white with reddish-orange, glabrous; column short, cylindrical, white, glabrous; column foot, slender, yellow, glabrous; anthercap, orbiculoid, white, glabrous; pollinia 4, waxy, cohering in 2 pairs, oblong, yellow, glabrous; stigmatic surface, obovoid, white, glabrous; ovary 6 ridge, oblongoid, creamy white, glabrous.

Specimens Examined: Chin State; Tedim Township; Kennedy; June 14, 2019; 23° 07′ 29.8″ N, 94° 01′ 40.8″E; Ah Nge Htwe and groups; collection No.2.

6. ***Dendrobium peguanum*** Lindl., J. Proc. Linn. Soc. Bot. 3:19. 1859.

Local Name - Unknown

Flowering period from September to February.

Sympodial epiphytes. Roots clinging, cylindrical, white, glabrous. Pseudobulbs 2-3 jointed, erect, oblong-conical, green, glabrous. Leaves alternate and distichous, linear-oblong, green, glabrous. Inflorescence terminal racemes, erect, 3-6 flowered; peduncular bracts, ovate-lanceolate, glabrous; peduncle, slender, brownish-green, glabrous. Flower 1.0-2.0 cm across at anthesis, sepals and petals white, dark purplish brown veins on light brown lip; floral bracts, ovate, green, glabrous; pedicels, slender, brown, glabrous; dorsal sepal, oblong-ovate, white, glabrous; lateral sepals, oblong, white, glabrous; lateral petals, oblanceolate, white, glabrous; lip, oblong-orbicular, dark purplish brown veins on light brown lip, glabrous; spur, conical, green, glabrous; column, stout, green, glabrous; column foot, green, glabrous; anthercap, oblongoid, white, glabrous; pollinia 4, waxy, hard, ovoid, yellow, glabrous; stigmatic surface, oblong, white, glabrous; ovary, oblongoid, green, glabrous.

Specimen Examined; Chin State; Tedim Township; Kennedy Mountain; February 7.2020; 23° 07′ 03″N, 93° 45′ 42″ E; Ah Nge Htwe and groups; collection No.9.

7. ***Dendrobium pendulum*** Roxb. Hort. Beng. 63. 1814.

Local Name - Mya-sity-kyo, Pan-kyan-sit

Flowering periods from February to April.

Sympodial epiphytes. Roots clinging, cylindrical, white, glabrous. Pseudobulbs 8-12 jointed, fleshy, pendulous, swollen internode, cylindrical, pale-green, glabrous. Leaves alternate and distichous, leafless at anthesis, 7.0-10.0cm long and 2.0-3.0cm wide, linear *lanceolate*, margins entire, tips slightly bi-lobed, sub-coriaceous, green, glabrous. Inflorescence lateral racemes on swollen internode of pseudobulbs, erect, 1-3 flowered; peduncular bracts, broadly ovate, pale-brown, glabrous; peduncle short, slender, green, glabrous. Flower 3.0-3.5cm across at anthesis, white sepals and petals with tips purple, lip pubescent with deep yellow blotch at base and lavender tip; floral bracts, ovate-oblong, pale-brown, glabrous; pedicels, slender, creamy-white, glabrous; dorsal sepal, oblong, margins entire, tips acute, white with tips purple, glabrous; lateral sepal, oblong, white with tips purple, glabrous; lateral petal 1.8-2.5cm long and 1.2-1.7cm wide, ovate, margins entire, tips obtuse, white with tip purple, glabrous; lip, ovate-orbicular, margins ciliate, tips obtuse, deep yellow blotch at base and purple tip, pubescent; spur, saccate, purple, glabrous; column, stout, white, glabrous; column foot 0.1-0.2cm long and wide, oblong, purple, glabrous; anthercap, oblongoid, white, glabrous; pollinia 4, waxy, ovoid, yellow, glabrous; stigmatic surface, oblongoid, white, glabrous; ovary, oblongoid, greenish-purple, glabrous.

Specimen Examined: Chin State; Tedim Township; Kennedy Mountain; February 7.2020; 23° 19′ 03″N, 93° 45′ 42″ E; Ah Nge Htwe and groups; collection No.8.

8. ***Dendrobium sutepense*** Rolfe Kew Bull. p.374. 1925.

Local Name - Chin-dewi

Flowering periods from December to January.

Sympodial epiphytes. Roots clinging, cylindrical, white, glabrous. Pseudobulbs 3-5 jointed, erect, terete, pale-green, pubesecent with black. Leaves alternate and distichous, oblong, upper the dark green and beneath the pale green, glabrous. Inflorescence terminal racemes, erect, 2-3 flowered; peduncular bracts, pale-brown, glabrous; peduncle, terete short, green, glabrous. Flower

1.5-3.0cm across at anthesis, white flower, lip white with yellow patch tip the base; floral bracts, ovate-elliptic, pale-brown, glabrous; pedicels, slender, greenish-white, glabrous; dorsal sepal, oblong, white, glabrous; lateral sepal, oblong-lanceolate, margins entire, tips acuminate, coriaceous, white, glabrous; lateral, oblong-lanceolate, margins undulate, tips acuminate, white, coriaceous, glabrous; lip distinctly 3-lobes, white with yellow stain; sidelobes, oblanceolate, margins sinuate, tips truncate, creamy white with yellow streak, coriaceous, glabrous; midlobe, oblong, creamy white with stain, glabrous; spur, funnel-shaped, tips tubular, coriaceous, creamy-white, glabrous; column short, slender, white, glabrous; column foot, oblong, creamy white, glabrous; anthercap, ovoid, white, glabrous; pollinia 4, waxy, cohering in 2 pairs, oblong, yellow, glabrous; stigmatic surface, ovate, white, glabrous; ovary 6-ridges, oblongoid, white, glabrous.

Specimens Examined: Chin State; Tedim Township; Kennedy; March 14.2019; 23° 07′ 29.8″ N, 94° 01′ 40.8″E; Ah Nge Htwe and groups; collection No.3.

Subfamilies Vandoideae

Key to the genera

1. Sepal and petal with tessellation; color bluish-lavender; spur funnel-shaped----------***Vanda coerulea***

1. Sepal and petal without tessellation; color green sepal and petal with reddish brown stipes, midlobe of lip red purple; spur conicle-shaped----------***Pelatantheria insectifera***

9. ***Pelatantheria insectifera*** (Rchb. f.) Ridl., J. Linn. Soc.32: 373. 1896.

Local Name - Unknown

Flowering periods from December to January.

Monopodial epiphytes. Roots clinging, cylindrical, greenish-white, glabrous. Stem leafy, erect, cylindrical, green, glabrous. Leaves alternate and distichous, oblong, green, glabrous. Inflorescences axillary racemes, erect, 2 to 4 flowered; peduncular bracts absent; peduncles short, stout, green, glabrous. Flower 1.3-1.5cm across at anthesis, green sepals and petals with reddish-brown stipes, midlobe of lip red purple; floral bracts, cuneate, brown, glabrous; pedicels, cylindrical, pale-green, glabrous; dorsal sepal, oblong, margins entire, tips obtuse, green with reddish-brown stripes, glabrous; lateral sepals, elliptical, green with reddish-brown stripes, glabrous; lateral petals, linear, green with reddish-brown stripes, glabrous; lip shallowly 3 lobed, cuneate, reddish-purple, margins undulate, tips acute, glabrous; spur, conical, tips obtuse, greenish-white, glabrous; column short, stout, white, glabrous;anthercap 2, ovoid, white, glabrous; pollinia 2, waxy, 1.0-2.0mm long and wide, globose, yellow, glabrous; caudicle absent; visidium 1, ovoid, white, glabrous; stigmatic surface, oblongoid, pale-green, glabrous; ovary 3, narrowly oblongoid, pale green, glabrous.

Specimen Examined: Chin State; Tedim Township; Kennedy; January 5.2020; 23° 07′ 29.8″ N, 94° 01′ 40.8″ E; Ah Nge Htwe and groups; collection No.5.

10. ***Vanda corulea*** Grift. ex. Lindl., Bot. Reg. Sub. t. 30.1847.

Local Name - Moe-Lone-Hmine

Flowering periods from November to January.

Monopodial epiphytes. Roots long clinging, vermiform, white, glabrous. Stem densely leafy, erect, cylindrical, green, glabrous. Leaves alternate and distinchous, flat, recurved, oblong, green,glabrous. Inflorescences axillary racemes, erect, 1-3 flowered; peduncular bracts 2-4,

sheathing, ovate, brownish-green, glabrous; peduncle, cylindrical, green, glabrous. Flower 6.5-7.1cm across at anthesis, fleshy, color bluish-lavender, tessellated; floral bracts, broadly ovate, brownish-green, glabrous; pedicels, slender, white, glabrous; dorsal sepal, ovate, flat, margins sinuate, tips rounded, bluish-lavender, glabrous; lateral sepal, flat, suborbicular, margins entire, tips rounded, bluish-lavender, glabrous; lateral petal, flat, elliptic-ovate, margins sinuate, tips rounded, bluish- lavender, glabrous; lip bi-lobed, erect, linear-oblong, purple, glabrous; spur, funnel-shaped, white to purple, tips obtuse, glabrous; column short, stout, white, glabrous; anthercap, ovoid, white, glabrous; pollinia 2, waxy, obovoid, yellow, glabrous; caudicle, cylindrical, white, glabrous; visidium, orbicular, white, glabrous; stigmatic surface, orbicular, white, glabrous; ovary, oblongoid, white, glabrous.

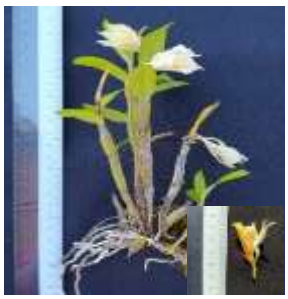
Specimen Examined: Chin State; Tedim Township; Kennedy Mountain; December 17.2019; 23° 07′ 29.8″ N, 94° 01′ 40.8″E; Ah Nge Htwe and groups; collection No.4.

A. *Coelogyne viscosa* Rchb.f.

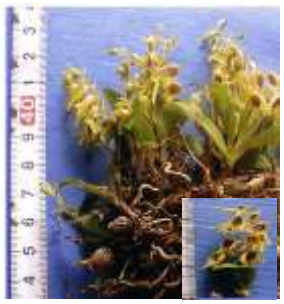
B. *Pleione praecox* (Sm.) D.Don

C. *Bulbophyllum pictum* Par. & Rchb.f.

D. *Dendrobium bellatulum* Rolfe



E. *Dendrobium cariniferium* Rchb. f.



F. *Dendrobium pegunum* Lindl.

G. *Dendrobium pendulum* Roxb.

H. *Dendrobium sutepense* Rolfe. ex Dowine

I. *Pelatantheria insectifera* Rchb.f.

J. *Vanda coerulea* Grift. ex Dowine

Discussion

The present study deals with taxonomic study on some wild orchids in Kennedy Mountain, Tedim Township, Chin State. The research work consists of 6 genera and 10 species including 9 species epiphytes and 1 species lithophyte. According to the distribution of vegetation types in Myanmar by Krees *et al*. (2003), the study area is covered by evergreen forests and semi-evergreen forest.

The sub-family Epidendroideae are the major orchid group, with more than half of all orchid species in this two sub-family. Most are epiphytes with some terrestrial. The popular ***Dendrobium*** sp., ***Coelogyne*** has with large, showy flowers. The ever-popular ***Dendrobium*** is one of the most recording orchids to grow, being handsome, "floriferous" and hardy. Pseudobulbs some in all sorts of shapes. They are long vertical cones up to 1.5m in length in the horn ***Dendrobium***, dangling pendulous rods in the noble ***Dendrobium*** round. The members of orchids are belonging to ***Bulbophyllum pictum***, ***Dendrobium bellatulum*** and ***Dendrobium sutepense*** are not reported in this study. Therefore, I have reported these 3 species in Tedim Township, Northern Chin State.

Five species of ***Dendrobium*** was collected in this area. Dorsal sepals of ***Dendrobium pendulum*** and ***Dendrobium sutepense*** are oblong but these of rest species ***Dendrobium bellatulum***, ***Dendrobium cariniferum*** and ***Dendrobium pegunum*** are ovate-lanceolate, ovate and oblong-ovate. Ovary of ***Dendrobium sutepense*** and ***Dendrobium cariniferum*** are white and creamy-white; ***Dendrobium bellatulum*** and ***Dendrobium peguanum*** are pale-green and green but the rest of one species is ***Dendrobium pendulum***.

One species of ***Bulbophyllum pictium*** is found in the study area. The distinctive characters are 1-leaves at apex pseudobulbs, lip auricles and anther with a horn at apex but ***Ceologyne viscosa*** is distinctive 2-leaves at apex pseudobulbs, lip oblong and anther without a horn at apex. Only one species of lithophyte is found in the study area. This species is defined as ***Pleione praecox*** Lind., Wall. Cat. 1965. The distinctive characters are roots fibrous, 2 leaves plicates, stigmatic surface quadrangular and ovary trigonous. This sub-families Vandoideae consists of ***Pelatantheria insectifera*** and ***Vanda coerulea*** species are 2 pollinia but rest of another sub-families Epidendroideae species are 4 pollinia in the study area.

In Chin State, orchid plants that are pride of the state and once thrived abundantly are now endangered due to over collection and deforestation. It is needed to conserve the orchid's resources from extinctions of rare species. The loss of national resources of Myanmar could be prevented by prohibiting collection and selling of orchids without due consideration, protecting and conserving the forests is a National duty for the state and future generation.

Orchids are not only significant worldwide in the horticulture industry, but in many countries, they are valued locally for their medicinal, national and ornamental qualities. Therefore, it is inevitably our sole duty at least to record the orchid flora and to prevent the loss of Myanmar treasure plant, the orchid.

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SPECIES DIVERSITY AND STAND STRUCTURE OF DRY ZONE FOREST IN SHINMA TAUNG HILL, YESAGYO TOWNSHIP, MAGWAY REGION

Pa Pa Win [1] Hnin Ei Shwe Sin Myint [2]

Abstract

A quantitative study of tree species was carried out in Shinma taung hill, Yesagyo Township. To find out the diversity of trees and to know the forest stand structure, 20 quadrates [20m x 20 m] in the study site were established and studied. Species diversity was presented by diversity index. In the study area, Shannon Wiener's index and Simpsom index were 4.24 and 0.74. In the present study, 54 species, 44 genera belonging to 29 families were found in study area. Relative density, relative frequency, mean basal area and relative dominance were calculated. IVI values of major tree species were presented by ranking order. In this study area, ecologically the most successful species were *Tectona hamiltoniana* Wall. (Dahat) 58.13%, *Terminalia oliveri* Brandis *(*Than) 47.88%, *Hiptage benghalensis* (L) Kurz. (Bein nwe) 14.86%, *Bridelia retusa* (L.)A.Juss (Seik chi) 11.95% and *Capparis flavicans* Wall (Saung gyan) 9.17%. It was influenced by *Tectona hamiltoniana Wall.* (Dahat) and *Terminalia oliveri* Brandis *(*Than) in this region.

Keywords: species diversity, forest stand structure, frequency class

Introduction

Biological diversity is fundamental to our life. Not only does it secure our material needs, it also provides valuable services that humans require from their environment, such as food, clothing, clean drinking water, and medical care. It ensures the stability of our habitats and is an essential basis of our culture and civilization. Biodiversity also means genetic diversity and is our best safeguard against environmental changes. No one knows how many species of organisms exist in the world. According to the World Wide Fund for Nature (WWF), 34,000 terrestrial and marine species are currently endangered, and the number of extinctions per day is estimated between 2 and 130. This global mass extinction is the cumulative result of local extinction events, which lead to a decrease in the diversity of local ecosystems long before these species are definitively extinct. Today the biosphere's greatest problems are the human-caused landscape changes that can be observed around the globe, the climate changes, and the accompanying loss of biological diversity. Basically three different aspects of biological diversity can be distinguished: firstly, biodiversity as a product of evolution, which has brought forth and continues to develop a diversity of populations and species; secondly, biodiversity as a resource for humans; thirdly, biodiversity as a prerequisite for the functioning of ecosystems.

Yesagyo Township is situated in the easternmost part of Magway Region in central Myanmar. The studied area was in Yesagyo Township. Shinma taung hill is an isolated hill in central flat land. The elevation of Shinma taung hill peak point is 525 meter above the sea level. The road was also constructed around the area of the hill. The structure of this hill forest was consequently changing due to the factor of over exploitation (eg. Thanakha). In study area, density of individual trees and number of species were decreasing over time and floristic composition and forest stand structure had altered. The degradation of forests and destruction of habitat occurred in the study area due to overexploitation by humans and climate changes. Degradation of forest ecosystem was the major cause of declination to the plant diversity.

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Forest stands can be described by horizontal and vertical structure. Horizontal structure represents a spatial distribution of individual trees and vertical structure describes the spatial vertical distribution of canopy. The vertical structure of tree communities is formed by the variations in growth forms or tree physiognomy (Kimmins, 1997). Stand level spatial distribution is a fundamental part of forest structure that influences many ecological processes and ecosystem functions. Vertical and horizontal spatial structure provides key information for forest management. Although horizontal stand complexity can be measured through stem mapping and spatial analysis, vertical complexity within the stand remains a mostly visual and highly subjective process. Stand structure is defined as the spatial arrangement of the components of vegetation (Lincoln *et al*. 2003). Stand structure is not static; it is a constantly changing part of forest ecosystem. Forests are continuously subject to disturbances at many scales, ranging from death of an individual tree within a stand to high severity fires that wipe out large forest communities. The diversity of type and frequencies of natural disturbances lead to a high diversity of structural conditions: from even-aged, single species stands to multi-age, compositionally diverse, multilevel canopy forest structures. Stand structure reflects ecosystem's resistance and resiliency to disturbance. An ecosystem's recovery from a disturbance is one of the factors that determine the structural arrangement of trees as the stand progresses through the stages of development from stem initiation to stem exclusion, to vertical and horizontal diversification (Van Pelt 2007). This study was conducted to achieve following objectives are to assess the diversity of growing trees, to describe the stand structure and species composition, to understand the relationship between plant species and their environment and to know the calculation of diversity indices.

Materials and Methods

Study area

Yesagyo Township is situated in the easternmost part of Magway Region in central Myanmar. It is located between north latitude 21° 08′ to 21° 51′ and east longitude 95° 01′ to 95° 20′. The total area of Yesagyo Township is 99000 ha or 989.99 km^2 (244634.328 acres). Shinma taung hill is an isolated hill in central flat land. The elevation of Shinma taung hill peak point is (525 meters) above sea level.

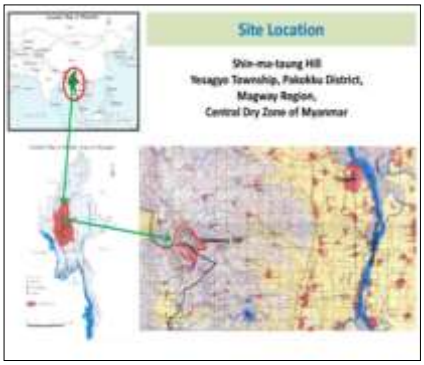


Figure 1 Location map of study area in Yesagyo Towbnship

Figure 2 Location map of

Data collection

The field investigation was carried out from December, 2018 to June, 2019. In the study area, 20 sample plots (20 × 20 m) located at various elevations from 233 m to 433 m were laid out systematically for sampling the forest. The spatial location (latitude, longitude and altitude) of each quadrat was collected by using a Global Positioning System (GPS). The girths of sampled

plants (≥ 10 cm girth at breast height) at breast height (GBH) and height were systematically measured.

Jackknife estimate of species richness

Species richness is the simplest measure of biodiversity and is simply a count of the number of different species in a given area. Heltshe and Forrester (1983) proposed the formula of Jackknife estimate of species richness;

$$\hat{S} = S + \left(\frac{n-1}{n}\right)^{k}$$

$\hat{S}$ = Jackknife estimate of species richness

S = observed total number of species in "n" sample plots

n = total number of plots sample

k = number of unique species

Calculation of species diversity and evenness

Species diversity indices and Evenness or equitability were calculated to determine for each species. Species diversity expresses the degree of evenness of the mixture of species. Species diversity was measured by Shannon-Wiener (1963) and Simpson (1949) indices.

$$H = -\sum_{i=1}^{S}(p_i)(\log_2 p_i) \qquad D = 1 - \sum_{i=1}^{S}(p_i)^2$$

H = Shannon-Wiener's index of species diversity

S = number of species

P_i = proportion of total sample belonging to the i^{th} species

D = Simpson's index of species diversity

Evenness

Two components of diversity are combined in the Shannon-Wiener function: (1) number of species (2) equitability or evenness of allotment of individuals among the species diversity.

$$E = \frac{H}{H_{max}} \qquad H_{max} = \log_2 S$$

E = evenness (range 0-1)

H = index of species diversity

H_{max} = species diversity under conditions of maximal equitability

S = number of species

Evaluation of relative density, relative frequency and relative dominance

Vegetation was quantitatively analysed for relative density and mean basal area following Curtis & McIntosh (1950).

$$\text{Relative Density (R.D)} = \frac{\text{No. of individuals of the species}}{\text{No. of individuals of all the species}} \times 100$$

$$\text{Relative Frequency (R.F)} = \frac{\text{No. of occurrences of the species}}{\text{No. of occurrences of all the species}} \times 100$$

$$\text{Mean basal area (MBA)} = \frac{\text{Total basal area}}{\text{Number of trees}}$$

The basal coverage or the area covered by a species is used to express dominance. The Relative Dominance (R.Dm) of a species is calculated by-

$$\text{Relative Dominance (R.Dm)} = \frac{\text{Total basal area of the species}}{\text{Total basal area of all the species}} \times 100$$

Investigation of Importance Value Index (IVI)

The index was calculated by summing the three relative values, viz., relative density, relative frequency and relative dominance as per the methods of Curtis (1959). This total value out of 300 is called Importance Value Index (IVI) of the species.

Tree species distribution by frequency classes

The law of frequency analysis (Raunkiaer,1934) was used to assess the rarity commonness of the tree species. In this classification the percentage frequency of the species was classed as A, B, C, D and E; Where A represents rare (1-20%), B is low frequency (21-40%), C is intermediate frequency (41-60%) , D is moderate high frequency (61-80%) and E is high frequency or common (81-100%). These frequency classes were used to determine whether the vegetation of the study area is homogeneous or heterogeneous.

Stratification: Horizontal and Vertical Stand structure

Population structure of tree species were analysed across fixed girth classes (Horizontal structure). Species and their corresponding individuals were proportionately analysed by height class intervals (Vertical structure).

Methodology

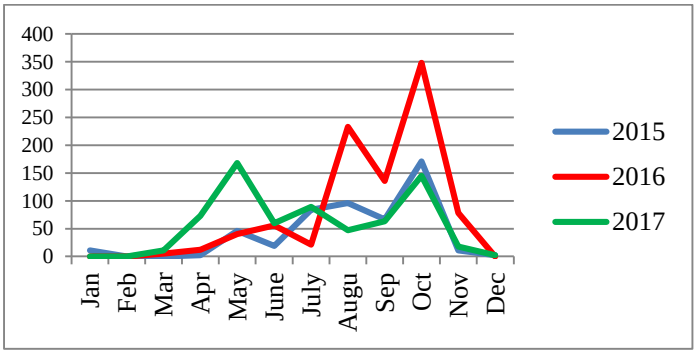
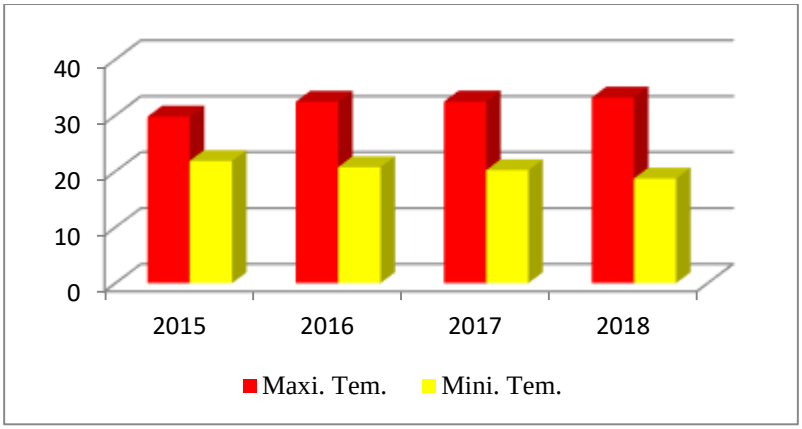


Figure 3 Monthly mean rain fall



Source: Department of Meteorology and Hydrology, Yesagyo

Figure 4 Monthly mean Temperature

Results

Plant species diversity

In the present study, a total of tree individuals, representing 54 species, 44 genera belong to 29 families and a total of 2680 tree individuals were recorded. The most number of growing species in the number of 10 species was possessed by family Fabaceae, the second were Euphorbiaceae, Rubiaceae with 4 species in the study site. The most number of growing individuals 700 was possessed by Verbenaceae, the second was Combretaceae with 512 individuals and the third one was Fabaceae with 410 in the study site.

Table 1 Number of families, genera and species of trees in study area

Taxonomic rank	Number
Family	29
Genus	44
Species	54
Individual	2680

Jacknife estimate of species richness

Result data show that Jackknife estimate of species richness was 54.9 (Table. 2). In the study area, 54 species belonging to 29 families were recorded and 2 species possessed only one individual.

Table 2 Jackknife estimate of tree species richness

	Quantitative estimate of species richness
Total no. of species (s)	54
Total individual in all sample plots	2680
Total no. of unique species (k)	2
Jackknife estimate of species richness ($\hat{S}$)	54.90

Shannon-Wiener (H), evenness (E) and Simpson index (D)

Diversity indices are better measure of the species diversity of a forest and these indices were used to compare among diversities of communities. According to Magurran (1988), species diversity is often expressed by two indices: Shannon-Wiener index (H), Eveness (E) and Simpson Index (D). According to the results of the study area, the species diversity were Shannon-Wiener index (H) 4.24, evenness (E) 0.74 and Simpson Index (D) 0.89.

Table 3 Tree species diversity indices in study area

Diversity category	Study site
Jackknife estimate of species richness (Ŝ)	54.90
Shannon-Wiener Index (H)	4.24
Shannon- Wiener Evenness (E)	0.74
Simpson Index (D)	0.89

Tree species distribution by frequency classes

According to Raunkiaer (1934), five frequency classes of species frequency distribution were found in the study area. 2 species were in highest frequency class E (81-100%), 2 species were found in frequency class D (61- 80%), 7 species were found in the frequency class C (41-600%), 9 species belong to frequency class B (21- 40%) and 34 species belong to lowerest frequency class A (1-20%) in the study area.

Table 4 Tree species distribution by frequency classes in study area

	Frequency class	Frequency range	No. of species	% total species Frequency distribution
1	A	1-20%	34	62.96
2	B	21-40%	9	16.67
3	C	41-60%	7	12.96
4	D	61-80%	2	3.70
5	E	81-100%	2	3.70

Importance Value Index (IVI)

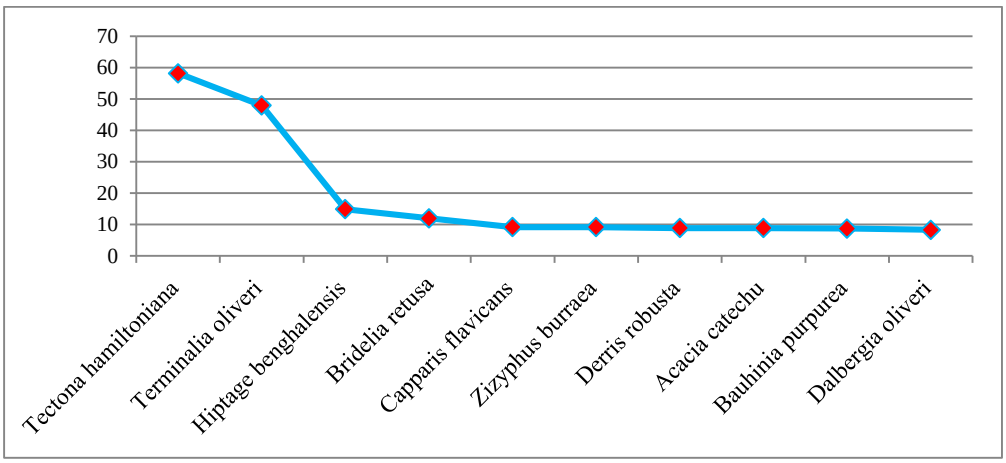


Figure 3 Importance Value Index value of top ten species

Horizontal stand structure

The horizontal species occurrence and horizontal structure of the study sites were shown in Table (5).

In the study area, two species were observed to be the biggest trees (<90≥70cm GBH) which were 3% of the total species. These species were *Tectona hamiltoniana* Wall. (80 cm) and *Terminalia oliveri* Brandis. (70 cm). There were 53 species in lower class (<30cm GBH) which were 98.1% of the total species. In the stand portion of site, there were 2680 trees per hectare and basal area varied from 0.001 to 2.697. Total mean basal areas per hectare were 0.222 m^2 ha^- . The number of basal area per hectare with < (30) cm GBH was relatively more abundant than other GBH. (Table. 5.)

Table 5 Tree species distribution by DBH classes (cm) in site

No.	DBH Classes	No. of individuals	No. of species	Species %
1	<30	2327	53	98.1%
2	<50 ≥ 30	330	34	62.9%
3	<70 ≥ 50	18	9	16.7%
4	<90 ≥ 70	5	2	3.7%
5	<110 ≥ 90	-	-	-

Vertical stand structure

Different growth forms determine the stratification, or vertical layering of the community. Because plant populations have a limited height range, plant communities have a vertical structure, whose nature depends upon the height ranges of the different species it includes. The vertical structuring of communities is an important component affecting how communities function at the level of photosynthesis in plants.

Species occurring in all storeys

In the study site, eight species were found in all storeys. These species were *Acacia catechu* (L.f.) Brandis, *Azidirachta indica* A. Juss, *Boscia variabillis* Collett & Hemsl, *Dalbergia oliveri* Gamble, *Lannea coromandelica (*Houtt.) Mrr., *Shorea robusta* Gaertn., *Tectona hamiltoniana* Wall. *and Terminalia oliveri* Brandis.

Table 6 Tree species distribution by height classes (m) in site

Sr. No	Height class	No. of individuals	No. of species
1	<5	2041	53
2	<7 ≥ 5	625	35
3	<9 ≥ 7	14	8
4	<11 ≥ 9	-	-

Discussion and Conclusion

In the study area, among 54 tree species, only a few species abundantly distributed and the most species were lower in number. The species richness was moderate but many rare and unique species occurred in this forest stand. Jackknife estimate of species richness was 54.90 (table 3). Results from this study indicated that evenness (E) for tree species was 0.74 in site. This data was

shown that the forest has lower equitability in individual number of each species. Shannon-Wiener (H) and Simpson index (D) calculated for this site was 4.24, 0.89 (Table 3).

A total of 2680 tree individuals, representing 54 species, 44 genera belong to 29 families were recorded in the study area. The most number of growing species in the number of 10 species was possessed by family Fabaceae, the second was Euphorbiaceae and Rubiaceae with 4species and the third was Apocynaceae with 3 in the study area. The most number of growing individuals 700 was possessed by Verbenaceae, the second was Combretaceae with 512 individuals and the third was Fabaceae with 410 in the study area. 2 unique species were observed. These are *Carissa carandas* L. (Khan) and *Syzygium cumini* (L.) Skeels (Thaphay- phyu).

Timber and fuel-wood cutting, overgrazing and construction of hill road also known as anthropogenic effect occurred in the study area; these effects caused habitat degradation and reduced plant species diversity. According to this research data, it can be concluded that this site has the lower diversity index due to the habitat degradation by anthropogenic effect. And forest fires also caused deforestation. The conservation of natural habitat and the protection of biological diversity were important for the stability of this forest stand.

Tectona hamiltoniana Wall (Dahat) and *Terminalia oliveri* Brandis (Than) have the highest relative density value in both sites. It means that those species occurred more number of individuals in this environment. These species were found in more places because they can be distributed more than other species in this forest. Important value index (IVI) is imperative to compare ecology significance of species (Lamprecht, 1989). The highest IVI value possessed 2 species which were *Tectona hamiltoniana* Wall. (Dahat) and *Terminalia oliveri* Brandis (Than) in the study area. These data indicated that these top 2 species were predominantly growing and the most ecologically important in this environment. The highest IVI of those species indicated their dominance and ecological success, in the form of its better regeneration and greater ecological amplitude. This result indicates that the most trees were small in size and the larger trees were cut down for various uses. If the small tree could not reach the reproductive stage it would not produce fruits and the succeeding generation of this species could disappear near future.

Shinma taung hill used to be covered with good dry forest until 1960. One of the famous species from this region is *Hesperethusa crenulata* (Roxb.) M. Roem. locally called Thanakha. The species diversity of Shinma taung hill area was crucial for forest conservation and management for this area. In a region, the more diversed wild and domesticated forms of plants existed, the more sources for food, medicine and housing for human near villages were supported. The diversity of tree was primarily important to total forest biodiversity because trees provided resources and habitat for almost all organisms. The information on distribution and abundance of tree species gave fundamental support in planning and management of biodiversity conservation. The structure of this hill forest was consequently changing due to the factor of over exploitation (eg. Thanakha) (Ba Kaung, 2013). Now, the collaboration of forest staffs and villagers were leading to re-planting the trees. So juvenile trees were found in the bottom of the hill. Now, it was also found that a lot of Thanakha were cultivated in the bottom of the hill. Key factors of diversity such as density of individual trees and number of species were decreasing over time and floristic composition and forest stand structure were altered. The degradation of forests and destruction of habitats occurred in the study area due to the overexploitation by humans and climate changes. Degradation of forest ecosystem was the major cause of declination to the plant diversity.

According to the above results, Shannon-Wiener index (H) and evenness (E), similarly Simpson Index (D) were relatively lower in number. It was also found that a greater number of individuals and species decrease in these forests therefore species diversity indices (D), (H) and equitable distribution among species (E) also decrease. The result data indicate that Verbenaceae was ecologically significant family and *Tectona hamiltoniana* Wall. was ecologically significant

species in this area. If the afforestation work is carried out in this area, these species must be aware to be successful replantation. The combination of this data indicated that status of trees in this forest was very poor and forest conservation was urgently needed before this forest disappeared. The quantitative analysis of a certain forest provides extended knowledge to meet the current and future challenges in the data management for complex tropical forest data. Although many forestry data collected in Myanmar have been altered due to the anthropogenic effects and unlawful extraction of many goods, it is statistically helpful in future data complication and provides more precise data management. This will undoubtedly improve the rigor inherent in dry zone forest data analysis as well as ascertaining elements of precision and correctness in data analysis during several decades. The results of this study may provide insight into forest management and ecological study that would be applicable to other tropical region.

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TAXONOMY AND POLLEN MORPHOLOGY OF GENUS *DESMODIUM* IN PAPILIONOIDEAE

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Abstract

Taxonomy and pollen morphology of 8 species belonging to genus *Desmodium* of Papilionoideae were studied. The specimens were collected from Magway Region during 2019-2020. Taxonomic descriptions, artificial key to the species and photographs of each species were presented. According to the resulting data, *Desmodium confertum* DC., *D. jucundum* Thw, are perennial and the remaining species are annual. The unifoliolate leaves occurred in *D. renifolium* (L.) Schindl., *D. teres* Wall., *D. velutinum* (Willd.) DC. and the remaining species are trifoliolate. Pollen morphology of each species was studied. Pollen grains found are monad and aperture colporate. All study species were found tricolporate and pori lalongate. Pollen of *D. confertum* DC., *D. velutinum* (Willd.) DC. are small sized and the remaining species are medium. Three types of exine sculpture (psilate, reticulate, microreticulate) were found. *D.confertum* DC. is reticulate; *D. griffithianum* Benth. is psilate and the remaining species are microreticulate. Pollen key to the species were constructed on different palynological characters.

Keywords: Taxonomy, pollen morphology, genus *Desmodium*

Introduction

Fabaceae are the third largest family of flowering plants. The species of this family are found throughout the world, growing in many different environments and climates. Fabaceae has been divided into three subfamilies Caesalpinioideae, Mimosoideae and Papilionoideae. The Fabaceae is very large group with worldwide distribution and the important plant groups, being the source of numerous pulses, soil rotation plants, oil, timber trees, gums and dyes (Simpson 2006). Legumes include a large number of crops for human and animal consumption as oil, fiber, fertilization, timber and medicine (Martin *et al.* 2006).

Pollen grains have a number of morphological features. These palynological features have provided characters that have been important in inferring phylogenetic relationship of plants (Simpson 2006). Palynology is the study of pollen, fine structure of their wall, particularly of its outermost layer, the exine. (Erdtman1985). The exine is the outer layer of pollen. It is composed of sporopollenin. Sporopollenin is very chemically stable and it is resistant to almost all kinds of environmental damage (Briggs & Brady 2000). The examination of pollen grains, both recent and ancient, can be of value in scientific studies. Taxonomy, genetic, evolutionary studies, honey studies, forensic science, tracing vegetation history, climate change studies (Moore *et al.* 1991). The palynological research can be either basic or applied. Basic aspects belongs to pollen morphology in relation to taxonomy, applied aspects belong to geopalynology, aeropalynology, iatropalynology and melitopalynology (Bhojwanii &Bhatnagar 2005).

The purpose of this research is to record the taxonomy and pollen morphology of genus *Desmodium* in Papilionoideae, to know palynological features of the *Desmodium* spp. to give the knowledge of pollen features and identification of the key, to fulfill the information concerning with pollen morphology.

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Materials and Methods

The plants were collected from Magway Region 2019- 2020. All the collected species were recorded by photographs during flowering times. Identification of collected specimens was carried out by using floristic literature of Dassanayake (1991, 1997), Kress *et al*. (2003) and Langran *et al*. (2010). Pollen samples were collected from the anther of blooming flowers and were acetolysed by Erdtman method (1952).The pollen samples in a glass vials were crushed with a glass rod and 1cc of acetic acid was added. The mixture of glass vial was transferred into a test tube and drops of concentrated sulphuric acid were added then put into a water bath. The material was transferred to a centrifuge. After centrifuging, the polliniferous material was transferred to be stored in a bottle and labeled. Pollen sample were mounted and observed by Light microscope. The identification of pollen is referred to Erdtman (1969), Erdtman (1971), Erdtman (1985), Paldat (2005), Hesse *et al*. (2009).

Results

Taxonomy and pollen morphology of 8 species in genus *Desmodium* of Papilionoideae has been studied.

A. Artificial key to the species

1. Perennial ----------2
1. Annual ----------3
    2. Flower white ----------1.*Desmodium confertum*
    2. Flower purple----------3. *D. jucundum*
3. Leaves trifoliolate ----------4
3. Leaves unifoliolate----------6
    4. Herbs----------2. *D. griffithianum*
    4. Shrub or under shrub ----------5
5. Leaflets cuneate at the base ----------4. *D.laxiflorum*
5. Leaflets obtuse at the base ----------6. *D.spirale*
    6. Flower pale greenish yellow----------7. *D. teres*
    6. Flower pale purple, bluish purple or pale blue----------7
7. Stem glabrous----------5. *D. renifolium*
7. Stem pubescent----------8.*D. velutinum*

B. Artificial pollen key to the species

1. Pollen size small ----------2
1. Pollen size medium ----------3
    2. Amb rounded ----------1.*Desmodium confertum*
    2. Amb triangular ----------8.*D. velutinum*
3. Sculpture psilate ----------2.*D. griffithianum*
3. Sculpture microreticulate----------4
    4. Shape oblate ----------4. *D. laxiflorum*
    4. Shape subprolate or prolate spheroidal----------5
5. Grain size more than 37µm in length ----------7. *D. teres*

5. Grain size less than 32µm in length ----------6
   6. Colpi less than 19 µm in length ----------3. *D. jucundum*
   6. Colpi more than 22 µm in length ----------7
7. Pori less than 8 µm in length ----------6. *D. spirale*
7. Pori more than 11 µm in length ----------5.*D. renifolium*

1. *Desmodium confertum* DC., Ann. Sc. Nat. Ser. 1.4:101.1825. (Fig.1, A)

Myanmar name : Unknown

English name : Unknown

Flowering period : June-September

Perennial, small tree; stems and branches terete, green, pubescent. Leaves trifoliolate compound, alternate; stipules linear-lanceolate, 0.5-0.9 cm long; petioles 1.5-3.0 cm long, green, pubescent, pulvinous; stipels lanceolate, 0.3-0.5 cm long, pubescent; leaflets ovate-oblong, 5.0-12.0 cm by 7.0-8.5 cm, terminal leaflets large, acute at the base, entire along the margin, rounded or mucronate at the apex, glabrous above, pubescent beneath. Inflorescences axillary racemes, few-many flowered; peduncles terete, pale green, pubescent. Flowers bisexual, zygomorphic, pentamerous, white, 1.2-1.5 cm in diameter at anthesis, pedicels terete, 0.2-0.4 cm long, pale green, pubescent; bracts lanceolate, deciduous, pale green, pubescent; bracteoles lanceolate, pubescent. Calyx campanulate, 5-lobed; tube 0.1-0.2 cm, pubescent; lobes lanceolate, 0.2-0.3 cm long, green, pubescent. Corolla papilionaceous; standard obovate to nearly orbicular, 1.0-1.2 cm by 0.9-1.0 cm; clawed 0.1-0.2 cm long; wings oblong lanceolate, 0.8-0.9 cm by 0.3-0.4 cm, adherent to the keel; keels oblong, 0.8-1.0 cm by 0.4-0.5 cm, clawed, glabrous. Stamens 10, diadelphous, staminal tube 0.8-1.0 cm long; anthers dithecous, basifixed, longitudinal dehiscence. Ovary oblong, superior, unilocular with few ovules on the marginal placentae; style terminal, stigma simple. Pods compressed, 1-4 jointed, indehiscent, 1-2 cm long, hairy.

Description of pollen morphology (Figure 1. B, C)

Tricolporate, subprolate, small, 21.25-25.0 × 18.75-22.5 µm in length and breadth; amb rounded, angulaperturate; colpi longicolpate, 18.75-22.5 × 2.5-3.75 µm in length and breadth; pori lalongate, 5.0-6.25 × 7.5-10.0 µm in length and breadth; exine 2.5-3.75 µm thick; sexine thicker than nexine; sculpturing reticulate; lumina heterobrochate, 0.6-1.25µm width; muri simplibaculate, about 0.3 µm wide.

2. *Desmodium griffithianum* Benth., in Miq., PL. Fungh. 222. 1825. (Fig.1, D)

Myanmar name : Unknown

English name : Unknown

Flowering period : September to November

Annual, erect herbs; stems and branches terete, appressed white hairs. Leaves trifoliolate compound, alternate; stipules lanceolate, 0.5-1.0 cm long, green, tomentose; petioles terete, 1-3 cm long, green, tomentose, slightly canaliculate above; stipels linear, green, tomentose; petiolules terete, green, tomentose; leaflets obovate, 1.5-5.5 cm by 1-2 cm, green, obtuse at the base, entire along the margin with ciliate, truncate or emarginate at the apex, glabrous above, appressed tomentose beneath. Inflorescences terminal and axillary raceme, many-flowered; peduncles terete, brownish, appressed tomentose. Flowers bisexual, zygomorphic, pentamerous, pale purple, 0.5-0.8 cm in diameter at anthesis; bracts lanceolate, brownish, tomentose, deciduous;

pedicels 0.3-0.5 cm long, brown, tomentose, deflexed at the tip; ebracteolate. Calyx campanulate, 4- lobed; tube 0.2-0.3 cm long, green, sparsely pubescent; lobes deltoid, 0.2-0.3 cm long, green, setaceous. Corolla papilionaceous; standard obovate, 0.5-0.8 cm by 0.3-0.5 cm, pale purple, glabrous; wings oblong, 0.4-0.6 cm by 0.3 cm, pale purple, glabrous; keels oblique, 0.4-0.6 cm by 0.3 cm, whitish purple, glabrous. Stamens 10, diadelphous, filaments filiform, white, glabrous; anthers uniform, dithecous, basifixed, longitudinal dehiscence. Ovary oblongoid, green, tomentose, unilocular with few ovules on the marginal placentae; style filiform, white, sparsely pubescent; stigma subcapitate. Pods linear-oblongoid, 1.5-3.5 cm long, 4-8 jointed, indehiscent, green, densely tomentose, lower suture straight the upper slightly indented. Seeds kidney-shaped, small, glabrous, brown.

Description of pollen morphology (Fig. 1, E, F)

Tricolporate, suboblate, medium, 25.0-31.3×30.0-37.5 µm in length and breadth; amb triangular, angulaperturate; colpi longicolpate, 18.8-23.8×2.5-5.0 µm in length and breadth; pori lalongate, 12.5-15.0 ×16.5-22.5 µm in length and breadth; exine 1.3-1.9 µm thick, sexine as thick as nexine, sculpturing psilate.

3. *Desmodium jucundum* Thw., Enum. Pl. Zeyl. 411. 1864. (Fig. 1, G)

Myanmar name : Unknown

English name : Unknown

Flowering period : October to December

Perennial, erect shrubs; stems and branches subterete, green, sparsely pubescent. Leaves trifoliolate compound, alternate; stipules lanceolate, 1.0-1.9 cm long, green, ribbed, pubescent, persistent; petioles 2-4 cm long, green, canaliculate above, densely velutinous; stipels linear, 0.5-0.6 cm long, green, pubescent, persistent; petiolule terete, 0.2-0.3 cm long, green, pubescent; rachis 1.0-1.5 cm long, green; leaflets obovate, terminal one longer than lateral, 6-22 cm by 3-11 cm, obliquely acute at the base, entire and ciliate along the margin, acute at the apex, densely appressed seriaceous above, glabrous beneath, green. Inflorescences axillary or terminal raceme, many-flowered; peduncles terete, green, densely pubescent. Flowers bisexual, zygomorphic, pentamerous, 0.7-0.8 cm in diameter at anthesis, pale purple; bracts lanceolate, 0.5-0.7 cm long, green, pubescent, deciduous; pedicel very short, green, pubescent; bracteoles linear, 0.2-0.3 cm long, green, pubescent. Calyx campanulate, 5-lobed; tube 0.1-0.2 cm long, green, pubescent; lobes linear, 0.2-0.3 cm long, green, pubescent. Corolla papilionaceous; standard elliptic or obovate, 0.6-0.7 cm by 0.7 cm, pale purple; wings obliquely oblong, 0.6-0.7 cm by 0.3 cm, pale purple, adnate to the keel, wholly clawed; keel beaked, 0.5-0.6 cm by 0.2 cm, white, connate above, long spurred at the base. Stamens 10, diadelphous, inserted; staminal tube 0.5-0.7 cm long, glabrous, white; anthers uniform, dithecous, basifixed, longitudinal dehiscence. Ovary superior, oblong, 0.5-0.6 cm long, green, 5-10 ovules on the marginal placentae; style curved, 0.2-0.3 cm long, white; stigma simple. Pods linear, fattened, constrict, 5-10 seeded, tip linear, 2.5-4.0 cm long, 4-6 jointed, indehiscent, dark brown, densely white appressed. Seeds minute, sericeous, dark brown, densely white appressed, smooth.

Description of pollen morphology (Fig.1, H, I)

Tricolporate, prolate spheroidal, medium, 27.5-30.0×25.0-28.75 µm in length and breadth; amb triangular, angulaperturate; colpi ¾ way up to the pole, 16.25-18.75 × 3.8-5.0 µm in length and breadth; pori lalongate, 6.25-8.75×13.8-16.25 µm in length and breadth; exine 1.25-1.85 µm thick, sexine thicker than nexine; sculpturing microreticulate.

4. *Desmodium laxiflorum* DC. in Ann., Sci. Nat. Paris 1, 4:100. 1825. (Fig.1, J)

Myanmar name : Ywet kat

English name : Unknown

Flowering period : August- November

Annual, under shrub; stems and branches obscure angular, pubescent. Leaves trifoliolate compound, alternate; stipules lanceolate, about 0.1 cm long; petioles terete, 2-3 cm long, canaliculate above, with longitudinal ribs, sericeous, densely yellowish brown hairy; leaflets ovate-elliptic, 5-10 cm by 3.5-8.5 cm, nearly cuneate at the base, entire or sinuate along the margin, acuminate at the apex, sparsely pubescent above, densely pubescent beneath. Inflorescences axillary or terminal racemes, with fascicles of flowers, 2-7 flowered. Flowers bisexual, zygomorphic, pentamerous, hypogynous, pale purple, about 0.6 cm in diameter at anthesis; pedicel 0.5-1.0 cm long, green, densely hair pubescent; bracts lanceolate, minute, caducous. Calyx campanulate, 5-lobed, with dense hooked hairs; tube 0.2 cm long, green; lobes setaceous, deltoid, densely villous. Corolla papilionaceous; standard broadly obovate or orbicular, 0.5-0.6 cm by 0.3-0.4 cm, yellowish brown hairy, short clawed; wings auriculate, oblong, about 0.4 cm long, tomentose, clawed, keel oblong, about 0.4 cm long, violet, clawed, tomentose. Stamens 10, diadelphous; staminal tube 0.3-0.4 cm long; anthers uniform, basifixed, longitudinal dehiscence. Ovary superior, densely white pubescent, green; style long, slightly hairy at the base, glabrous at the apex; stigma capitate. Pods linear, with dense minute short hairs, 2.5-3.0 cm long, 4-12 jointed, slightly constricted lower suture, densely hooked hairy. Seed globoid, dark brown.

Description of pollen morphology (Fig.1, K, L)

Tricolporate, oblate, medium, 25.0-31.25 × 28.75-35.0 µm in length and breadth; amb rounded triangular, angulaperturate; colpi ¾ way up to the pole, 17.5-23.75 × 2.5-5.0 µm in length and breadth; pori lalongate, 3.75-6.25 × 8.75-12.5 µm in length and breadth; exine 0.6-1.3 µm thick, sexine as thick as nexine; sculpturing microreticulate.

5. *Desmodium renifolium* (L.) Schindl. Repert. Spec.Nov. Regni Veg.22: 262.1926(Fig.2, A)

***Hedysarum renifolium* L. Syst. Nat. ed. 10, 2:1169. 1759**

Myanmar name : Unknown

English name : Unknown

Flowering period : October-January

Annual herbs; stems and branches terete, green, glabrous. Leaves unifoliloate, alternate; stipules linear, about 0.2 cm long, pubescent, caducous; petioles terete, 1-2 cm long, green, glabrous, slightly canaliculate above; stipels green, glabrous; blades oval-reniform, 2-4 cm by 3.5-5.0 cm, subcoriaceous, slightly cordate at the base, entire along the margin, truncate or emarginate at the apex, green, glabrous on both surfaces. Inflorescences axillary racemes, many-flowered; peduncles terete, green, glabrous. Flowers bisexual, zygomorphic, pentamerous, pale blue, 0.2-0.3 cm in diameter at anthesis; pedicels terete, 0.6-0.8 cm long, green, pubescent; bracts linear, green, glabrous, deciduous. Calyx campanulate, 5-lobed; tube 0.2-0.3 cm long, subglabrous, green; lobes setaceous, glabrous. Corolla papilionaceous; standard obovate, 0.3-0.4 cm long, bluish-white, glabrous; wings oblong, 0.2-0.3 cm long, adherent to keel, glabrous; keel obovate, about 0.2 cm long, glabrous. Stamens 10, diadelphous, filaments filiform, 0.3-0.4 cm long, glabrous; anthers dithecous, basifixed, uniform, longitudinal dehiscence, Ovary superior, oblongoid, glabrous, unilocular with marginal placentae; style filiform, 0.2-0.3 cm long, glabrous,

green; stigma capitate. Pods compressed, lomentum, 3-5 jointed, indehiscent, 1.5-2.5 cm long, glabrous. Seeds compressed, about 0.2 cm long, black.

Description of pollen morphology (Fig.2, B, C)

Tricolporate, subprolate, medium, 28.75-31.25 × 25-30 µm in length and breadth; amb rounded triangular, angulaperturate; colpi ¾ way up to the pole, 21.25-23.75 × 2.5-5.0 µm in length and breadth; pori lalongate, 11.25-15.00× 12.5-18.75 µm in length and breadth; exine 1.3-2.5 µm thick, sexine thicker than nexine; sculpturing microreticulate.

6. *Desmodium spirale* DC., Prod. 2:332.1825. (Fig.2, D)

Myanmar name : Unknown

English name : Unknown

Flowering period : September to November

Annual, erect shrubs; stems and branches terete, brownish green, longitudinal ribs, hirsute. Leaves trifoliolate compound, alternate; stipules linear-lanceolate, 0.5-0.8 cm long, green, brownish pubescent; petioles terete, 2-4 cm long, canaliculate above, green, pubescent; stipels filiform, green, pubescent; petiolules terete, brownish green, pubescent; leaflets obovate, 3-8 cm by 2-4 cm, green, obtuse at the base, entire along the margin with ciliate, emarginate at the apex, terminal leaflets larger than lateral, sparsely pubescent above, short hooked hairs beneath. Inflorescences axillary and terminal racemes, many-flowered; peduncles terete, brownish green, densely hooked hairs. Flowers bisexual, zygomorphic, pentamerous, bluish purple, 0.7-1.0 cm in diameter at anthesis; bracts lanceolate, deciduous, pubescent; pedicels filiform, 0.4-0.9 cm long, brown, sparsely pubescent; bracteoles minute, fugacious, caducous. Calyx campanulate, 5-lobed; tube 0.1-0.2 cm long, brownish green, puberulous; lobes deltoid, 0.2-0.4 cm long, brownish green, puberulous. Corolla papilionaceous; standard obovate, 1.0-1.2 cm by 0.9-1.0 cm, bluish purple, with white tinged base, glabrous; wings oblong, 0.3-0.4 cm by 0.5-0.6 cm, bluish purple, adhering to the keel, glabrous; keel obtuse, 0.3-0.4 cm by 0.6-0.7 cm, violet, glabrous. Stamens 10, diadelphous, inserted; filaments filiform, white, glabrous; anthers uniform, dithecous, basifixed, longitudinal dehiscence. Ovary oblongoid, pale green, sparsely pubescent, unilocular, marginal placentae; style curved; white; stigma capitate. Pods 3-6 jointed, 0.8-1.0 cm long, elliptic, curved, indehiscent, sparsely hooked hairs, brown when mature. Seeds terete, small, brown, glabrous.

Description of pollen morphology (Fig.2, E, F)

Tricolporate, prolate spheroidal, medium, 28.75-31.25×26.25-30.0 µm in length and breadth; amb triangular, angulaperturate; colpi ¾ way up to the pole, 22.5-23.75 ×2.5-5.0 µm in length and breadth; pori lalongate, 5.0-7.5×11.25-15.0 µm in length and breadth; exine 1.3-1.9 µm thick, sexine thicker than nexine; sculpturing microreticulate.

7. *Desmodium teres* Wall., Cat. 5694.1832. (Fig.2, G)

Myanmar name : Unknown

English name : Unknown

Flowering period : September to November

Annual, erect herbs; stems and branches angles, solid, woody, pubescent. Leaves unifoliolate; alternate; stipules linear lanceolate, 0.7-1.2 cm long, green, pubescent, persistent; petioles terete, about 0.7 cm long, green, slightly canaliculate above, pubescent; stipels linear,

green, pubescent; leaf blades oblong-lanceolate, 6-15 cm by 2.3-6.5 cm, green, obtuse or rounded at the base, wavy and ciliate along the margin, acuminate at the apex, sparsely pubescent on both surfaces. Inflorescences terminal racemes, many-flowered; peduncles angular, green, hispid. Flowers bisexual, zygomorphic, pentamerous, pale greenish yellow, 0.3-0.4 cm in diameter at anthesis; bracts linear, pale green, caducous, pubescent; pedicel terete, 0.2- 0.3 cm long, green, with hooked pubescent; bracteoles absent. Calyx campanulate, 5-lobed; tube about 0.2 cm long, green, pubescent; lobes deltoid, green, setaceous. Corolla papilionaceous, exserted; standard obovate, 0.5-0.6 cm by 0.4-0.5 cm, pale greenish-yellow, glabrous; wings oblong, about 0.4 cm by 0.2 cm, white, glabrous; keel oblong, about 0.4 cm by 0.2 cm, purplish white, glabrous. Stamens 10, diadelphous; filaments filiform, white, glabrous; anthers dithecous, uniform, dorsifixed, longitudinal dehiscence. Ovary superior, oblongoid, unilocular with many-ovuled on the marginal placentae; with hooked hairs; style white, glabrous; stigma capitate. Pods 5-10 jointed, 2-5 cm by 0.2-0.4 cm, compressed, indehiscent, very narrowly turgid, acute at the tip, with hooked hairs, slightly constricted at both suture. Seeds small, pale brown, elliptic.

Description of pollen morphology (Fig.2, H, I)

Tricolporate, prolate spheroidal, medium, 37.5-42.5×32.5-38.75 µm in length and breadth; amb triangular, angulaperturate; colpi ¾way up to the pole,18.75-22.5×2.5-5.0 µm in length and breadth; pori lalongate, 6.25-7.5×10-15 µm in length and breadth; exine 1.25-1.85 µm thick, sexine as thick as nexine; sculpturing microreticulate.

8. *Desmodium velutinum* (Willd.) DC. Prodr. 2:328. 1825. (Fig.2, J)

***Desmodium latifolium* (Roxb.) DC., Prodr. 2:328. 1825.**

***Hedysarum velutinum* Willd., Sp. Pl. 3:117. 1803**

Myanmar name : Kyo pan

English name : Unknown

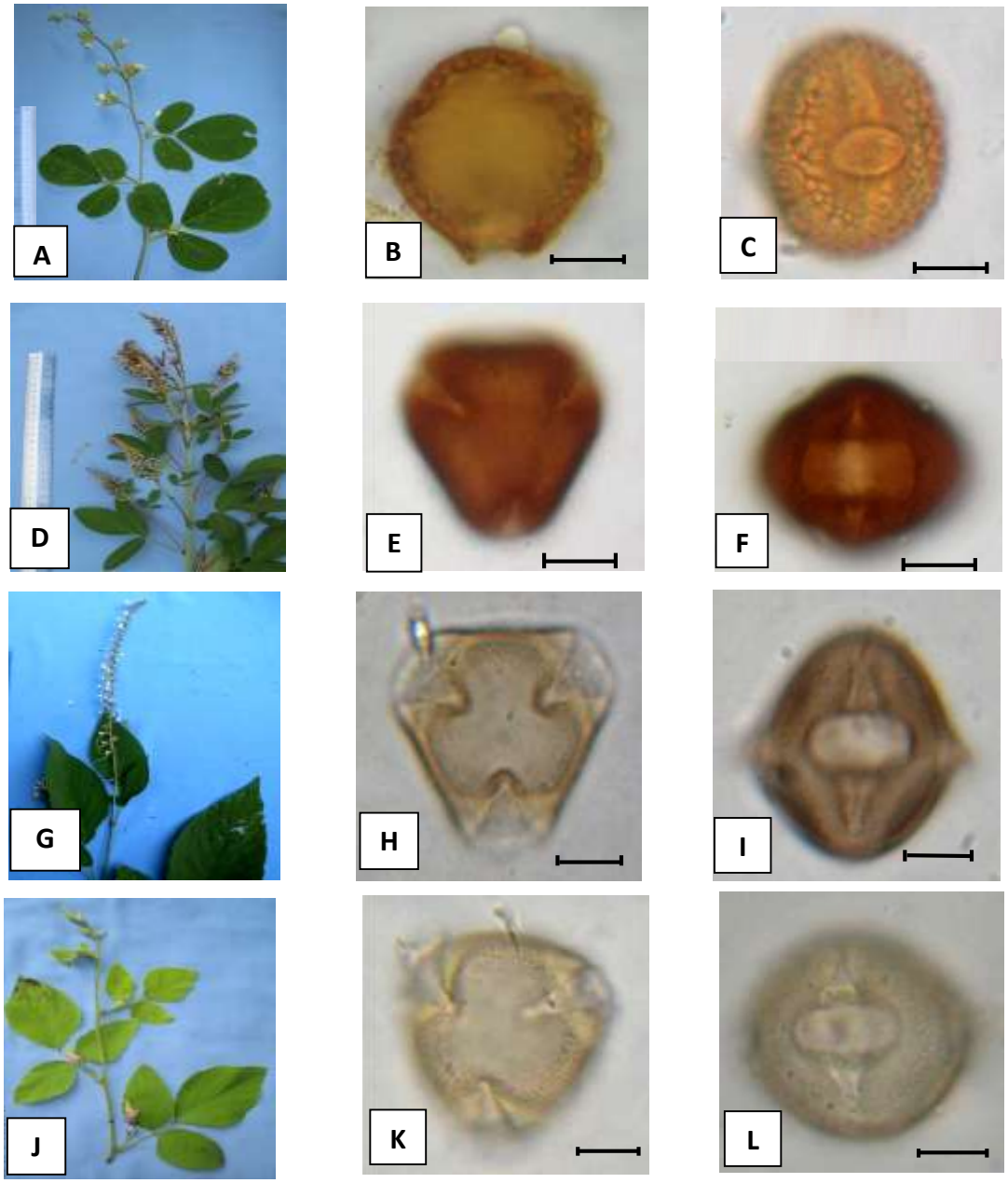
Flowering period : October to December

Annual, erect herbs; stems and branches terete, green, brownish pubescent. Leaves unifoliolate; alternate; stipules linear, 0.2-0.3 cm long, brown, tomentose; stipels linear, tomentose; petiolules terete, 0.2-0.4 cm long, brown, tomentose; petioles terete, 0.5-1.0 cm long, greenish brown, tomentose; leaf blades broadly ovate, 4-10 cm by 2.5-7.5 cm, obtuse or truncate at the base, entire along the margin with ciliate, rounded to acute at the apex, pubescent on both surfaces. Inflorescences terminal or axillary dense racemes, many flowered; peduncles terete, green, brown pubescent. Flowers bisexual, zygomorphic, pentamerous, pale purple, 0.2-0.4 cm in diameter at anthesis; bracts lanceolate, caducous, densely hispid; pedicels terete, 0.1-0.2cm long, green, pubescent; ebracteolate. Calyx campanulate; tube about 0.2 cm long, green, pubescent; lobes lanceolate, green, pubescent. Corolla papilionaceous; standard ovate, 0.5-0.6 cm by 0.4-0.5 cm, pinkish purple, shortly clawed, pubescent; wings oblong, 0.3 - 0.4 cm by 0.2 cm, pinkish purple, pubescent; keel obtuse, 0.3-0.4 cm long, purple, glabrous. Stamens 10, diadelphous; filaments filiform, white, glabrous; anthers dithecous, uniform, basifixed, longitudinal dehiscence. Ovary superior, linear-oblong, densely white pubescent, unilocular, marginal placentae; style filiform, brownish white, glabrous, stigma capitate. Pods lomentum, 3-6 jointed; indehiscent, pubescent, the lower suture straight, the upper suture slightly indented. Seeds small, elliptic, pale brown.

Description of pollen morphology (Fig.2, K, L)

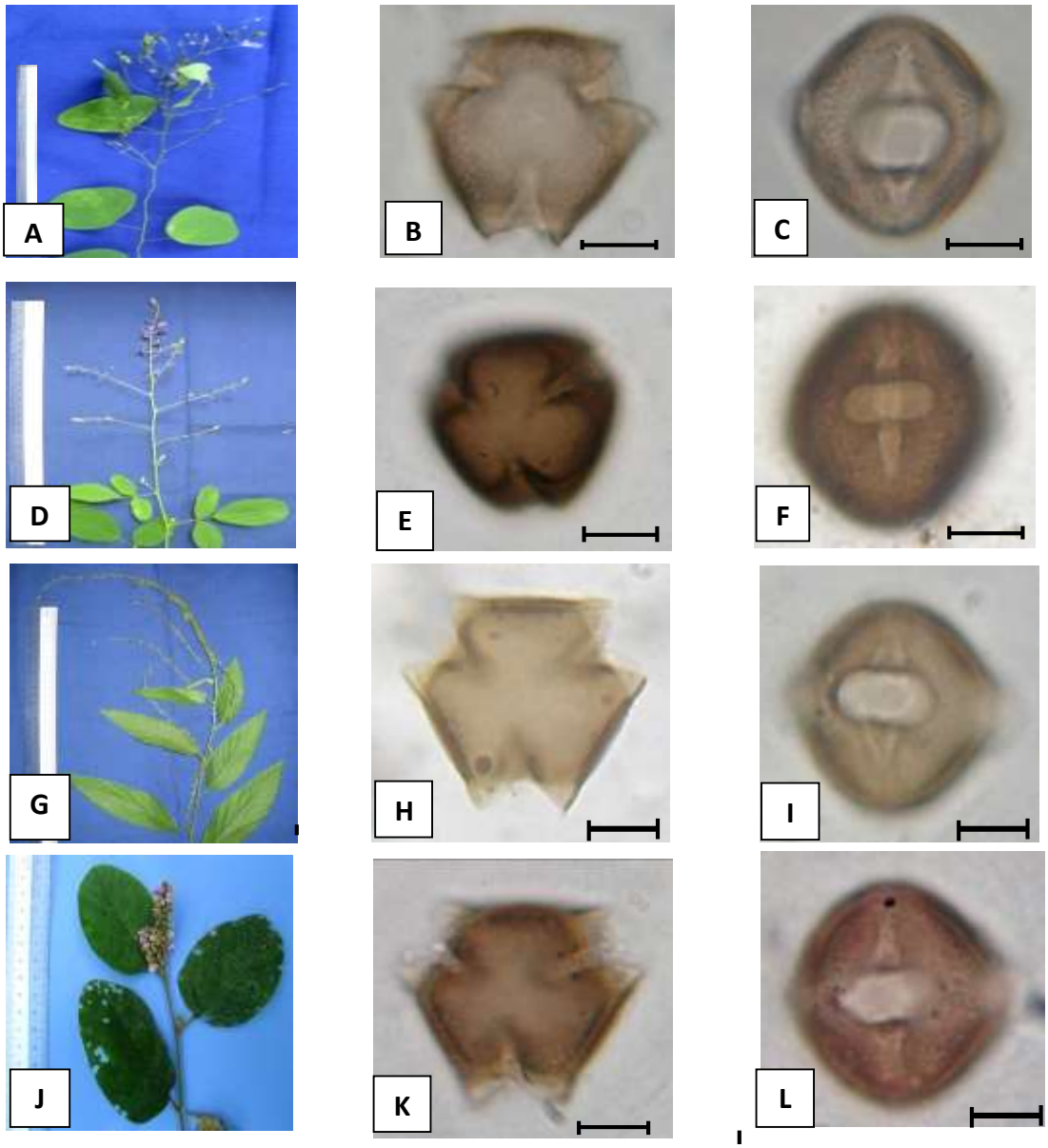
Tricolporate, subprolate, small, 17.5-22.5×15.0-18.75 µm in length and breadth; amb triangular, angulaperturate; colpi longicolpate, 15.0-18.75 ×2.5-3.75 µm in length and breadth; pori

lalongate, 6.25-8.75×8.75-12.5 µm in length and breadth; exine 1.3-1.9 µm thick, sexine thicker than nexine; sculpture microreticulate.



Scale bar = 10 µm

Figure 1 A. Inflorescences of *Desmodium confertum* DC.
B &C Polar & Equatorial view pollen of *D. confertum* DC.
D. Inflorescences of *Desmodium griffithianum* Benth.
E &F Polar & Equatorial view pollen of *D. griffithianum* Benth.
G. Inflorescences of *Desmodium jucundum* Thw.
H & I Polar & Equatorial view pollen of *D. jucundum* Thw.
J. Inflorescences of *Desmodium laxiflorum* DC.
K &L Polar & Equatorial view pollen of *D. laxiflorum* DC.



Scale bar = 10 µm

Figure 2 A. Inflorescences of *Desmodium renifolium* (L.) Schindl.
B &C Polar & Equatorial view pollen of *D. renifolium* (L.) Schindl.
D. Inflorescences of *Desmodium spirale* DC.
E &F Polar & Equatorial view pollen of *D. spirale* DC.
G. Inflorescences of *Desmodium teres* Wall.
H & I Polar & Equatorial view pollen of *D. teres* Wall.
J. Inflorescences of *Desmodium velutinum* (Willd.) DC.
K &L Polar & Equatorial view pollen of *D. velutinum* (Willd.) DC.

Discussion and Conclusion

In this research, taxonomy and pollen morphology of 8 species belonging to genus *Desmodium* of the subfamily Papilionoideae were studied. According to the resulting data, *Desmodium confertum* DC., *D. jucundum* Thw. are perennial and the remaining species are annual. *D. confertum* is small tree and remaining species are herbs or shrubs. The unifoliolate leaves occurred in *D. renifolium* (L.) Schindl., *D. teres* Wall., *D. velutinum* (Willd.) DC. and the remaining species are trifoliolate. The inflorescences types of studied species are raceme and Papilionaceous flowers. Stamens are found in diadelphous, ovary marginal placentation and indehiscent fruits.

Pollen morphology was classified on the basic of size, shape and sculpturing pattern. The resulting data of the pollen morphology were presented Figure 1- 2.

In this research, the types of pollen are found monad and aperture colporate. The sizes of pollen grains are small and medium. *Desmodium confertum* DC., *D. velutinum* (Willd.) DC., are small sized and the remaining species are medium sized. All study species are tricolporate pollen and pori lalongate. Colpi of *Desmodium confertum* DC., *D. griffithianum* Benth., *D. velutinum* (Willd.) DC. are longicolpate and the other species are colpi ¾way up to the pole. The shapes of pollens are prolate spheroidal, subprolate, suboblate, oblate and oblate spheroidal. Oblate shape is found in *D. laxiflorum* DC.; suboblate in *D. griffithianum* Benth.; subprolate are *D. confertum* DC., *D. velutinum* (Willd.) DC., *D. renifolium* (L.) Schindl and the remaining species are prolate spheroidal. Sculpture patterns are reticulate, psilate and microreticulate. Reticulate sculpture occurred in *Desmodium confertum* DC.; psilate is *D. griffithianum* Benth. and the remaining species are microreticulate.

In the present research, pollen grains of *Desmodium* species are tricolporate, small to medium sized, pori lalongate, suboblate, subprolate, oblate spheroidal, prolate-spheroidal, oblate shape; amb triangular, rounded; sculpture psilate, microreticulate and reticulate. Mitra & Mondal (1982) mentioned that pollen grains of *Desmodium* are tricolporate, medium to large sized, spheroidal to oblate-spheroidal, amb subangular and sculpture rugulose, verrucose, obscure or microreticulate to reticulate; which characters are similar to the present research. Butt (1989) stated that the pollen grains of *Desmodium* spp. are tricolporate, small to medium, psilate to finely granulate, granulate to finely reticulate, which characters are agreed with present research.

Faegri *et al*. (1964) stated that the structure and sculpturing of the exine provide characters of great diagnostic value. There are many other characters which may be of equal or even greater importance in the identification of pollen grains.

In this research is provided the knowledge of pollen morphology of genus *Desmodium* to botanist and others scientists who are interested. Pollen characters will be supported for identification and classification of the plants. Pollen characters are now being used as important taxonomic tool for reassessing different types of plant groups. The present research, to give the taxonomic information and pollen morphological data for the future studies.

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POLLEN MORPHOLOGY OF SOME *BLUMEA* SPECIES DISTRIBUTED IN MANDALAY AREA

Aye Aye Thin[1] & Soe Myint Aye[2]

Abstract

The pollen morphology of some *Blumea* species distributed in Mandalay Area was undertaken. The samples of 8 species belonging to 1 genus under 1Tribes were collected from January to June during the year 2018. The morphological characters of pollen grains of each species were also studied. The types of pollen grains were tricolpate, triporate, tricolporate and tetracolporate. Three species were tricolpate and triporate. Only one species were tricolporate and tetracolporate. The exine ornamentation in pollen was found to be all echinate. The shapes of pollen were spheroidal, prolate spheroidal, oblate spheroidal and subprolate. Four species were spheroidal, two species were subprolate, only one species were prolate spheroidal and oblate spheroidal. The size of pollen were small or medium. Five species were medium and three species were small. An artificial key to the species were also constructed. The pollen photomicrographs of each species were presented by polar view and equatorial view.

Keywords*: Blumea*, Tricolpate, Triporate, Tricolporate, Tetracolporate, Exine, Echinate.

Introduction

Blumea DC. is one of the largest genera in the Tribe Inuleae which includes approximately 100 species worldwide. *Blumea* is primarily distributed in tropical Asia and Africa while its highest diversity is in tropical Asia (Peng *et al.* 2020). Seventeen species were recorded in Myanmar (Kress *et al.* 2003).

Palynology is the study of spores and pollen grains. Spores and pollen grains have a number of morphological and ultrastructural features. Pollen wall structure refers to the internal form of the pollen grain wall. Mature pollen walls almost always consists of two major layers; intine and exine. The exine is the outermost layer which is composed of primarily of cellulose and pectins. The exine is the hard, outermost desiccation-resistant wall layer that provides the major structural support for the cytoplasm (Simpson 2006). Two basic types of pollen grains are the porate and colpate. Furrows are elongate and boat shaped and ends are more or less sharp. Pollen grains that have one long furrow are called monocolpate. Tricolpate pollen grains have three long furrows (Yamada & Iwanmai 1988).

Pollen grains carry the male gametes of seed plants. Pollen biology has a strong impact in agriculture, biotechnology forestry and genetics (Meo 2009). There are many disciplines associated with plant taxonomy which are used by taxonomists to improve the identification, classification and systematic position of plant taxa. Among these disciplines palynology is one of the most significant tools used by modern taxonomist to identify and differentiate closely related taxa. The study of pollen has direct relevance in agriculture, horticulture, foresty, plant breeding and biotechnology (Zafar *et al.* 2007). Dinis and Pereira (2007) described pollen morphology of Inuleae Tribes are porilolongate, sculpture echinate. Pornpanggrueng (2016) stated that shape of *Blumea* species were prolate spheroidal, oblate spheroidal and subprolate, spine length 1.3-6µm long.

The aim and objectives of this research to investigate on type, shape, size and sculpture of pollen and to provide the valuable information of pollen morphology that will be useful in plant identification.

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Materials and Methods

The specimens were collected in Mandalay Area from January to June during the year 2018. Identification of genera and species were carried out by referring to Dassanayake (1980), Backer (1965). Myanmar and English names were checked by referring Hundley and Chit Ko Ko (1989), Kress *et al*. (2003). All the fresh pollen were collected from the anther of mature flowers and stored in glass vial with glacial acetic acid.

The pollen samples were acetolysed by the method of Erdtman (1960). The samples in glass vial were put into a test tube then crushed with a glass rod. Acetolysis solution was mixed by 9 parts of glacial acetic acid and 1 part of concentrated sulphuric acid. The acid was dropped gently down the side of the tube. The 1cc of acetolysis mixture was poured into the test tube containing the pollen sample and stirred with a glass rod. The test tube was heated in a water bath 70º-80ºC for 25-30 minutes. The test tube was allowed to cool, and the sample diluted with distilled water and centrifuged for 20-30 minutes at 3000 rpm. This was repeated twice decanting the water each time. Dilute glycerin solution was added to the residue then transferred and stored in air tight glass vial and labeled.

The mounted slides were observed under light microscope to study the pollen morphology. For each species more than 10 pollen grains were measured and recorded. The terminology used in the accordance with Erdtman (1971) and Hesse *et al.* (2009).

Results

Pollen morphology of 8 species of *Blumea* has been studied. The lists of collected species were as shown in Table 1.

Table 1 List of Collected Species

Tribe	No	Scientific Name	Myanmar Name
Inuleae	1	*Blumea barbata* DC.	Ya nat
	2	*Blumea hieraciifolia* (D. Don) DC.	Unknown
	3	*Blumeajun ghuhniana* (Miq.) Boerl.	Unknown
	4	*Blumea laciniata* (Wall. ex Roxb.) DC.	Mmo
	5	*Blumea megacephala* (Randeria) C. C. Chang & Y. Q. Tseng	Pme lan
	6	*Blumea pterodonta* DC.	Unknown
	7	*Blumea sericans* (Kurz) Hook. f.	Kadu
	8	*Blumea tenella* DC. ex Decne	Unknown

1. *Blumea barbata* DC. in Wight, Contrib, Bot. India 14. 1834.

Myanmar name : Ya nat

English name : Unknown

Flowering period : December to June

Pollen morphology

Triporate, spheroidal, medium, 30.0 - 32.4 µm in diameter; amb rounded triangular: pori lolongate, 15.6 - 18.0 × 1.8 - 2.4 µm in length and breadth; exine 6.0 - 8.4 µm thick, sexine 4.8 - 6.0 µm thick, sexine thicker than nexine; sculpturing echinate; spine 3.6 - 6.0 µm long, basal cushion about 6.0 µm in width, about 3.6 µm in height, interspinal space about 1.8 µm wide.

Specimens examined: Madaya Township, Sedawgyi; 22° 28' N and 96° 20' E; 9 March, 2018; Aye Aye Thin, collection no 4.

2. *Blumea hieraciifolia* (D.Don) DC., in Wight, Contrib. 15. 1834.

Erigeon hieraciifolius D.Don, Prodr. Fl. Nepal. 172. 1825

Blumea flexuosa, C. B. Clarke, Comp. Ind. 86. 1876.

Myanmar name : Unknown

English name : Unknown

Flowering period : October to April

Pollen morphology

Tetracolporate, spheroidal, medium, 31.2 - 32.4 μm in diameter; amb rounded quadrangular; colpi ½ way up to the pole, 15.6 - 16.2 × 3.6 - 4.8 μm in length and breadth; pori circular, 2.4 - 3.6 μm in diameter; exine 4.8 - 5.8 μm thick, sexine about 4.6 μm thick, sexine thicker than nexine; sculpturing echinate; spine 3.6 - 4.8 μm long, basal cushion 4.8 - 6.0 μm in width, about 3.6 μm in height, interspinal space about 6.0 μm wide.

Specimens examined: Amarapura Township, Shanlaykyun; 22° 55' N and 96° 03'E; 13 March, 2018; Aye Aye Thin, collection no. 5.

3. *Blumea junghuhniana* (Miq.) Boerl., Fl. Ind. Bat. 1858.

Conyza junghuhniana Miq., Fl. Ind. Bat. 2: 55. 1856.

Conyza dasycoma var. *pinnatifida* (Miq) Boerl. Handl. Fl. Ned. Ind. 1:239. 1891.

Myanmar name : Unknown

English name : Unknown

Flowering period : September to March

Pollen morphology

Tricolporate, subprolate, small, 20 - 24 × 18.0 - 19.2 μm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 14.4 - 15.6 × 1.2 - 2.4 μm in length and breadth; pori lolongate, 4.8 - 7.2 × 1.8 - 2.4 μm length and breadth; exine about 5.4 μm thick, sexine about 3.0 μm thick, sexine thicker than nexine; sculpturing echinate; spine about 4.8 μm long, basal cushion about 2.4 μm in width, about 1.8 μm in height, interspinal space about 3.6 μm wide.

Specimens examined: Mahaaungmyay Township, Mandalay University Campus; 96º 03' N and 96º 08' E; 28 February, 2018; Aye Aye Thin, collection no. 2.

4.*Blumea laciniata* (Wall. ex Roxb.) DC., Prodr. 5: 436. 1836.

Conyza laciniata Wall.ex Roxb., Fl. Ind. 3:427. 1832.

Blumea glandulosa Benth., Fl. Hongk. 177.1861.

Myanmar name : Unknown

English name : Cut leaf Blumea

Flowering period : October to March

Pollen morphology

Tricolpate, spheroidal, small, 22.8 - 24.0 μm diameter; amb rounded triangular; colpi ¾ way up to the pole, 15.6 -18.0 × 2.4 - 4.8 μm in length and breadth; exine 3.6 μm thick, sexine about 2.4 μm thick, sexine thicker than nexine; sculpturing echinate; spine 2.4 - 3.6 μm long, basal cushion 2.4 - 3.6 μm in width, about 2.4 μm in height, interspinal space about 6.0 μm wide.

Specimens examined:Chanmyathazi Township, Myothit; 21° 55' N and 96° 5' E; 1 March, 2018; Aye Aye Thin, collection no.3.

5. ***Blumea megacephala*** (Randeria) C. C. Chang & Y. Q. Tseng, Acta Phytotax. Sin 12 (3): 310. 1974.

Blume ariparia DC.var. *megacephala* Randeria, Blumea 10 (1): 215. 1960.

Myanmar name : Pme lan

English name : Unknown

Flowering period : January to May

Pollen morphology

Triporate, prolate spheroidal, medium, 28.8 - 30.0 × 25.2 - 28.0 μm in length and breadth; amb rounded triangular; pori lolongate, 6.0 - 7.2 × 4.8 - 5.4 μm in length and breadth; exine 6.6 μm thick, sexine about 5.16 μm thick, sexine thicker than nexine; sculpturing echinate; spine about 6.0 μm long, basal cushion about 2.4 μm in width, about 2.4 μm in height, interspinal space 6.0 μm wide.

Specimens examined: Chanmyathazi Township, Myothit; 21° 55' N and 96° 5' E; 13 March, 2018; Aye Aye Thin, collection no. 8.

6. ***Blumea pterodonta*** DC. in Wight, Contrib. 16. 1834.

Laggera pterodonta (DC.) Benth. & Hook.f., Gen. Pl. 2:290.1873.

Myanmar name : Unknown

English name : Unknown

Flowering period : February to May

Pollen morphology

Triporate, oblate spheroidal, medium, 25 - 32 × 30 - 31 μm in length and breadth; amb rounded triangular; pori circular, 6 - 7 μm in diameter; exine 4.2 - 4.8 μm thick, sexine about 3.6 μm thick, sexine thicker than nexine; sculpturing echinate; spine 2.4 - 4.8 μm long, basal cushion about 3.6 μm wide, about 3.6 μm in height, interspinal space about 2.4 μm wide.

Specimens examined: Amarapura Township, Shanlaykyun; 22° 55' N and 96° 03' E; 13 May, 2018; Aye Aye Thin, collection no. 6.

7. ***Blumea sericans*** (Kurz) Hook. f., Fl. Brit. India.3: 262. 1881.

*B. barbata*var. *sericans* Kurz, As. Soc. Bengal.46:188.1877.

Myanmar name : Kadu

English name : Unknown

Flowering period : January to March

Pollen morphology

Tricoplate, spheroidal, small, 19.2 - 24.0 µm in diameter; amb rounded triangular; colpi ¾ way up to the pole, 15.6 - 16.8 × 2.4 - 4.8 µm in length and breadth; exine about 3.6 µm thick, sexine about 2.4 µm thick, sexine thicker than nexine; sculpturing echinate; spine 1.2 - 2.4 µm long, basal cushion about 1.8 µm in width, about 1.2 µm in height, interspinal space about 1.2 µm wide.

Specimens examined: Madaya Township, Sedawgyi; 22° 28' N and 96° 20' E; 13 March, 2018; Aye Aye Thin, collection no.7.

8. ***Blumea tenella*** DC. ex Decne in Wight, Contrib. Bot. Ind: 411. 1834.

Conyza tenella (Decne) DC.ex Miq., Fl. Ned. Ind. 240. 1856.

Myanmar name : Unknown

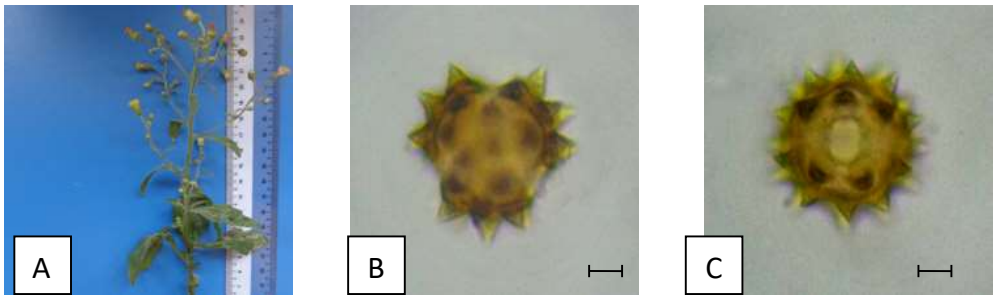
English name : Unknown

Flowering period : January to March

Pollen morphology

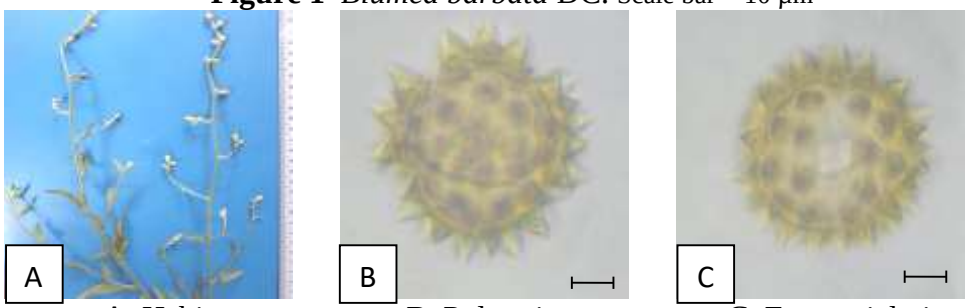
Tricolpate, subprolate, medium, 36.0 - 38.4 × 31.2 - 32.4 µm in length and breadth; amb rounded triangular; colpi ½ way up to the pole, 18.0 - 19.2 × 2.4 - 3.6 µm in length and breadth; exine 4.8 - 6.0 µm thick, sexine about 4.8 µm thick, sexine thicker than nexine; sculpturing echinate; spine about 3.6 µm long, basal cushion about 2.4 µm in width, about 2.4 µm in height, interspial space about 6.0 µm wide.

Specimens examined: Chanmyathazi Township, Myothit; 21° 55' N and 96° 5' E; 10 January, 2018; Aye Aye Thin, collection no. 1



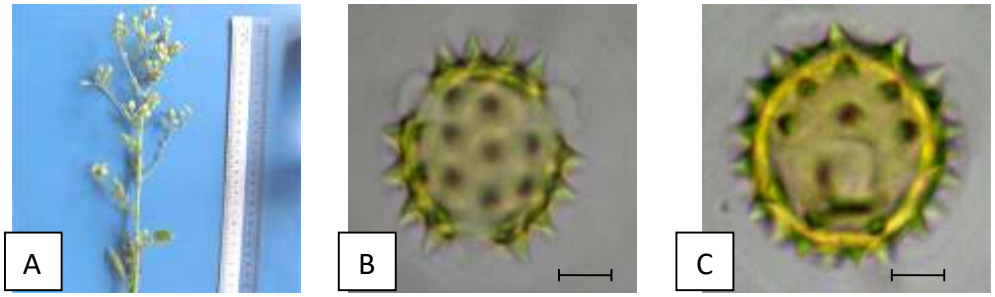
A. Habit B. Polar view C. Equatorial view

Figure 1 *Blumea barbata* DC. Scale bar = 10 µm



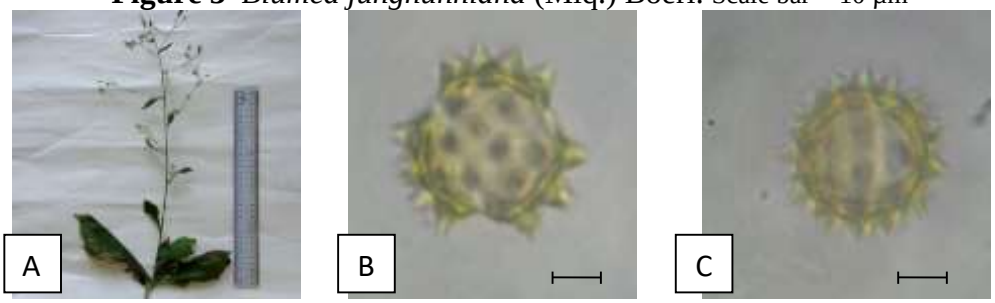
A. Habit B. Polar view C. Equatorial view

Figure 2 *Blumea hieraciifolia* (D. Don) DC. Scale bar = 10 µm



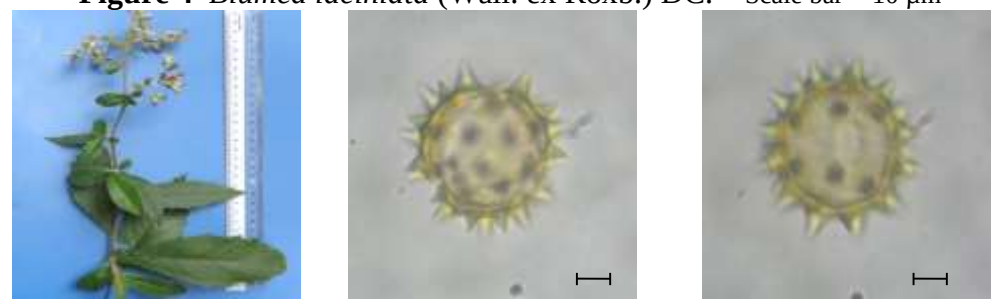
A. Habit B. Polar view C. Equatorial view

Figure 3 *Blumea junghuhniana* (Miq.) Boerl. Scale bar = 10 µm



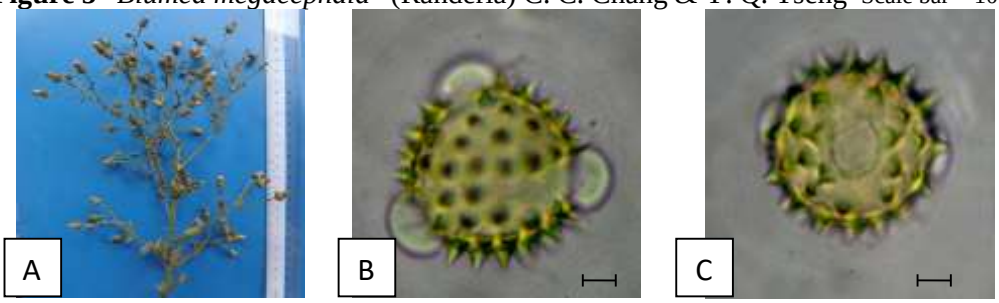
A. Habit B. Polar view

Figure 4 *Blumea laciniata* (Wall. ex Roxb.) DC. Scale bar = 10 µm



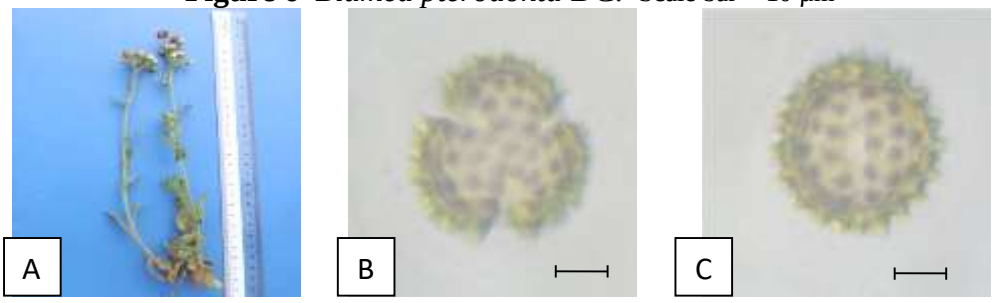
A. Habit B. Polar view C. Equatorial view

Figure 5 *Blumea megacephala* (Randeria) C. C. Chang & Y. Q. Tseng Scale bar = 10 µm



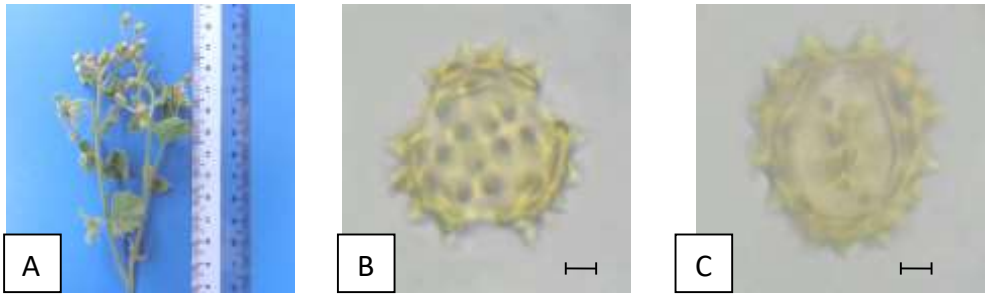
A. Habit B. Polar view C. Equatorial view

Figure 6 *Blumea pterodonta* DC. Scale bar = 10 µm



A. Habit B. Polar view C. Equatorial view

Figure 7 *Blumea sericans* (Kurz) Hook. f. Scale bar = 10 µm



A. Habit B. Polar view C. Equatorial view

Figure 8 *Blumea tenella* DC. ex Decne Scale bar = 10 µm

Table 2 Pollen Morphology of 8 Species of *Blumea*

No.	Scientific Name	Type of Aperture	Shape of EV	Size of EV	Colpi	Pori	Exine thickness	Size of pollen grains	Sculpture	Spine length
1	*Blumea barbata* DC.	P_3	S	30.0-32.4	-	Lo	6.0-8.4	Me	echi	3.6-6.0
2	*Blumea hieraciifolia* (D. Don) DC	C_4P_4	S	31.2-32.4	½	Cir	4.8-5.8	Me	echi	3.6-4.8
3	*Blumea junghuhniana* (Miq.) Boerl.	C_3P_3	Subpro	20-24×18.0-19.2	¾	Lo	5.4	Sm	echi	4.8
4	*Blumea laciniata* (Wall. ex Roxb.) DC.	C_3	S	22.8-24.0	¾	-	3.6	Sm	echi	2.4-3.6
5	*Blumea megacephala* (Randeria) C. C. Chang & Y. Q. Tseng	P_3	Pros	28.8-30.0×25.2-28.0	-	Lo	6.6	Me	echi	6.0
6	*Blumea pterodonta* DC.	P_3	Obls	25-32×30-31	-	Cir	4.2-4.8	Me	echi	2.4-4.8
7	*Blumea sericans* (Kurz) Hook. f.	C_3	S	19.2-24.0	¾	-	3.6	Sm	echi	1.2-2.4
8	*Blumea tenella* DC. ex Decne	C_3	Subpro	36.0-38.4×31.2-32.4	½	-	4.8-6.0	Me	echi	3.6

C = Colpate P_3 = Triporate ¾ = ¾ way up to the pole Pros = Prolate Spheroidal
P = Porate Cir = Circular Pro = Prolate Obls = Oblate Spheroidal
Subpro = Subprolate Lo = Lolongate S = Spheroidal ½ = ½ way up to the pole

Artificial key to the species

1. Aperture colporate or porate ---------- 2
1. Aperture colpate ---------- 6
   2. Tricolporate or tetracolporate ---------- 3
   2. Triporate ---------- 4
3. Pollen shape spheroidal, colpi ½ way up to the pole, pori circular ----------
   ---------- 2. *Blumea hieraciifolia*
3. Pollen shape subprolate, colpi ¾ way up to the pole, pori lolongate---
   ---------- 3. *Blumea junghuhniana*
   4. Pori circular, exine 4.2 - 4.8µm thick------ 6. *Blumea pterodonta*
   4. Pori lolongate, exine 6.0-8.4- 4.8µm thick---------- 5
5. Pollen shape spheroidal---------- 1. *Blumea barbata*
5. Pollen shape prolate spheroidal----------5. *Blumea megacephala*

6. Colpi ½ way up to the pole---------- 8. *Blumea tenella*

6. Colpi ¾ way up to the pole----------7

7. Spine length more than 2.4 ---------- 4. *Blumea laciniata*

7. Spine length less than 2.4 ---------- 7. *Blumea sericans*

Discussion

Pollen morphology was classified on the basic of aperture type, shape, size and sculpture pattern of the pollen. In this research 8 species belonging to 1 genus under 1 tribes were studied. In this study, apertures of pollen grain were tricolpate, triporate, tricolporate and tetracolporate, 3 species were tricolpate and triporate and only one species were tricolporate and tetracolporate.The shape of pollen grains were spheroidal, prolate spheroidal, oblate spheroidal and subprolate, 4 species were spheroidal, 2 species were subprolate, only species were prolate spheroidal and oblate spheroidal. The sizes of pollen grains were found to be small and medium, 3 species were small and 5 species were medium. The sculptures of pollen grains were all echinate.

In the present study, the pollen grains of Tribes Inuleae are echinate and the pollen grains of *Blumea. barbata* DC., *B. junghuhniana* (Miq.) Boerl., *B. megacephala* (Randeria) C. C. Chang & Y. Q. Tsengare pori lolongate. Dinis and Pereira (2007) reported that the pollen grains of Tribes Inuleae were echinate and porilolongate. Therefore, the resulting character is agreed with previous finding. In the present study, the spine lengths of *Blumea* specieswere 1.3-6.0 µm long and the shape of *Blumea junghuhniana* (Miq.) Boerl., *B. megacephala* (Randeria) C. C. Chang & Y. Q. Tsengand *B. pterodonta* DC. were prolate spheroidal, oblate spheroidal and subprolate. Pornponggrungrueng (2016) described that the pollen grains of this species were prolate spheroidal, oblate spheroidal and subprolate and the spine length of *Blumea* species were 1.3-6.0µm long which character is agreed with (Pornponggrungrueng 2016).

According to resulting data, spines, sculpture, shape and size are useful taxonomical character in systematic study of *Blumea* species.

Conclusion

It was concluded that the size, shape, sculpture and aperture of pollen grains were varied from species to another. The results showed that the form of pollen were useful as any other characters in the identification of plants.

By studying on pollen morphology of *Blumea*, it was observed that the members of *Blumea* possess the peculiar sculpture of pollens among species.

Thus, the present study will be useful information for the palynological characters which can be used in identification of some species of *Blumea.*

Acknowledgements

We would like to thank the Rector Dr Thura Oo, Monywa University for his permission this research. We would like to express our gratitude to Dr Tin Tin Nyunt, Professor and Head, Department of Botany, Monywa University for her encouragement, reviewing and providing necessary suggestions in this research. We are also thankful to Dr Theingi Htay, Professor, Department of Botany, Monywa University, for her invaluable advice. We would like to express my heartfelt gratitude and thank to Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay, for her very kind help.

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EVALUATION ON POSTHARVEST SEED QUALITY OF THREE SELECTED LABLAB BEAN CULTIVARS

Hlaing Hlaing Myint*

Abstract

The study was carried out the seedling growth of three selected lablab bean cultivars *Lablab niger* Medik var. *lignosus* Prain cv. Pe-maung-makhaw (V_1), cv. Pe-sau (Yin-mar) (V_2) and cv. Pe-gyi (Thet-yin) (V_3) effected by different duration of seeds storage and storage materials. Postharvest storage of seeds were intended to save seeds and otherwise they were be destroyed mainly by insects and pests. Treatments included four different storage materials bottles, tins, penan bags, plastic bags were used for the seed storage of lablab bean under room temperature for 6 months. The result showed that the highest seed germination and survival percentages were obtained in cv. Pe-maung-makaw (V_1) and the lowest percentage in cv. Pegyi (V_3) after 2, 4 and 6 months storage. Before storage, increased shoot length 7 DAS and 14 DAS, root length 14 DAS, seedling vigor index 7DAS and seedling vigor index 14 DAS were found in cv. Pegyi (V_3). After 2, 4 and 6 months storage, increased shoot length 7DAS and 14 DAS, seedling vigor index 7DAS and seedling vigor index 14 DAS were found in cv. Pe-maung-makaw (V_1) and decreased in cv. Pegyi (V_3). Before storage, increased root length 14DAS was found in cv. Pesau (V_2) and cv. Pegyi (V_3) than the cv. pe-maung-makaw (V_1). Among the different storage materials, seeds stored in bottles gave many benefits to be the best performance of seedling such as the highest percentage of seed germination and survivals. Bottles should be used as storage container during storage.

Keywords: seed growth, duration of seed storage, storage materials

Introduction

Pulses are grain legumes or food legumes belonging to the family Fabaceae. These are protein rich staple food, and important source of human food and animal feed. High quality seed leads to excellent seedling performance in the field. It is the ultimate basis of successful companies that breed crop plants for seed production.

Quality seeds are characterized by maintaining a high germination rate and stable content after storage. However, seeds gradually lose quality and viability after harvest (Coolbear, 1995). In addition, environmental stresses in the field or during harvest can compromise seed quality and storability. Besides decreasing germination, the undesirable consequences of seed deterioration include unpalatable food and inferior products.

Storage and upkeep of agricultural products are very important postharvest activities. Considerable amount of food grains is being spoiled after harvest due to lack of sufficient storage and processing facilities (Singh and Satapathy, 2003).The storage is an important aspect of post-harvest management. Therefore, the supply has to be maintained by proper storage throughout the year.

Storage protects the quality of grains from deterioration and help in stabilization of prices by regularizing demand and supply. It has been reported that the storage loss caused by insects, rodents and microorganisms are maximum. Lack of storage facilities forced the farmers to sell their product at low price. (Pangale, 1976). Storage practices vary and there are small or big storehouses, indoor or outdoor, temporary or permanent and individual or community storage structure (Jain *et al.,* 2004).

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Packaging is a practice to protect the product from any damage during storage and protect the quality. Improve conservation and storage structures to reduce postharvest losses, improve nutrition standard; bring a better price to the grower (Canada, 2006).

In the present work, a detailed study has been undertaken evaluation of storage condition of three lablab bean cultivars *Lablab niger* Medik var. *lignosus* Prain cv. Pe-maung-makhaw (V_1), cv. Pe-sau (Yin-mar) (V_2) and cv. Pe-gyi (Thet-yin) (V_3). In the study, treatments include four different storage materials bottles, tins, penan bags and plastic bags were used for under room temperature for 6 months.

Materials and Methods

Time and Place of the Study

The study was carried out in Campus of Hinthada University, Hinthada District from April to October 2014.

Preparation of Materials and Methods

The seeds for this experiment were collected from the field of Department of Botany, University of Hinthada. During the experiment, temperature of storage room was 23.5 –32.5°C and relative humidity (RH) was 69-90%.

Determination of Seed germination and Survival percentages after Seed storage

Lablab bean seeds stored with different packaging materials for 0, 2, 4 and 6 months were planted humus with sand in pots. A total 25 seeds per treatment were sown in each pot. Each treatment consisted of three replications. The germination percentage was determined in all treatments at one week after sowing seeds. The survival percentage was determined in all treatments at two weeks after sowing seeds.

Data Collection

Relative humidity (RH) and temperature were recorded at 0, 2, 4 and 6 months interval during storage under room temperature. Germination and survival percentages, shoot length, root length, seedling vigor index were recorded at 0, 2, 4 and 6 months, respectively.

Experimental Design and Statistical Analysis

This experiment consisted of 4 treatments as bottle, tin, penan bag, plastic bag with 3 replications using completely randomized design (CRD). The data were statistically analyzed by SAS software. Means of all treatments were compared using LSD (Least Significant Differences) at 5% level of significance.

Methods

Germination and Survival Percentages

The percentages of germination and survival were calculated by the following formula (ISTA,1987).

$$\text{Germination (\%)} = \frac{\text{Total number of germinating seeds}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Total number of survived plants}}{\text{Total number of sown seed}} \times 100$$

Determination of Seedling Vigor Index

Seedling vigor index was determined using the following formula (ISTA, 1987).
Seedling Vigor Index = germination (%) x shoot length (cm)

Results

Storage Room Temperature and Relative Humidity

During seeds storage, the lowest storage room temperature was determined as 23.5ºC in April and the highest room temperature 32.5° C also occurred in April among the records within seven months. However, RH at storage room was high in percentage in August 90% and minimum in percentage was obtained 69% in April, 2014.

Table 1 The storage room temperature and relative humidity data during the growing period of lablab beans

Parameters	Storage period						
	April	May	June	July	August	September	October
Maximum Temperature(ºC)	32.5	31.5	30.6	30	29.3	31.2	31.4
Minimum Temperature (ºC)	23.5	24	24.4	24	24	23.5	23.7
Relative Humidity (%)	69.00	70.00	85.00	87.50	90.00	86.00	80.12

Table 2 Germination percentage of three selected lablab bean cultivars after storing room temperature

Cultivars and Treatments	Seed Germination (%)				Mean
	0 month	2 months	4 months	6 months	
Cultivars					
Pe-maungmakaw (V_1)	100 a	96.67 a	89.00 a	87.33 a	93.25
Pesau (Yinn-mar) (V_2)	100 a	79.67 b	72.67 b	77.00 b	82.33
Pegyi (Thet -yin) (V_3)	100 a	75 .33 b	70.67 b	67.00 c	78.25
F-test	Ns	*	**	**	
LSD (5%)	-	10.9	3.78	4.5	6.30
CV (%)	-	11.46	4.3	5.15	6.97
Treatment					
Bottle	-	90.22 a	85.33 a	81.78 a	85.78
Tin	-	84 .44ab	76.44 ab	78.67 a	79.85
Penan Bag	-	83.11 ab	76.00 ab	75.56 a	78.22
Plastic Bag	-	77.78 b	72.00 b	72.44 a	74.07
F-test	-	*	*	Ns	
LSD (5%)	-	9.55	12.02	14.77	12.11
CV (%)	-	11.49	15.67	19.35	15.50

Means with the same letter are not significantly different. **p ≤0.01; *p ≤0.05 ns = non significant

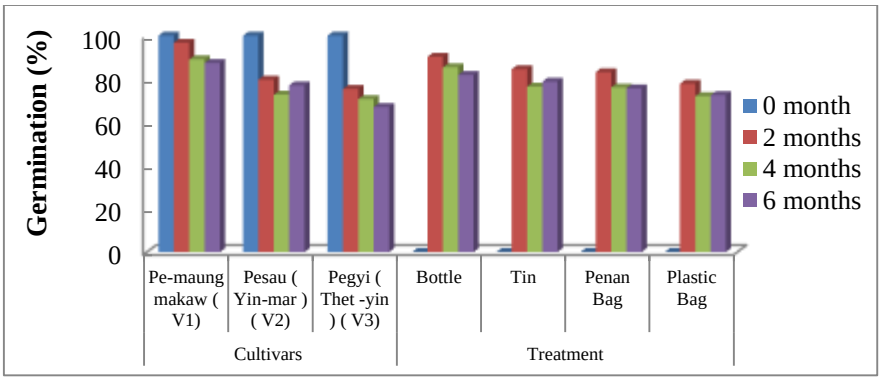

Figure 1 Germination percentage of three selected lablab bean cultivars after storing room temperature

Table 3 Significant levels and interaction for germination percentage of three selected lablab bean cultivars after storing room temperature

Interaction For Factors	2 Months	4Months	6 Months
Cultivar (var)	1525.78 *	1213.78**	1240.44 **
Treatment (trt)	235.11 *	284.89 ns	145.19 ns
Var x trt	117.78 ns	81.33 ns	215.85 ns

@ values are the mean square for the factor divided by the total sum squares. ** P ≤ 0.01, *P≤ 0.05 , ns = non significant. cv = lablab bean cultivars, trt= Bottle , Tin , Penan bag and Plastic bag

Table 4 Survival percentage of three selected lablab bean cultivars after storing room temperature

Cultivars and Treatments	Seed Survival (%) at 14 DAS				Mean
	0 month	2months	4 months	6 months	
Cultivars					
Pe-maungmakaw (V_1)	100 a	96.67 a	89.00 a	84.67 a	92.59
Pesau (Yinn-mar) (V_2)	100 a	79.67 b	72.67 b	78.67 a	82.75
Pegyi (Thet -yin) (V_3)	100 a	75 .33 b	70.67 b	65.00 b	77.75
F-test	ns	*	**	*	
LSD (5%)	-	10.9	11.11	11.25	11.09
CV (%)	-	11.46	4.30	13.03	9.60
Treatment					
Bottle	-	90.22 a	85.33 a	81.78 a	85.78
Tin	-	84 .44ab	76.44 ab	78.67 a	79.85
Penan Bag	-	83.11 ab	76.00 ab	73.33 a	77.48
Plastic Bag	-	77.78 b	72.00 b	70.67 a	73.48
F-test	-	*	*	ns	
LSD (5%)	-	9.55	12.02	13.16	11.57
CV (%)	-	11.49	15.68	17.46	14.88

Means with the same letter are not significantly different. **p ≤0.01; *p ≤0.05 ns = non significant.

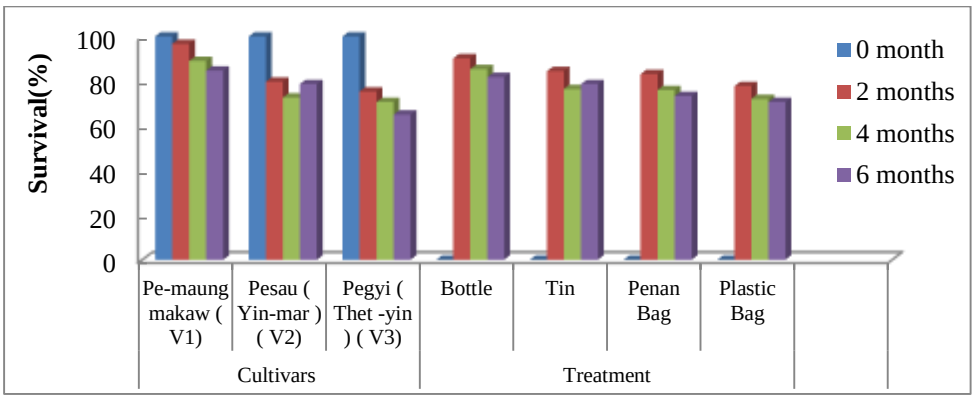

Figure 2 Survival percentage of three selected lablab bean cultivars after storing room temperature

Table 5 Significant levels and interaction for survival percentage of three selected lablab bean cultivars after storing room temperature

Interaction For Factors	2 Months	4Months	6 Months
Cultivar (var)	1525.78 *	1213.78**	1219.11 *
Treatment (trt)	235.11 *	284.89 ns	228.00 ns
Var x trt	117.78 ns	81.33 ns	367.55 ns

@ values are the mean square for the factor divided by the total sum squares. ** P ≤ 0.01, *P≤ 0.05 , ns = non significant. cv = lablab bean cultivars, trt= Bottle , Tin , Penan bag and Plastic bag

Table 6 Shoot length of three selected lablab bean cultivars after storing room temperature at 7DAS

Cultivars and Treatments	Shoot Length (7DAS) (%)				Mean
	0 month	2 months	4 months	6 months	
Cultivars					
Pe-maungmakaw (V_1)	16.92 a	22.48 a	22.97 a	21.71 a	21.02
Pesau (Yinn-mar) (V_2)	17.36 a	20.23 ab	19.16 b	19.20 b	18.99
Pegyi (Thet -yin) (V_3)	22 a	19.79 b	17.04 c	16.80 c	18.91
F-test	Ns	**	**	**	
LSD (5%)	-	2.28	1.6	1.86	1.91
CV (%)	-	9.64	7.18	8.52	8.45
Treatment					
Bottle	-	23.82 a	22.41 a	21.94 a	22.72
Tin	-	22.99 a	20.09 b	20.03 a	21.04
Penan Bag	-	19.38 b	18.64 bc	17.54 b	18.52
Plastic Bag	-	17.15 b	17.60 c	17.44 b	17.40
F-test	-	**	**	**	
LSD (5%)	-	2.64	1.78	2.23	2.22
CV (%)	-	12.81	9.14	11.69	11.21

Means with the same letter are not significantly different. **p ≤0.01; *p ≤0.05 ns = non significant

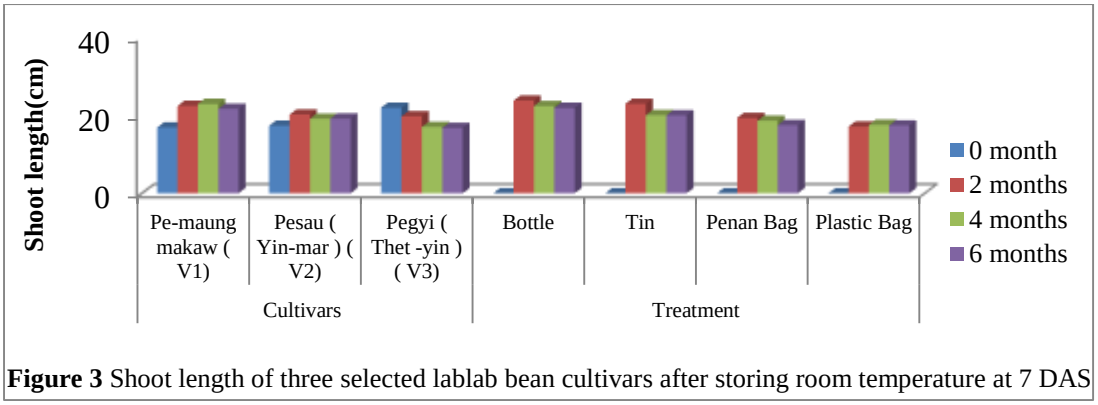

Figure 3 Shoot length of three selected lablab bean cultivars after storing room temperature at 7 DAS

Table 7 Significant levels and interaction for shoot length of three selected lablab bean cultivars after storing room temperature at 7DAS

Interaction For Factors	2 Months	4Months	6 Months
Cultivar (var)	25.13**	109.12 **	72.41 **
Treatment (trt)	87.65 **	39.07 **	42.12 **
Var x trt	4.56 ns	17.06 **	12.12 ns

@ values are the mean square for the factor divided by the total sum squares. ** P ≤ 0.01 , ns = non significant. cv = lablab bean cultivars, trt= Bottle , Tin , Penan bag and Plastic bag

Table 8 Shoot length of three selected lablab bean cultivars after storing room temperature at 14 DAS

Cultivars and Treatments	Shoot Lenglth (14DAS) (%)				Mean
	0 month	2 months	4 months	6 months	
Cultivars					
Pe-maungmakaw (V_1)	29.25 a	32.53 a	33.75 a	34.26 a	32.45
Pesau (Yinn-mar) (V_2)	35.05 a	31.48 a	29.96 b	33.90 a	32.60
Pegyi (Thet -yin) (V_3)	43.5 a	30.67 a	29.46 b	28.47 b	33.03
F-test	ns	Ns	*	**	
LSD (5%)	-	3.26	3.44	2.82	3.17
CV (%)	-	9.12	9.78	7.73	8.88
Treatment					
Bottle	-	33.65 a	31.80 a	36.52 a	33.99
Tin	-	31.67 ab	31.67 a	32.05 b	31.80
Penan Bag	-	31.44 ab	31.14 ab	30.48 bc	31.02
Plastic Bag	-	29.49 b	29.63 b	29.78 c	29.63
F-test	-	*	**	**	
LSD (5%)	-	2.52	1.74	1.81	2.02
CV (%)	-	8.04	5.63	5.65	6.44

Means with the same letter are not significantly different. **p ≤0.01; *p ≤0.05 ns = non significant.

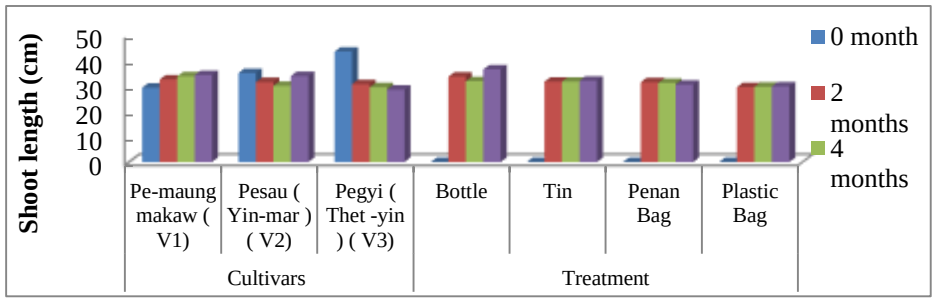

Figure 4 Shoot length of three selected lablab bean cultivars after storing room temperature at 14 DAS

Table 9 Significant levels and interaction for shoot length of three selected lablab bean cultivars after storing room temperature at 14DAS

Interaction For Factors	2 Months	4Months	6 Months
Cultivar (var)	913674.79 *	575074.43 *	2188635.87**
Treatment (trt)	621923.55 **	529023.3 *	1555538.99**
Var x trt	315673.27 **	146480.90ns	722395.68*

@ values are the mean square for the factor divided by the total sum squares. ** P ≤ 0.01, *P≤ 0.05 , ns = non significant. cv = lablab bean cultivars, trt= Bottle , Tin , Penan bag and Plastic bag

Table 10 Root length of three selected lablab bean cultivars after storing room temperature at 14 DAS

Cultivars and Treatments	Root Length (14DAS)				Mean
	0 month	2 months	4 months	6 months	
Cultivars					
Pe-maungmakaw (V1)	4.56 a	5.78 a	6.25 a	6.10 a	5.67
Pesau (Yinn-mar) (V2)	6.8 a	5.43 a	6.11 a	6.04 a	6.1
Pegyi (Thet -yin) (V3)	6.8 a	4.92 a	5.09 a	6.01 a	5.71
F-test	ns	ns	ns	ns	
LSD (5%)	-	0.96	1.19	0.52	0.89
CV (%)	-	15.61	18.04	7.60	13.75
Treatment					
Bottle	-	5.67 a	6.61 a	6.59 a	6.59
Tin	-	5.58 a	5.74 b	6.38 a	5.90
Penan Bag	-	5.40 ab	5.52 b	6.15 a	5.69
Plastic Bag	-	4.85 b	5.40 b	5.09 b	5.11
F-test	-	**	**	*	
LSD (5%)	-	0.69	0.58	0.96	0.74
CV (%)	-	12.82	9.96	16.03	12.94

Means with the same letter are not significantly different. **p ≤0.01; *p ≤0.05 ns = non significant.

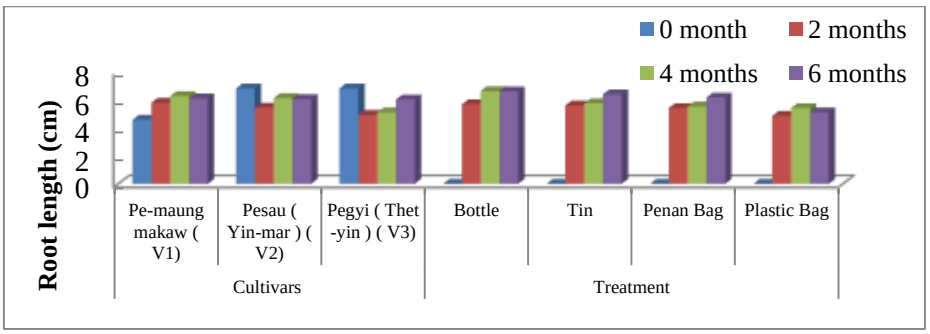

Figure 5 Root length of three selected lablab bean cultivars after storing room temperature at 14 DAS

Table 11 Significant levels and interaction for root length of three selected lablab bean cultivars after storing room temperature at 14DAS

Interaction For Factors	2 Months	4Months	6 Months
Cultivar (var)	2.23 ns	4.80 ns	0.02 ns
Treatment (trt)	1.21 **	2.69 **	4.0 *
Var x trt	1.17 ns	2.42 **	4.62 **

@ values are the mean square for the factor divided by the total sum squares. ** P ≤ 0.01, *P≤ 0.05, , ns = non significant. cv = lablab bean cultivars, trt= Bottle , Tin , Penan bag and Plastic bag

Table 12 Seedling vigor of three selected lablab bean cultivars after storing room temperature for at 7DAS

Cultivars and Treatments	Seedling Vigor Index (7 DAS)				Mean
	0 month	2 months	4 months	6 months	
Cultivars					
Pe-maungmakaw (V_1)	1692.0 a	1877.0 a	1621.10 a	1685.74 a	1718.93
Pesau (Yinn-mar) (V_2)	1736.67 a	1700.2 a	1515.90 ab	1448.19 b	1600.24
Pegyi (Thet -yin) (V_3)	2200 a	1645.2 a	1396.88 b	1287.48 b	1632.39
F-test	ns	ns	*	*	
LSD (5%)	-	340.24	141.01	191.78	224.34
CV (%)	-	17.24	8.23	11.48	12.32
Treatment					
Bottle	-	2154.5 a	1701.4 a	1714.7 a	1856.87
Tin	-	1926.0 b	1699.8 a	1627.9 a	1751.23
Penan Bag	-	1591.5 c	1397.0 b	1303.0 b	1430.50
Plastic Bag	-	1291.4 d	1247.0 b	1249.6 b	1262.67
F-test	-	**	**	**	
LSD (5%)	-	219.01	255.67	271.08	248.59
CV (%)	-	12.7	17.08	18.57	16.12

Means with the same letter are not significantly different. **p ≤0.01; *p ≤0.05 ns = non significant

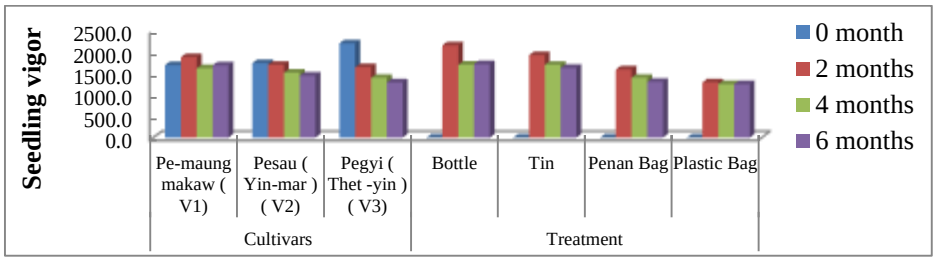

Figure 6 Seedling vigor of three selected lablab bean cultivar after storing room temperature at 7 DAS

Table 13 Significant levels and interaction for seedling vigor of three selected lablab bean cultivars after storing room at 7DAS

Interaction For Factors	2 Months	4Months	6 Months
Cultivar (var)	176048.84 ns	151019.21*	481733.74 *
Treatment (trt)	1289147.86 **	463725.95**	483602.15**
Var x trt	106796.19 ns	159946.40ns	182044.58ns

@ values are the mean square for the factor divided by the total sum squares** P ≤ 0.01 , . *P≤ 0.05 , ns = non significant. cv = lablab bean cultivars, trt= Bottle , Tin , Penan bag and Plastic bag

Table 14 Significant levels and interaction for seedling vigor of three selected lablab bean cultivars after storing room temperature at 14 DAS

Cultivars and Treatments	Seedling Vigour Index (14 DAS)				Mean
	0 month	2 months	4 months	6 months	
Cultivars					
Pe-maungmakaw (V_1)	2925.33 a	2953.7 a	2623.5 a	2910.7 a	2853.31
Pesau (Yinn-mar) (V_2)	3505.33 a	2526.8 b	2371.8 ab	2214.4 b	2654.58
Pegyi (Thet -yin) (V_3)	4354.67 a	2437.3 b	2187.4 b	2134.2 b	2778.39
F-test	Ns	*	*	**	
LSD (5%)	-	400.75	335.29	383.56	373.2
CV (%)	-	13.39	12.33	13.98	13.2333
Treatment					
Bottle	-	2829.9 a	2697.4 a	2905.3 a	2810.87
Tin	-	2829.3 a	2416.5 ab	2625.3 a	2623.7
Penan Bag	-	2625.4 a	2356.1 ab	2103.6 b	2361.7
Plastic Bag	-	2272.3 b	2107.0 b	2044.9 b	2141.4
F-test	-	**	*	**	
LSD (5%)	-	279.69	343.14	465.49	362.773
CV (%)	-	13.39	14.47	19.42	15.76

Means with the same letter are not significantly different. **p ≤0.01; *p ≤0.05 ns = non significant.

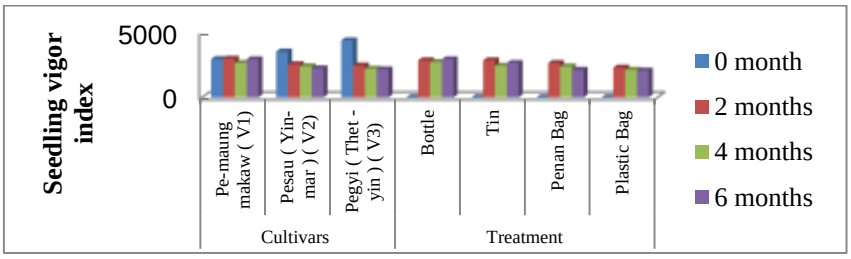

Figure 7 Seedling vigor of three selected lablab bean cultivar after storing room temperature at 14 DAS

Table 15 Significant levels and interaction for seedling vigor of three selected lablab bean cultivars after storing room temperature at 7DAS

Interaction For Factors	2 Months	4Months	6 Months
Cultivar (var)	913674.79 *	575074.43 *	2188635.87**
Treatment (trt)	621923.55 **	529023.3 *	1555538.99**
Var x trt	315673.27 **	146480.90ns	722395.68*

@ values are the mean square for the factor divided by the total sum squares. ** P ≤ 0.01 , *P≤ 0.05 , ns = non significant. cv = lablab bean cultivars, trt= Bottle , Tin , Penan bag and Plastic bag

Correlation Analysis Between the cultivars and Treatments duration of

Seed Storage on Seedling Growth of Three selected Lablab bean cultivars after 2 months

Correlation analysis showed that seedling vigour index (14DAS) had significantly positive correlation with shoot length (7DAS) (r = 0.9547*) at 5% level and root length (14 DAS) (r = 0.9918**) at 1 % level.(Table 16)

Seedling vigour index (7DAS) had significantly positive correlation with germination percentage and survival percentage (r = 0.9624*), shoot length (7DAS) (r =0.9892*) at 5 % level.

Table 16 Correlation matrix on seedling growth of three selected lablab bean cultivars after 2 months storage

Parameter	Gp%	SVP%	SL (cm) 7DAS	SL (cm) 14DAS	RT(cm) 14DAS	SVI 7DAS	SVI 14DAS
Gp%	1						
SVP%	1						
SL (cm)(7DAS)	0.9137ns	0.9137ns	1				
SL (cm)(14DAS)	0.9984**	0.9984**	0.8961ns	1			
RT(cm) (14DAS)	0.9173ns	0.9173ns	0.9353ns	0.9185ns	1		
SVI (7DAS)	0.9624*	0.9624*	0.9892*	0.9492ns	0.9398ns	1	
SVI (14DAS)	0.8845ns	0.8845ns	0.9547*	0.8801ns	0.9918**	0.9416ns	1

value represents for the r value of correlation relationship, ** =1% level of significant, *=5 % level of significant ns =nonsignificant. Gp=Germination percentage,SVP=Survival percentage, SL= Shoot length, RT= Root length, SVI= Seedling vigour index

Correlation Analysis Between the cultivars and Treatments duration of Seed Storage on Seedling Growth of Three selected Lablab bean cultivars after 4 months

Correlation analysis showed that seedling vigour index (14DAS) had significantly positive correlation with germination percentage and survival percentage (r = 0.9745*) shoot length (7DAS) (r = 0.9723*) at 5 % level. (Table 17)

Root length (14DAS) had significantly positive correlation with germination percentage and survival percentage (r = 0.9786*), shoot length (7DAS) (r =0.9681*) at 5 % level. Shoot length (7 DAS) had significantly positive correlation with germination percentage and survival percentage (r = 0.9651*) at 5 % level.

Table 17 Correlation matrix on seedling growth of three selected lablab bean cultivars after 4 months storage

Parameter	**Gp%**	**SVP%**	**SL (cm) 7DAS**	**SL (cm) 14DAS**	**RT(cm) 14DAS**	**SVI 7DAS**	**SVI 14DAS**
Gp%	1						
SVP%	1	1					
SL(cm)(7DAS)	0.9651*	0.9651*	1				
SL(cm)(14DAS)	0.7677ns	0.7677ns	0.8296ns	1			
RT(cm)(14DAS)	0.9786*	0.9786*	0.9681*	0.6814ns	1		
SVI (7DAS)	0.7606ns	0.7606ns	0.8912ns	0.9214ns	0.7530ns	1	
SVI(14DAS)	0.9745*	0.9745*	0.9723**	0.8919ns	0.9298ns	0.8606ns	1

value represents for the r value of correlation relationship, * =5 % level of significant, ns =nonsignificant. Gp=Germination percentage, SVP=Survival percentage, SL= Shoot length, RT= Root length, SVI= Seedling vigour index

Correlation Analysis Between the cultivars and Treatments duration of Seed Storage on Seedling Growth of Three selected Lablab bean cultivars after 6 months

Correlation analysis showed that seedling vigour index (14DAS) had significantly positive correlation with germination percentage (r = 0.9634*), survival percentage (r = 0.9871*) at 5% level, shoot length (7DAS) (r = 0.9952**) and seedling vigour index (7 DAS) (r = 0.9915**) at 1 % level. (Table 18)

Therefore, results indicated that seedling vigour index (14DAS) had high and positively correlation with germination percentage, survival percentage shoot length (7DAS) and seedling

vigour index (7 DAS). Seedling vigour index (7 DAS) had significantly positive correlation with germination percentage (r =0.9578*), survival percentage (r = 0.9868*), shoot length (7DAS) (r =0.9743*) at 5 % level.

Shoot length (7 DAS) had significantly positive correlation with germination percentage (r =9538*) and survival percentage (r = 0.9747*) at 5 % level. Survival percentage had significantly positive correlation with germination percentage (r =0.9916**) at 1% level.

Table 18 Correlation matrix on seedling growth of three selected lablab bean cultivars after 6 months storage

Parameter	Gp%	SVP%	SL (cm) 7DAS	SL (cm) 14DAS	RT(cm) 14DAS	SVI 7DAS	SVI 14DAS
Gp%	1						
SVP%	0.9916**	1					
SL(cm)(7DAS)	0.9538*	0.9747*	1				
SL(cm)(14DAS)	0.9289ns	0.9198ns	0.9579ns	1			
T(cm)(14DAS)	0.9167ns	0.8795ns	0.7575ns	0.7326ns	1		
SVI (7DAS)	0.9578*	0.9868**	0.9743*	0.8804ns	0.818ns	1	
SVI(14DAS)	0.9634*	0.9871*	0.9952**	0.9315ns	0.7920ns	0.9915**	1

value represents for the r value of correlation relationship, ** =1 % level of significant, *=5 % level of significant, ns =nonsignificant. Gp=Germination percentage, SVP=Survival percentage, SL= Shoot length, RT= Root length, SVI= Seedling vigour index

Discussion and Conclusion

This study was carried out to investigate the storage conditions of lablab bean seeds under room temperature for 6 months using the different storage materials such as bottle, tin, penan bag and plastic bag respectively.

According to the results of temperature and relative humidity (RH) during seed storage, the storage room temperature was observed in 23.5 - 32.5ºC. The ranges of room RH during seed storage were obtained 69 – 90% during seed storage. Thus, RH was significantly higher in (90)% during seed storage.

This result was in agreement with Canada (2006) who reported that the seeds stored within 85 – 90% RH may lose their germination capacity due to the pathogenic action of molds. Thus seeds were no longer store under high RH. He also described that the temperature is an important abiotic factor governing the condition of pulses in store. In addition, he reported that the optimum temperature for breeding of most insects in storage ranges between 27 - 37 ºC and 70% RH.

Coolbear (1995) stated that low storage temperature slows down the rate of aging. In the study, seeds stored in bottles found 90.22% of seed germination after 2 months storage among the cultivars. Among the cultivars, cv. Pemaungmakaw (V_1) observed the highest seed germination percentage 96.67 % after 2 months of storage periods.

However, the rest of the seed storing materials such as tin, penan bags and plastic bags were observed poor seed germination percentage were obtained in the study below 85% seed germination percentages. Therefore, these results were in agreement with Hanson et al. (2006). He also revealed that germination percent should not be below 85%for cultivated species. These results were also in agreement with Genchev (1997) who reported that the poor storage conditions to cause 10% loss in seed quality in the tropics.

After 2 months,4 months and 6 months storage increased shoot length 7 DAS and 14 DAS, seedling vigor index 7 DAS and seedling vigor index 14 DAS were found in cv. pe-maungmakaw (V_1) and decreased in cv. pegyi (V_3). According to the correlation relationship between cultivars

and treatments (different seed storage materials), there were no interaction relationship in seed germination and survival percentages.

However, the result of shoot length, root length and seedling vigor index were observed the significant interaction relationships between cultivars and treatments in this study. Thus, the seedling growth of lablab bean seeds will be increased depending on age of plants. As a result, the increase germination percentage and survival percentage were positively correlated with increase the seedling vigor index.

In conclusion, lablab bean seeds should be stored in bottles for 2 months compared to the others seed storage materials such as tin, penan bags and plastic bags according to this study. After storing of seeds in bottles, seed germination and survival percentages and seedling vigor index were obviously the highest among the cultivars and treatments.

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INVESTIGATION OF MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS, PRELIMINARY PHYTOCHEMICAL EXAMINATION AND ANTIMICROBIAL ACTIVITYON LEAVES OF *MUNTINGIA CALABURA* L.

Htay Htay Lwin[1], Yadana[2], Aye Aye Aung[3]

Abstract

The plant *Muntingia calabura* L., Myanmar name "Hnget-thagya" belongs to the family Muntingiaceae. The plants are widely distributed in Myanmar. It was collected from Hpa-an township, Kayin State from October to December (2019). In this study, morphological and histological characters, phytochemical properties and antimicrobial activity of *Muntingia calabura* L. were investigated. In morphological study, the plant is small trees, leaves alternate and simple. Inflorescences solidary cymes. Flower white. In histological study, anisocytic stomata present on lower surface of lamina. Unicellular simple, stellate and multicellular head glandular trichomes are also present. Type of Vascular bundles in lamina, midrib and petiole are collateral type. The powdered sample has been investigated and presented as diagnostic characters for the standardization of powdered drugs. Phytochemical tests were done at Department of Botany, Hpa-an University. Phytochemical tests of *Muntingia calabura* L. leaves showed the presence of alkaloid, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid. Antimicrobial activity of leaves of *Muntingia calabura* L. was carried out at Botany Department, University of Yangon by using different solvent extracts (petroleum ether, ethyl acetate, acetone, ethanol, methanol and water). The extracts of *Muntingia calabura* L. leaves indicated antimicrobial activity against *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Echerichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, methonolic extracts showed the most significant antimicrobial activity against *Aspergillus flavus* and *Candida albicans* as well as ethanolic extract on *Candida albicans*. Peteroleum ether and aqueous extract showed antimicrobial activity on all microorganisms. *Muntingia calabura L.* leaves are effective on protection of diseases which caused by microorganisms.

Keyword: *Muntingia calabura* L., morphological and histological characters, phytochemical test and antimicrobial activity.

Introduction

The plant *Muntingia calabura* L.belongs to the family Muntingiaceae (Zakaria, *et al*., 2016). This family is small recently recognized neotropical family with genera formerly included in Tiliaceae (Heywood, *et al*., 2007). In Myanmar, it is locally known as Hnget-thagya and calabur; cherry tree; cotton candy berry; Jamaican cherry; Panama berry; Panama cherry; strawberry tree in English. It is native to the American continent and is widely cultivated in warm areas of Asian region (Chin, 1989). It is widely cultivated in warm areas in India and Southeast Asia such as Malaysia, Indonesia, and the Philippines (Morton, 1987; Zakaria, *et al*., 2010; Sani, *et al*., 2012 and Yusof, *et al*., 2013). In Philippines Islands, the flowers are made into infusion, used after the manner of lime flower in Europe for head-aches (Burkill, 1935). Medicinal plants are known for their rich sources of secondary metabolites such as triterpene glycosides, flavonoids, tannins alkaloids and other aromatic compounds (Sindhan, *et al.,* 1999).

Muntingia calabura L. leaves extracts also possesses antibacterial activity (Zakaria, *et al*., 2006). In *Muntingia calabura* L. leaves, flowers, barks and roots have been used as a folk remedy

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to treat headaches, fever and incipient cold. According to Peruvian folklore, the leaves are used to provide relief from gastric ulcers and to reduce swelling of the prostate gland. Besides, they are also employed as antiseptic, antispasmodic and antidyspeptic agent (Zakaria, *et al*., 2006), antitumor (Kaneda, *et al*., 1991 and Su B-N, *et al*., 2003), antibacterial and antinociception (Zakaria, *et al*., 2006), anti-inflammatory, antipyretic (Zakaria, *et al*., 2007), antioxidant and antiproliferative activities exhibited by the leaves of *Muntingia calabura* (Zakaria, *et al*., 2011).

Many plant extracts show their antimicrobial traits, which is due to the presence of compounds synthesized in the secondary metabolism of the plant. These secondary metabolites mainly consists of phenolics including polyphenols, flavonoids, tannins and quinones known for their potent antioxidant, cytotoxic and antimicrobial activities (Dai, *et al*., 2010, Pahari, *et al*., 2012 and Wong, *et al*., 2014). In this paper, morphological characters, phytochemical test and antimicrobial activity of *Muntingia calabura* L. were carried out.

The aim and objectives are to determine the morphological and histological characters, preliminary phytochemical tests and to examine the antimicrobial activities from the different solvent extracts by using on six types of microorganisms.

Materials and Methods

Collection and identification of *Muntingia calabura* L.

The plant *Muntingia calabura* L. was collected from Hpa-an township, Kayin State, from October to December (2019). The collected plant was identified with the available literatures of Hundley and Chit Ko Ko, (1961), Kress, *et al*., (2003), Heywood, *et al*., (2007) and Mahmood, *et al*., (2014).

Histological study of *Muntingia calabura* L.

In histological studies, free hand section of leaves (lamina, midribs, petioles) from the fresh specimens were prepared by using chloral hydrate solution for clearing reagents, safranin for testing lignin. These characters were determined according to the literatures of Metcalfe and Chalk (1950); Wallis (1967) and Pandey (1996).

Preparation of powdered samples of *Muntingia calabura* L. leaves

The collected samples were washed with water to remove impurities. After washing the samples cut into small pieces then air dried at room temperature when constant weight was obtained the dried plant material were homogenized by blender to get powder and stored in air tight containers to prevent moisture changes and contamination. Diagnostic characters of powder were examined to get standardization of powdered drug in traditional medicine.

Phytochemical investigation of *Muntingia calabura* L. leaves

In this investigation, the powdered *Muntingia calabura* L. leaves were tested to find out the presence or absence of chemical constituents such as alkaloid, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid compounds. Preliminary phytochemical tests were carried out according to the methods of Marini Bettolo, *et al*., (1981), Central Council for Research in Unani Medicine (1987) and Sasikala and Sundaraganapathy (2017).

Antimicrobial activities of different solvent extracts from *Muntingia calabura* L. leaves

Antimicrobial activities of different solvent extracts of *Muntingia calabura* L. leaves were tested on six pathogenic microorganisms by using paper disc diffusion method at the Department of botany, University of Yangon.

Preparation of crude extracts

The powdered of *Muntingia calabura* L. leaves were extracted with various solvents such as petroleum-ether, ethyl-acetate, acetone, ethanol, methanol and water. The filtrates were evaporated by using water bath.

Preparation of sample for testing antimicrobial activity

Screening of Antimicrobial activity of crude extracts had been done by paper disc diffusion method. Paper disc having six millimeter diameter were utilized for antimicrobial test. Assay medium was prepared according to the method described by Cruickshank (1975). Assay medium was boiled and 20- 25 ml of the medium was poured into each conical flask, plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the conical flasks were cooled down to 40- 45°C and each of 0.1- 0.2 ml of test organisms were also added into the flask and then, poured into sterilized petridishes. After solidification, paper disc impregnated with sample were applied on the agar plates and incubated at 37°C for 24 hours. Then the diameter of inhibitory zone was measured with the help of a transparent ruler.

Results

Morphological characters of *Muntingia calabura* L.

Small trees, with spreading branches. The leaves are simple, alternate, distichous, oblong or lanceolate, long pointed at the apex, oblique at the base with dark green color and stellate, simple and glandular hair on the both surfaces. Inflorescences solidary or two to three flowers. The flowers are ebracteate, ebracteolate, pedicellate, complete, bisexual, actinomorphic, regular, 5-merous, hypogynous. Calyx (5), fuse at the base, sepaloid, lanceolate, hairy, persistent. Petals 5, white, caducous. Stamens numerous, apostamenous, filament filiform, anther yellow, dithecous, dorsifixed, longitudinal dehiscence. Carpel (5), pentacarpellary, syncarpous, pendulous placentation, numerous ovules, style stout and thick, stigma capitate. The fruits are abundant, in round shape, with red or yellow, smooth, soft, juicy pulp, with very sweet and filled with exceedingly tiny, yellowish seeds.

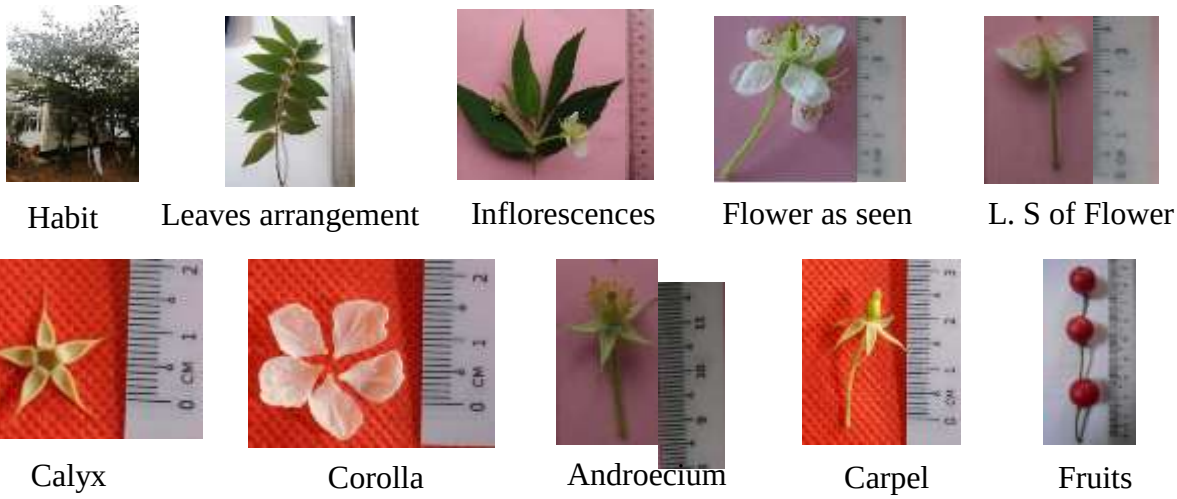


Figure 1 Morphological characters of *Muntingia calabura* L.

Histological characters of leaves on *Muntingia calabura* L.

Lamina

In surface view, the cuticle is thin, the epidermal cells both surfaces are thin- walled parenchymatous and the anticlinal wall of the upper surface is straight and lower surface is wavy. Anisocytic stomata are present only on the lower surface. The stomata are oval in outline with two-reniform shaped guard cells and contain abundant chloroplasts. Cluster of calcium oxalate crystals are present on both surfaces. Unicellular stellate, simple with multicellular head glandular trichomes are present on the both surfaces.

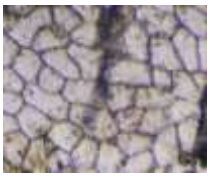
In transverse section, cuticle layer is thin on both surfaces. Both upper and lower epidermal cells are barrel shaped, thin-walled, parenchymatous cells. Palisade parenchyma found beneath the upper epidermis is 1 – 2 layers. These cells are vertically elongated and compactly arranged, with abundant chloroplast. The spongy mesophyll cells are 1 – 2 layers, loosely arranged, irregular in shape, with many intercellular spaces. Vascular bundles are embedded in the mesophyll cells. They are collateral type. Cluster of calcium oxalate crystal are abundantly present among the mesophyll cells.

Midrib

In transverse section, the cuticle layer is thin. Both upper and lower epidermal cells are more or less barrel shaped, thin-walled, parenchymatous cells. Below the epidermis, 2 – 4 layers of collenchymatous cells are present towards the upper surface and 2 – 3 layers the lower surface. Inner to the upper and lower collenchymatous layers consists of parenchymatous cells are 4 – 6 layers, rounded to oval in shape. Collateral type vascular bundles are embedded in the parenchymatous layers. Cluster of calcium oxalate crystals are present in cortex layers and vascular bundles. Unicellular simple, stellate and multicellular head glandular trichomes are present on the both surfaces.

Petiole

In transverse section, the cuticle layer is thin. The epidermal cells of both surfaces are more or less barrel shaped, with thin-walled, parenchymatous cells. The collenchymatous cells are 2 – 3 layers, rounded to polygonal shaped. The parenchymatous cells between two collenchymatous layers are 5 – 7 layers and are polygonal to isodiametric in shape. Vascular bundles are collateral type and embedded in the parenchymatous layers. Cluster of calcium oxalate crystals are present in cortex layers and vascular bundles. Unicellular simple, stellate and multicellular head glandular trichomesare present.



Upper surface view of lamina(X400)

Upper surface view of lamina showing stellate trichomes (X100)

Lower surface view of lamina (X400)

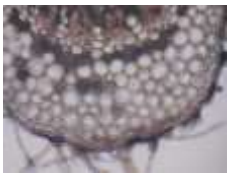
Transverse section of lamina (X100)

Transverse section of lamina showing trichomes (X100)

Transverse section of midrib (X100)

Transverse section of midrib showing upper cortex (X200)

Transverse section of midrib showing vascular bundle (X200)



Transverse section of midrib showing lower cortex (X200)

Transverse section of petiole in outline (X40)

Transverse section of petiole showing cortex layer (X400)

Transverse section of petiole showing vascular bundle (X100)

Figure 2 Microscopical characters of leaves of *Muntingia calabura* L.

Diagnostic characters of powdered plant of *Muntingia calabura* L.

The powdered *Muntingia calabura* L. was green coloured and odourless. It was slightly bitter taste. It consists of fragment of lamina, unicellular simple trichomes, stellate and glandular trichomes, fragment of upper epidermal cells with chloroplast, stomata, spiral vessels, annular vessels, fibres, tracheids and cluster of calcium oxalate crystals.

Table 1 Sensory characters of powder leaves of *Muntingia calabura* L.

Characters	**Powdered leaves of *Muntingia calabura* L.**
Colour	Green
Odour	Odourless
Taste	Slightly bitter
Texture	Fibrous with sticky

Fragment of lamina (X 400)

Various types of trichomes (X 400)

Fragment of epidermal cells (X 400)

Fragment of stomata (X 400)

Annular vessel (X 200)

Spiral vessel (X 200)

Glandular trichome (X 400)

Simple trichome (X 100)

Multicellular glandular trichomes (X 200)

Figure 3 Diagnostic characters of powdered leaves of *Muntingia calabura* L.

Phytochemical investigation of *Muntingia calabura* L. leaves

Preliminary phytochemical tests indicated the presence of alkaloid, α-amino acids, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid of *Muntingia calabura* L. leaves. The experimental results were shown in Table (2).

Table 2 Phytochemical test of *Muntingia calabura* L. leaves

No.	Test	Extract	Test reagents	Observation	Results
1.	Alkaloid	EtOH	1.Dragendorff's reagent 2. Mayer's reagent 3.Wagner's reagent 4. Hager's reagent	Orange brown ppt White ppt Reddish brown ppt Yellow ppt	+ + + +
2.	α-amino acids	H_2O	Ninhydrin reagent	Pink spot	+
3.	Carbohydrate	H_2O	α-napthol+conc:H_2SO_4	Red ring	+
4.	Flavonoid	EtOH	HCl / Mg	Pink color	+
5.	Glycoside	EtOH	H_2O + NaOH	Yellow color	+
6.	Phenolic compound	EtOH	H_2O + 10% $FeCl_3$	Greenish blue color	+
7.	Protein	H_2O	Millon's reagent	White ppt turns red on heating	+
8.	Reducing sugar	H_2O	Fehling's A and B	Brick red ppt	+
9.	Saponin	H_2O	H_2O	Frothing	+
10.	Starch	H_2O	Iodine solution	Blue black	+
11.	Steroid	EtOH	$CHCl_3$ + conc:H_2SO_4	Green color	+
12.	Tannin	H_2O	5% $FeCl_3$ + H_2SO_4	Yellow brown ppt	+
13.	Terpenoid	EtOH	$CHCl_3$ + conc:H_2SO_4	Pink color	+

(+) = Present

Antimicrobial activities of different solvent extracts of *Muntingia calabura* L. leaves by using paper disc diffusion method

Table 3 Antimicrobial activities of different solvent extracts from *Muntingia calabura* L. leaves against (6) tested organism

No	Solvents	*A. flavus*	*B. subtilis*	*C. albicans*	*E. coli*	*P.fluorescen*	*X. oryzae*
1.	Acetone	16 mm	14 mm	14mm	8mm	8 mm	10mm
2.	Ethyl acetate	14 mm	10mm	14mm	8 mm	8 mm	8 mm
3.	Ethanol	14mm	12mm	20mm	8 mm	16 mm	14 mm
4.	Methanol	20 mm	14mm	20mm	8mm	16 mm	14 mm
5.	Pet- ether	8mm	8mm	10mm	8 mm	8 mm	8 mm
6.	Aqueous	12mm	10 mm	10mm	8 mm	10 mm	10 mm

Paper disc size = 6 mm

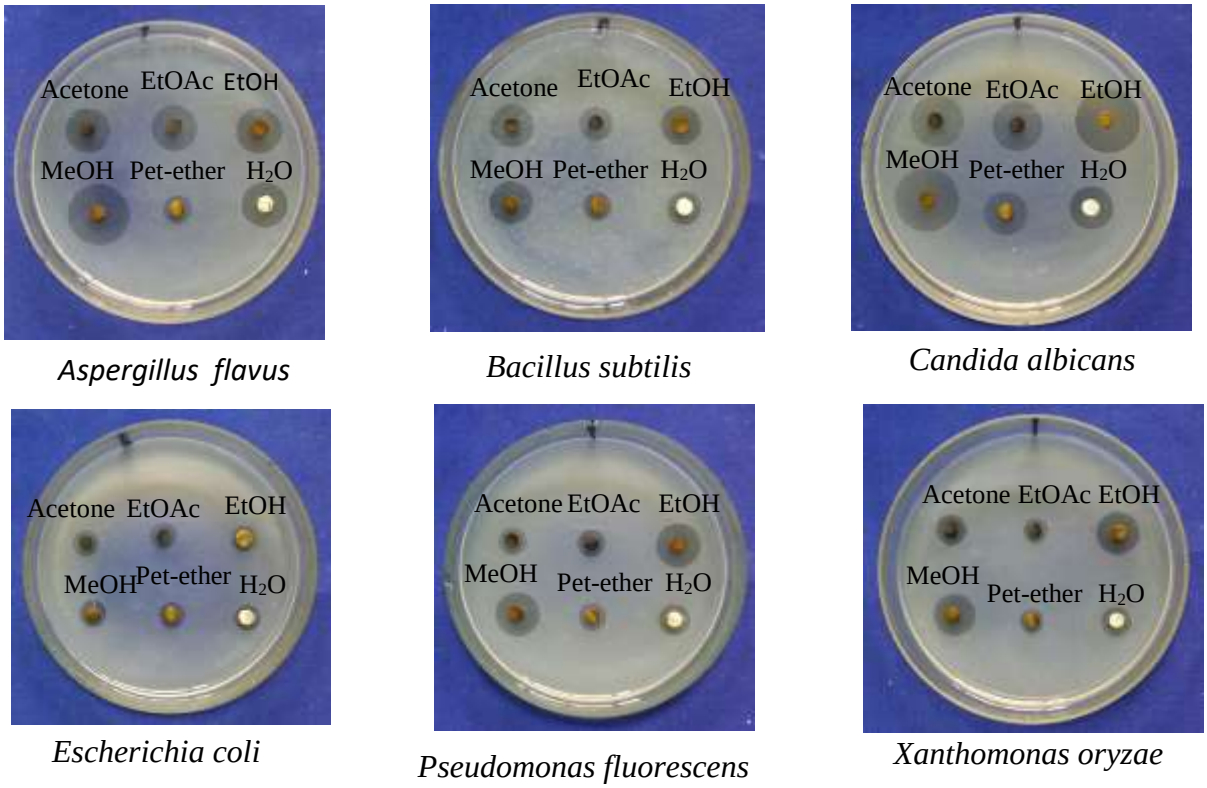


Figure 4 Antimicrobial activities of *Muntingia calabura* L. leaves

Discussion

In this investigation, morphological and histological characters, phytochemical test, and antimicrobial activity of *Muntingia calabura* L. leaves were carried out.

In morphological study, this plant is small trees, with spreading branches. The leaves are simple, alternate, distichous, stellate, simple and glandular hair on the both surfaces. Inflorescences solidary or two to three flowers. The flowers are bisexual, actinomorphic. Calyx (5), fuse at the base. Petals 5, white, caducous. Stamens numerous. Carpel (5), pentacarpellary, syncarpous, pendulous placentation, numerous ovules, style stout and thick, stigma capitate. The fruits are abundant, in round shape, with red or yellow with very sweet. These characters were agreed with those described by Heywood, *et al*., (2007) and Mahmood, *et al*., (2014).

In histological study, the epidermal cells the upper surface is straight and lower surface is wavy. Anisocytic stomata present only on the lower surface. Vascular bundles of lamina, midrib and petiole are collateral type. Cluster of calcium oxalate crystals present in mesophyll layer of lamina and cortex layer and vascular bundle of midrib and petiole. Unicellular simple, stellate, and glandular hair with multicellular head present in lamina, midrib and petiole. These characters were agreed with those described by Metcalfe and Chalk (1950) Wallis (1967) and Pandey (1996).

Singh, *et al*., (2017) reported that phytochemical screening of *Muntingia calabura* L. extracts revealed the presence of sterols, flavonoids, alkaloids, and tannins.

Minh, *et al*., (2019) reported that *Muntingia calabura* L. leaf contains different phytochemicals that include terpenoids, reducing sugars, flavonoids, saponins, tannins, phenols and carbohydrates.

Zakaria, (2007) mentioned that cherry leaf contains flavonoid, tannin, triterpene, saponin and polifenol as antioxidants.

In this research, the powdered sample of *M. calabura* L. contained alkaloid, α- amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid.

Ramasamy, *et al*., (2017) stated that the methanol leaf extract of *Muntingia calabura* L. inhibited the growth against *Xanthomonas oryzae, Erwinia amylovora* and *Agrobacterium tumefaciens.*

Buhian, *et al*., (2016) mentioned that methanolic and acetate fractions of the leaf crude extracts were shown to inhibit the growth of *Staphylococcus aureus*.

Pujaningsih, *et al*., (2018) reported that the extract from the cherry leaf produced higher inhibitory resistance to *S. aureus* and *E. coli*.

In this research, the different solvent extracts (petroleum ether, acetone, ethyl-acetate, ethanol, methanol and distilled water) of *Muntingia calabura* L. leaves were tested on six pathogenic microorganisms such as *Aspergillus flavus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens, Xanthomonas oryzae* by using paper disc diffusion method. According to this experiment, all extracts (petroleum ether, acetone, ethyl-acetate, ethanol, methanol and aqueous) showed antimicrobial activity all test organisms. Among them, methanolic extract showed the most significant antimicrobial activity against *Aspergillus flavus* (20mm), acetone, ethyl acetate, ethanolic extracts showed the more significant antimicrobial activity (14 – 16 mm) and petroleum ether and aqueous extracts showed antimicrobial activity against *Aspergillus flavus* (8 –12 mm). Acetone and methanolic extract showed the more significant antimicrobial activity on *Bacillus subtilis* (14 mm), ethyl acetate, ethanol, petroleum ether and aqueous extracts showed antimicrobial activity against *Bacillus subtilis* (8 –12 mm). Ethanolic and methanolic extracts showed the most significant antimicrobial activity against *Candida albicans* (20mm), acetone and ethyl acetate showed more significant antimicrobial activity (14 mm) and petroleum ether and aqueous extracts showed antimicrobial activity against *Candida albicans* (10 mm). Ethanolic and methanolic extracts showed more significant antimicrobial activity against *Pseudomonas fluorescens* (16 mm) and *Xanthomonas oryzae* (14 mm), acetone, ethyl acetate, petroleum ether and aqueous extracts showed antimicrobial activity against *Pseudomonas fluorescens* and *Xanthomonas oryzae* (8 –10 mm). All extracts showed antimicrobial activity against *Escherichia coli* (8 mm).

Conclusion

The plant *Muntingia calabura* L. belongs to family Muntingiaceae. It is a small tree, with spreading branches. The leaves are simple, alternate, distichous. In histological characters, anisocytic stomata present on lower surface. Simple, stellate, and glandular trichomes present on lamina, midrib and petiole.

Due to the presence of active constituents such as alkaloid, α-amino acid, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, steroid, tannin and terpenoid these documents were highlighted to know effective medicinal bioactivities of *Muntingia calabura* L. leaves.

The extracts of *Muntingia calabura* L. leaves indicated antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Candida albicans, Echerichia coli, Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, acetone extract showed more significant antimicrobial activity against *Aspergillus flavus, Bacillus subtilis* and *Candida albicans* (14 – 16 mm), antimicrobial activity against *Escherichia coli, Pseudomonas fluorescens and Xanthomonas oryzae* (8 –10 mm). Ethyl acetate extract showed more significant antimicrobial activity on *Aspergillus flavus* and *Candida albicans* (14 mm) and *Bacillus subtilis, Escherichia coli,*

Pseudomonas fluorescens and Xanthomonas oryzae on antimicrobial activity (8 – 10 mm). Ethanolic extract showed the most significant antimicrobial activity on *Candida albicans* (20 mm), more significant activity on *Aspergillus flavus, Pseudomonas fluorescens and Xanthomonas oryzae* (14 – 16 mm) and antimicrobial activity on *Bacillus subtilis, Escherichia coli* (8 – 10 mm). Methanolic extract showed the most antimicrobial activity against *Aspergillus flavus* and *Candida albicans* (20 mm) and more significant activity on *Bacillus subtilis, Pseudomonas fluorescens and Xanthomonas oryzae* (14 – 16 mm) and antimicrobial activity on *Escherichia coli* (8 mm). Petroleum ether and aqueous extracts showed antimicrobial antimicrobial activity against *all microorganisms.*

Therefore, extracts of *Muntingia calabura* L. leaves is effective in protecting against bronchitis caused by *Aspergillus flavus*, endocarditis, meningitis, infection of wounds, ears, eyes, respiratory tract, urinary tract and gastrointestinal tract caused by *Bacillus subtilis* alimentary tract infection, cardiac infection, sores and inflammation by *Candida albicans*, diarrhoea, dysentery by *Echerichia coli*. Fever, nausea and vomiting and rapid heart rate in human and leaf blight caused by *Pseudomonas fluorescens*. Extracts of *Muntingia calabura* L. leaves can prevent rice blight caused by *Xanthomonas oryzae.* So, *Muntingia calabura* L. leaves is effective on protection of diseases which caused by microorganisms.

Acknowledgement

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ANTIBACTERIAL ACTIVITY OF ISOLATED ACTINOMYCETES FROM COLLECTED SOIL SAMPLES AT MAGWAY REGION

Khaing Zar Lwin[1] and Moe Moe Aye[2]

Abstract

In this study, fifteen actinomycetes were isolated from the four soil samples collected at Pwint Phyu Township, Magway Region. Soil samples were carried out from June 2016 to July 2016. The isolation of actinomycetes was undertaken by the method of Chemical Treatment Dilution Method and Physical Treatment Serial Dilution Method. Soil actinomycetes were tested the antibacterial activities with five test organisms by using paper disc diffusion assay method. These isolated actinomycetes showed the antibacterial activities on test organisms. Among them, actinomycete A-01 showed the highest antibacterial activity on Bacillus subtilis (23.41 mm). Therefore, this actinomycete A-01 was selected for further investigation. In the fermentation studies of A-01, it was found that 46 hrs ages and 2% sizes of inoculum were optimized to produce the antibacterial metabolites.

Keywords: isolation, antimicrobial activity, ages and sizes of inoculum

Introduction

The Actinomycetes are aerobic, Gram-positive bacteria, which produce extensive branching vegetative (substrate) mycelium and aerial mycelium bearing chains of arthrospores. The substrate mycelium and spores can be pigmented, but also diffusible pigments are produced. On agar plates, they form lichenoid, leathery or burnous colonies. The GC-content of the DNA is 69-78 % (Williams *et al*., 1989).

Soil samples can be considered as a new source for isolation of microorganisms because there is much possibility of finding new microorganisms. Microorganisms have significant function in ecosystems and are found in all kinds of habitats. They produce numerous antimicrobial agents, including organic acids, enzymes and antibiotics. Microorganisms that live in the soil are essential to life on earth. The soil sample is the most effective and popular materials for the isolation of fungi and actinomycetes (Harayama & Isono, 2002).

Microbial secondary metabolites are important sources of natural compounds with potential therapeutic applications. As one of the versatile microorganisms, the streptomyces are the potent producers of secondary metabolites (Wux *et al*., 2007).

The percentage of actinomycetes and fungal strains which are showing antimicrobial activities. In standard agar diffusion assays ranges between 30- 80% depending on the ecological or taxonomic groups (Demain, 1999). Fermentation producers have to be developed for the cultivation of microorganisms under optimal condition and for the production of desired metabolites or enzyme by the microorganisms (Yamaie, 1984).

Antibiotic are the best known products of actinomycete. The present study in an attempt to produce antibiotics from actinomycetes, isolated from soil, by fermentation and the determination of their antimicrobial activity. *Bacillus subtilis* used as test organisms cause the food damage and fever.

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Materials and Methods

Collection of soil samples

Four different places of soil samples were collected at Pwint Phyu Township, Magway Region and were utilized for the isolation of soil actinomycetes. Soil samples were carried out from June 2016 to July 2016. In every collection site, soils were collected sample from different depths, between 1-6 inch. Four different places of soil samples were used for the isolation of actinomycetes and their location, soil pH and soil types are shown in Table 1.

Table 1 Soil samples collected at different places (Pwint Phyu Township)

Soil No.	Collected place		Texture	pH
S-1	Min Hla village	N 20º 21′ 45″ E 94º 40′ 08″	Silt Loam	7.2
S-2	Kwat Thit village	N 20º 21′ 43″ E 94º 40′ 08″	Silt Loam	7.4
S-3	Ywa Htaung village	N 20º 21′ 44″ E 94º 40′ 07″	Silt Clay Loam	7.8
S-4	Shwe Hlay village	N 20º 21′ 44″ E 94º 40′ 07″	Silt Clay Loam	7.9

Isolation of microorganisms from different soil samples

At the collecting different soil samples were dried at room temperature in the laboratory. Isolation of soil actinomycetes was undertaken by the methods of Chemical Treatment Dilution Method and Physical Treatment Serial Dilution Method (Phay and Yamamura, 2005).

Chemical treatment dilution method (Phay and Yamamura, 2005)

Soil sample 0.1 gram was added into the 9 mL of 1.5% phenol solution tube and shaking for 15 min. After shaking, 0.1 mL suspension was transferred to the 5 mL of sterile water tube. After that 0.5 mL of this suspension was transferred to the 4.5 mL sterile water tube and then 1 mL of this suspension was transferred to the 4 mL sterile water tube respectively. After taking 30 µL of the final soil suspension was placed on GSE medium and incubated at the temperature of 27°C. The inoculated plate was incubated for about 14 day.

Physical Treatment Serial Dilution Method

(Phay and Yamamura, 200 5)

One gram of soil was added into the 100 mL of water and heated at water batch (60°C) for 10mins. After that 0.1mL was transferred to 9.0 mL saline solution and then 1 mL was transferred to 9.0 mL of saline solution. In this way a series of 10 dilutions were prepared. After taking 0.1 mL of the final soil suspension tube was placed on GSE medium and incubated at the temperature of 27°C. The inoculated plate was incubated for about 14 day.

Medium Used for Screening of Actinomycetes

GSE Agar medium

Glucose	1.0g
Soy bean flour	0.5g
Nalidixic acid	0.01g
Humic acid	0.001g
$CaCO_3$	0.02g
NaH_2PO_4	0.5g
KCl	1.7g
$FeSO_47H_2O$	0.01g
Agar	1.8g
Soil extract + DW	50+50 mL
pH	7.2

After autoclaving, cycloheximide was added to this medium.

Preliminary study for antimicrobial activities by paper disc diffusion assay (NITE, 2005)

The isolated actinomycetes were grown at 27˚C for 14 days on ISP II medium. And then actinomycetes were inoculated on seed medium (glucose 2.0 g, Yeast extract 1.0 g, peptone 0.5 g, KNO_3 0.1g, K_2HPO_4 0.001 g, DW 100 mL at pH 7) and incubated at 27˚C for 3 days. Seed culture (4.0%) was transferred into the fermentation medium (glucose 2.0 g, glycerol 1.0 g, yeast extract 1.5 g, polypeptone 1.2 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.1 g, DW 100 mL at pH 7.2) and incubated at 27˚C for 7 days. After seven days 30 µL sample was put on paper disc and dry. And then placed on assay agar plate containing test organism (Paper disc size = 8 mm). The test organisms used in paper disc diffusion assay were as followed.

Test organisms used in antibacterial activities

Five pathogenic bacteria, including two Gram positive bacteria (*Bacillus subtilis* IFO 90571, *Micrococcus luteus* NITE 83297) and three Gram negative bacteria (*Agrobacterium tumefaciens* NITE 09678, *Pseudomonas fluorescens* IFO 94307, *Escherichia coli* AHU 5436) were used to determined the antibacterial activity of the isolated actinomycetes strains.

Study on the Ages and Sizes of Inoculum for the Fermentation

A slant culture (8 days old) was transferred into seed medium. Seed cultures of 38 hrs, 42 hrs, 46 hrs, 50 hrs, 54 hrs and 58 hrs incubation was inoculated into the flasks containing fermentation medium. In the study of sizes of inoculum, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% of seed culture at 46 hrs were transferred into the flasks containing the fermentation medium. Fermentation was carried out 7 days and antibacterial activity was tested by paper disc diffusion assay method (Omura, 1985).

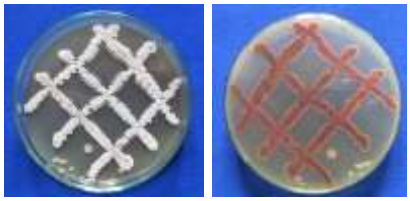
Results

Isolation of microorganisms from different soil samples

In this study, 15 kinds of soil actinomycetes were isolated from four different places of soil samples from Pwint Phyu Township, Magway Region, as shown in Table 2.

Table 2 Actinomycetes isolated from four different soil samples by Chemical and Physical Treatment Methods

Soil Sample No.	Collected Places	Chemical Treatment	Physical Treatment	No.
S-1	Min Hla village	2	2	A- 01,02,03,04
S-2	Kwat Thit village	2	1	A-05,06,07
S-3	Ywa Htaung village	3	1	A-08,09,10,11
S-4	Shwe Hlay village	3	1	A-12,13,14,15
Total Isolated Actinomycetes		10	5	15

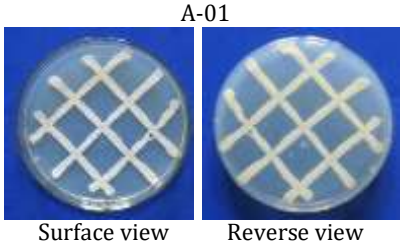


Surface view pigmentation Reverse view pigmentation

A-01

Surface view pigmentation Reverse view pigmentation

A-02



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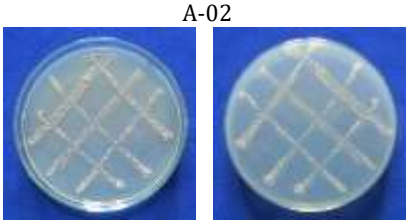

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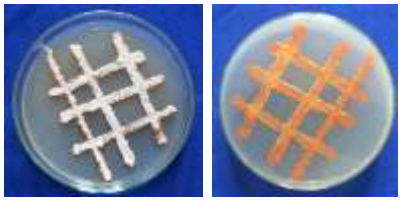
A-04



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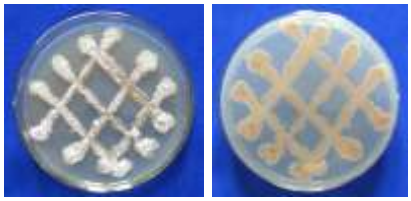
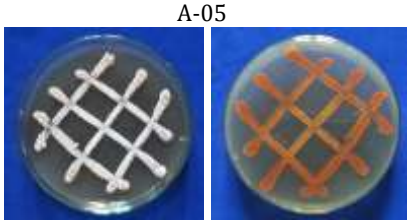
A-05



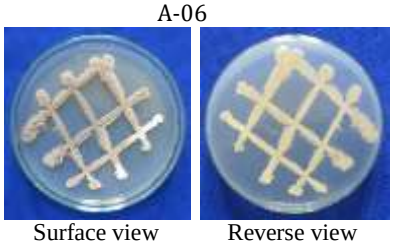
Surface view pigmentation Reverse view pigmentation

A-06



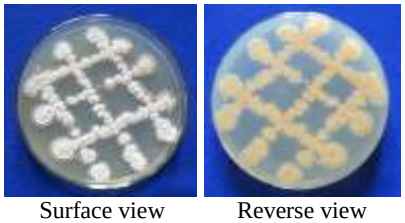
Surface view pigmentation Reverse view pigmentation

A-07



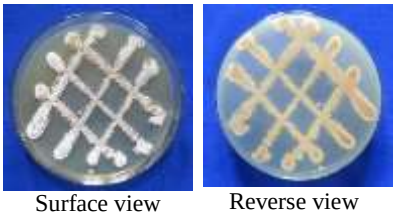
Surface view pigmentation Reverse view pigmentation

A-08



Surface view pigmentation Reverse view pigmentation

A-09



Surface view pigmentation Reverse view pigmentation

A-10

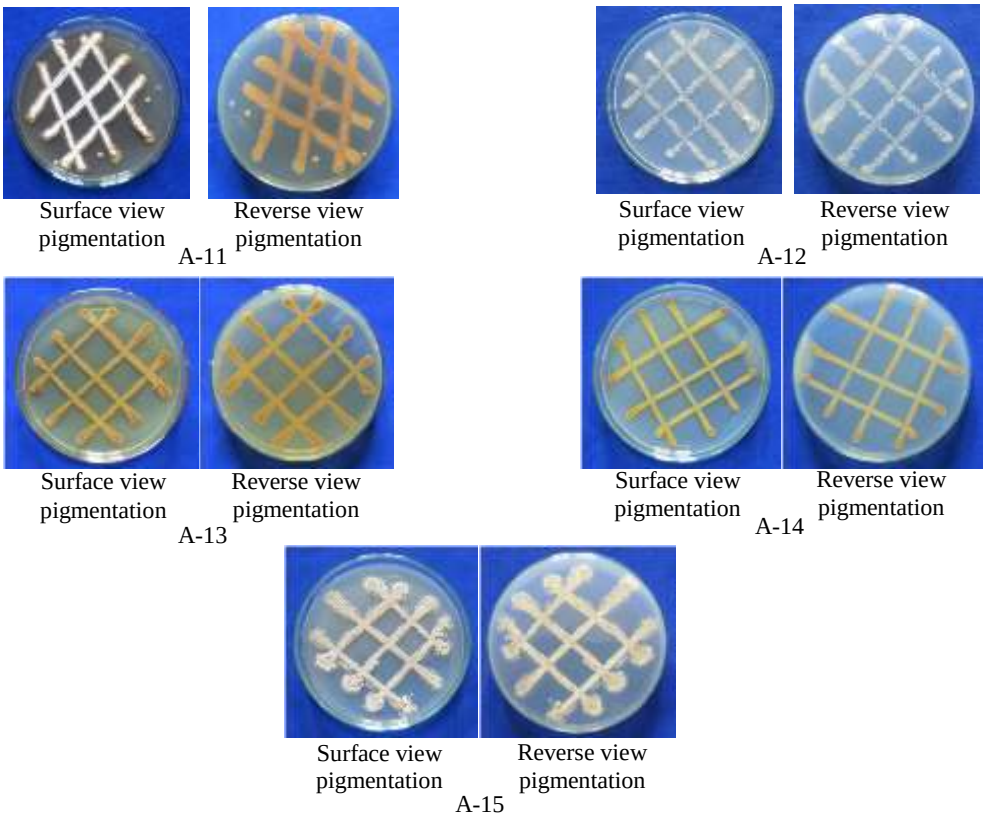


Figure 1 Aerial mass colour of actinomycetes strains on ISP II medium (7-14 days old culture)

Study on the antibacterial activities

In the study of antibacterial activitites of soil actinomycetes on five test organisms, A-01 and A-06 showed the activity against *Bacillus subtilis*. A-01 and A-08 showed the activity against *Agrobacterium tumefaciens,* A-04 and A-12 showed the activity against *Escherichia coli*. A-10 and A-15 showed the activity against *Pseudomonas fluorescens*. Among them, A-01 showed more highly antibacterial activity than others.

Table 3 Antimicrobial activities of isolated actinomycetes

Isolated Actinomycetes	*B. subtilis*	*M. luteus*	*A. tumefaciens*	*P. fluorescens*	*E. coli*
A-01	**23.41**	-	17.13	-	-
A-02	-	-	-	-	-
A-03	-	-	-	-	-
A-04	-	-	-	-	12.15
A-05	-	-	-	-	-
A-06	19.71	-	-	-	-
A-07	-	-	-	-	-
A-08	-	-	13.41	-	-
A-09	-	-	-	-	-
A-10	-	-	-	13.58	-
A-11	-	-	-	-	-
A-12	-	-	-	-	12.27
A-13	-	-	-	-	-
A-14	-	-	-	-	-
A-15	-	-	-	12.80	-

Figure 2 Antibacterial activity of isolated actinomycetes A-01 and A-06 against *Bacillus subtilis*

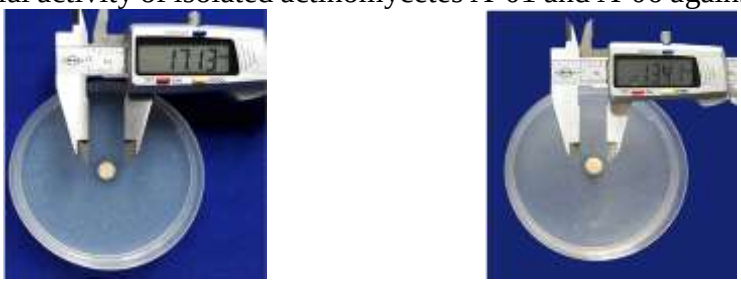


Figure 3 Antibacterial activity of isolated actinomycetes A-01 and A-08 against *Agrobacterium tumefaciens*

Figure 4 Antibacterial activity of isolated actinomycetes A-04 and A-12 against *Escherichia coli*

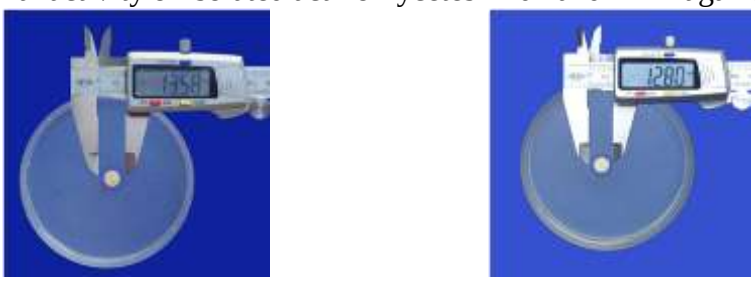


Figure 5 Antibacterial activity of isolated actinomycetes A-10 and A-15 against *Pseudomonas fluorescens*

Effect of ages of inoculums on the fermentation

In the investigation of the age of inoculums, six different hours of 38 hrs, 42 hrs, 46 hrs, 50 hrs, 54 hrs and 58 hrs were used and the results showed the inhibitory zone of 19.57 mm, 20.96 mm, 22.47 mm, 21.32 mm, 20.87 mm and 18.21 mm (Table 4 and Figure 6). According to these results, 46 hrs of culture was selected for the fermentation.

Table 4 Effect of Ages of Inoculums on Fermentation antibacterial activity against *Bacillus subtilis*

Culture Times (Ages of Culture, hrs)	Antibacterial activity (Clear zone, mm)
38	19.57
42	20.96
46	**22.47**
50	21.32
51	20.87
58	18.21

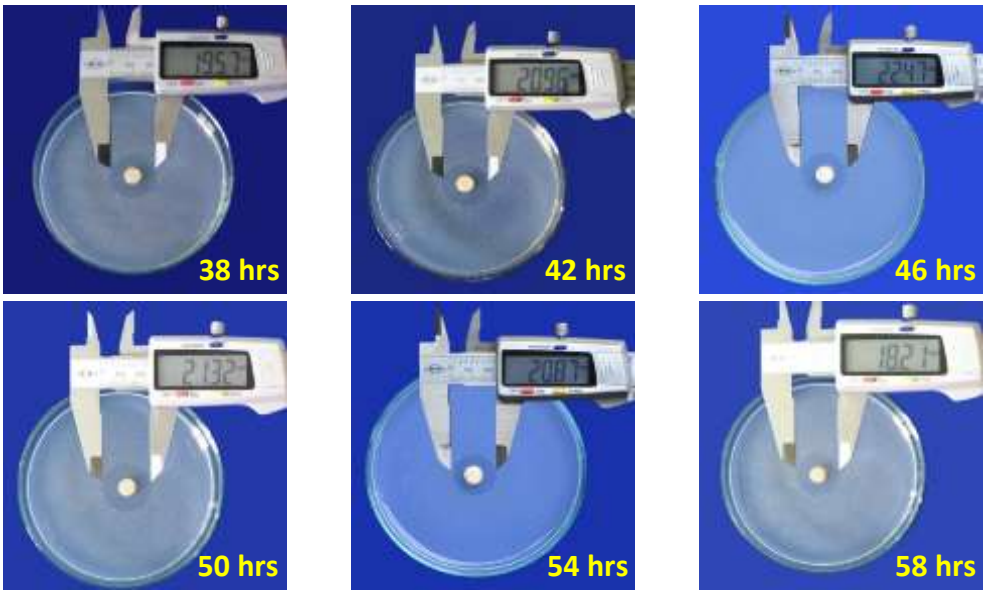


Figure 6 Effect of ages of inoculums on fermentation antibacterial activity against *Bacillus subtilis*

Effect of size of inoculums on the fermentation

The study of the size of inoculums 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% six different percentages were used and the results showed the inhibitory zone of 18.23 mm, 20.15 mm, 21.79 mm, 22.63 mm, 20.00 mm and 19.59 mm respectively (Table 5 and Figure 7). Depending on the results, it was determined that 2.0% inoculums was optimization for fermentation to produce the antibacterial metabolites.

Table 5 Effect of Sizes of Inoculums on Fermentation antibacterial activity against *Bacillus subtilis*

Culture Times (Sizes of Culture, %)	Antibacterial activity (Clear zone, mm)
0.5 %	18.23
1.0 %	20.15
1.5 %	21.79
2.0 %	**22.63**
2.5 %	20.00
3.0 %	19.59

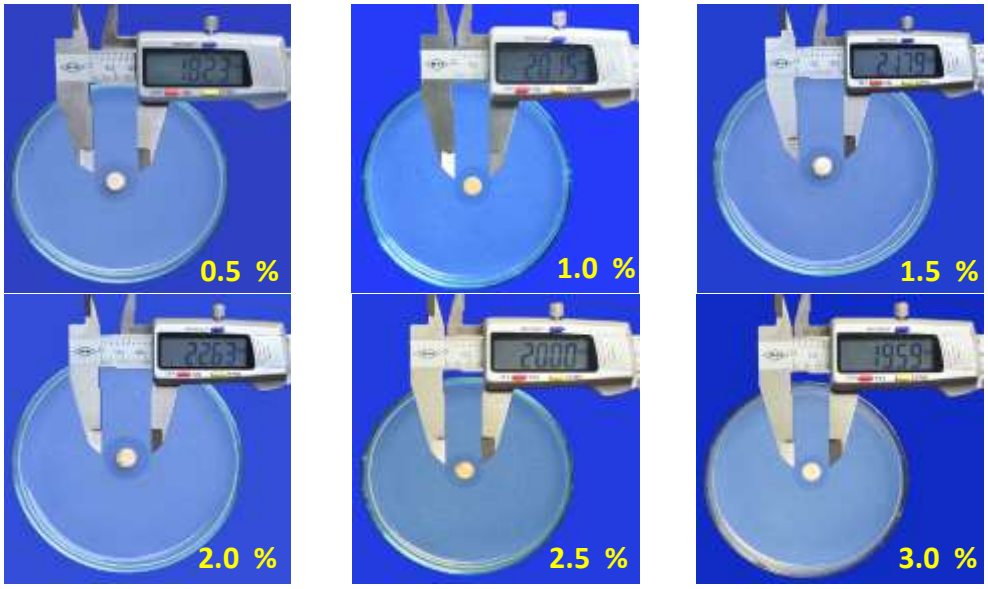


Figure 7 Effect of sizes of inoculums on fermentation antibacterial activity against *Bacillus subtilis*

Discussion and Conclusion

In the investigation for antibacterial metabolite producing actinomycetes, fifteen actinomycetes were isolated from the four soil samples collected at Pwint Phyu Township, Magway Region. Isolation of soil actinomycetes was undertaken by the methods of Chemical Treatment Dilution Method and Physical Treatment Serial Dilution Method (Phay and Yamamura , 2005). Actinomycets A-01, 02, 03 and 04 were isolated from Min Hla soil sample. A- 05, 06 and 07 were isolated from Kut Thit soil sample. A- 08, 09, 10 and 11 were isolated from Ywa Htaung soil sample. A- 12, 13, 14 and 15 were isolated from Shwe Hlay soil sample.

In the present work, 10 actinomycetes were isolated by Chemical Treatment Dilution Method and 5 actinomycetes were isolated by Physical Treatment Serial Dilution Method. By the result, the numbers of isolated actinomycete were greatly different between these two methods. Phay and Yamamura, 2005 reported that Chemical Treatment Dilution Method was more effective than Physical Treatment Serial Dilution Method for isolation of actinomycetes because the actinomycetes were released from the substrates depending on the isolation methods. So, similar result was obtained in this study. In the study of antibacterial activities, actinomycets A-01 and 06 showed antibacterial activities against *Bacillus subtilis.*

A-01 and A-08 showed antibacterial activities against Agrobacterium tumefaciens. A-04 and A-12 showed antibacterial activities against Escherichia coli. A-10 and A-15 showed antibacterial activities against Pseudomonas fluorescens. Among them, actinomycete A-01 exhibited the highest antibacterial activity against on Bacillus subtilis (23.41 mm). Therefore, this strain A-01 was selected for further studies such as ages of culture and sizes of inoculum. This actinomycete A-01 was isolated from the Min Hla village soil (Silt Loam, pH-7.2) of Pwint Phyu Township, Magway Region. In the study of the effects of ages and sizes of inoculum, it was observed that 46hrs seed culture and 2.0% sizes of inoculum were optimized for the fermentation. In conclusion, the isolated actinomycete A-01 will be intended to utilize in further processes like extraction, purification and identification of new bioactive metabolites.

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BIO COMPOST AND BIOFERTILIZER ON GERMINATION, GROWTH AND YIELD OF *ABELMOSCHUS ESCULENTUS* (L.) MOENCH. (OKRA)

Khin Lay Nandar Aung[1], Su Latt Win[2] and Ngwe Sandar[3]

Abstract

Experimental study was conducted to assess response of different types of bio composts and biofertilizers on germination, growth and yield of *Abelmoschus esculentus* (L.) moench. Five treatments: Bokarshi compost, *Spirulina* biofertilizer, Hatake biofertilizer, Vermicompost and control were comprising in germination and pot experiment. All of the experiments were done in Kyauk Tan Township, February to April in 2020 by using Randomized Completely Block Design (RCBD) with four replications in pot experiment under greenhouse condition. The results indicated that germination, growth and yield parameters treated with all types of bio compost and biofertilizer were found superior than control. Among the treatments, the results of Bokarshi compost were significantly affected than other treatment in cultuvaion of okra.

Keywords: Germination, pot, bio compost, biofertilizer.

Introduction

Abelmoschus esculentus (L.) moench. (okra) is a family of Malvaceae and important vegetable for human consumption which supplies many sectors for nutrition. Today, over-dose application of agro-chemicals increased to food productivity but also created to several environmental problems and consequently to serious negative effect to people. That habitat likely decreased in soil fertility, food nutritional quality, and polluted to various ecosystem.

Biofertilization has become a positive alternative to chemical fertilizer in last few decades. It is beneficial in nitrogen fixation, enhancing nutrients uptake, also secrete higher amounts of hormones, vitamins and antibiotics (Kannaiyan, 2002). Biofertilizers are environmentally friendly fertilizers that non only prevent damages to natural sources but, help in cleaning the nature (FAO, 2008).

Bokashi is a Japanese word that means “fermented organic matter.” Most Bokashi sites state that the inoculant (usually called EM or Effective Micro-organisms). This medium is inoculated with beneficial microbes that flourish in anaerobic, acidic environments, natural anaerobic conditions (www.planetnatural.com). *Spirulina plantensis* was a photosynthetic blue green micro alga. It can be used as a beneficial biofertilizer and has been largely studied in cultivation of various crops due to eco-agronomical importance.

Dominguez (2004) described that “Vermicompost is a nutrient rich, microbiologically-active organic amendment that results from the interaction between earthworms and microorganisms during the breakdown of organic matter”. Hatake biofertilizer is a product that contains pure *Bacillus Amyloliquefaciens* D 203 strain discovered from the microflora of marine environment in Japan. The strain showed excellent plant pathogen fighting ability and high organic matter degradation activities (http://hatake-global.com/product).

The application of bio composts and biofertilizers in crop cultivation was suitable for soil health, provided to good agricultural practice and adopted among the farmers in Myanmar. So, the effect of bio composts and biofertilizers were necessary to assess which kind will be effective in okra cultivation. This study was aimed to undertake a sustainable agricultural method of

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horticulture with the use of bio compost and biofertilizer in okra cultivation. The objectives of this research are to study germination of okra seeds with application of bio composts and biofertilizers, and to compare the impacts of growth and yield of okra treated with different types of bio compost and biofertilizers under greenhouse condition.

Materials and Methods

Preparation on Materials in Germination and Pot Experiment of Okra

Okra seeds, Bokarshi and Vermicompost were bought from Department of Vegetable and Fruit Research and Development Center, Ministry of Agriculture, Liver stock and Irrigation, Hlegu Township, Yangon Region. *Spirulina* biofertilizer was kindly provided by June Pharmaceutical CO. Ltd. Hatake biofertilizer was bought from Lawkanat Myanmar Group Co. Ltd. The basal cultured soil was collected from Kyauk Tan Township. The colorful baskets (21″×14″×6″) were used for germination experiment and the pots with 10″ height and 10″ width in diameter were used to grow okra plants.

The Methods Applied in Germination and pot Experiment

The experiments were conducted in Kyauktan Township, during February and April, 2020. The baskets and pots were well perforated to allow excess water at the base and filled with 10 kg of soil in each. Five treatments comprising: T1 (control), T2 (100 g *Spirulina*), T3 (100 g Hatake), T4 (1 kg Vermicompost) and T5 (1 kg Bokarshi) per basket in germination experiment. Twelve seeds of okra were sown in each treatment and number of seed germinated were counted on 7th day. The germination percentage and vigor index were finally determined by the following formula of Harris (1996), and Abdul - Baki and Anderson (1973). The shoot and root length of okra seedlings were also measured.

$$\text{Germination percentage (\%)} = \frac{\text{No.of germinated seeds}}{\text{No.of total seeds in sowing}} \times 100$$

$$\text{Vigor index} = \text{Mean shoot length (cm)} + \text{Mean root length (cm)} \times \text{Percent of seedling germination (\%)}$$

Pot experiment consists of five treatments comprising: T1 (control), T2 (1 kg Bokarshi), T3 (100 g *Spirulina*), T4 (100 g Hatake) and T5 (1 kg Vermicompost) per pot. Okra seeds were pre-soaked in water for 12 hours to avoid the incidence of any plant disease. Three seeds were sown in each pot and out of three seedlings only one healthy plant was allowed to grow to maturity. Randomized Completely Blocked Design was used with four replications. Two liters of water per pot were poured to provide moisture for plants. The plant height, number of leaves, flowers and fruits per plant were recorded. All of collected data in pot experiment were subjected to the analysis of variance by using IRRISTAT software and mean values were separated by the least significant differences 5 % levels.

Results

Effect of Bio composts and Biofertilizers on Germination of Okra

In this experiment, the different types of bio composts and biofertilizers were used in germination test and their effect on germination and seedling growth of okra was described in Table (1). All of the biofertilizer treatments were higher in germination percentage than the control.

Among them, T5 treatment has the highest germination percentage and followed by T2, T4, and T3. The mean germination percentage of T5 were 91.67 % and the second highest germination percentage was found in T2 and T4 treatments, 83.33 %. The germination percentage of T3 was 75% while that of T1 was 58.33%.

Table 1 Effect of bio composts and biofertilizers on germination of okra.

Treatment	Mean Germi - nation (%)	Mean Shoot length(cm)	Mean root length (cm)	Vigor index
T1	58.33	13.57	3.81	3015.76
T2	83.33	23.25	7.07	13697.58
T3	75.00	21.42	7.36	11823.84
T4	83.33	23.38	6.88	13404.00
T5	91.67	25.54	8.01	18753.43

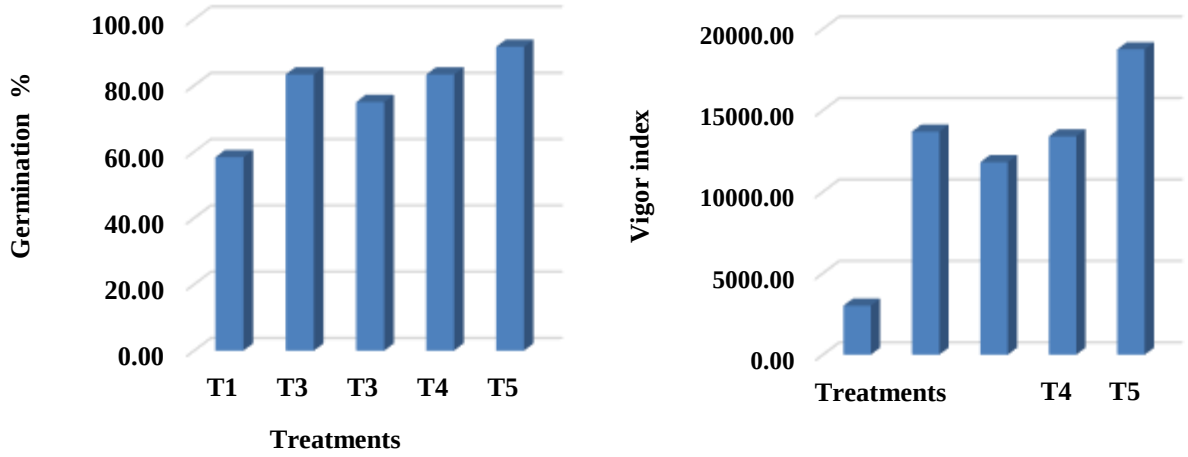

Figure 1 Germination percentage and vigor index of okra treated with bio composts and biofertilizers.

According to the results, the vigor index of T5 treatment in okra seedlings were significantly higher than other treatments. The highest vigor index value, 18753.43 was produced by T5 treatment and followed by T2 and T4 treatments, 13697.58 and 13404.00. The vigor index value of T3 treatment were 11823.84. The lowest vigor index value, 3015.76 was obtained by T1 treatment.

It was found that T5 treatment produced the highest shoot length, 25.54 cm and followed by T2 and T4 treatments. The mean shoot length of T2 and T4 were not clearly different, 23.25 cm and 23.38 cm. The shoot length of T3 was 21.42 cm. Among these treatments, T1 treatment produced the lowest shoot length, 13.57 cm. The highest root length, 8.01 cm was possessed by T5 treatment. The root length of other treatment was presented as descending order: T3,7.36 cm; T2,7.07 cm and T4, 6.88 cm, respectively. Among these, T1 treatment had the lowest root length, 3.81 cm.

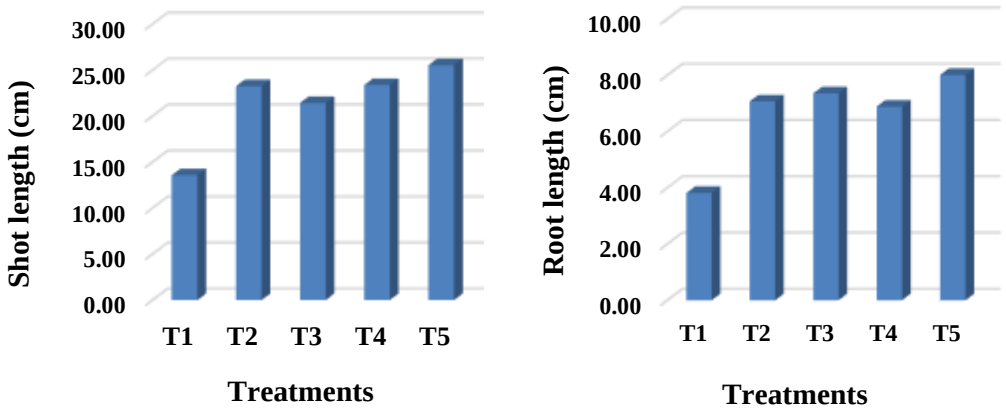

Figure 2 Shoot length and root length of okra treated with bio composts and biofertilizer.

Effect of Bio composts and Biofertilizers on Growth and Yield in Okra

Growth and yield parameters in all treatements were recorded to 6 WAS depending upon weather condition. It was found that the data were satistically significantly difference among treatments. T2 treatment produced the highest plant height, 33.50 cm while that of T1 treatment was recorded as the least plant height, 19.18 cm (Table 2 and Figure 3).

Table 2 Effect of bio composts and biofertilizers on plant height (cm) of okra.

Treatment	1WAS	2WAS	3WAS	4WAS	5WAS	6WAS
T1	3.30	7.18	11.13	15.80	18.25	19.18
T2	5.10	8.43	14.50	27.30	32.25	33.50
T3	4.68	7.98	14.13	25.50	29.50	30.50
T4	3.88	7.98	13.18	21.70	24.13	25.63
T5	4.78	7.58	13.68	23.05	25.15	28.13
F test	1.71	0.89	1.03	4.64	5.35	5.59
5 % LSD	ns	ns	ns	*	*	**
CV%	25.9	12.8	19.4	18.3	18.0	16.8

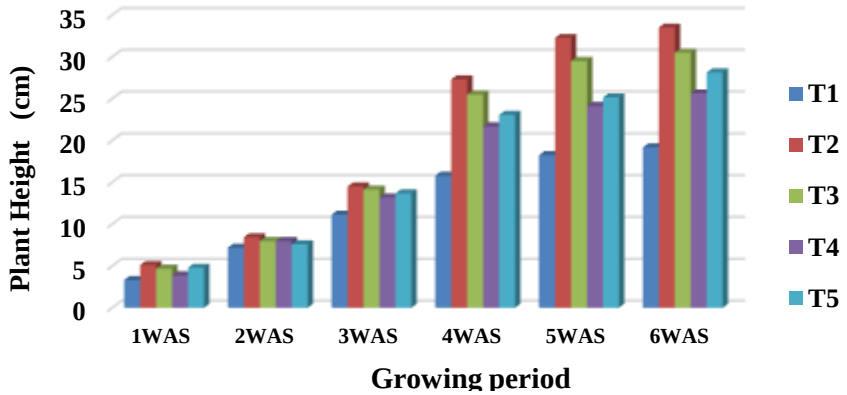

Figure 3 Plant height treated with bio composts and biofertilizers in okra.

The mean number of leaves were not clearly different in initial growth (1WAS). The number of leaves were increased enentually at mature. T2 treatment obtained the highest leave number 10.25, followed by T3, T5 and T4 while T1 treatment recorded the least leave number (Table 3 and Figure 4).

Table 3 Effect of bio composts and biofertilizers on number of leaves of okra.

Treatments	1WAS	2WAS	3WAS	4WAS	5WAS	6WAS
T1	2.00	2.75	3.50	4.00	4.00	5.50
T2	2.00	5.25	5.75	8.00	8.50	10.25
T3	2.00	3.75	5.75	6.50	7.75	9.25
T4	2.00	3.00	4.50	5.25	5.75	7.75
T5	2.50	3.50	5.50	5.75	6.00	8.50
F test	3.00	3.14	3.97	4.75	3.55	4.58
5% LSD	ns	ns	*	*	ns	*
CV%	12.3	31.5	19.7	23.1	29.4	20.3

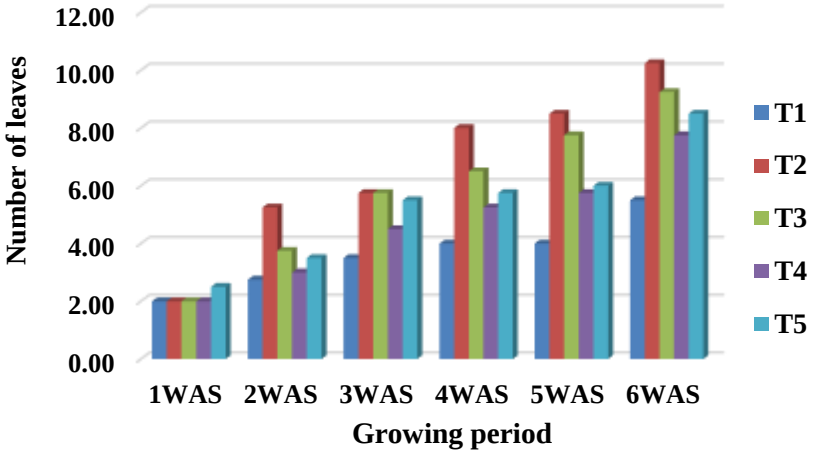

Figure 4 Number of Leaves treated with bio composts and biofertilizers in okra.

Flower formation was occurred in 2 WAS in all treatments. Among these, T2 treatment showed better results in flowering, followed by T3, T5 and T4. T1 treatments had the lowerst flower number than other treatments (Table 4 and Figure 5).

Table 4 Effect of bio composts and biofertilizers on number of flowers of okra.

Treatments	2WAS	3WAS	4WAS	5WAS	6WAS
T1	1.00	2.00	2.00	2.75	4.25
T2	3.00	3.50	4.25	5.75	7.50
T3	2.00	2.75	3.50	4.75	7.00
T4	2.00	2.50	3.25	3.75	5.50
T5	2.00	2.25	3.25	4.75	6.00
F test	15.00	7.35	32.43	14.22	10.08
5% LSD	**	**	**	**	**
CV%	18.3	23.7	11.8	14.1	13.3

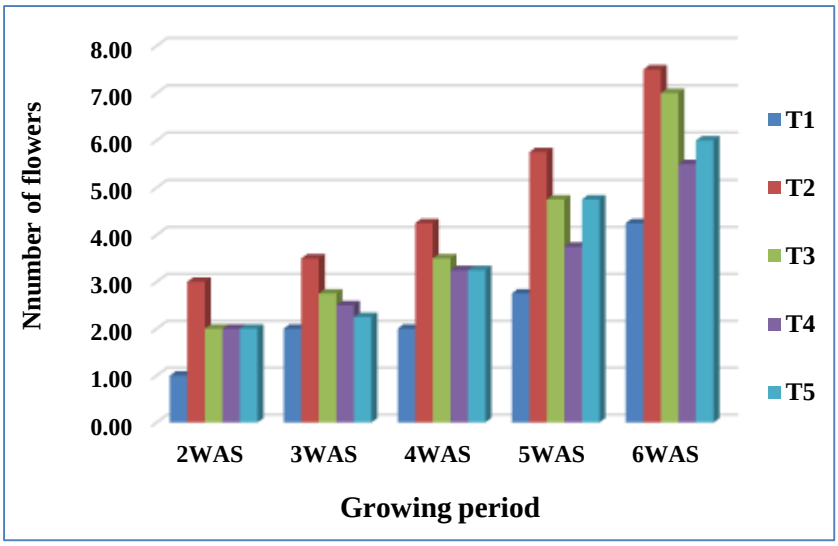

Figure 5 Number of flowers treated with bio composts and biofertilizers in okra.

Similarly, the number of fruits per plant was the highest in T2 treatment, 7.00 until harvest time (6WAS) and the second highest fruiting was observed in T3 treatment, 6.00. The result of T4 and T5 treatments were not significantly different, 4.75 and 5.25. Among these, T1 possessed lowest fruit number, 3.25 (Table 5 and Figure 6).

Table 5 Effect of bio composts and biofertilizers on number of fruits of okra.

Treatments	3WAS	4WAS	5WAS	6WAS
T1	1.50	2.75	2.50	3.25
T2	3.50	4.25	5.00	7.00
T3	3.25	4.00	4.75	6.00
T4	2.50	3.25	3.50	4.75
T5	3.00	3.75	4.25	5.25
F test	5.77	5.31	10.83	17.83
5% LSD	**	*	**	**
CV%	23.9	17.8	17.1	12.7

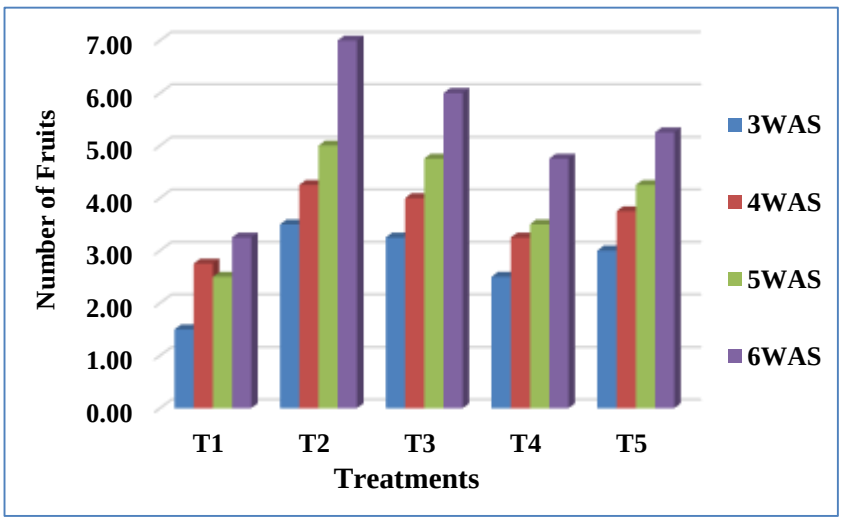

Figure 6 Number of fruits treated with bio composts and biofertilizers in okra.

Figure 7 Okra seeds, bio composts and biofertilizers

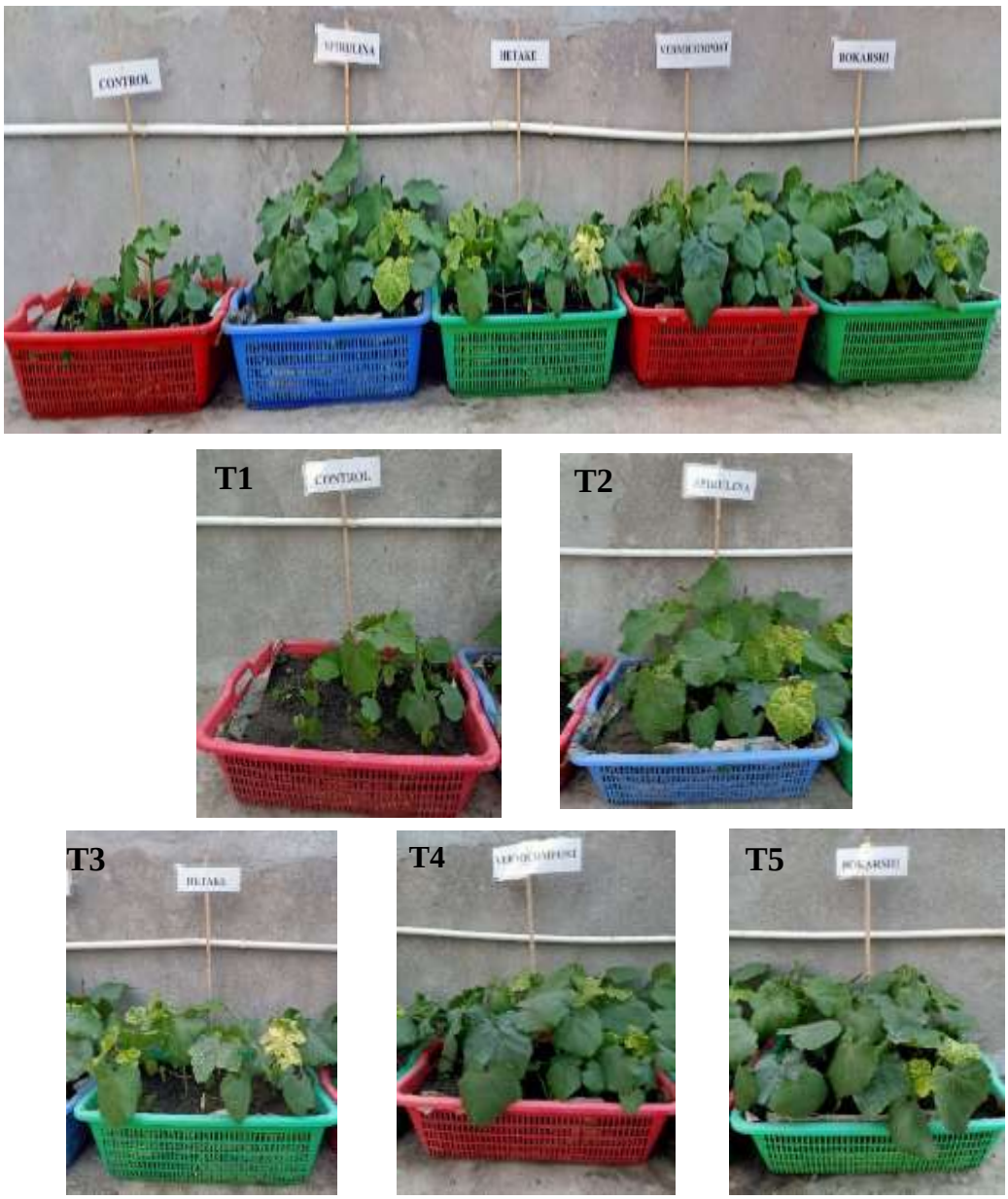

Figure 8 Germination experiment of okra

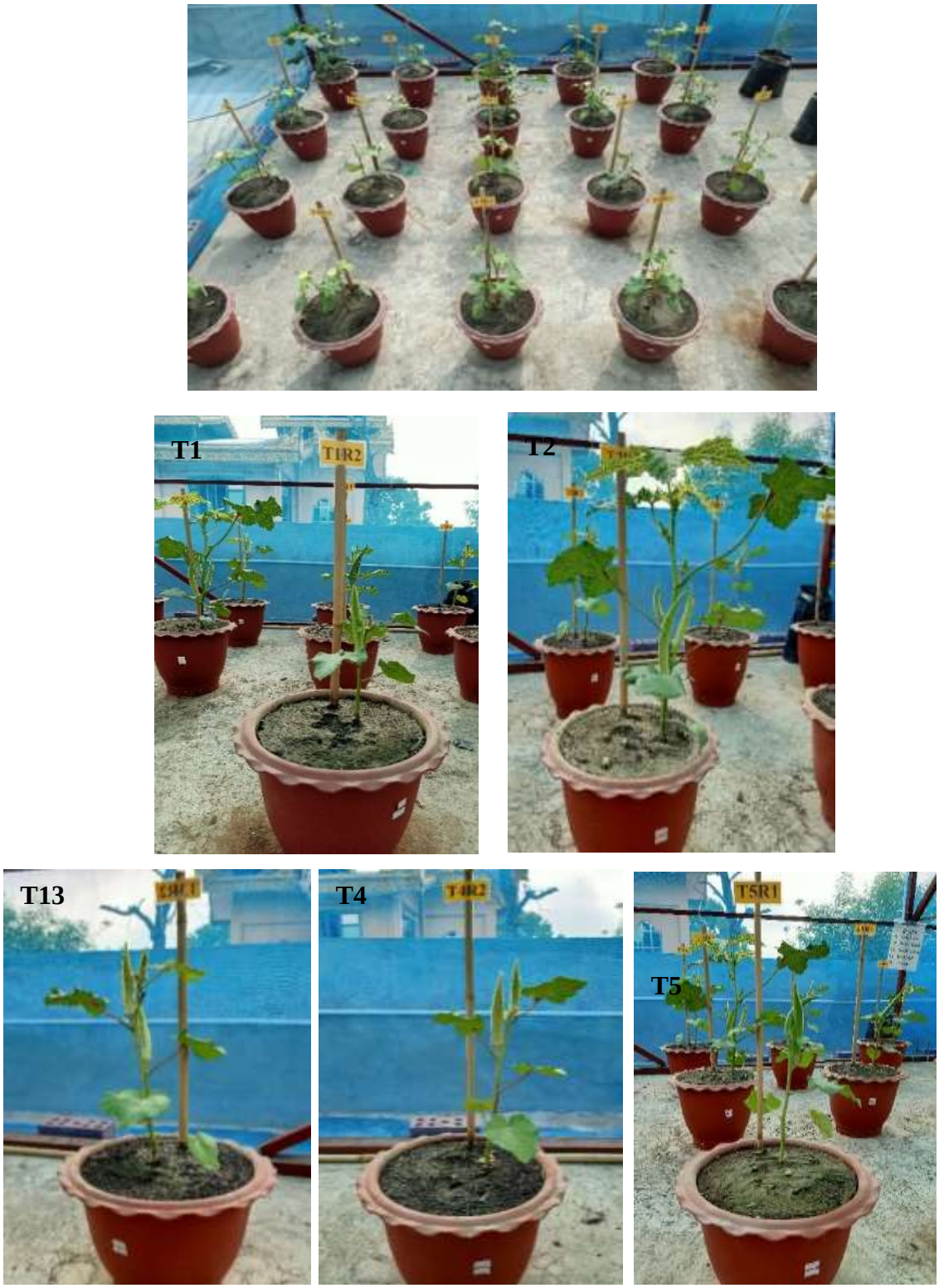

Figure 9 Okra plants treated with bio composts and biofertilizers in pot experiment.

Discussion and Conclusion

The effective utilization of bio composts and biofertilizers for crop production will not only to safe food production and but also provide avoidance to various health hazards of the people. Kannaiyan (2002) stated that "the beneficial effect of organic and biofertilization on enhancing soil fertility and the uptake of different nutrients surely reflected on stimulating growth characters and nutritional status of the trees in favour of producing more fruits".

This study has clearly described that the application of bio composts and biofertilizers make significant difference in germination, growth and yield of okra. Among these, germination percentage and all of measuring parameters were distinctly affected in T5 (Bokarshi) treatment. Ncube *et al*.,(2011) stated that "the application of Bokarshi appeared to promote early fruiting and root growth in tomato". Christel (2017) explained that "Bokarshi may be a feasible soil ferility amendment to be used as an alternative or supplement to compost". Naik *et al*., (2020) found that "foliar application of EM gave higher pod yields in okra compare to control".

Spirulina biofertilizer has a good response in okra cultivation and it has the second highest values in growh and yield parameters. Ahmed *et al*., (2011) presented that "Enriched organic fertilizers with biofertilizers especially *Spirulina platensis* algae was beneficial in improving yield quantitively and qualitatively rather than application of organic fertilizer alone". The plant height, number of leaves, flowers and fruits of Vermicompost treatment and *Spirulina* biofertilizer treatment were nearly difference.

According to the results, the germination percentage of okra treated with Vermicompost was 83.33 % and their height was 25.15 cm at 5 WAS. Tensingh and Muthulakshmi (2017) found that "the rate of seed germination was higher in okra plants treated with Vermicompost, 80 %. Agarwal and Sinha (2010) presented that "80 % of seed germination of okra was found to be numerically higher in pots with Vermicompost treatment in summer. They also stated that the plant height of okra in Vermicompost treatment was 24.13 cm after 45 days. Edwards *et al*., (2004) stated that "Vermicompost has a positive influence on vegetative growth, stimulating shoot growth and root development". In 2007, stimulated seed germination in green gram, tomato and petunia after imbedding vermicompost in soil has been documented by Zaller.

Among the treatments, Hatake biofertilizer showed better results in root length. Hatake biofertilizer improved the growth rate of plants, enhancing the microbial activities of soil, may be improved to root biomass and increasing plant uptake on soil nutrients. It is 100 % organic and will not contaminate the soil, water streams or environment and even safe for human ingestion (http://hatake-global.com/product).

Therefore, the results of this study were agreed with findings of previous researchers. The results showed that application with Bokarshi, Vermicompost, *Spirulina* biofertilizer and Hatake biofertilizer have been shown to have several agronomic positive impacts on germination, growth and yield of okra under greenhouse condition. Thus, these bio composts and biofertilizers were determined as promising beneficial fertilizers in organic agriculture and can be used as an alternative to inorganic fertilizers.

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PRELIMINARY PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF RHIZOME EXTRACT OF *KAEMPFERIA CANDIDA* WALL.

Khin Myo Aye[1], Khin Hnin Yee[2], Khin Soe Aye[3]

Abstract

Morphological characters of rhizome of *Kaempferia candida* Wall. belonging to the family Zingiberaceae was reported. This specimens had been collected from Mansan Fall, Lashio Township, during July to August 2019.In addition, this rhizome was studied by using phytochemical, antimicrobial tests and then antioxidant activity. The phytochemical tests indicated that alkaloids, glycosides, phenols, polyphenols, reducing sugars and tannins were present in this rhizome. Furthermore, ethanol extract of rhizome has been tested for their antimicrobial activities by using agar-well diffusion method. They were found to be antimicrobial activity against five different types of microbes such as *Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus, Candida albicans* and *Escherichia coli*. The antioxidant activity of rhizomes extracts of *Kaempferia candida* Wall. was carried by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay. Strong antioxidant activity of *Kaempferia candida* Wall. was shown in this experiment compared with the control ascorbic acid.

Keywords: *Kaempferia candida*, Phytochemicals test, Antimicrobial characters, Antioxidant activity, DPPH Free radicals

Introduction

Human beings are dependent upon the plants and plant products for their basic needs, and in modern civilization, they still need the plants and plant products in daily life, as sources of food, oxygen, wood, drugs, many fibres, fossil fuel, insecticides, biofertilizers, ornamentals, as well as rubber and other products.

Most of the members of the Zingiberaceae have been used worldwide in traditional medicines for the treatment of diseases. Herbal medicine is a traditional medicine or folk medicine based on the use of plant parts and plant extracts. The herbal medicines have been recognized as a valuable and readily available resource of primary health care (Cruickshank, 1970).

The family Zingiberaceae comprises of about 47 genera and 1400 species distributed in tropical and subtropical regions of the world (Hutchinson, 1967; Lawrence, 1964). Hooker (1894) reported that the family comprises of 40 genera and 400-500 species. 42 genera and 750 species were recorded by Rendle (1930). According to Hundley and Chit Ko Ko (1987), 125 species belonging to 18 genera are represented in the Union of Myanmar.

The family is taxonomically characterized by the presence of the leaves which are distichous or in spiral, the sheathing petioles usually opened, rarely closed, the presence of aromatic oils, ligulate; zygomorphic, trimerous flowers with the marked differentiation of the outer perianth series from the inner; the single fertile stamen, the large, usually petaloid staminodium, the inferior ovary and the seeds with copious hard or mealy endosperm.

The phytochemistry has developed with the chemical aspects of various metabolite processes taking place in plants. The phytochemicals are plant chemicals that they have health enhancing effects (British Pharmacopoeia, 1968).

There is an increasing interest in plants secondary metabolites like polyphenols, because of their therapeutic effects. Polyphenols or phenolic compounds form a large group of secondary

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compounds including phenolic acids, carotenoids, flavonoids, tannins, flavones glycosides, etc. These phenolic compounds have the property of quenching oxygen-derived free radicals by donating a hydrogen atom or an electron (Madhu, K., 2013).

Phenolic compounds are responsible for antioxidant property. Antioxidants are classified into two major categories, natural and synthetic antioxidant. Plants are prospective source of natural antioxidants. The natural antioxidants are safer and environment friendly than synthetic antioxidants. Plants are a potential source of natural antioxidants which are secondary metabolites of plant, that exhibit a wide range of biological effects like antibacterial, anti-inflammatory, anti-allergic, anti-hepatotoxic and anti-thrombotic activities and prevention of cardiovascular diseases (Sadeghi, Z., Valizadeh. J., 2015).

Antioxidants are capable of blocking the effect of the Reactive Oxygen Species (ROS). In living organisms, the imbalance in the production and detoxification of free radicals by the biological system causes oxidative stress. Free radicals are generated by different types of exogenous chemicals and a number of endogenous metabolic processes oxidize the bio molecules leading to cell death and tissue damage. The organism must keep free radicals at relatively low concentrations using different defence systems and antioxidant molecules (Bhattacharyya, A., Chattopadhyay, R., Mitra, S, 2014).

Production of high amount of reactive oxygen species overcomes inbuilt antioxidant system and damages the cells, tissues and organs and hence, there is a need to develop new drugs from traditional medicine to protect and support the biological system to avoid serious disorders of liver, cardiac and cancer diseases etc (Shivasharanappa, K., Londonkar, R., 2014).

Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals (Walton and Brown, 1999).

The present research deals with the phytochemical and antimicrobial tests and antioxidant activity of rhizomes of *Kaempferia candida* Wall. (Pan-u) belonging to family Zingiberaceae.

Finally, the aims of this research were to provide the information of morphological characters; to observe the preliminary phytochemical findings; to interest the pharmacological active compounds and antimicrobial effects; to use the ethno medicine for treatment of bacterial infections.

Materials and Methods

Plant Material Collection

The specimens had been collected from Mansan Fall, Lashio Township, during July to August 2019. The collected specimen was recorded by photographs while flowering and fruiting periods. The specimens were collected during the extended field study by GPS (Global Positioning System). The collected specimens were identified by referring to Flora of British India (Hooker, 1894), Flora of Java (Backer, 1968), Flora of Ceylon (Dassanayake, 1976).

Preparation of Plant Extract

The collected specimens were washed to remove dust and rinsed again with distilled water. The plant samples were air dried at room temperature 35ºC-40ºC for one month. After drying the samples, they were ground to get powder and stored in air-tight containers for further chemical analysis.

Preparation of Ethanolic Extract

The dried powder of rhizomes 100g were percolated with 500 ml of ethanol for one week and filtered with filter paper for three times respectively. The filtrates were evaporated by removing the solvent under reduced pressure using rotary evaporators at 50ºC. Then, the filtrates were dried in a beaker placed on a water bath at 60ºC. The dried extract was stored in the desiccator for further analysis.

Phytochemical Screening

The preliminary phytochemical screening for bioactive compounds was carried out by the standard methods (Harbone, 1998). The preliminary phytochemical screening of *Kaempferia candida* rhizome extracts was carried out for the presence or absence of alkaloids, flavonoids, glycosides, phenols, polyphenols, saponins, reducing sugars, carbohydrates and tannins.

Test for Alkaloids

Two grams of dried powdered sample was boiled with few ml of 1% HCL for 10 min, allowed to cool and filtered. The filtrate was tested with Wagner's reagent, Dragendroff's reagent, and Mayer's reagent. The reddish brown, orange and the white of creamy precipitate indicated as positive according to the test in the respective reagent.

Test for Flavonoids

Two grams of dried powdered sample was boiled with ethanol (5ml) for 10 min and filtered. The filtrate was placed in a test tube. Then, 0.5 ml of concentrated hydrochloric acid and a few pieces of magnesium (Mg) were added and the mixture was boiled for a few minutes. If pink or red colour develops, presence of flavonoids is noted.

Test for Glycosides

One gram of dried powdered rhizomes was boiled with distilled water for about 10 minutes, allowed to cool and filtered. The filtrate was treated with 10 % lead acetate solution. If the white precipitates were traced, it showed the presence of glycosides.

Test for Phenolic Compounds

Two grams of dried powdered sample was boiled with distilled water for 10 min and filtered. The filtrate was tested with 5% $FeCl_3$ solution. The dark green colour indicates the presence of phenolic compounds.

Test for Polyphenols

Two grams of dried powdered sample was boiled with distilled water for 10 min and filtered. The filtrate was tested with 5% $FeCl_3$ solution and 1% potassium ferricyanide ($K_3Fe(CN)_6$). The dark green blue colour indicates the presence of phenolic compounds.

Test for Saponins

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. The formation of froth or persistent foam indicated that saponins were present.

Test for Reducing Sugars

Two grams of dried powdered sample was boiled with distilled water for 10 min, the solution was cooled and filtered. The filtrate was tested fehlling A and B solution. The red precipitate indicates the presence of reducing sugars.

Test for Carbohydrates

The water extract of the sample was put into a test tube and 2 drops of 10 % napthanol was added to it. Then concentrated sulphuric acid was gradually poured down into the side of the test tube and allowed to stand. The formation of violet red ring indicates the presence of carbohydrates.

Test for Tannins

Dried plant materials (about 2g) were introduced into a test tube and was boiled with distilled water (10 cm^3) for about 20 minutes and filtered. The filtrate was treated with 3ml of 10 % lead acetate solution. The white precipitate shows the presence of tannins.

Antimicrobial Tests

Tested microorganisms: The antimicrobial screening was carried out, using the following organisms; *Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus, Escherichia coli* and a fungus *Candida albicans*.

Determination of Antimicrobial Activity

The agar well diffusion method was used for antimicrobial activity, evaluation by modifying the method described by Schlegel. Tested microorganisms were inoculated in Muller Hinton Broth at 37ºC for overnight. On the next day, the overnight broth culture was diluted with Normal saline to obtain the OD_{600} at 0.08 to 0.1 with the approximate cell density of $1.5X10^8$ CFU/ml. Muller Hinton Agar plates were prepared and sterilized by autoclaving at 121ºC for 15 min. The broth inoculums were evenly spread out with sterile cotton swabs on the Muller Hinton Agar plates to obtain the uniform inoculums. After the plate was inoculated, 8-mm diameter wells were made on the agar medium by using a sterile cork borer. Each 50ul of plant extract (500ug/50ul) was introduced into each well labelled. Chlorempheniсol 30ug/well was used as the positive control. Then, the plates were placed in an incubator at 37ºC for 16 to 18 hours. After incubation, the plates were examined and zone diameters of complete inhibition were measured and recorded to the closest milimeter.

Positive control : Chlorempheniсol

Negative control : 70% EtOH

Antioxidant Activity

The ethanolic extract of Pan-u was determined its antioxidant activity by DPPH Scavenging Assay.

Chemical and materials: Reagents and chemical used in this experiment were of the highest analytical grade. The antioxidant activity of plant extracts were determined by the DPPH free radical scavenging assay according to Lee *et.al*. The samples were dissolved in DMSO (10mg/ml) and the dissolved samples were diluted with 50% EtOH for various concentrations. Briefly, the reaction mixture containing 50µl of diluted test sample of various concentrations and 50 µl of DPPH (300 µml) dissolved in ethanol, was taken in a 96-well microliter plate and kept standing at 37ºC for 30 min. The absorbance was measured at 517 nm by using 96-well microplate reader (Spectrostar Nano, BMG Labtech Microplate reader). Ascorbic acid was used as positive control.

50% EtOH was used as negative control and added to the 96-well plate instead of the sample. Percent Radical Scavenging Activity (% RSA) was calculated by using the following formula:

%RSA = [1-(ABS test compound/ABS control)] x100

ABS = Absorbance

RSA = Radical Scavenging Activity

Statistical Analysis

The experimental work was performed by triplicate test. The results were reported as mean±standard deviation (SD). Calibration curve was obtained by plotting percentage inhibition against standard concentration. The IC_{50} value was calculated from linear regression analysis using Microsoft excel.

Results

Morphological Characters

Kaempferia candida Wallich, Pl.Asiat. Rar.1:47.1830.

Myanmar Names : Padatsa; Pan-u; Pan-u-phyu

English Name : Narrow-leaf peacock ginger

Family : Zingiberaceae

Flowering period : June to November.

Perennial erect shrubs, 6.0-9.0 cm tall. Rhizomes tuberous, ovoid, brown without and white within. Leaves mostly 2, alternate, broadly elliptic, 10-12 cm long and 6.0-10 cm wide, obtuse at the bases, more or less undulate the margins, acute at the apex. Inflorescences terminal spikes; bracts variable, the two outer ones large, enclosing the inflorescences, ovate to lanceolate, the two inner floral ones linear. Flowers infundibuliform, 4.0-6.0 cm long and 2.3-3.0 cm wide, white. Calyx tubular, 1.5-2.0 cm long and 3-5 mm wide; tubes 1.5-1.7 cm long and 3-5 mm wide; lobes tooth-like, about 1 mm long and wide. Corolla infundibuliform; tubes 2.0-2.4 cm long and 2.0-3.0 mm wide, white; lobes linear, 1.5-1.9 mm long and wide. Fertile stamen one, erect, 0.5-1.0 cm long and 1.0-2.0 mm wide, pale yellow; filament flattened; anther lobes linear-oblongoid; lateral staminodes obovate, 1.5-1.7 cm long and 1.0-1.3 cm wide, white; basal staminodes two, linear-acicular, 2-5 mm long and 0.4 mm wide, white; labellum obovate, 1.7-2.2 cm long and 2.0-2.3 cm wide, white. Ovary oblongoid, 2-4 mm long and 2-3 mm wide, trilocular, one ovule in each locule on the axile placenta, style filiform; stigma globoid.

Specimen examined: Mansan Fall, Lashio Township, Northern Shan State, 20 July, 2019, Khin Myo Aye, Collection No.1.

Figure 1 *Kaempferia candida* Wall., natural habit of a plant

Figure 2 *Kaempferia candida* Wall., flower as seen

Figure 3 *Kaempferia candida* Wall., plant rhizomes

Preliminary Phytochemical Analysis

The preliminary phytochemical studies for the ethanol extract of rhizomes of *Kaempferia candida* Wall., show the presence of alkaloids, glycosides, phenols, polyphenols, reducing sugars and tannins and the absence of flavonoids, saponins, and carbohydrates. The phytochemical constituents of the plants investigated are summarized in Table 1.

Table 1 Preliminary phytochemical test of rhizomes of ethanol extract for *Kaempferia candida* Wall.

No	Chemical Constituents	Chemical Reagents	Observations	Results (+/-)
1.	Alkaloids	Wagner's	Reddish brown	+
		Dragendroff's	Orange	+
		Mayer's	Creamy ppt	+
2.	Flavonoids	Mg + HCL	No Pink	-
3.	Glycosides	10% Lead acetate	White ppt	+
4.	Phenolic Compounds	10 % $FeCL_3$	Dark green	+
5.	Polyphenols	10% $FeCL_3$+ 1% $K_3Fe(CN)_6$	Dark green blue	+
6.	Saponins	Water (H_2O)	No Foam	-
7.	Reducing Sugars	Fehlling A+ B	Red ppt	+
8.	Carbohydrates	10 % Napthanol	No Red ring	-
9.	Tannins	10 % Lead acetate	White ppt	+

+ = presence of chemical constituents, - =absence of chemical constituents

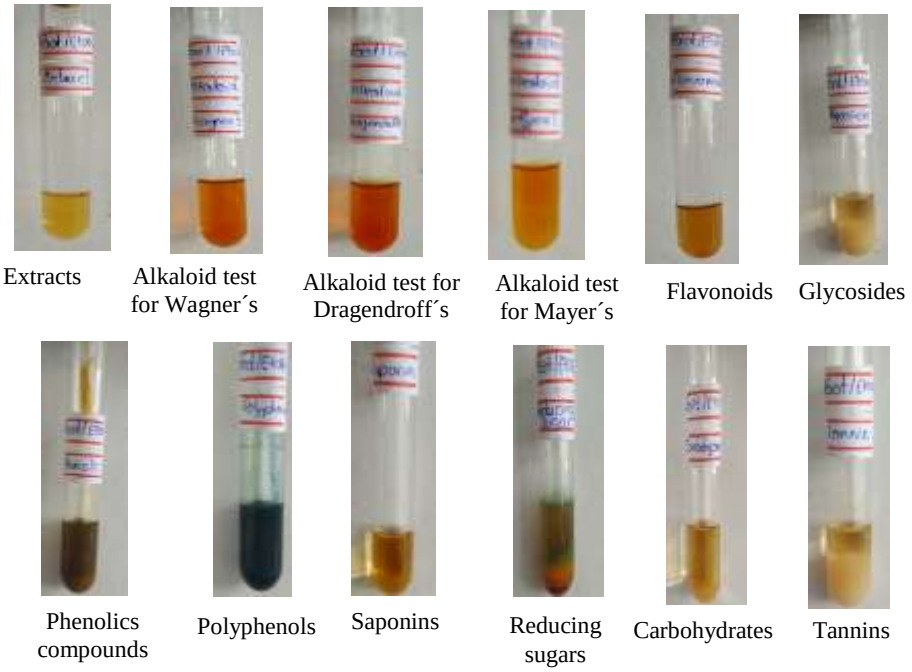

Figure 4 Preliminary Phytochemical tests of rhizomes of ethanol extract for *Kaempferia candida* Wall.

Antimicrobial Activity

One Gram-negative bacterium (*Escherichia coli*), three Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis and Bacillus cereus*) and one fungal strain (*Candida albicans*) were used as the tested microorganisms for this experiment.

Table 2 Antimicrobial activity of ethanol extract from powdered rhizomes of *Kaempferia candida* Wall.

Sample	Inhibition Zone Diameter(mm)				
	Escherichia coli	***Enterococcus faecalis***	***Staphylococcus aureus***	***Bacillus cereus***	***Candida albicans***
Panu	0	11	16	17	14
Chloremphenicol	40	35	40	28	32

The ethanol extract of *Kaempferia candida* (Pan-u) shows antimicrobial activities against *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans* but it does not show inhibition zone against *Escherichia coli*.

(A) *Escherichia coli*

(B) *Enterococcus faecalis*

(C) *Staphylococcus aureus*

(D) *Bacillus cereus*

(E) *Candida albicans*

Figure 5 Antimicrobial activities of ethanol extract of *Kaempferia candida* Wall.

Antioxidant Activity

The antioxidant activity of Pan-u was carried out by DPPH scavenging assay. Strong antioxidant activity of (IC_{50}: 96.8 ±4.15) µg/ml was shown in this experiment compared with the control ascorbic acid. (IC_{50}: 84.78 ± 0.39) µg/ml.

Table 3 The antioxidant activity of Pan-u was carried out by DPPH scavenging assay

Sample (Concentration ug/ml)	**1000**	**500**	**250**	**125**	**62.5**	**31.25**	**15.63**	**IC(ug/ml)±SD**	**Method**
DPPH Scavenging (%)±SD	>100	86.64±3.60	84.68±1.18	64.64±3.28	32.42±1.23	1.77±0.68	-	96.8±4.15	DPPH Radical Scavenging Assay

All data were represented as Mean±SD from triplicate experiments. Ascorbic acid was used as a positive control for DPPH Radical Scavenging Assay. Ascorbic acid shows 94.63± 0.34% inhibition at 500µg/ml and IC_{50} of Ascorbic acid is 84.78± 0.39µg/ml. The concentration of DPPH used for this experiment was 0.3mM.

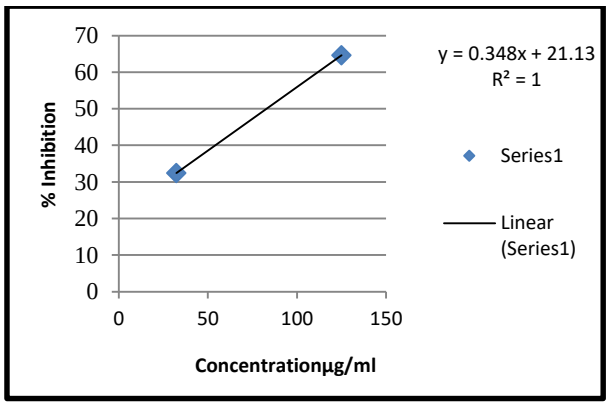

Figure 6 Calibration Curve

Discussion

Kaempferia candida Wall. are characterized by perennial underground stems (rhizomes), simple and distichous leaves. Petioles are long and the sheathing petioles are usually opened, rarely closed, ligulate. The flowers are irregular, trimerous, with single fertile stamen and two to five petaloid staminodes. The ovary was inferior, trilocular, axile placentation. These morphological characteristics were in accordance with those described by Rendle(1930), Lawrence (1964), Nyunt Nyunt San (1992).

Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer (Hossain MA, Nagooru, MR,2011).

In the present study,the phytochemical screening carried out on the ethanol extract of rhizomes of *Kaempferia candida* Wall.,show the presence of alkaloids, glycosides, phenolic compounds, polyphenols, reducing sugar and tannins and the absence of flavonoids, saponins, and carbohydrates.

The ethanol extract of rhizomes of *Kaempferia candida* (Pan-u) shows antimicrobial activities against *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans* but it does not show inhibition zone against *Escherichia coli.*

Cruickshank (1970) stated that soft tissues infections and skin diseases are caused by *Bacillus cereus. Staphylococcus aureus* is the cause of inflammation,burns and wound infections. *Candida albicans* can also cause sores, many skin diseases.

According to the results of this research,ethanol extract of rhizome of *Kaempferia candida* Wall.,will be useful in curing the diseases caused by the microbes mentioned above.

The antioxidant activity of Pan-u was carried out by DPPH scavenging assay. Strong antioxidant activity of (IC_{50}: 96.8 ±4.15) µg/ml was shown in this experiment compared with the control ascorbic acid (IC_{50}: 84.78 ± 0.39) µg/ml.

Conclusion

The extracts of rhizomes of *Kaempferia candida* plant contains chemical compounds such as phenolic compounds and polyphenols that are responsible for its antioxidant and antimicrobial activity. This result showed that rhizomes of *Kaempferia candida* extracts have interesting pharmacological active compounds and antimicrobial effects, and such could be used in ethno medicine for treatment of bacterial infections and ailments.

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INVESTIGATION OF MORPHOLOGICAL, HISTOLOGICAL CHARACTERS AND QUALITATIVE ANALYSIS OF *ROTHECA SERRATA* (L.) STEANE & MABB.

Khin San Nu[1], Htay Htay Lwin[2]

Abstract

Rotheca serrata (L.) Steane & Mabb. (Family Lamiaceae) is an important medicinal plant growing in the tropical and warm temperate regions. This plant is locally known as Yin-bya in Myanmar. It was collected from Hpa-an University Campus, Hpa-an Township, Kayin State from March to September 2019. The flowering and fruiting period is from May to September. In morphological study, the plant is perennial shrub, stem bluntly quadrangular. Leaves opposite and decussate, sharply serrate margin. Flower numerous, showy, dichotomous cymes. Fruits black in mature, seeds pyrene. In histological characters, diacytic stomata, uniseriate glandular and non glandular trichomes were present. Calcium oxalate crystals were present in mesophyll tissue of lamina and acicular crystals in petiole and stem. The vascular bundles were collateral and close type in the midrib, collateral and open type in petiole and stem. The present of alkaloid, flavonoid, phenolic compound, starch, reducing sugar, glycoside, saponin, tannins, α-amino acid and carbohydrates were found in phytochemical investigation. In physicochemical properties, the solubility of powdered leaves was found to be most soluble in polar solvent.

Keywords: *Rotheca serrata* (L.) Steane & Mabb., morphological, histological characters, phytochemical and physicochemical analysis.

Introduction

According to World Health Organization (WHO), about 80% of individuals from developed countries use traditional medicine. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Baker *et. al.,* 1995 and Reddy *et. al.,* 2001).

Lamiaceae are comparatively a large family composed of about 200 genera and 3300 species (Trease and Evans, 2002). The largest genera are *Rotheca* (400) (Cronquist, 1981).

The genus *Rotheca* (L.) Steane & Mabb. [Family Lamiaceae (Verbenaceae)] is very widely distributed in tropical and subtropical regions of the world. Estimates of number of species *in Rotheca* vary widely, about 450 (Rahman *et.al.*, 2007).

Rotheca serrata (L.) Steane & Mabb. is a genus of flowering plants in the Verbenaceae family. The plant *Rotheca serrata* (L.) Steane & Mabb. is commonly known as Yin-bya-net in Myanmar and Blue glory in English.

According to traditional uses the roots and leaf extracts of *Rotheca serrata* (L.) Steane & Mabb. has been used for the treatment of rheumatism, asthma and other inflammatory diseases (Hazekamp *et. al.,* 2001). The roots of the plant have been claimed to be used in dyspepsia, seeds in dropsy and leaves as a febrifuge and in cephalalgia and ophthalmia (Anonymous, 1992). Aqueous extracts of leaves of *Rotheca serrata* (L.) Steane & Mabb. possess bronchodilator property (Kirtikar and Basu, 1935 and Steane *et. al.,* 1999).

The aims of the present study are to identify and confirm the morphological characters, to investigate the phytochemical constituents and to determine the physicochemical properties of this plant.

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Materials and Methods

Collection and identification of *Rotheca serrata* (L.) Steane & Mabb.

The specimens used in this research were collected from the surrounding area of Hpa-an University Campus, Hpa-an Township, Kayin State during flowering period from May to September in 2019. The vegetative and reproductive parts of the fresh specimens were identified by using available literatures such as Hooker, 1885; Kirtikar and Basu, 1935; Backer and Brink, 1965; Cronquist, 1981; Dassanayake, 1983; Hundley and Chit Ko Ko, 1983; Hu Qi-ming and Wu De-Lin, 2009)

Histological study of *Rotheca serrata* (L.) Steane & Mabb.

Free hand sections of fresh specimen from lamina, midrib, petiole, stem and root were made and studied under microscope. Chloral-hydrate solution B.P as clearing reagent, solution of phloroglucinol B.P followed by with concentrated hydrochloric acid for lignin, acetic acid and 80% sulphuric acid B.P for calcium oxalate crystals, iodine solution B.P for starch, sudan III and IV for oil and ethanol for mucilage were used to examine for free hand sections and the powdered samples.

Preliminary phytochemical test on leaves of *Rotheca serrata* (L.) Steane & Mabb.

In this qualitative analysis, the air dried powdered of the leaves were tested at Department of Botany, Hpa-an University by the method of Vogel, 1966; British Pharmacopoeia, 1968; Marini Bettolo *et. al*., 1981; Robinson, 1983; Central Council for Research in Unani Medicine, 1987; Harborne, 1993; Trease and Evans, 2002.

Physicochemical properties on powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

The physicochemical characters such as moisture content, extractive values for the various solvents were determined according to British Pharmacopoeia, 1968 and WHO, 1998, at Department of Botany, Hpa-an University.

Results

Morphological characters of *Rotheca serrata* (L.) Steane & Mabb.

Habit; perennial shrub, scarcely woody, not much branched. Stem; bluntly quadrangular. Leaves; simple, opposite and decussate, apically clustered and variable in size, lamina oblong or elliptic about, acute, coarsely and sharply serrate margin, glabrous, base acute, petiolate, about. Inflorescence; terminal or axillary, green, quadrangular, in lax pubescent, dichotomous cymes, with a pair of acute bracts at each branching and a flower in the fork, each in the axil of a large leafy bract and collectively forming a long lax terminal usually pyramidal erect panicle about. Flower; showy, pale blue, bracteate, bracteolate, pedicellate, glabrous, complete, bisexual, irregular, zygomorphic, pentamerous, cyclic, hypogynous. Calyx; 5, synsepalous, puberulous, cup-shaped, connate at the base, enlarge at the middle, apex deeply five-lobe, lobe elliptic, inferior. Corolla; (1+4), synpetalous, the lower larger lobed, bluish purple, ovate to lanceolate, corolla tube slender, hairy within the tube, inferior. Stamen; 4, epipetalous, filament much more curved, densely hair at the base, didynamous, white, anther dark purple, dithecous, dorsifixed, longitudinal dehiscence, inferior. Ovary; 2, syncarpous, globose, false septum, each with two ovules, axile placentation. Style; filiform, white colour, glabrous, stigma bifid, purple, disc present, superior. Fruits; broadly obovoid, succulent, dark green in immature, black in matures, glabrous. Seeds; normally four lobed with one pyrene in each lobe, dark purple.

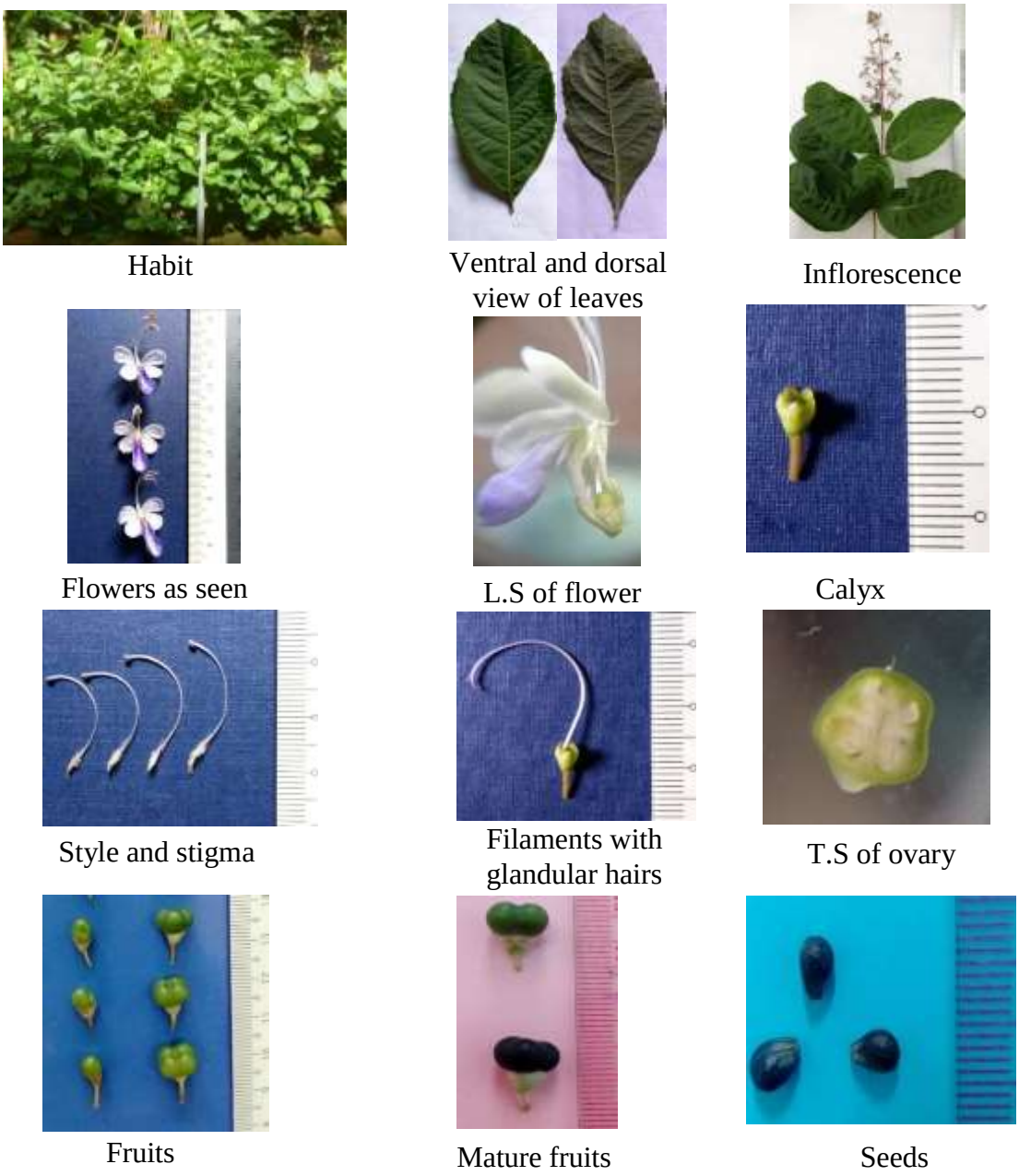


Figure 1 Morphological characters of *Rotheca serrata* (L.) Steane & Mabb.

Histological characters of *Rotheca serrata* (L.) Steane & Mabb.

Lamina

In surface view, stomata were present on both surfaces but abundant on the lower surfaces. They were diacytic type, elliptic shape in outline.

In transverse section, both the upper and lower epidermis is one layer thick and covered with thin cuticle. The epidermal cells are parenchymatous, barrel shaped and compactly arranged. In mesophyll cells are composed of palisade and spongy mesophyll cells. The palisade mesophyll cells were made up of 2 to 3 layers of vertically elongated cylindrical cells which are closely packed with one another. The spongy mesophyll layers are composed of 4 to 5 layers of parenchymatous

cells, which are irregular to oval in shaped and loosely arranged. Abundant calcium oxalate crystals are found in this region. The vascular bundles were embedded in mesophylls cells.

Midrib

In surface view, the epidermal cells are thin-walled parenchymatous and rectangular to polygonal in shape, elongated along the length of the midrib. Anticlinal walls are straight.

In transverse section of midrib, the apical portion was convex on both surfaces; the epidermis was covered with smooth cuticle. The upper epidermal cells were barrel shaped and lower epidermal cells were oval shaped, compactly arranged. The cortex was made up of two different types of cells, lamellar collenchymatous cells and thin walled parenchymatous cells. The lamellar collenchymatous cells are 3 to 4 layers' thickness towards the upper surfaces and 1 to 2 layers in thickness towards the lower surfaces. The parenchyma cells were absent towards the upper surfaces and 2 to 3 layers in thickness toward the lower surfaces. The vascular bundles are in the form of ring and collateral type. In apical regions of the leaf only one vascular bundle is left surrounded by prominent bundle sheath.

Petiole

In surface view, the epidermal cells are thin walled and rectangular to polygonal in shape with straight wall.

In transverse section, the petiole was slightly convex on the upper side and prominently rounded on the lower side. The cuticle layer was thin. The epidermal cells were barrel shaped and compactly arranged. The cortex was made up of two different types of tissue. The lamellar collenchymatous cells 3 to 4 layers and parenchymatous cells 4 to 5 layers towards the upper region and 6 to 8 layers towards the lower region and rounded to polygonal, thin walled parenchymatous cells 6 to 15 layers in thickness at the center. Acicular crystals (raphides and styloid) were scattered in the parenchymatous cells. The vascular bundles were circular in arrangement, bundles were collateral and open type.

Stem

In surface view, the epidermal cells were rectangular to polygonal-shape parenchymatous cells, thin walled, compactly arranged, anticlinal walls straight. Glandular and non-glandular uniseriate trichomes were present.

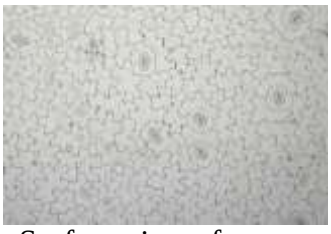
In transverse section, the young stem was quadrangular in outline. The cuticle layer was thin. The epidermal cells were thin walled, barrel shaped parenchymatous cells and one layer thick. The cortex region was made up of collenchymatous tissue and parenchymatous tissue. The collenchymatous tissues were lamellar types and consisted of 3 to 5 layers in thickness below the epidermis. The parenchymatous tissue consists of 4 to 5 layers, thin-walled, isodiametric to rounded in shape, beneath the collenchymatous tissues and the vascular bundles were surrounded the pith region, which composed of thin-walled parenchymatous cells. The vascular bundles were collateral and open type.

Root

In surface view, the epidermal cells were thin walled, rectangular in shape and compactly arranged

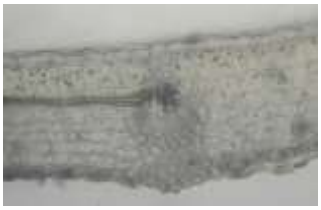
In transverse section, the roots were circular in outline. The epidermal cells were disorganized and displaced by periderm which consists of phloem or cork, the phellogen or cork cambium and phelloderm or secondary cortex. Phellem or cork cells consist of 1-2 layered,

irregular to round in shape. Phellogen or cork cambium was 2-3 layered and polygonal to round in shaped. Phelloderm or secondary cortex made up of 6-10 layered thickened, the parenchymatous cells oval to rounded, secretory ducts between them. Cortex, endodermis, pericycles and vascular bundle were clearly differentiated. Endodermis was a single layered, thin-walled parenchymatous cells and barrel shaped. Pericycle was lying internal to the endodermis thin-walled parenchymatous cells and barrel shaped. The vascular bundles were arranged in concentric ring. Xylem towards the inner and phloem outside the xylem. The xylem is endarch.



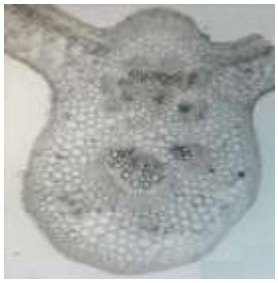
Surface view of upper epidermis (X 200)

Surface view of lower epidermis (X 400)



T.S of lamina (X 100)

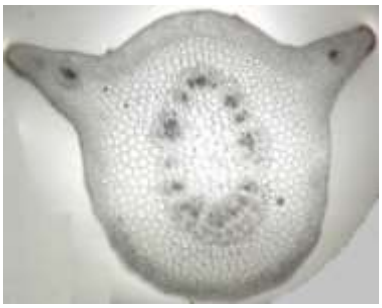
Surface view of midrib (X 400)



T.S of midrib (X 40)

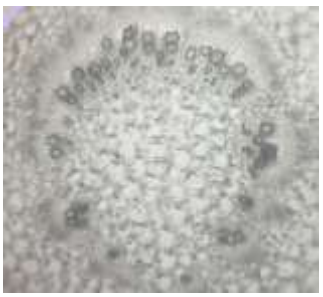
Closed up view of cortical layer and vascular bundle of midrib(X 200)

Surface view of petiole (X 400)



T.S of petiole (X 40)

Closed up view of cortical layer of prtiole (X 400)



Closed up view of vascular bundle and crystals of petiole (X 200)

Surface view of stem (X 400)

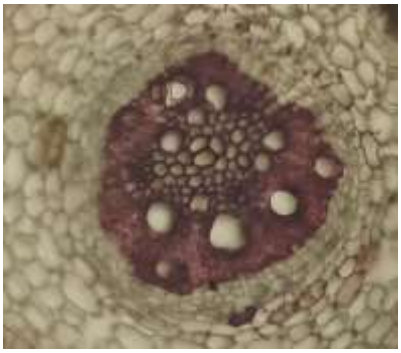
T.S of stem (X 40)

Closed up view of cortex layer, vascular bundle and crystals of stem (X 200)

Surface view of root (X 400)

T.S of the root (X 40)

Closed up view of cortex region and vascular bundle (X 200)



Closed up view of vascular bundle (X 200)

Figure 2 Microscopical characters of *Rotheca serrata* (L.) Steane & Mabb.

Preliminary phytochemical test of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

The phytochemical tests of powdered leaves of *Clerodendrum serratum* (L.) Moon indicated that the presence of alkaloid, flavonoids, phenolic compound, starch, reducing sugar, glycoside, saponins, tannins, α-amino acid and carbohydrates. The results were shown in Table (1).

Table 1 Phytochemical test of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

No.	Test	Extracts	Test reagents	Observation	Results
1.	Alkaloid	1% HCL	Dragendorff's Mayer's Wagner's Hager's	Orange ppt White ppt Brown solution Yellow ppt	+ + + +
2.	Flavonoid	EtOH	HCL / Mg	Pink	+
3.	Phenolic compound	H_2O	5% $FeCl_3$ solution	Brownish ppt	+
4.	Starch	H_2O	10% Iodine solution	Bluish black	+
5.	Reducing sugar	H_2O	Benedict solution	Orangr red	+
6.	Glycoside	H_2O	10% Lead acetate	White ppt	+
7.	Saponin	H_2O	Distilled water	Frothing	+
8.	Tannins	H_2O	5% $FeCl_3$	Brownish black ppt	+
9.	α-amino acid	H_2O	Ninhydrin reagent	Purple	+
10.	Carbohydrates	H_2O	10% α-napthol and concentrated H_2SO_4	Red ring	+

Present = (+)

Physicochemical properties of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

In physicochemical properties, the moisture content was usually determined by drying to constant weight and taking the loss in weight as moisture. The solubility of powdered leave was investigated to determine the amount of total solids soluble in various solvents. The solubility of powdered leaves was found to be mostly soluble in distilled water, moderately soluble in ethanol soluble and methanol soluble, least soluble in petroleum ether. The results were shown in Table (2).

Table 2 Physicochemical properties of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

No.	Physicochemical properties	Content (%)
1	Moisture content	16.6
2	Total ash	14.6
3	Distilled water soluble content	6.67
4	Acetone soluble content	3.3
5	Chloroform soluble content	3.3
6	Ethanol soluble content	5.0
7	Ethyl-acetate soluble content	2.67
8	Methanol soluble content	5.3
9	Petroleum ether soluble content	2.0

Discussion

In this research, the morphological studies on vegetative and reproductive parts, histological characters as well as phytochemical, physicochemical properties the leaves have been studied and described.

The plant of *Rotheca serrata* (L.) Steane & Mabb. are perennial shrubs, bluntly quadrangular. The leaves are simple, opposite and decussate, apically clustered, sharply serrate margins and glabrous. The inflorescences are both axillary and terminal cymes, solitary flower, in lax pubescent, dichotomous cymes with a pair of acute bracts at each branching **(**Kirtikar and Basu, 1935; Backer and Brink, 1965).

The flowers of these plants are pale blue, showy, bisexual, irregular, zygomorphic and hypogynous (Backer and Brink, 1965; Dassanayake, 1983; Hu Qi-ming and WU De-Lin, 2009).

The calyx is (5), synsepalous. The corolla consists of (1+4) petals, synpetalous, the lower larger lobed, bluish purple, hairy within the tube, inferior. The ovary is superior, each with two ovules, axile placentation, disc present. The fruits are broadly obovoid, succulent, dark green in immature, black in matures, glabrous. The seeds are dark purple. These characters are in agreement with those reported by (Hooker, 1885; Kirtikar and Basu, 1935; Backer and Brink, 1965; Dassanayake, 1983; Hu Qi-ming and WU De-Lin, 2009).

In histological studies, the surface views of upper and lower epidermis of the leaves have the epidermal cells with wavy anticlinal walls. The stomata are distributed on both surfaces of the leave and diacytic type. Calcium oxalate crystals are found in mesophyll cells. The vascular bundles were embedded in mesophylls cells.

In transverse section of petiole, the epidermal cells were barrel shaped. The cortex was made up of lamellar collenchymatous and polygonal thin walled parenchymatous cells. Acicular crystals (raphides and styloid) were scattered in the parenchymatous cells. The vascular bundles were collateral and open type. Patches of phloem fibers surrounded the collateral vascular bundles.

In transverse section of stem, the young stem was quadrangular in outline. The vascular bundles were collateral and open type. Acicular crystals were found in the parenchymatous cells.

In transverse section of roots were circular in outline. The epidermal cells were disorganized and displaced by periderm. The vascular bundles were arranged in concentric ring. The xylem is endarch. The histological characters of leaves; petiole, stem and root are in agreement with Esau, 1965; Pandey, 1978; Metcalfe and Chalk, 1979 and 1989; Trease and Evans, 2002.

According to the result of phytochemical studies, chemical constituents such as alkaloid, flavonoid, phenolic compound, starch, reducing sugar, glycoside, saponin, tannins, α-amino acid and carbohydrates were isolated from the leaves of *Rotheca serrata* (L.) Steane & Mabb. These researches were agreed with those reported by Prasad, 2012. The main properties of flavonoid include antioxidant activity (Belinha *et al*., 2007).

In physicochemical studies, showed that the powdered leaves were mostly soluble in polar solvent. These solubility properties are considered for the preparation of crude drugs in British pharmacopoeia (1968).

Conclusion

Rotheca serrata (L.) Steane & Mabb. has played an important role in Indian system of medicine. In addition to the common local use in respiratory diseases, other ethnomedicinal uses include treatment of pain, inflammation, rheumatism and fever especially malarial fever.

Therefore, the present research deals to provide a comprehensive overview of the traditional and ethnomedicinal uses, phytochemistry of *Rotheca serrata* (L.) Steane & Mabb. So, this plant should be investigation of effective pharmacological research in the coming future.

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EFFECT OF CARBON AND NITROGEN SOURCES ON ANTIBACTERIAL METABOLITE PRODUCTION BY ENDOPHYTIC FUNGUS *ASPERGILLUS CANDIDUS* AGAINST *AGROBACTERIUM TUMEFACIENS*

Khin Thida Swe[1], Yin Yin Mya[2]

Abstract

In present study, endophytic fungus was isolated from the petiole of *Vigna mungo* (L.) Hepper in Thae Phyu Village, Hinthada Township, Ayeyawady Region during June in 2018. The endophytic fungus was isolated by surface sterilization method. The effect of various carbon and nitrogen sources were observed for the colony characters, growth condition and maximum antibacterial metabolite production of isolated fungus. The isolated fungus was identified as *Aspergillus candidus* based on morphological-microscopical characters and references key. The antibacterial activities of *Aspergillus candidus* were studied by agar well diffusion assay method. The result showed that maximum production of antibacterial metabolite (20.69 mm) was obtained when medium was supplemented with rice powder as carbon source. Fermentation medium containing yeast extract as nitrogen source showed the highest antibacterial metabolite (22.80 mm) production. This study reveals that endophytic fungus serves as a potential source for the production of an effective compound.

Keywords: Endophytes, *Aspergillus candidus*, Antibacterial activities

Introduction

Plants are a tremendous source for the discovery of new products of medicinal value for drug development. Today several distinct chemicals derived from plants are important drugs currently used in one or more countries in the world. The evolving commercial importance of secondary metabolites has in recent years resulted in a great interest in secondary metabolism, particularly in the possibility of altering the production of bioactive plant metabolites (Alexopoulos *et al*., 1996).

Carbon is the most important medium component as it is an energy source for the microorganisms and plays an important role in the growth as well as in the production of primary and secondary metabolite. The rate at which the carbon source is metabolized can often influence the formation of biomass and /or the production of primary or secondary metabolites. Like carbon, the selection of nitrogen source and its concentration in the medium

also play a crucial role in metabolite production. The microorganism can utilize both inorganic and/or organic sources of nitrogen. Use of specific amino acids can increase the productivity in some cases and conversely, unsuitable amino acids may inhibit the synthesis of secondary metabolites (Marwick *et al*., 1999).

For medium formulation, carbon and nitrogen sources showing enhancement effect on the desired metabolite production in supplementation experiments are generally tried to be used as a whole carbon and nitrogen source (Niwas *et al*., 2013).

Given this in view, nutrients type and their concentrations in the medium play an important role in commencing the production of primary and secondary metabolites as limited supply of an essential nutrient can restrict the growth of microbial cells or product formation. Generally, carbon

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and nitrogen sources present in the medium can influence the metabolite production (Singh *et al*., 2017).

In present study, the influence of different carbon and nitrogen sources on the colony morphology, growth conditions and antibacterial metabolite production by *Aspergillus candidus*. The aim and objectives of the study are to take a comprehensive look at most recent research on antibacterial metabolite produced by endophytic fungus *Aspergillus candidus.*

Materials and Methods

Collection of plant specimen

Vigna mungo (L.) Hepper plant sample was collected at Thae Phyu Village, Hinthada Township, Ayeyawady Region during June in 2018. Healthy plant sample was placed in plastic bags and labelled with date and site of collection until isolation procedure was completed. The identification of these plant was referred by Dassanayake, 1991; Wu *et al*., 2010 and Heuze´ *et al*., 2016.

Isolation of antibiotic producing fungus

The endophytic fungus was isolated from petiole of *Vigna mungo* (L.) Hepper by using surface sterilization method (NITE, 2004). Plant sample was washed thoroughly in running tap water and air dried before it was processed. The material was then surface sterilized by immersing them sequentially in 70% ethanol for 1 minute and then, also immerse 10% sodium hypochloride for 1 minute and rinsed thoroughly with sterile distilled water. Then, with a sterile scalpel, outer tissues were removed and the inner tissues of 0.5 cm size were carefully dissected and placed on petri-disc containing Czapek–Doz Agar (CZA) medium. The medium was supplemented with chloramphenicol to suppress bacterial growth. The Petri-disc was incubated at room temperature for three to seven days until fungal growth appeared.

Identification of antibiotic producing fungus

The morphological and microscopical characters of antibiotic producing fungus was observed by the methods of Ando and Inaba 2004, Ando 2006, Domsch *et.al.*, 1993 and Watanabe 2002 and 2010. Microscopical characters were studied by microscope (Olympus; Sato Shouji Microscope Attached Camera). Comparison of these characters with reference keys was undertaken to identify according to Ando and Inaba, 2004;Ando, 2006; Domsch *et al.*, 1993; Watanabe 2002 and 2010.

Carbon sources (each 1.0 g) such as rice powder, potato, tapioca powder, fructose, glucose, sucrose, maltose, oat, corn, lactose, glycerol, soluble starch, carrot were used with agar 1.8 g and DW 100 mL for studying the colony characters and growth conditions of selected fungus.

Nitrogen sources (each 1.0g) such as yeast extract, poly peptone, meat extract, peptone, malt extract, casein, peanut cake, soybean, gelatin, fish cake, potassium nitrate, ammonium sulphate and ammonium chloride were used with agar 1.8 g and DW 100 mL for studying the colony characters and growth conditions of selected fungus.

Test bacterial strain

The bacterial strain such as *Agrobacterium tumefaciens* (NITE 09678) was received from B.R.B.D.C (Biological Resources and Biotechnology Development Centre, Pathein University).

Screening for antibacterial activities (NITE, 2005)

The isolated fungus was grown on CZA medium at room temperature for 4 days. After incubation period, these fungus inoculated into the seed medium (glucose 2.5g, yeast extract 0.8g, $MgSO_4 7H_2O$ 0.01g, K_2HPO_4 0.01 g and DW 100 mL at pH 6.5) for 3 days at room temperature. After three days, the seed medium (2%) was transferred into the basal fermentation medium (glucose 1.0 g, yeast extract 0.5 g, Soluble starch 0.3 g, $MgSO_4 7H_2O$ 0.02 g, K_2HPO_4 0.01 g and DW 100 mL at pH 6.5) and carried out for 4 - 10 days and evaluated the antibacterial activities by agar well diffusion method.

Screening of antibacterial activities by agar well method (Collins,1965)

The antibacterial activities of *Aspergillus candidus* isolate was tested by using agar well diffusion method. Two days old culture test broth (0.1 mL) was added to 25 mL of assay medium and thoroughly mixed and poured into plate. After solidification, a well of about 8 mm with sterile cork borer was aseptically punched on each agar plate. The fermentation broth of *Aspergillus candidus* (20 µL) was carefully added into the wells and incubated at room temperature for 24 - 48 hours. The diameter of the zones of inhibition around each well was measured and recorded after 24 - 48 hours incubation.

Effect of different carbon utilization on fermentation

To determine the effect of carbon sources on antibacterial production in *Aspergillus candidus,* different carbon sources such as rice powder, potato, tapioca powder, fructose, glucose, sucrose, maltose, oat, corn, lactose, glycerol, soluble starch and carrot were employed. 100 mL of basal fermentation broth medium with different carbon sources (1%) in 250 mL conical flask that autoclaved at 121 ºC for 45 minutes. The inoculated flasks were incubated at room temperature for 4 to10 days under stationary condition. The fermented broth was tested by using agar well diffusion method for antibacterial activities against *Agro. tumefaciens*.

Effect of different nitrogen utilization on fermentation

The optimization of nitrogen source used in fermentation broth during antibacterial metabolite production was carried out by employing various nitrogen sources such as yeast extract, poly peptone, meat extract, peptone, malt extract, casein, peanut cake, soybean, gelatin, fish cake, potassium nitrate, ammonium sulphate and ammonium chloride. 100 mL of basal fermentation broth medium with different nitrogen sources (1 %) in 250 mL conical flask that autoclaved at 121 ºC for 45 minutes. The inoculated flasks were incubated at room temperature for 4 to10 days under stationary condition. The fermented broth was tested by using agar well diffusion method for antibacterial activities against *Agro. tumefaciens.*

Results

Collection of plant specimen

In present endeavor, plant sample of the *Vigna mungo* (L.) Hepper was collected from Thae Phyu Village (Hinthada Township), Ayeyawady Region.

Isolation of antibiotic producing fungus

In this research work, endophytic fungus was isolated from petiole of *Vigna mungo* (L.) Hepper. The endophytic fungus was isolated by surface sterilization method. The surface view is pale yellow in the center and edge white colour and reverse view is pale orange colour Figure 1.

Surface view Reverse view

Figure1 Colony morphology of isolated fungus on Czapek–Doz Agar (CZA) medium

Carbon sources utilization for growth

The effect of different carbon sources on morphological growth by isolated fungus is presented in figure 2. The results obtained in carbon sources study indicated that rice powder, potato, oat and carrot were excellent growth and tapioca powder was moderate growth and then fructose, glucose, sucrose, maltose, corn, lactose, glycerol and soluble starch showed poor growth Figure 2 and Table 1.

Culture on Rice powder Culture on Potato Culture on Tapioca powder

Culture on Fructose Culture on Glucose Culture on Sucrose

Culture on Maltose Culture on Oat Culture on Corn

Culture on Lactose Culture on Glycerol Culture on Soluble Starch Culture on Carrot

Figure 2 Morphological growth of isolated fungus on different carbon sources

Table 1 Growth of isolated fungus on different carbon sources

Sr. No.	Carbon sources	Growth
1.	Rice powder	6.1 cm (Excellent)
2.	Potato	6.2 cm (Excellent)
3.	Tapioca powder	3.6 cm (Moderate)
4.	Fructose	1.5 cm (Poor)
5.	Glucose	1.2 cm (Poor)
6.	Sucrose	1.0 cm (Poor)
7.	Maltose	2.0 cm (Poor)
8.	Oat	7.0 cm (Excellent)
9.	Corn	1.5 cm (Poor)
10.	Lactose	1.9 cm (Poor)
11.	Glycerol	1.0 cm (Poor)
12.	Soluble Starch	1.0 cm (Poor)
13.	Carrot	8.6 cm (Excellent)

1.0 to 3.0 cm - Poor 3.1 to 6.0 cm - Moderate 6.1 cm above – Excellent

Nitrogen sources utilization for growth

In the study on the utilization of nitrogen sources, moderate growth on yeast extract, poly peptone, meat extract, peptone, casein, peanut cake, soy bean, gelatin, fish cake and potassium nitrate; malt extract, ammonia sulphate and ammonia chloride indicated poor growth Figure 3 and Table 2.

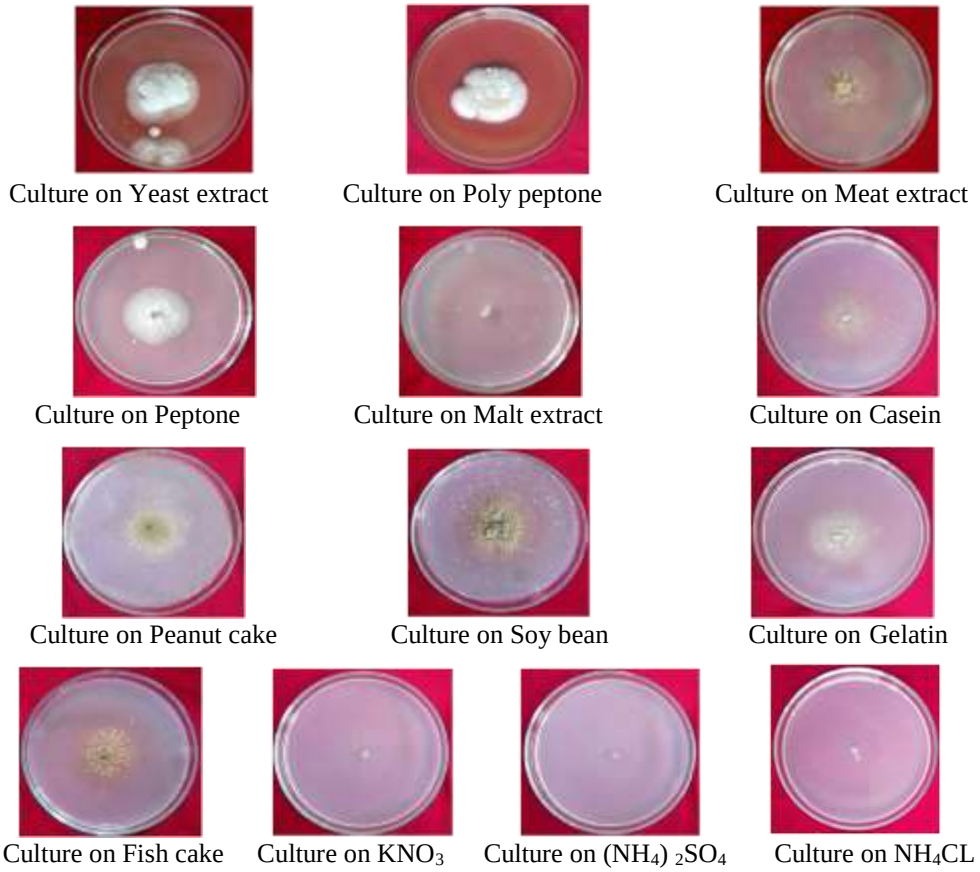

Figure 3 Morphological growth of isolated fungus on different nitrogen sources

Table 2 Growth of isolated fungus on different nitrogen sources

Sr. No.	Nitrogen sources	Growth
1.	Yeast extract	4.2 cm (Moderate)
2.	Poly peptone	4.3 cm (Moderate)
3.	Meat extract	4.1 cm (Moderate)
4.	Peptone	3.7 cm (Moderate)
5.	Malt extract	1.2 cm (Poor)
6.	Casein	3.4 cm (Moderate)
7.	Peanut cake	3.5 cm (Moderate)
8.	Soy bean	4.9 cm (Moderate)
9.	Gelatin	3.4 cm (Moderate)
10.	Fish cake	4.2 cm (Moderate)
11.	KNO_3	3.5 cm (Moderate)
12.	$(NH_4)_2SO_4$	1.2 cm (Poor)
13.	NH_4CL	1.0 cm (Poor)

1.0 to 3.0 cm - Poor 3.1 to 6.0 cm - Moderate 6.1 cm above – Excellent

Identification of antibiotic producing fungus

The colony morphologies of endophytic fungus from petiole of *Vigna mungo* (L.) Hepper, macroscopic and microscopic observations were identified by using references keys of Ando and Inaba, 2004; Ando, 2006; Domsch *et al*., 1993; Watanabe, 2002 and 2010. The isolated fungus was identified as *Aspergillus candidus* using colony morphology, colour of colony and the sporulating structure Figure 4.

Figure 4 Macroscopic and microscopic characters of isolated fungus

Effect of different carbon utilization on fermentation

Rice powder as a carbon sources resulted in the maximum antibacterial activities showed the highest inhibition zone reached (20.69 mm), followed by potato (20.35 mm), tapioca powder (19.75 mm), fructose (19.52 mm), glucose (19.05 mm), sucrose (19.04 mm), maltose (18.84 mm), oat (18.62 mm), corn (17.72 mm), lactose (17.36 mm), glycerol (16.66 mm), soluble starch (16.03 mm) and carrot (16.02 mm) Figure 5 and Table 3.

Rice powder Potato Tapioca powder

Fructose Glucose Sucrose

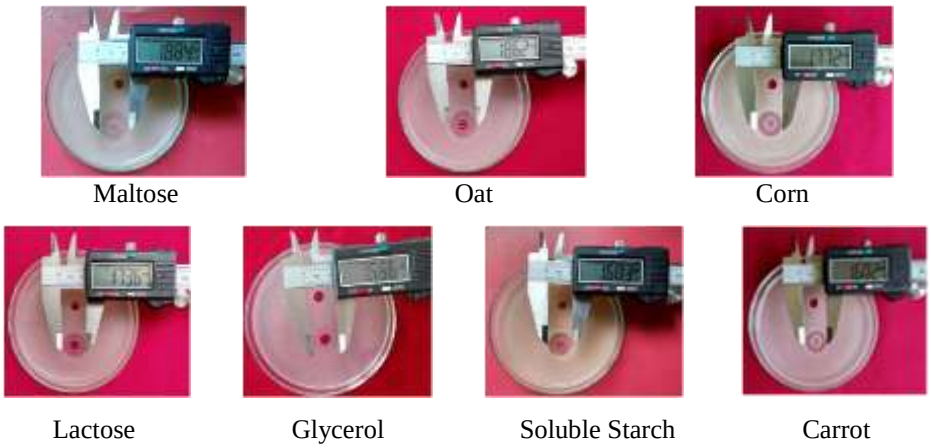

Figure 5 Anitibacterial activities of *Asp.candidus* on different carbon sources fermentation against *Agro.tumefaciens*

Table 3 Anitibacterial activities of *Asp.candidus* on different carbon sources fermentation against *Agro.tumefaciens*

Sr. No.	Carbon sources	Antibacterial Activities Inhibitory zone (mm)
1.	**Rice powder**	**20.69**
2.	Potato	20.35
3.	Tapioca powder	19.75
4.	Fructose	19.52
5.	Glucose	19.05
6.	Sucrose	19.04
7.	Maltose	18.84
8.	Oat	18.62
9.	Corn	17.72
10.	Lactose	17.36
11.	Glycerol	16.66
12.	Soluble Starch	16.03
13.	Carrot	16.02

Effect of different nitrogen utilization on fermentation

Furthermore, in the study of nitrogen sources, maximum antibacterial activities of yeast extract (22.80 mm), poly peptone (21.14 mm), meat extract reached (20.45 mm) followed by peptone (20.09 mm), malt extract (19.08 mm), casein (19.05 mm), peanut cake (18.94 mm), Soy bean (18.88 mm), gelatin (18.75 mm), fish cake (18.94 mm), potassium nitrate (18.74 mm), ammonia sulphate (17.50 mm), ammonia chloride (15.98 mm) were respectively Figure 6 and Table 4.

The maximum production of antibacterial metabolite as nitrogen sources (22.80 mm to 15.98 mm). There was a reduction in antibacterial activities when ammonia sulphate and ammonia chloride were used as nitrogen sources.

Figure 6 Anitibacterial activities of *Asp.candidus* on different nitrogen sources fermentation against *Agro.tumefaciens*

Table 4 Anitibacterial activities of *Asp.candidus* on different nitrogen sources fermentation against *Agro.tumefaciens*

Sr. No.	Nitrogen sources	Antibacterial Activities Inhibitory zone (mm)
1.	Yeast extract	22.80
2.	Poly peptone	21.14
3.	Meat extract	20.45
4.	Peptone	20.09
5.	Malt extract	19.08
6.	Casein	19.05
7.	Peanut cake	18.94
8.	Soy bean	18.88
9.	Gelatin	18.75
10.	Fish cake	18.94
11.	KNO_3	18.74
12.	$(NH_4)_2SO_4$	17.50
13.	NH_4CL	15.98

Discussion and Conclusion

Culture medium components such as carbon and nitrogen sources may effect metabolites production in isolated fungus. Carbon or nitrogen sources which are quickly metabolized will increase the mycelium growth but it potentially inhibits secondary metabolite production (Kumar *et al*., 2012).

In present research work isolated endophytic fungus *Aspergillus candidus* from the *Vigna mungo* (L.) Hepper plant showing considerable antibacterial activity against *Agrobacterium tumefaciens.* The antibacterial spectrum of the *Aspergillus candidus* was tested against *Agrobacterium tumefaciens* through agar well diffusion assay method.

In this study, different carbon and nitrogen sources supplement to the culture broth strongly influenced the growth and biosynthesis of active metabolite by *Aspergillus candidus.* On studying the effect of different carbon source, the results indicated that rice powder affected the antibacterial production by *Aspergillus candidus.* As Moe Moe Aye 2019 reports that rice power is the best other than carbon sources.

Other than the carbon, the source of nitrogen is important for the production of antibiotic substances. Nitrogen is known as one of major component of complex macromolecule within living organism. It is an essential component of amino acid required for biosynthesis of various bioactive compounds. In this study, medium with yeast extract as nitrogen source showed the highest activity followed by poly peptone. S Aung Myo Htay 2017 has reported similar results that promoted the biosynthesis of secondary metabolite.

The results of antibacterial susceptibility tests indicated that maximum antibacterial production was obtained in culture supplemented with rice powder as carbon source and yeast extract and poly peptone as nitrogen source. Studies using one single fungus cultivated under different culture conditions are not only suitable to produce different compounds, but also provide conditions to guide the production of a specific compound and become easier for the purification process. In conclusion, carbon and nitrogen sources play an important role for the production of various bioactive metabolites.

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SPORE- TETRAD AND POLLEN FERTILITY OF TEN *Pisum sativum* L. CULTIVARS

Kyi Kyi Win[1], Soe Soe Hlaing[2], Tin Lay Mar[3], Ah Nge Htwe[4]

Abstract

The studied ten *Pisum sativum* L. cultivars were commercially cultivated all over in various part of North Western Myanmar. They were grown abundantly in Kalay, Kalay-wa Township in Sagaing Region. The spore- tetrad and pollen fertility of ten *Pisum sativum* L. cultivars showed micronuclei per spore tetrad is less than 3 mean micronuclei per spore tetrad. The pollens normality have more than 69 % in all the cultivars and lines. These ten cultivars showed the percentage of normal pollen was greater than abnormal pollen. Thus, to improve their fertility and yield characters, it is essentially needed to understand pollen mother cell cycle that produces pollen and spore- tetrad.

Keywords: *Pisum sativum* L, Ten cultivars, spore- tetrad, pollen

Introduction

Pisum sativum L. was probably originated in South-West Asia, now cultivated in many temperate countires, as a cool-season crop in the subtropics and at higher altitudes in the tropics (Maesen, 1992).The pea is the oldest among of domesticated plants.

Currently the *Pisum sativum* L. is one of the world's most important grain legumes, serving a variety of roles as a source of both human food and animal feed. Its seed is harvested either as the dry mature form or immature state. The precise stage of maturity varying according to the end use. *Pisum sativum* L. is a diploid species 2n=14 which has been in cultivation for food since date back to 3000 years. Thus, it showed the basic chromosome of each set is 7 (Colin, 1956).

The spore- tetrad that has only one large nucleus and has no small nucleus was recorded as normal spore-tetrad. Abnormal spore-tetrad was observed many micronuclei and large normal nucleus. Number of micronuclei per spore-tetrad were also counted and recorded. Normal pollen has one generative nucleus and one vegetative nucleus in normal condition. Abnormal pollen were observed lack one or both tube nucleus and only the generative nucleus. Normal and abnormal pollen were also counted and recorded.

Peas cultuvars that grown in Myanmar shows variation in size, shaped, colour and other morphological forms. This work is tend to investigate the performance of spore-tetrad and pollen fertility.

Material and Methods

Materials

Ten *Pisum sativum* L. cultivars of family Fabaceae namely Pe-kyauk-sane, Kalay-sadaw-pe. Myitkyina-maw-lue, Shwebo-thann-lann, Ta- kaung,Kyun-lone-thae, Kanada, Ta-yoke-phwe-thae, Ta-yoke-phyu and Shwe-pe-thee were collected from Sagaing Township.

Methods

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The samples of pollen mother cells (PMCs) of the sample species were collected from ten collection cultivars. The flower buds start from the very beginning of the appearance till somewhat maturation buds were collected early in the morning round about 6.00 to 8.00 AM. The anther (s) from the collected flower were trace out and they were immediately fixed in freshly prepared 3:1 alcohol: acetic solution (Carnoy's solution), that filled in the brown glass vials were used in order to protect from the light that will give somewhat changes to PMCs. They were stored in the cool and dry place till they were used.

Cytological Analysis

Thirty tetrad cells were examined at the tetrad stage for numbering the number to micronuclei per tetrad cell. Hundred pollen grains for each cultivar were studied for normal and abnormal pollen. This was prepared by using 1% acetocarmine squarsh method and observed under the Olympus light microscope.

Slide preparation

After fixing for three days in the fixative solution, one of the flower bud were selected and place on the glass slide. By the help of needle pointer a pair of forceps, the anther from the flower removed and the remaining parts of the flowers were discarded, one to two drops of acetocarmine barax staining solution was added on the dissected anther. The anther were crushed by using specially prepared silver knife. The undesired materials were then again removed from the glass slide the sample was covered with thin glass cover slip. Using the unique thumb pressure, the cover slip was gently pressed to obtain well spread slide. In this way the slide was ready to observed the PMCs character under the microscope (Beeks, 1955).

Statistical analysis

Spore-tetrad studies were test with student 't' test as by Steel and Torrie (1960). The data of normal and abnormal pollen of 10 cultivars were compared using Chi-square test as stated by Steel and Torrie (1960).

Results

Tetrad Characters

All the cultivars possessed more than 0.87 mean micronuclei per spore-tetrad were recorded. The mean range of 0.87 – 2.2 in the occurrence of micronuclei per spore-tetrad of the ten *Pisum sativum* L. cultivars. They were not significantly differently from one to another but Shwebo-thann-lann possessed the highest mean number of micronuclei 2.200 ± 2.626 , thus cultivar Pe-kyauk-sane has significantly inferior to the remaining cultivars at 1 % and 5 % level respectively (Table 1).

Pollen Characters

Occurrence of normal pollen per cell studied among the 10 cultivars studied ranged from 69 % to 86 % normal but did not exhibited a single significantly superior or inferior number of pollen. Abnormal pollen was also observed non significant in any of the comparisons (Table 1).

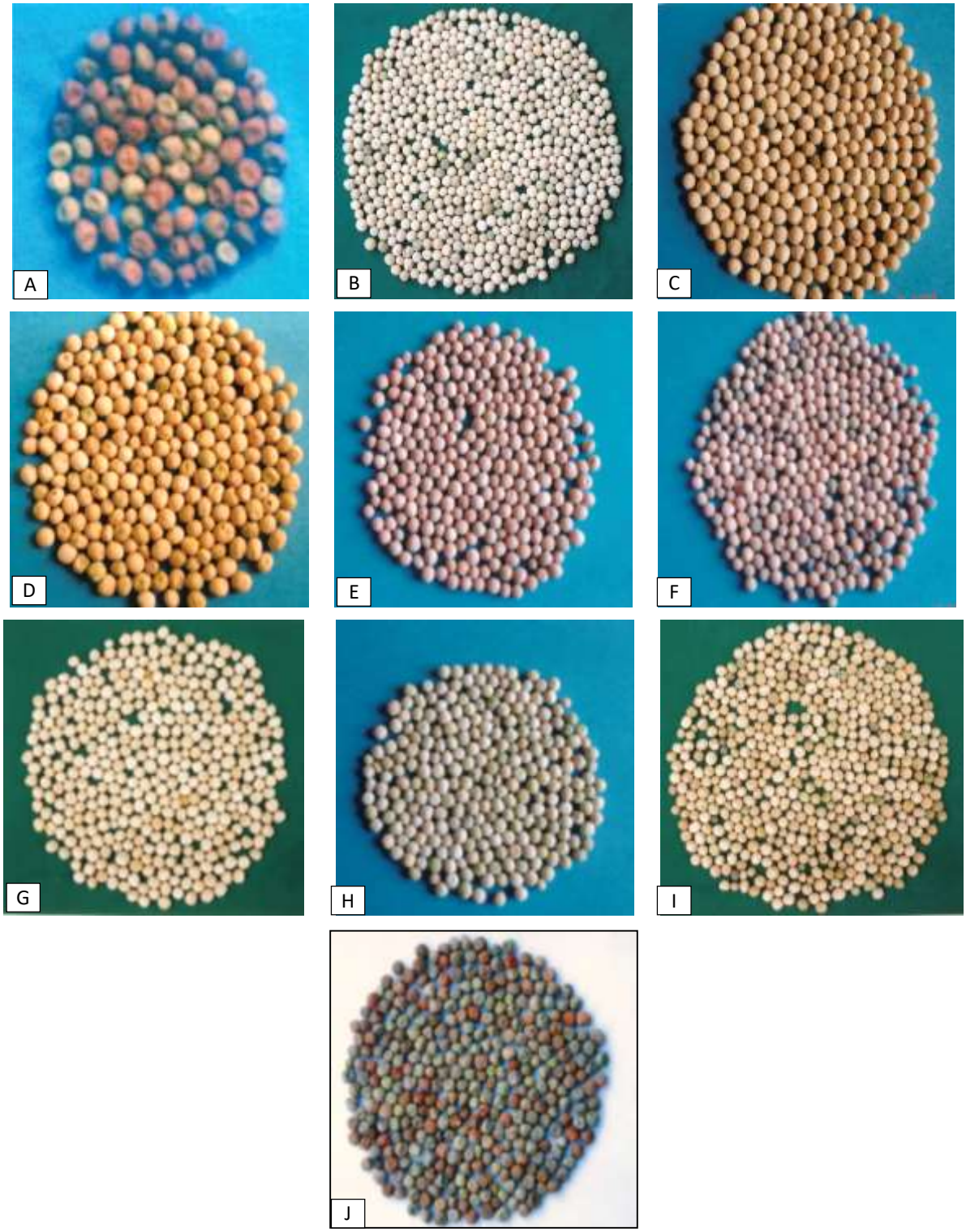

Figure 1 Seed characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane
B. Kalay-sadaw-pe
C. Shwebo-thann-lann
D. Myitkyina-maw-lue
E. Ta-kaung-pe
F. Kyun-lone-thae
G. Kanada
H. Ta-yoke-phwe-thae
I. Ta-yoke-phyu
J. Shwe-pe-thee

Table 1 Comparison on occurrence of micronuclei per spore-tetrad, pollen normality and abnormality of 10 *Pisum sativum* L. cultivars

Identity / Comparison	Micronuclei		normal pollen %	Arc-sine value	abnormal pollen %	Arc-sine value	χ^2 value
	Mean ± S.D	't' value					
Pe-kyauk-sane	0.87 ± 1.454	-1.828^{ns}	80	63.44	20	26.56	0.251^{n-s}
Vs							
Kalay-sadaw-pe	1.77 ± 2.216		74	59.34	26	30.66	
Pe-kyauk-sane	0.87 ± 1.454	-2.386*	80	63.44	20	26.56	0.117$^{n.s}$
Vs							
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Pe-kyauk-sane	0.87 ± 1.454	-2.089*	80	63.44	20	26.56	0.117$^{n.s}$
Vs							
Myitkyina-maw-lue	1.73 ± 1.672		83	65.65	17	24.35	
Pe-kyauk-sane	0.87 ± 1.454	-2.162*	80	63.44	20	26.56	0.251^{ns}
Vs							
Ta-kaung	1.83 ± 1.894		74	59.34	26	30.66	
Pe-kyauk sane	0.87 ± 1.454	- 0.783^{ns}	80	63.44	20	26.56	0.025^{ns}
Vs							
Kyun-thone-thae	1.17 ± 1.462		81	64.16	19	25.84	
Pe-kyauk-sane	0.87 ± 1.454	-2.230*	80	63.44	20	26.56	0.396^{ns}
Vs							
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Pe-kyauk-sane	0.87 ± 1.454	-2.339*	80	63.44	20	26.56	0.291^{ns}
Vs							
Ta-yoke-phyu-thae	1.87 ± 1.784		85	67.21	15	22.79	
Pe-kyauk-sane	0.87 ± 1.454	-1.273^{ns}	80	63.44	20	26.56	0.597^{ns}
Vs							
Ta-yoke-phyu	1.40 ± 1.705		69	56.17	31	33.83	
Pe-kyauk-sane	0.87 ± 1.454	-3.156**	80	63.44	20	26.56	0.248^{ns}
Vs							
Shwe-pe-thee	2.17 ± 1.675		73	58.69	27	31.31	
Kalay-sadaw-pe	1.77 ± 2.216	-0.674^{ns}	74	59.34	26	30.66	0.686^{ns}
Vs							
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Kalay-sadaw-pe	1.77 ± 2.216	0.078^{ns}	74	59.34	26	30.66	0.686^{ns}
Vs							
Myitkyina-maw-lue	1.73 ± 1.672		83	65.65	17	24.35	
Kalay-sadaw-pe	1.77 ± 2.216	-0.111^{ns}	74	59.34	26	30.66	0.004^{ns}
Vs							
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Kalay-sadaw-pe	1.77 ± 2.216	1.217^{ns}	74	59.34	26	30.66	0.414^{ns}
Vs							
Kyun-lone-thae	1.17 ± 1.462		81	64.16	19	25.84	
Kalay-sadaw-pe	1.77 ± 2.216	0.199^{ns}	74	59.34	26	30.66	1.275^{ns}
Vs							
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Kalay-sadaw-pe	1.77 ± 2.216	- 0.139^{ns}	74	59.34	26	30.66	1.220^{ns}
Vs							
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	

Table 1 Continue

Identity / Comparison	Micronuclei		normal pollen %	Arc-sine value	abnormal pollen %	Arc-sine value	χ^2 value
	Mean ± S.D	't' value					
Kalay-sadaw-pe	1.77 ± 2.216		74	59.34	26	30.66	
Vs		0.713^{ns}					0.575^{ns}
Ta-yoke-phyu	1.40 ± 1.705		69	56.17	31	33.83	
Kalay-sadaw-pe	1.77 ± 2.216		74	59.34	26	30.66	
Vs		0.775^{ns}					0.092^{ns}
Shwe-pe-thee	2.17 ± 1.675		73	58.69	27	31.31	
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Vs		-0.775^{ns}					0.005^{ns}
Myitkyina-maw-lue	1.73 ± 1.672		83	65.65	17	24.35	
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Vs		0.615^{ns}					0.482^{ns}
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Vs		1.846^{ns}					0.013^{ns}
Kyun-lone-thae	1.17 ± 1.462		81	64.16	19	25.84	
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Vs		0.935^{ns}					0.139^{ns}
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Vs		0.559^{ns}					0.062^{ns}
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Vs		0.052^{ns}					0.255^{ns}
Ta-yoke-phyu	1.40 ± 1.705		69	56.17	31	33.83	
Shwebo-thann-lann	2.20 ± 2.626		83	65.65	17	24.35	
Vs		0.213^{ns}					0.225^{ns}
Shwe-pe-thee	2.17 ± 1.675		73	58.69	27	31.31	
Myitkyina-maw-lue	1.73 ± 2.892		83	65.65	17	24.35	
Vs		-0.213^{ns}					0.482^{ns}
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Myitkyina-maw-lue	1.73 ± 2.892		83	65.65	17	24.35	
Vs		1.358^{ns}					0.013^{ns}
Kyun-thone-thae	1.17 ± 1.462		81	64.16	19	25.84	
Myitkyina-maw-lue	1.73 ± 2.892		83	65.65	17	24.35	
Vs		0.141^{ns}					0.139^{ns}
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Myitkyina-maw-lue	1.73 ± 2.892		83	65.65	17	24.35	
Vs		-0.308^{ns}					0.062^{ns}
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	
Myitkyina-maw-lue	1.73 ± 2.892		83	65.65	17	24.35	
Vs		0.744^{ns}					1.124^{ns}
Ta-yoke phyu	1.40 ± 1.705		69	56.17	31	33.83	
Myitkyina-maw-lue	1.73 ± 2.892		83	65.65	17	24.35	
Vs		-1.001^{ns}					0.225^{ns}
Shwe-pe-thee	2.17 ± 1.675		73	58.69	27	31.31	

Table 1 Continue

Identity / Comparison	Micronuclei		normal pollen %	Arc-sine value	abnormal pollen %	Arc-sine value	χ^2 value
	Mean ± S.D	't' value					
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Vs		1.483^{ns}					0.414^{ns}
Kyun-lone-thae	1.17 ± 1.462		81	64.16	19	25.84	
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Vs		0.351^{ns}					1.275^{ns}
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Vs		-0.083^{ns}					1.220^{ns}
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Vs		0.908^{ns}					0.092^{ns}
Ta-yoke-phyu	1.4 ± 1.705		69	56.21	31	33.83	
Ta-kaung	1.83 ± 1.899		74	59.34	26	30.66	
Vs		-0.723^{ns}					0.574^{ns}
Shwe-pe-thee	2.17 ± 1.675		73	56.17	27	31.31	
Kyun-lone-thae	1.17 ± 1.462		81	64.14	19	25.86	
Vs		-1.259^{ns}					0.309^{ns}
Kanada	1.67 ± 1.556		86	58.69	14	21.97	
Kyun-lone-thae	1.17 ± 1.462		81	64.14	19	25.86	
Vs		-1.634^{ns}					0.203^{ns}
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	
Kyun-lone-thae	1.17 ± 1.462		81	64.14	19	25.86	
Vs		-0.551^{ns}					0.782^{ns}
Ta-yoke-phyu	1.40 ± 1.705		85	56.17	31	33.83	
Kyun-lone-thae	1.17 ± 1.462		81	64.14	19	25.86	
Vs		-2.422*					0.344^{ns}
Shwe-pe-thae	2.17 ± 1.675		73	58.69	27	31.31	
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Vs		-0.455^{ns}					0.003^{ns}
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Vs		0.629^{ns}					1.844^{ns}
Ta-yoke-phyu	1.40 ± 1.705		69	56.17	31	33.83	
Kanada	1.67 ± 1.556		86	68.03	14	21.97	
Vs		-1.178^{ns}					1.143^{ns}
Shwe-pe-thee	2.17 ± 1.675		73	58.69	27	31.31	
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	
Vs		-1.026^{ns}					1.573^{ns}
Ta-yoke-phyu	1.40 ± 1.705		69	56.17	31	33.83	
Ta-yoke-phwe-thae	1.87 ± 1.784		85	67.21	15	22.79	
Vs		-0.660^{ns}					0.931^{ns}
Shwe-pe-thee	2.17 ± 1.675		73	58.69	27	31.31	
Ta-yoke-phyu	1.40 ± 705		69	56.17	31	33.83	
Vs		-1.735^{ns}					0.108^{ns}
Shwe-pe-thae	2.17 ± 1.675		73	58.69	27	31.31	

n.s = non-significant S.D = Standard deviation

*,** = statistically significant at 5% and 1% level respectively

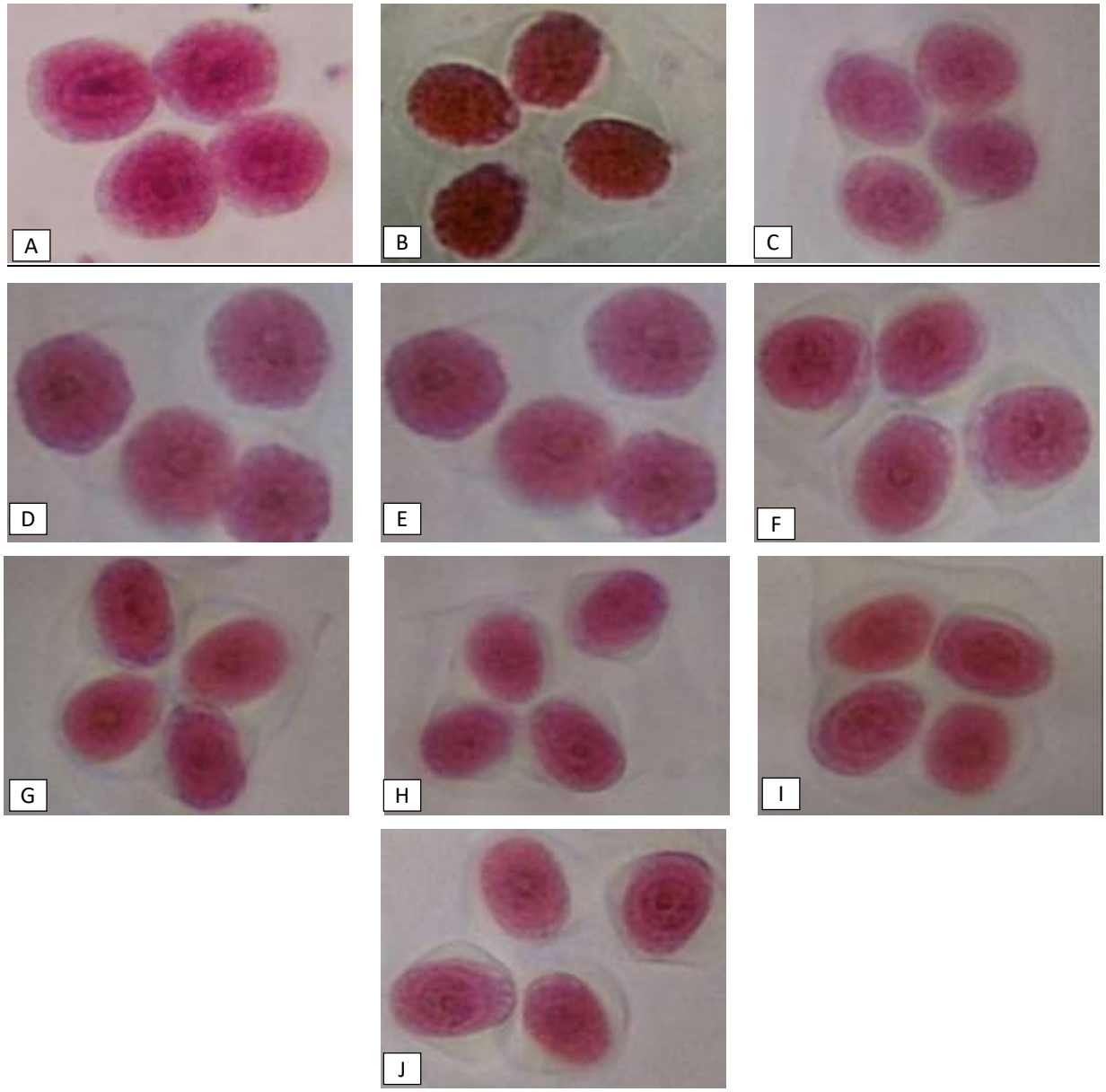

Figure 2 Spore-tetrad characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane	B. Kalay-sadaw-pe	C. Shwebo-thann-lann
D. Myitkyina-maw-lue	E. Ta-kaung	F. Kyun-lone-thae
G. Kanada	H. Ta-yoke-phwe-thae	I. Ta-yoke-phyu
J. Shwe-pe-thee		

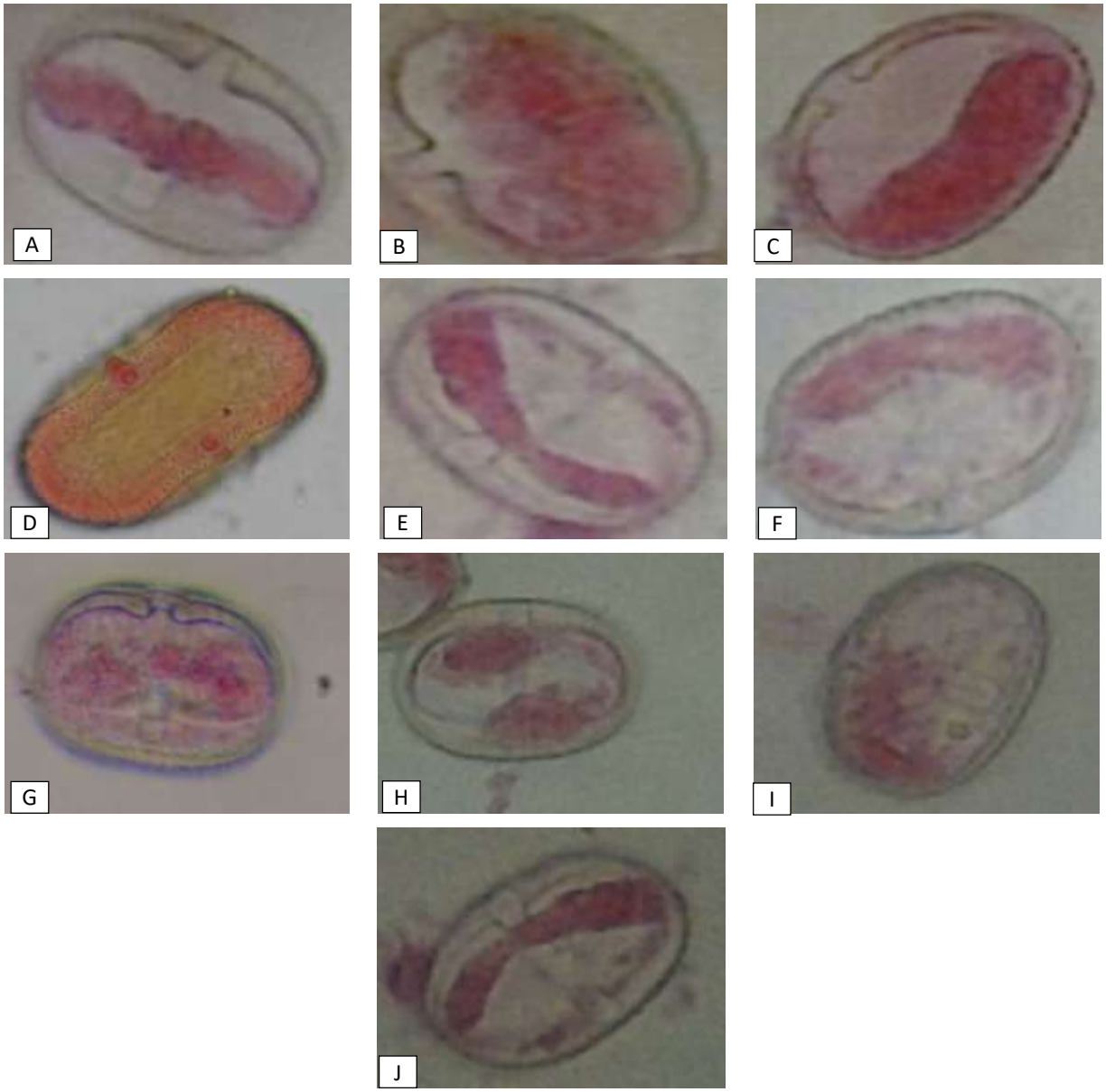

Figure 3 Normal pollen characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane B. Kalay-sadaw-pe C. Shwebo-thann-lann
D. Myitkyina-maw-lue E. Ta-kaung F. Kyun-lone-thae
G. Kanada H. Ta-yoke-phwe-thae I. Ta-yoke-phyu
J. Shwe-pe-thee

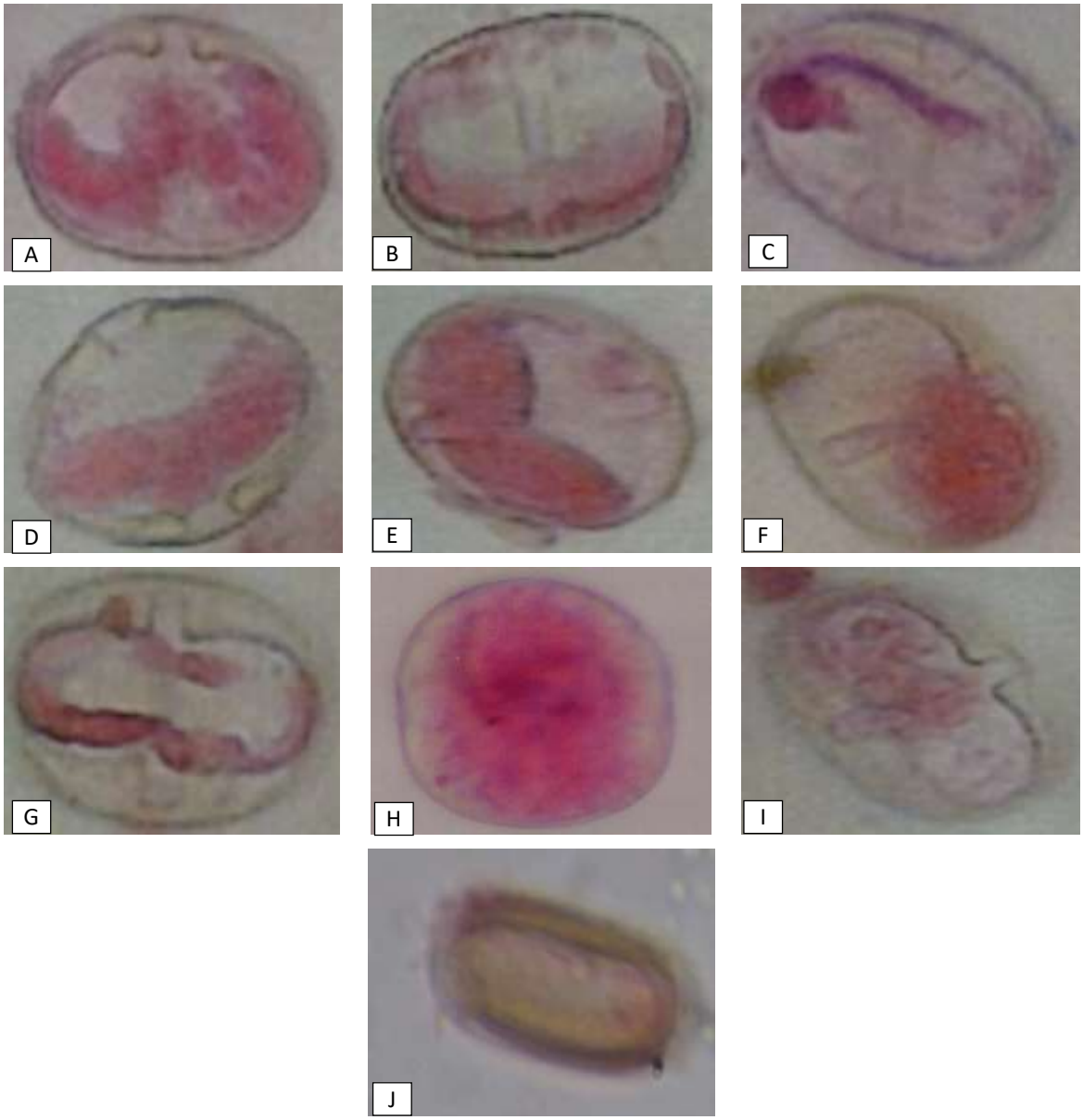

Figure 4 Abnormal pollen characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane B. Kalay-sadaw-pe C. Shwebo-thann-lann
D. Myitkyina-maw-lue E. Ta-kaung F. Kyun-lone-thae
G. Kanada H. Ta-yoke-phwe-thae I. Ta-yoke-phyu
J. Shwe-pe-thee

Discussion and Conclusion

As peas are diploid and self- fertilizing, the breeding strategies adapted to improve the crop have been those conventionally adapted for such species. The extent of natural out crossing has been estimated to be less than 1%. Hybridization among cultivars or between cultivars, land races and primitive forms followed by pedigree, bulk or backcross methods of selection, has been traditionally used; more recently, single seed descent methods have also been evaluated (Bown, 1992) as three generations can be generated each year (Snoad, 1980).

In the present study, spore-tetrad formation and pollen fertility were observed and recorded.

Hockett (1984) stated that different genes for male sterility are responsible for different of pollen abortion that give rise low fertility and yield.

In the present study, it was observed that chance of the cccurrence of micronuclei per spore-tetrad is very low, only one cultivar i.e., Shwebo-thann-lann have more than 2.2 micronuclei in mean number, while the remaining cultivars were less than that. Pollen mother cells (PMCs) of each of the ten *Pisum sativum* L. cultivars were also collected from ten collections sites for spore-tetrad and pollen fertility study. Micronuclei per spore-tetrad, normal and abnormal pollen were also varied from cultivar to cultivar. These differences were also analysed by the help of acetocarmine squash method.

In the present study, among the 10 cultivars studied ranged from 69 % to 86 % normal but did not exhibited a single significantly superior or inferior number of pollen. Abnormal pollen was also observed non significant in any of the comparisons These results showed that the studied cultivars may be possessed genetic stability and highest pollen fertility. Thus, these cultivars and lines are good to conserved for long pea cultivation.

Acknowledgements

I would like to express my hearfelt thanks to Dr. Thar Htun Maung, Rector of Kalay University of giving permission and encouragement. I am very thankful to Dr. Khin Thida Soe, Professor and Head, Department of Botany, Kalay university for her various help and suggestion throughout this work.

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MORPHOLOGICAL, ANATOMICAL CHARACTERS, PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITIES OF *CROTALARIA JUNCEA* L.

Moe Moe Lwin[1]

Abstract

In the present study, *Crotalaria juncea* L. belongs to the family Fabaceae which is grown in Kyaing Tong University Campus. In the present study, morphological characters, anatomical characters, physicochemical properties and antimicrobial activity were carried out. In morphological characters, erect, simple or branched, appressed hairy, annual herbs, stem conspicuously striated, leaves oblong to oblanceolate. Flowers terminal racemes, bracts and bracteoles linear, corolla golden yellow; pods cylindrical, brown silky. In anatomical characters, the stomata anisocytic types which abundant on both surfaces and unicellular, uniseriate trichomes abundant on lower surface. Leaves and stem shows non-glandular, unicellular and uniseriate trichomes. In physicochemical properties, the aqueous extracts showed more soluble than other solvents. In antimicrobial activities, results revealed that *Crotalaria juncea* L. possess significant antimicrobial activities against *Aspergillus flavous* and *Bacillus subtilis.*

Keywords: Morphological, Anatomical, Physicochemical Properties, Antimicrobial Activities

Introduction

Crotalaria juncea L. is a tropical Asian plant of the legume family Fabaceae. It is considered to have originated in India. It is widely grown throughout the tropics and subtropics as a source of green manure, fodder and liginified fiber obtained from its stem. Sunn hemp is also being looked at as a possible bio-fuel. Doubtfully wild; cultivated extensively for fibre in Parkistan; India; Burma; Malay Isles, Austrial; Russia; introduce in Tropical Africa. Fabaceae consists of about 643 genera, 18000 species. Fabaceae is a very large group with a worldwide distribution (Website-1).

A native of India but widely distributed elsewhere in the tropics. Widely cultivated as a cover crop and for fibre (Dassanayake, 1981). The family Fabaceae as here narrowly defined consists of about 400 genera and 10000 species, widespread in temperate and cold as well as tropical regions. The large genera *Crotalaria*, 500 (Cronquist, 1981).

The plant is native in Asia especially Asia tropical (Bangladesh; Bhutan; India). It is now widely cultivated in the drier areas of the tropics and subtropics and in many temperate areas with a hot summer. It often escaped from cultivation, naturalizes easily and grows in many areas as a ruderal plant. *Crotalaria juncea* L. is recorded in many countries across the African continent from the Atlantic coast to the Red Sea, from Tunisia to South Africa and in the Indian Ocean islands. It is used for medicinal, edible and culinary purposes by many tribal communities. It is traditionally used as blood purifier, abortificient, astringent, demulcent, emetic, purgative, in the treatment of anaemia, impetigo, menorrhagia and psoriasis (Ali Esmail Al-Snafi, 2016).

'Tropic Sun' or Sunn hemp is principally used as a green manure crop for soil improvement. It is an excellent, rapid-growing green manure to be included in rotation with vegetable, ornamental, and other plants to add nitrogen and organic matter to suppress weeds, and to reduce root-knot nematodes. To achieve maximum benefits, plantings should be made at regular intervals in a planned crop rotation scheme (Peter and Robert, 1983).

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Crotalaria juncea L. is grown mainly in India, Brazil, and West Pakistan for its fiber. It is used in the production of twine, rug yarn, tissue paper, fish nets, sacking, canvas, and cordage. *Crotalaria juncea* L. is used as a nitrogen-fixing green manure to improve soil quality, reduce soil erosion, conserve soil moisture, suppress weeds a nematodes, and recycle plant nutrients (Duke, 1983). The aim and objectives are to study the morphological characters of *Crotalaria juncea* L., to know the anatomical characters of *Crotalaria juncea* L., to investigate the physicochemical properties and to examine the antimicrobial activities of *Crotalaria juncea* L.

Materials and Methods

In this study, the specimens of *Crotalaria juncea* L. were collected from Kyaing Tong University Campus. After collected specimens were recorded in detailed for taxonomic description and identification were carried out by (Hooker, 1885, Backer *et al*., 1965, Cronquist, 1981 and Dassanayake, 1991). Microscopical characters of lamina, midrib, petiole and stem were examined by preparing freehand sections from the fresh specimens. The sections were carefully separated with the help of needle, washed with water and cleared in chloral hydrate solution for microscopical studies (Wallis, 1967; Sandara Rajan, 2000 and Trease and Evans, 2002). The diagnostic characters of the powdered drugs were made by using the powders of the leaves.

Physicochemical properties of the leaves of *Crotalaria juncea* L.

The physicochemical properties of the leaves of *Crotalaria juncea* L. were determined according to "The British Pharmacopoeia" 1968 as follows:

Ethanol-soluble matter

Ten gm of air-dried sample was soaked with 200 ml of 95% alcohol in a closed flask for 72 hrs and kept over three nights. The mixture was filtered rapidly taking precautions against loss of alcohol and filtrate was evaporated in a weighed petridish on a boiling water bath, until it was completely dried. The evaporated residue together with the petridish was weighed. The procedure was repeated until a constant weight was obtained. The difference in weights gave the alcohol-soluble extractive value.

Petroleum ether, Ethyl acetate, Acetone, Methanol and Distilled water soluble matter

The above procedure repeated with 200 ml of petroleum ether, ethyl acetate, acetone, methanol and distilled water instead of alcohol.

Antimicrobial Activities of *Crotalaria juncea* L.

Paper disc Diffusion Assay

Isolated bacterial strains grown on nutrient agar were inoculated into 50ml conical flasks containing 10ml of sterile growth medium. Then, they were incubated at 30˚C for 72 hours on a reciprocal shaker at 200 rpm.

Test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. 3ml of test organisms was added to assay medium, then poured into plates. After solidification, paper discs impregnated with broth samples were applied on the test plates and these plates were incubated for 24-36 hours at 30˚C. After for 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive compounds which inhibit the growth of test organisms (Cruickshank, *et al*., 1975).

Assay medium (SY) for test organisms

Agar	- 2.0.g
Sucrose	- 1.0 g
Yeast extract	- 0.3 g
NaCl	- 0.1 g
Distilled waterq	- 100 ml
pH	- 7.0

Results

Scientific name - *Crotalaria juncea* L.

Family name - Fabaceae

Myanmar name - Pike-san

English name - Sunn Hemp, Indian Hemp, Brown Hemp, Madras Hemp,

Diagnostic Characters of *Crotalaria juncea* L.

Annual herbs, to about 2-5 m tall; stems erect, ribbed, subappressed- pubescent; stipules filiform, caducous, leaves simple, oblong to oblanceolate, obtuse at the base, glabrous or sparsely pubescent above, subsericeously beneath; inflorescence terminal, racemose, many-flowered; bracts lanceolate-oblong, acute; bracteoles linear, inserted at the base of calyx; flowers yellow; calyx sub-bilabiate, lobes lanceolate; corolla golden yellow with dark reddish or brown streaks; standard suborbicular, wing oblong, keel falcate, curved, twisted beaked. Stamen 10, monadelphous, anther dimorphic. Ovary oblongoid, unilocular with many ovules on the marginal placentation; style incurved, stigma simple; fruit sessile, oblong-cylindrical, 6-20 seeded; seeds reniform, light brown to black. The results are shown in Fig. 1.

Morphological Characters of *Crotalaria juncea* L.

Habit

Upper surface of Leaves

Lower surface of Leaves

Inflorenscence

Closed up view of flowers

Calyx

Standard

Wing

Keel

Androecium

Gynaecium

Fruits

Seeds

T.S of Ovary

Figure 1 Morphological Characters of *Crotalaria juncea* L.

Anatomical characters of *Crotalaria juncea* L.

Lamina

In surface view, the cuticles were present on both surfaces. The epidermal cells of both surfaces were thin-walled, parenchymatous. Anisocytic types of stomata on the upper and lower surface. Non-glandular (unicellular, uniseriate) trichomes are present on more lower surfaces than upper surface.

In transverse section, the cuticle is present on both surfaces. Cuticle layer was thin. Epidermal cells are parenchymatous with straight anticlinal wall and one-layered thick. Both

epidermises are barrel-shaped, thin-walled, compactly arranged. The mesophyll composed of palisade parenchyma and spongy mesophyll cells. The palisade parenchyma cells found below the epidermis was one-two layered thick, vertically elongated, cylindrical closely compacted. The spongy mesophyll cells situated below the palisade.

The vascular bundles of the lateral veins are embedded in mesophyll cells. Xylem composed of tracheids, fibre-tracheids, fibres and xylem parenchyma cells. Phloem consisted of sieve tubes, companion cells and phloem parenchyma cells.

Midrib

In surface view, the epidermal cells of both surfaces were made up of thin-walled, parenchymatous. They were rectangular and elongated along the length of the midrib, non-glandular (unicellular, uniseriate) trichomes were present on lower surface.

In transverse section, the midrib was curved inwards above, with a prominent ridge on the upper surface, both surfaces are covered with thin-cuticle. Epidermal cells one-layered, rectangular-shaped, compactly arranged, anticlinal walls straight. Collenchyma cells were 1-2 layers situated under the epidermal cells. The cortex was made up of thin-walled parenchyma cells on both sides. The Parenchyma cells were 10-19 layers in thickness towards the adaxial, 5-9 layers in thickness towards the abaxial side.

The vascular bundles are crescent-shaped and collateral type. Xylem cells were radial rows, lignified and thin-walled, composed of tracheids, fibres-tracheids, fibres and xylem parenchyma. The phloem cells were thin-walled and composed of sieve tubes and companion cells.

Petiole

In surface view, the epidermal cells of both surfaces were made of parenchymatous cells. They were thin-walled, rectangular to polygonal in shaped. Unicellular, uniseriate trichomes are present.

In transverse section, the petiole is concave an adaxial side and has cuticle, the epidermal cells are depressed, parenchymatous polygonal-shaped compactly arranged, unicellular, uniseriate trichomes are present on both sides of the petiole. Below the epidermis palisade parenchyma cells were one-layer and upper vascular bundles has 15-23 layers of parenchyma and lower vascular bundles has 13-20 layers of parenchyma, the cells are thin-walled, oval-rounded.

The vascular bundles are arc-shaped and bicollateral type. The xylem are lignified thick-walled composed of tracheids, fibres-tracheids, fibres and xylem parenchyma, phloem composed of sieve tubes, companion cells and phloem parenchyma.

Stem

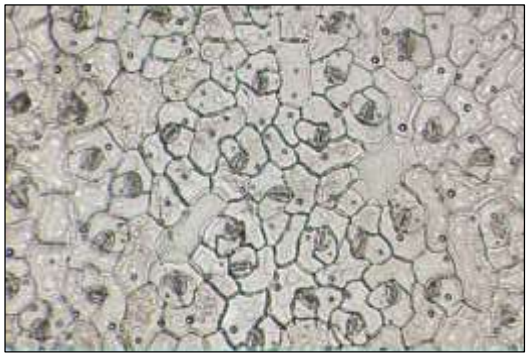
In surface view, the epidermal cells show wavy in outline with a cuticularized outer layer epidermis which is compactly arranged single row of rectangular cells and some cells provide unicellular, uniseriate non-grandular trichomes in young and old stem.

In transverse section, epidermis followed by cortex, which constituted hypodermis with 2-3 layers of collenchymatous cells and also patches of sclerenchymatous cells at the grooved. The cortex is made up of parenchymatous cells. The cells are thin-walled undifferentiated, irregular-shaped in young and old stem.

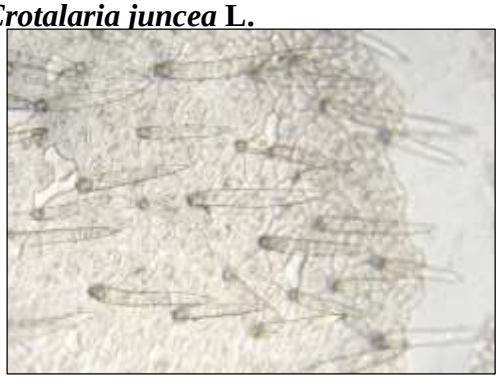
In the vascular bundles, in young and old stem, cortical vascular bundles are present. Endodermis is prominently single-layered composed of elongated irregular cells. Pericycle is single-layered shows presence of stone cells and contains isolated strands of fibers. The cells are

elongated, squarish and oval thick-walled. In xylem, vessels are mostly single or rarely group of 2-3, the smaller one constituting protoxylem lie towards center and bigger one constituting the metaxylem lie away from the center with thick lignified wall in young stem and old stem. Phloem is 3-5 layered, the cells are rectangular, elongated. Fibers thick-walled, radially arranged. Below the xylem 2-3 layers of thin-walled impregnated parenchymatous cells are present, it is compactly arranged, elongated or polygonal. In the centre, pith is large, which composed of compactly arranged parenchymatous cells in the young stem and pith is large, hollow in the old stem. The results are shown in Fig. 2.

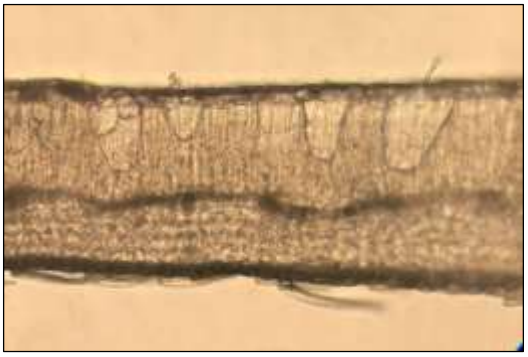
Anatomical Characters of *Crotalaria juncea* L.



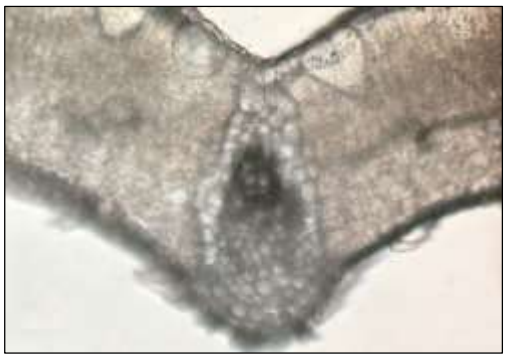
Upper epidermal cells with Anisocytic stomata (X100)



Lower epidermal cells with Anisocytic stomata and trichomes (X100)



T.S of lamina (X100)

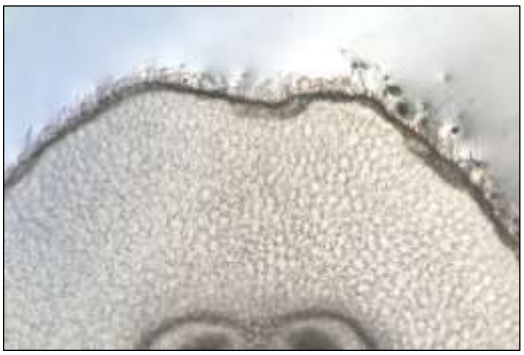


T.S of lateral veins showing vascular bundle (X400)

Surface view of midrib with numerous unicellular, uniseriate trichomes (X100)

T.S of midrib (X40)

Surface view of petiole with unicellular, uniseriate trichomes (X100)

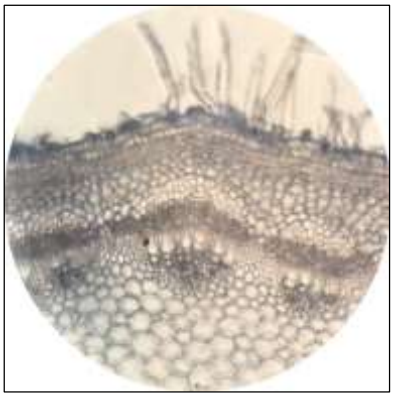


Transverse section of Petiole (X100)

Closed up view of vascular bundle (X100)

T.S of young stem (X100)

T.S of young stem pith (X100)



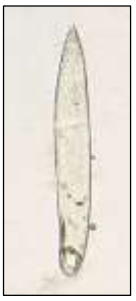
T.S of old stem (X100)

T.S of old stem with hollow pith (X100)

Fibre

Tracheid

Fibre-tracheid



Fragments of unicellular, uniseriate trichomes (X400)

Figure 2 Anatomical characters of *Crotalaria juncea* L.

Table 1 Epidermal characters of *Crotalaria juncea* L.

Scientific name	Stomata		Trichomes	Epidermal cells	
	Upper surface	Lower surface	Non-glandular	Upper	Lower
Crotalaria juncea L.	Numerous (Anisocytic)	Numerous (Anisocytic with trichomes)	Unicellular Uniseriate trichomes	Straight	Slightly wavy

Sensory Characters of the Powdered Leaves of *Crotalaria juncea* L.

Table 2 Sensory Characters of the Powdered Leaves of *Crotalaria juncea* L.

Characters	Leaves
Colour	Greenish
Odour	Aromatic
Taste	Slightly Bitter
Texture	Granular, fibrous

Antimicrobial Activities of *Crotalaria juncea* L. Leaves

In antimicrobial activity, results revealed that *Crotalaria juncea* L. various leaves extracts possess significant antimicrobial activity against *Aspergillus flavous* and *Bacillus subtilis.* The results are shown in Fig. 3 and Table. 3.

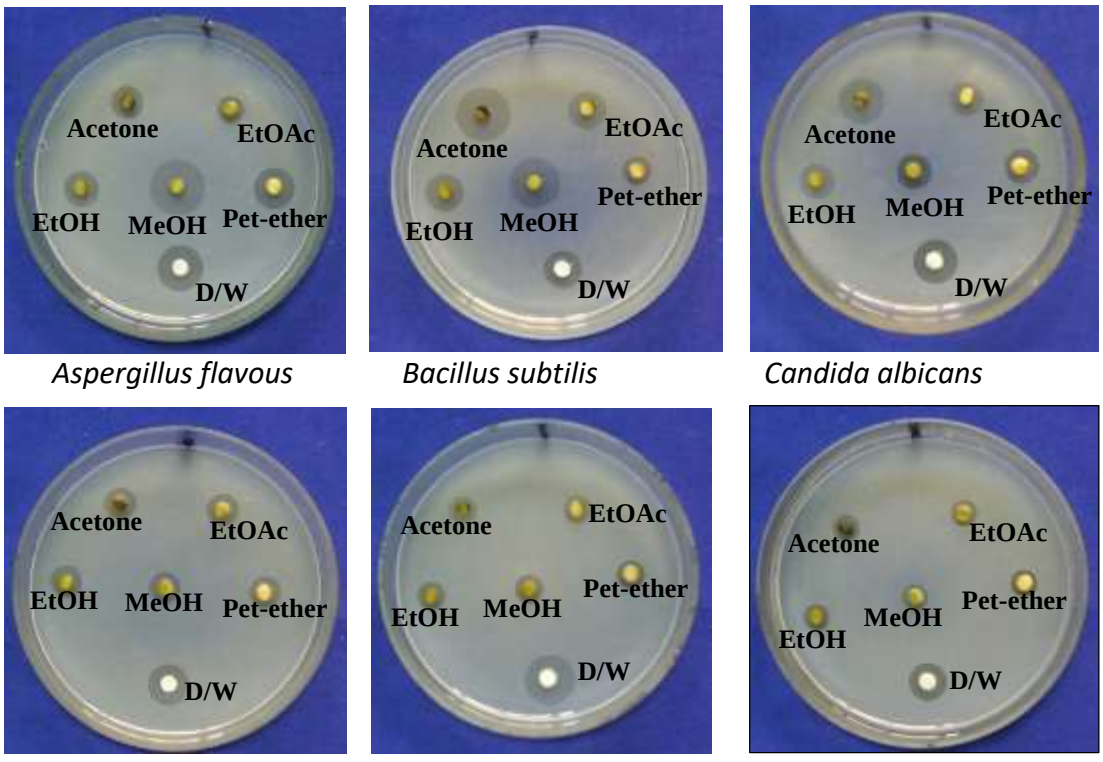


Figure 3 Antimicrobial Activities of *Crotalaria juncea* L. Leaves

Table 3 Antimicrobial Activities of *Crotalaria juncea* L. Leaves

No.	Test Organisms	Pet-ether	Ethyl Acetate	Acetone	Methanol	Ethanol	D/W
1	*Aspergillus flavous*	10	8	**12**	**18**	**14**	**16**
2	*Bacillus subtilis*	**16**	**10**	10	14	8	10
3	*Candida albicans*	12	8	8	8	8	10
4	*Escherichia coli*	8	8	8	8	8	12
5	*Pseudomonas fluorescens*	8	8	8	8	8	12
6	*Xanthomonas oryzae*	8	8	8	8	8	12

Physicochemical Properties of *Crotalaria juncea* L.

Solubility in different solvents

The solubility tests were carried out to find the amount of total solids soluble in solvents. In the *Crotalaria juncea* L. leaves were distilled water more soluble than other different solvents. The results are shown in Table. 4.

Table 4 Solubility Test of *Crotalaria juncea* L. Leaves

Solvents	Pet-ether %	EtOAc %	Acetone %	MeOH %	EtOH %	D/W %
Leaves Average %	3.0	7.0	4.0	14.0	4.0	22.0

Discussion and Conclusion

In morphological study, sunn hemp is erect herbs annual; stems cylindrical and ribbed with short appressed hairs. Leaves alternate, simple; stipules long, slender; petiole long; leaflets oblong-lanceolate, finely appressed pubescent. Inflorescence a leaf-opposed raceme, 6-20 flowered; bracts elliptical. Flowers in terminal racemes, bracts and bracteoles linear, corolla golden yellow, with elliptical standard reddish marked, wings a little shorter than keel, keel long, slightly incurved twisted beak; stamens 10, all joined in a sheath open at base; ovary superior, style curved, stigma small. Fruit a cylindrical pod, short, velvety hairy. Seeds oblique-cordiform, dark brown to black. These characters are in agreement with those given by (Hooker, 1885; Backer *et al*., 1965; Dassanayake, 1981; Ali Esmail Al-Snafi, 2016 and Sonje and Bhuktar, 2016 and Website 2).

In anatomical study, leaves and stems show presence of non-glandular, unicellular, uniseriate trichomes. Trichomes occur on abaxial and adaxial surfaces of leaves, lamina, midrib, petiole and stem (Esau, 1965; Metcalfe and Chalk, 1960; Pandey *et al*., 2011; Sundara Rajan, 2000 and Sonje and Bhuktar, 2016).

Sensory characters showed the colour of powdered leaves were greenish. The odour were aromatic in leaves. The taste of powdered leaves was slightly bitter. The texture was granular and fibrous in leaves.

In this study, the physicochemical properties of *Crotalaria juncea* L. leaves showed pet-ether extract value 3.0, ethyl acetate extract value 7.0, acetone extract value 4.0, methanol extract value 14.0, ethanol extract value 4.0, water soluble extract value 22.0. Sathis *et al*, 2011 and Ali

Esmail Al-Snafi, 2016 stated that ethanol soluble extract value 5.84, water soluble extract value 20.4.

In the antimicrobial activities, petroleum ether and ethyl acetate leaves extracts of *Crotalaria juncea* L. were found more antimicrobial activities in *Bacillus subtilis* than other microorganisms. Acetone, methanol, ethanol and D/W leaves extracts of *Crotalaria juncea* L. against more antimicrobial activities in *Aspergillus flavous* than other microorganisms. *Crotalaria juncea* L. various leaves extracts possess significant antimicrobial activity against *Aspergillus flavous* and *Bacillus subtilis.*

Bhakshul *et al*., 2008 explained that petroleum ether and ethyl acetate extracts of *Candida madurensis var. kurnoolica* leaves were found to be active on the tested microorganisms whereas, the alcoholic extract did not show any inhibitory activity. *Bacillus subtilis*, and *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and *Candida albicans* and *Candida tropicalis* were observed to be sensitive to the tested extracts. These results lend support the usage of *Candida madurensis var. kurnoolica* leaves by the local tribal population in using for wounds and skin diseases against bacterial and fungal infections.

In the further study, this plant review will highlight the chemical constituents will be extracted from methanol extracts and pharmacological activities of *Crotalaria juncea* L.

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POLLEN MORPHOLOGY AND PHYTOCHEMICAL INVESTIGATION OF LEAVES EXTRACT OF *TERMINALIA CATAPPA* L. FROM THE YAN LAW TRACT, KYAING TONG TOWNSHIP

Nwe Ni Tin[1], Nang Moon Sar[2], Aye Thida Hlaing[3]

Abstract

In this study an attempt has been made to identify and to authenticate the source pant *Terminalia catappa* L. In identifying the taxon, both external morphological and palynological characters are used as the taxonomic tools. The systematic treatment is worked out following the classification scheme of Byng J.W & M.W Chasem, 2016. The detection of phytochemical constituents present in leaf extract of *Terminalia catappa* L. has been done mainly based on the pharmacognostic methods. Characterization of major groups of plant compounds was carried out employing University of Yangon, Botany Department Laboratory. The investigation revealed the presence of glycosides, phenols,α-amino acid, saponin, tannin, flavonoid, steroid, terpenoid, reducing sugar and starch.

Keyword: *Terminalia catappa* L. Pollen Morphology and Phytochemical characterization.

Introduction

Terminalia catappa L. belong to the family Combretaceae. It is also known as Tropical almond is a large tree growing upto the height of about 10m with an upright closely spiral and symmetrical tiers of horizontal branches, thus named “umbrella tree”. It is widely cultivated as ornamental and shade - tree.

This Combretaceous plant can be recognized by its simple, entire, stipulate leaves which are arranged alternate or opposite to each other. Bractate, sessile flowers are of spike inflorescence. Drupaceous fruits are often winged. To establish the validity and identity of *Terminalia catappa* L. the systematic treatment is carried out according to APG IV classification system (Byng J.W & M.W. Chase, 2016).

The significance of pollen attributes in taxonomy has been widely realized. Recent palynological data are finding increasing application in construction of diagnoses of unknown taxa. Diagnoses based on pollen features have been found in agreement with those prepared on the basis of anatomical characters and data from other displines of Botany (Pritishukla, 1997).

In identifying *Terminalia catappa* L. both macro - morphology (external characters) and pollen morphology have been taken into consideration. The characterization of the chemical constituents of leaf extract was carried out according to pharmacognostic basis.

Leaf extracts have potentials in treatment as anti-oxidant, anti - cancer, anti - diabetic, anti - septic, cardiotonic and anti- inflammatory effects. It is also notable in assisting in wound healing. It is obvious that the chemical principles isolated from plants are compounded as drugs, and health supplements.

Asian nation such as Ayurveda, Indian traditional medicine, and traditional Chinese medicine have been extensively researched, standardized and are properly regulated. In constract, some other herbal medicinal practices do not have much achieved high commercial value due to lack of standardization and poor regulation for plant products. To enhance its worldwide

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acceptance, there is a need of identity and authenticity of the source plants concerned. The chemical characterization of the bioactive principles of leaf extract is also important.

This study is an attempt to ascertain the identity and the systematic of the source plant as *Terminalia catappa* L., to determine the phytochemical constituents, present in leaf extracts of *Terminalia catappa* L. and to provide a phytochemical characterization which may suggest a certain evidence for its ethno-medicinal use.

Materials and Methods

1. Plant material

The specimen upon which the present study is based were collected from plants mainly located at *Yan Law* tract, Kyaing Tong Township. The specimens were collected during the June to December in 2019 at the period of anthesis and fructification. Natural habit and vegetative and reproductive parts of the plants are documented by photography method. The plant materials were identified and specimens were mounted on herbarium sheets and were deposited in the Department of Botany, University of Kyaing Tong.

2. Collection of pollen samples

For pollen samples anthers were freshly collected from the mature flower buds or from partially opened flower to avoid contaminations. Collected pollen of each species was stored in small glass vials with 1 cc of glacial acetic acid and labeled.

3. Preliminary Phytochemical Examination of leaves of *Terminalia catappa* L.

Detection of the major Phytocomponents present in leaf extracts of *Terminalia catappa* L. employed the Department of Botany University of Yangon. The reagents and chemical used of this study are of analytical grade and are all provided by the Department of Botany (Yangon). The operational procedure for detection of leaf extracts were based on the methods described in British pharmacopoeia (1965) and those stated by Harbone (1973). Marini - Bettelo (1981) and Trease & Evans (2002).

The fresh leaves are thoroughly washed under tap water and finally will distilled water. Then they are air- dried for three weeks. Dried leaves were then milled to powder by grinding machine.

Using the corresponding solvent like that of 1% HCl, ethanol, methanol, hydro-chloric acid, pet-ether and water; the individual extracts was then subjected to qualitative analysis of Phyto-components.

In this study altogether thirteen major groups of Phytochemicals were characterized as shown in the Table.

1. Test for Alkaloid

The powdered sample 3g was boiled with 1% HCl 50 ml for about 20 minutes and filtered. The filtrate was divided into four portions and tested with modified Mayer's reagent, Wagner's reagent, Dragendroff's reagent and Hager's reagent. Treatment with the above-mentioned alkaloid reagents furnished turbid or brown precipitates, indicating the presence of alkaloid in the plant materials.

2. Test for Carbohydrates

The powdered sample 1g was boiled with 10 ml of distilled water and filtered. The filtrate was introduced into a test tube and a few drops of 10% α-napthol was added and shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of test tube. A red ring was formed between the two layers, showing the presence of carbohydrates.

3. Test for Glycosides

The powdered sample 1g was boiled with 10 ml of distilled water for about 10 minutes, allowed to cool and filtered. The filtrate was treated with 10% lead acetate solution. White precipitate took place on addition of the reagent.

4. Test for Phenolic Compound

The powdered sample 1g was boiled with 10 ml of distilled water for about 20 minutes and filtered. The filtrate was treated with 5% ferric chloride solution. Greenish brown pracipitates appeared showing the present of phenols.

5. Test for α-Amino Acid

The powdered sample 1g was boiled with 10 ml of distilled water for about 10 minutes and then filtered. An aliquot portion of filtrate was transferred to a filter paper with the help of the micropipette and allowed to dry. Then this filter paper was sprayed with ninhydrin reagent and allowed to dry at 110°C in an oven for a few minutes. The filter paper turned violet color spot.

6. Test for Saponin

The powdered sample 1g was put into a test tube and 10 ml of distilled water was added. Then the mixture was vigorously shaken for a few minutes. Frothings or persistent foams took place.

7. Test for Tannin

The powdered sample 2g was boiled with 20 ml of distilled water about 20 minutes and then allowed to cool and filtered. The filtrate was treated with a few drops of 1% gelatin with introduced and 10% NaCl solution. Formation of green or yellowish green color precipitate indicates the presence of tannins.

8. Test for Flavonoid

The aqueous extract residue of powdered sample was dissolved 70% ethanol. The alcoholic solution was then treated with 0.5g of magnesium turning and few drops of concentrated hydrochloric acid. Red coloration developed within three minutes.

9. Test for Steroid

Three gm of dried powdered samples were refluxed with benzene and the solvent was removed by distillation under reduced pressure. Acetic anhydride 3 dropswas added and the mixture was shaken. Then a few drops of concentrated hydrochloric acid were carefully added and shaken. The solution turned to blue color.

10. Test for Terpenoid

A few gm of petroleum ether extract was dissolved in 15 ml of chloroform. The chloroform extract and 0.3 ml of acetic anhydride were noted after the addition of a few drops of concentrated sulphuric acid. Formation of pink color indicates the presence of terpenoids.

11. Test for Reducing Sugar

The powdered sample 1g was boiled with 10 ml of distilled water and filtered. When the resulting solution was treated with Fehling's solution, it furnished brick red precipitates; indication and presence of reducing sugars.

12. Test for Starch

The powdered sample 2g was boiled with 20 ml of distilled water for about 10 minutes and filtered, 2 drops of iodine solution added to filtrate. Bluish black precipitates were observed.

13. Test for Cyanogenic Glycoside

The powdered sample 1g was mixed with distilled water in a conical flask. About 5 drops of concentrated sulphuric acid was added and sodium picrate paper trapped in a neck by means of a cork. The resulting mixture was heated by means of spirit burner. Sodium pictrate paper turned brick red.

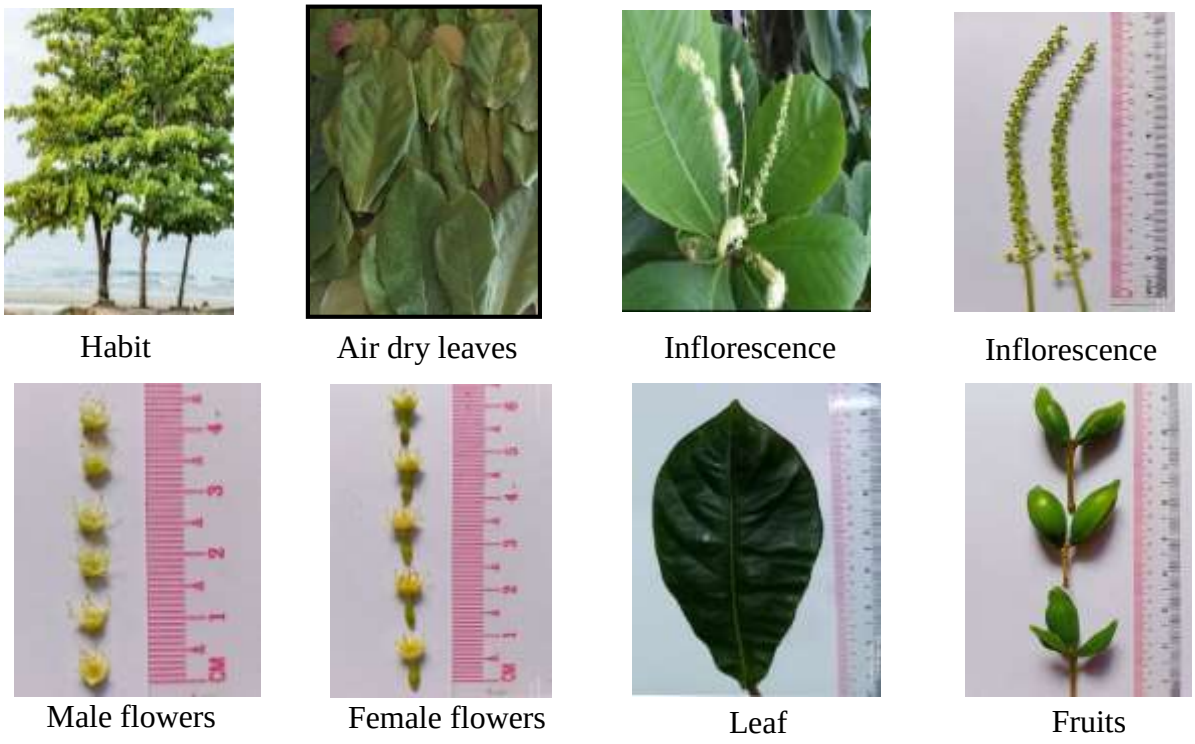
Results

1. Morphological characters of *Terminalia catappa* L. Syst, Na, 2: 674 (err.638). 1767, Clarke in Hook.f.,Fl.Br.Ind. 2: 444, 1878;Philcox Dassanayake, Fl. Cey.9:39.1995

Group	- Eudicots
Superorder	- Rosids
Order	- Myrtales
Family name	- Combretaceae
Vernacular name	- *Banda Pin*
Local name (Shan name)	-Twme – taung - tune
Flowering period	- August to October
English name	- India almond, Tropical almond, Singapore almond, Umbrella tree,
Locality	- *Yan –Law* Tract, Kyaing Tong

A large deciduous tree, up to 10 m high; stems and branches terete, densely yellow-brown sericeous- pubescent, glabrescent. Leaves simple, alternate, clustered at the ends of branches, exstipulate; petiole 5 – 12 mm long; blades obovate or elliptic- ovate, 13-22 cm by 7-12 cm subcordate at the base, entire along the margin, rounded or shortly acuminate apex, sessile gland on each side of the midrib, glossy when mature, finely verrucose on both surfaces. Inflorescence axillary, long spikes; peduncles 5-14 cm long. Flowers creamy- white, 5-6 mm in diameter at anthesis, bisexual in the lower portions, of upper ones many staminate, apetalous. Calyx 5- lobed, broadly deltoid, 2 mm by 1 mm, reflexed at maturity. Corolla (Petals) absent. Stamen 10 in 2 rows, adnate on the calyx tube, exserted; filaments filiform, 2-3 mm long, glabrous; anthers dithecous. Disk densely whitish- barbate. Ovary inferior, ellipsoid, 2 mm long, angular, glabrous, unilocular, with one ovule on the pendulous placenta; style filiform, 2 mm long, glabrous; stigma simple.

Fruits drupaceous, indehiscent, ellipsoid or ovoid, 3.5-5.5 cm by 2.5 cm, laterally more or less compressed, glabrous, ringed by a rigid wing or often without wing, green to yellow and red at maturity; stone surrounded by a thick layer of juicy flesh.



Habit | Air dry leaves | Inflorescence | Inflorescence

Male flowers | Female flowers | Leaf | Fruits

Figure 1 Morphology characters of *Terminalia catappa* L.

Pollen morphology

External morphology of pollen grains has been studied under the light microscope and was characterized as follows:

Tri- colporate with prominent psdeudo - colpi: isopolar; radiosymmetric; small size; equatorial view: prolate-spheroidal to sub- prolate; polar view: circular; colpi linear with acute ends and broad at middle; pseudo - colpi almost the size of colpi, fused at apocolpia; oralalongate; exine sculpture: microregulate.

Polar View | Equatorial view

Figure 2 Pollen morphology of *T. catappa* L.

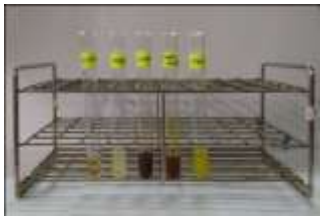
2. Preliminary Photochemical examination of leaves of *Terminalia catappa* L.

Preliminary phytochemical tests of the leaves of *Terminalia catappa* L. indicated the presence of alkaloids, carbohydrate, glycoside, phenolic compound, α-amino acid, saponin, tannin, flavonoid, steroid, terpenoid, reducing sugar and starch are present in the leaves and the test have shown that cyanogenic glycoside was absent found in *T. catappa* L. The experimental result was shown in table (1) and figure (3).

Table showing the detection of major phytochemical in leaf extracts of *T erminalia catappa* L.

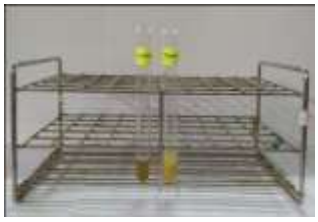
No.	Type of compound	Extract	Reagent used	Observation	Results
1.	Alkaloid	1%HCL	Mayer's reagent	Cream colour (turbid)	+
			Wagner's reagent	Brown ppt.	
			Dragendorff 's reagent	Brown ppt.	
			Hager's reagent	Yellow colour (turbid)	
2.	Carbohydrate	H_2O	10% α –naphthol & H_2SO_4(Conc:)	Red ring	+
3.	Glycoside	H_2O	10% Lead acetate solution	White ppt.	+
4.	Phenol	H_2O	5% $FeCl_3$ solution	Brownish green ppt.	+
5.	α-amino acid	H_2O	Ninhydrin reagent	Light purple colour	+
6.	Saponin	H_2O	H_2O	Persistent foam	+
7.	Tannin	H_2O	1% Gelatin & 10% NaCl solution	No ppt.	+
8.	Flavonoid	70%EtOH	Mg ribbon & Conc; HCl	Pink colour.	+
9.	Steroid	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Bluish green	+
10.	Terpenoid	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Pink colour.	+
11.	Reducing sugar	H_2O	Fehling's solution	Brick red ppt.	+
12.	Starch	H_2O	Iodine solution	Brown ppt.	+
13.	Cyanogenic Glycoside	Powder	H_2O,Conc; H_2SO_4, sodium picrate paper	No colour change	-

(+) = presence (-) = absence



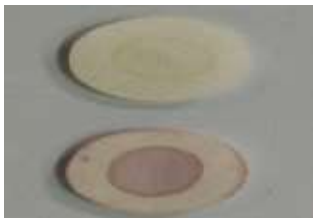
Test for Alkaloid

Test for Carbohydrate



Test for Glycoside

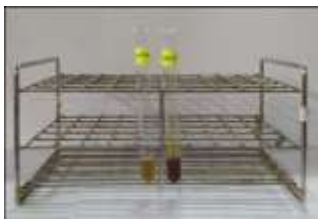
Test for Phenol



Test α- amino acid

Test for Saponin

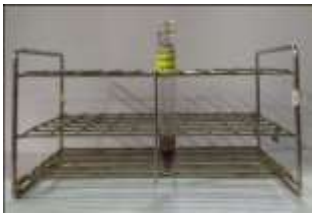
Test for Tannin



Test for Flavonoid

Test for Steroid &

Test for Reducing



Test for Starch

Test for Cyanogenic

Figure 3 Phytochemical test of leaves from the *Terminalia catappa* L.

Discussion and Conclusion

Terminalia catappa L. (Benda pin) collected from the Tract of Yan Law, Kyaing Tong District, Eastern of Shan State. In this study verification of nomenclature and systematic treatment of *Terminalia catappa* L. was done following the flora of British India (Hooker, 1881-87), Flora of Java (Backer, 1963), A Checklist of the Trees, Herbs and Shrubs of Myanmar, Kress.J. *etal*, 2003.

Distinguishing characters in delimiting *T.catappa* L. *are* trees up to 10m of height and large deciduous tree; Barks brownish black with longitudinal peelings without ridges; Young stems and leaves not hairy; Leaves simple, large, obavate, distichous turn red in senescence; inflorescence axillary, long spikes; flowers creamy - white, bisexual in the lower portions, of upper ones many staminate; petioles with 2 glands at the summit; stamen 10 in 2 rows, adnate on the calyx tube, exserted; filaments filiform; anthers dithecous; ovary inferior, ellipsoid, angular, glabrous, unilocular, with one ovule on the pendulous placenta; style filiform, glabrous; stigma simple; Fruits drupaceous, indehiscent, ellipsoid or oval, rounded to flattened, green red, two - ridged or two - winged.

Pollen morphological characters are used as a taxonomic device for identification of *T. catappa* L. Outstanding features of pollen grains are Tri- colporate with prominent psdeudo - colpi: isopolar; radiosymmetric; small size; equatorial view with prolate - spheroidal to sub - prolate; polar view with circular; colpi linear with acute ends and broad at middle; pseudo - colpi almost the size of colpi, fused at apocolpia; ora lalongate; exine sculpture with microregulate.

In this study isolation of leaf extracts showed the presence of twelve major components, viz: Alkaloid, Carbohydrate, Glycoside, Phenol, α-amino acid, Saponin, Tannin, Flavonoid, Steroid, Terpenoid, Reducing sugar, starch and Cyanogenic glycoside.

Four different kinds of solvents are used to isolate plant constituents from leaf extract as shown in the table. Water soluble extracts have the ability to isolate Carbohydrate, Glycoside, Phenol, α-amino acid, Saponin, Tannin, Reducing sugar and starch. Pet -ether extract revealed Steroid and Terpenoid; whereas 1% HCl extract shows the present of Alkaloids, while in 70% Ethenol flavonoids are present.

For global recognition of traditional medicinal practice extensive research standardization of plant product is required. While the knowledge of the therapeutic properties of plant extract was orally transmitted from mouth to mouth, identity and authenticity of source plants concerned are needed. The chemical characterization of the bioactive principles of plant extracts is also important.

Further investigation is needed for quantitative analysis of active phytocomponents of leaf extracts to determine their molecular formular, molecular weight, and relative amount in percentage in association with their usage in ethno- medicine.

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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *GMELINA ARBOREA* ROXB.

Thaw Maw Moe*

Abstract

Gmelina arborea Roxb. (White Teak) is a deciduous plant which belongs to the family Lamiaceae. This family is observed in Kyaing Tong University Campus area, Kyaing Tong Township. White teak is detected the morphological and organoleptic characters, medicinal uses, physicochemical properties, phytochemical constituents, elemental analysis and antimicrobial activity. Firstly, the morphological and organoleptic characters were presented. Medicinal and other uses were described from local people and practitioners. Secondly, the physicochemical properties showed that the solubility of the powdered samples was more soluble in ethanol, distilled water and methanol than other solvents. Phytochemical analysis of the powdered samples of leaves revealed the absence of steroid and others are presence in the different extract of the species. In the elemental analysis, percentage of calcium, silicon and potassium are higher than other elements. The antimicrobial activity against the tests organisms was founded. Especially pet ether, water extract and methanol extract were more effective than other solvent but possess the highest against on *Xanthomones oryzae*, *Pseumonous fluorescens* and *Aspergillus flavous*. These investigations may be helpful in development of herbal formulations.

Keywords: *Gmelina arborea* Roxb., morphological, physicochemical properties, phyto-chemical constituents, elemental analysis, antimicrobial activity, medicinal uses

Introduction

Gmelina arborea Roxb. (Verbenaceae) is native to Asia and known by various names, e.g. Yemane, Gamar, Gumhar and, Sor. This species has been introduced in several countries, particularly in West Africa and in Côte d'Ivoire and Nigeria, widespread in tropical and subtropical few temperate (Heywood, 1978). The family consist of 73 genera and over 3000 species and over 2600 species are herbs, shrubs, tree or climber. Often prickly and some are xerophytic in habit. The area of distribution includes Nepal, India, Pakistan, Bangladesh, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam and South China.

It has been introduced as a plantation species, and large plantations are found in South East Asia, West Africa and South America. (Ahiola, 2017).

Gmelina arborea is one of the important medicinal plants most widely propagated and cultivated species. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. The medicinal plant is highly used from time immemorial because of its vast medicinal properties. It is extensively used traditionally as antithelmintic, antimicrobial, antidiabetic, diuretic, hepatoprotective and antiepileptic agent Indian medicinal plant, 2011).

Ethnobotanical studies report that the species is widely used to treat many diseases including diarrhoea, hypertension and malaria, among others (Sharma & Balakrishnav, 1993).

Gmelina arborea is one among the most highly treasured medicinal plant species which is being used in the treatment of fever, heart diseases, nerve disorders, a number of digestive and reproductive disorders (Pemiah, 2014). This deciduous tree, indigenous to the tropical and subtropical region of Southeast Asia has widespread medicinal values embedded in all of its parts. (Asolkar, 1992).

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In general, secondary metabolites are an important source with a variety of structural arrangement and properties. The plant derived compounds have a long history of clinical use, better patient tolerance and acceptance (Kirtiar & Basu, 1994).

Therefore, the present study was undertaken with the objectives to assess the morphological characters, physicochemical investigation, phytochemical analysis, organoleptic characters, elemental analysis of leaf of *Gmelina arborea* Roxb. and its medicinal uses were described.

Materials and Methods

In the present study, specimen is collected during the field exploration. The field studies on both the season of flowering periods. The plant was identified at the Department of Botany, Kyaing Tong University, during from the February to May, 2019 with the help of available literatures (Hundley, H.G & Chit Ko Ko. 1961; Lawrence, 1969; Dassanayake,1983; Burmmitt, 1992; Hu Qi-ming, 2009).

The collected leaves were detached and washed with tap water and dried under shady place good ventilation for 15 days. Then the dried samples were powdered using kitchen blender and stored in air tight containers for further study. Physicochemical investigation of leaves was made according to The British Pharmacopoeia 1968.

The crude extracts were qualitatively tested for the presence of various secondary metabolites using standard established methods. The tests have been performed according to Trease and Evans, 2002.

The elemental analysis was analysed by using Energy Dispersive X-ray Florescence (EDXRF) spectrophotometer in URC, Monywa University.

Antimicrobial activities of crude extracts of sample were tested on six pathogenic microorganisms by using paper disc diffusion method described by Cruickshank, R.1975.

Results

Scientific Name - *Gmelina arborea* Roxb.

Vernacular Name - Ye-ma-ne

Family Name - Lamiaceae

English Name - White teak, Comb tree

Perennial deciduous large tree about 20 m high. Leaves simple, opposite and decussate, petiole long, glabrous, lamina broadly ovate, 10 - 25 × 7 - 17cm, cordate or truncate at the base with 2 glands at insertion of leaves, strongly toothed along the margin with young, margin entire, long acuminate at the apex, densely fulvous with stellate hair beneath. Inflorescences terminal and axillary paniculate cymes, peduncles 7 - 40 cm long. Flowers yellow to orange brown, about 2.5cm in diameter, fragrant. Sepals broadly campanulate, equally 5 toothed, densely fulvous-tomentose hair, tube with 2 glands without, teeth triangular, acute. Petals 5, funnelform, bilabiate, deeply 5 lobed, showy, fragrant, brownish yellow without and bright yellow within, 4 – 3.8cm long, oblique funnel form at throat, lobed unequal, upper lip orange pink, deeply divided 2 oblong, lower lip lemon yellow, about as long or twice as long as the upper and 3-lobed, ovate middle lobe much longer and broader than the ovate- rounded lateral ones. Stamens 4, didynamous, filament stout, yellow, anther dithecous, oblong- lanceolate. Style slender, stigma short, bifid.

Uses

The wood is used making for light construction and for pulp. Several parts of the tree are used for medicine.

The plant has been traditionally used for the treatment of various ailments. Leaves paste is applied to relieve headache and juice is used wash for ulcers and sunburn. Leaves juice are treat to leprosy and blood disorders. A juice made from the leaves is used to treat cough, diabetes, hypertension and skin diseases. Young leaves juice to cure diabetes. The seeds and leaves juice are considered a tonic for the heart, throat and for a variety of purposes. The paste made of leaves is applied to painful swelling and skin diseases.

Figure 1 Inflorescence

Figure 2 Flowers and petal

Figure 3 Sepal

Figure 4 Stamen

Figure 5 Style and stigma

Organoleptic characters of leaves of *Gmelina arborea* **Roxb.**

In the present study the powdered leaves of *Gmelina arborea* Roxb was greenish brown in colour, the odour was pungent and taste was tasteless and the fracture fibrous was investigated.

Figure 6 Leaves

Figure 7 Powdered

Determination of plant secondary metabolites

Gmelina arborea Roxb. revealed the presence of phytochemical constituents such as alkaloids, reducing sugar, glycosides, phenol, saponin, tannin, flavonoid, terpenoid and polyphenol were present and steroid was absent. Table (1)

Table 1 Preliminary phytochemical analysis from leaves of *Gmelina arborea* Roxb.

No	Test	Extract	Test Reagent	Observation	Result
1	Alkaloid	1 % HCl	Dragendorff's reagent	Orange ppt	+
2	Glycoside	H_2O	10% Lead acetate solution	White ppt	+
3	Flavonoid	EtOH	Mg. turning Conc. HCl acid	Pink color	+
4	Phenol	H_2O	5% Ferric chloride	Greenish black	+
5	Tannin	H_2O	1% Gelatin Solution	Green color	+
6	Reducing Sugar	H_2O	Fehling's solution	Brick red ppt	+
7	Saponin	H_2O	Distilled water	Frothings	+
8	Polyphenol	H_2O	1% $FeCl_3$, 1% K_3 Fe $(CN)_6$	Greenish blue	+
9	Steroid	P. E	EtOH, Conc H_2SO_4, $CHCl_3$	No change in color	-
10	Terpenoid	P. E	$CHCl_3$, Conc H_2SO_4	Reddish brown	+

The physicochemical properties showed that the solubility of the powdered samples was more soluble in ethanol, distilled water and methanol than other solvents. (Table 2)

Table 2 Physicochemical investigation of leaves of *Gmelina arborea* Roxb.

No	Physicochemical properties	Leaves Average %
1	Petroleum ether soluble content	**2.0**
2	Ethyl Acetate soluble content	**6.0**
3	Acetone soluble content	**4.0**
4	Methanol soluble content	**8.0**
5	Ethanol soluble content	**15.0**
6	Distilled water-soluble content	**10.0**

Table 3 Antimicrobial activities of leaves of *Gmelina arborea* Roxb.

	Organisms					
Solvents	***Bacillus subtillis***	***Aspergillus flavous***	***Pseudomonas fluorescens***	***Xanthomonas Oryzae***	***Candida albicans***	***E. coli***
Pet-ether	8mm(+)	14mm(+)	12mm(+)	16mm(+)	10mm(+)	10mm(+)
Acetone	8mm(+)	8mm(+)	8mm(+)	8mm(+)	8mm (+)	8mm(+)
MeOH	8mm(+)	12mm(+)	12mm(+)	14mm(+)	14mm(+)	8mm (+)
EtOAc	8mm(+)	10mm(+)	10mm(+)	10mm(+)	8mm (+)	10mm(+)
EtOH	8mm(+)	10mm(+)	12mm(+)	12mm(+)	10mm(+)	12mm(+)
D/W	12mm(+)	12mm(+)	14mm(+)	14mm(+)	14mm(+)	14mm(+)

Figure 8 *E. coli*

Figure 9 *Aspergillus flavous*

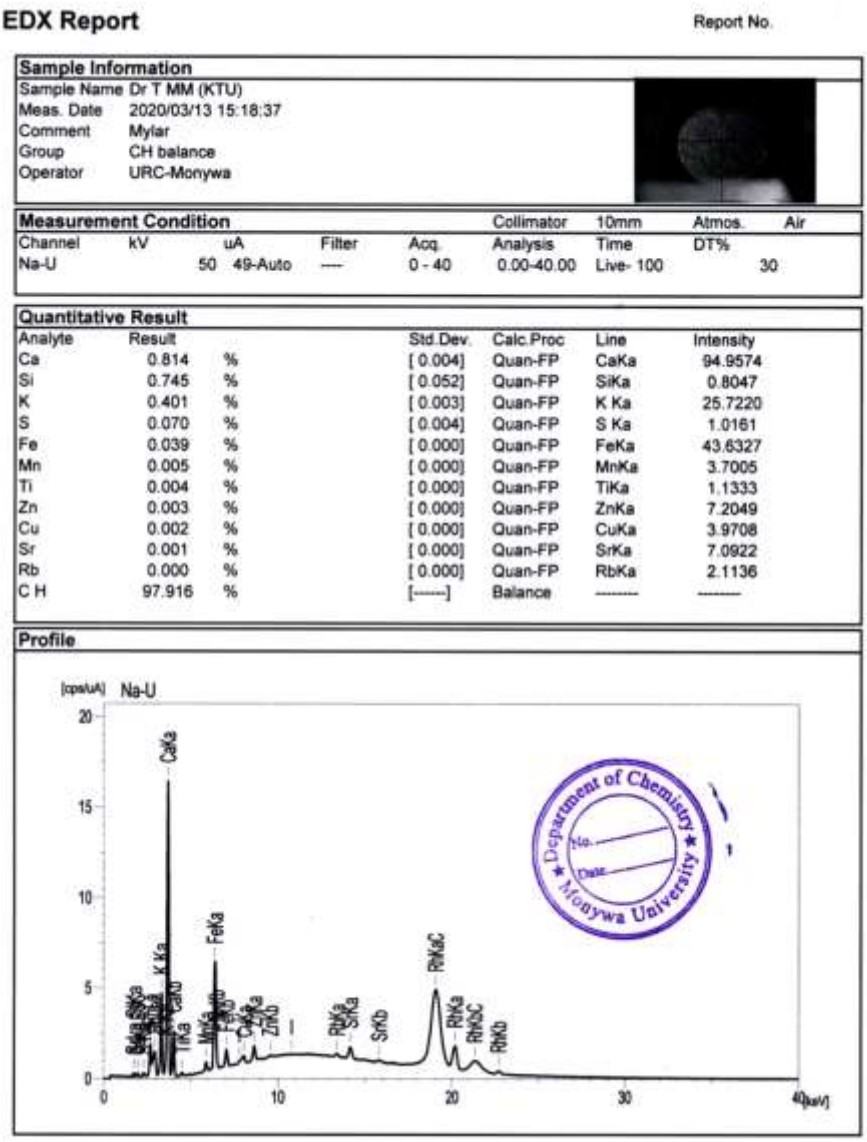
Figure 10 *Bacillus subtillis*

Figure 11 *Candida albicans*

Figure 12 *Pseudomonas fluorescens*

Figure 13 *Xanthomonas Oryzae*

1. Acetone
2. EtoAc
3. EtOH
4. MeOH
5. P/E
6. Water



EDX Report

Report No.

Sample Information

Sample Name	Dr T MM (KTU)
Meas. Date	2020/03/13 15:18:37
Comment	Mylar
Group	CH balance
Operator	URC-Monywa

Measurement Condition

Channel	kV	uA	Filter	Acq.	Collimator	10mm	Atmos.	Air
					Analysis	Time	DT%	
Na-U		50 49-Auto	----	0 - 40	0.00-40.00	Live- 100		30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
Ca	0.814	%	[0.004]	Quan-FP	CaKa	94.9574
Si	0.745	%	[0.052]	Quan-FP	SiKa	0.8047
K	0.401	%	[0.003]	Quan-FP	K Ka	25.7220
S	0.070	%	[0.004]	Quan-FP	S Ka	1.0161
Fe	0.039	%	[0.000]	Quan-FP	FeKa	43.6327
Mn	0.005	%	[0.000]	Quan-FP	MnKa	3.7005
Ti	0.004	%	[0.000]	Quan-FP	TiKa	1.1333
Zn	0.003	%	[0.000]	Quan-FP	ZnKa	7.2049
Cu	0.002	%	[0.000]	Quan-FP	CuKa	3.9708
Sr	0.001	%	[0.000]	Quan-FP	SrKa	7.0922
Rb	0.000	%	[0.000]	Quan-FP	RbKa	2.1136
C H	97.916	%	[------]	Balance	--------	-------

Profile

Figure 14 EDXRF Spectrum of leaves of *Gmelina arborea* Roxb.

In the elemental analysis of select plant, Ca, Si, K, S and Fe were present various percentages than another Mn, Ti, Zn, Cu and Sr in EDXRF spectrum. Toxic minerals such as Hg, Pb, Cd and as were not found.

Discussion and Conclusion

In the present study, morphological studies, organoleptic characters, physicochemical properties phytochemical constituents, elemental analysis, antimicrobial activity and uses of medicinal are described on leaves of *Gmelina arborea* Roxb. was carried out.

Leaves simple, opposite and decussate, densely fulvous with stellate hair beneath. Inflorescences terminal and axillary paniculate cymes. Flowers yellow to orange brown, fragrant. Sepals broadly campanulate, toothed, densely fulvous-tomentose hair, tube with 2 glands without. Petals funnel form, bilabiate, showy, fragrant, brownish yellow without and bright yellow within, oblique funnel form at throat. Stamens are didynamous, filament stout, yellow. Style slender, stigma bifid. These characters agreed with Hundley, H.G & Chit Ko Ko. 1961; Lawrence, 1969; Dassanayake, 1983; Burmmitt, 1992; Hu Qi-ming, 2009.

Leaves paste is applied to relieve headache and juice is used wash for ulcers and sunburn. Leaves juice are treat to leprosy and blood disorders. A juice made from the leaves is used to treat cough, diabetes, hypertension and skin diseases. Leaves juice are treat to leprosy and blood disorders. Young leaves juice to cure diabetes.

According to Navreet, 2018, leaves are used for treatment of headache and stomach ulcers, leprosy, hypertension, fruits are used as diuretic and for treatment of anaemia, leprosy and sexual debility in males.

The powdered leaf of *Gmelina arborea* Roxb was greenish brown in colour, the odour was pungent and taste was tasteless and the fracture fibrous was investigated.

The physicochemical properties showed that the solubility of the powdered samples was more soluble in ethanol, distilled water and methanol than other solvents.

Manasa *et al,* 2017, described that ethanol soluble extractive value 12.14%, alcohol soluble extractive value 1.14%, and water-soluble extractive value 11.03%. Shukla, 2010 *et al,* stated that root and leaves of *Gmelina arborea* were investigated and the methanolic extract and ethyl acetate fraction (4.0%, 5.5%) were used for evaluating the pharmacological activity.

Phytochemicals are non-nutritive compounds (secondary metabolites) that contribute to plants immunity, flavour and colour. In a general definition, they are the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Gibson,1998, Mathai, 2000).

In this study, phytochemical tests demonstrated the absence of steroid and glycosides, phenol, polyphenol, saponin, reducing sugar, terpenoid, alkaloid, flavonoid, tannin were present. Leaves of *Gmelina arborea* have phenolic compounds such as flavonoids contribute to increase plasma antioxidant capacity, decreased oxidative stress makers and reduced total and LDL cholesterol. It has potential to therapeutic effects in diabetes induced diabetic rats (Kalaivani, 2014).

In this study, the highest antimicrobial activity of pet ether, water and methanol extract was observed on *Xanthomonas oryzae, Pseudomonas fluorescens and Aspergillus flavous.* Deepthi *et al,* 2015, Walter, 2017 stated that study of leaf extracts showed significant activity against *E coli, Candida albicans, Xanthomonas oryzae, Klebsiella pneumoniae, Pseudomonous dysmetria* and *Salmonella typhi.*

Sevilla, 1999 stated that *Gmelina arborea* are non-toxic concentrations of tannic acid and coumarin as indicated by normal haematological values among sheep fed. All over the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases. Minerals are of critical importance in the diet, major minerals are those required in amounts greater than 150 mg per day and they represent 1% or less of body weight (Brook and Caldwell, 1954).

These include calcium, potassium, phosphorous, sulphur, chloride and magnesium. Essential trace elements are zinc, iron, copper, fluoride, iodine, chromium. The body required these minerals and vitamins for vital processing (Brooks and Caldwell, 1954).

According to EDXRF elemental analysis, Calcium (Ca), Potassium (K), Sulphur (S) and Iron (Fe) are found in powdered leaves. Percentage of calcium and potassium are higher than other elements.

Julian 1979, stated that nitrogen, phosphorous, zinc and boron level decreased and calcium and iron levels increased in the leaf.

Mineral matter is another essential class of food. Calcium is the most abundant mineral in the body. Ninety- nine percent of the calcium of the body is in the bones and teeth in the form of

chemical compounds which also contain phosphorus. The remaining one percent of this mineral is found in the blood stream and in the soft tissues. Calcium helps to maintain the normal functions of the nerves and muscles, including the regular beating of the heart. This mineral is necessary in the prevention of rickets. Approximately 99 percent of the body calcium is stored in the bones and teeth.

Potassium ions are necessary for the function of all living cells, Potassium ions shifts across nerve cell membranes are necessary for normal nerve transmission: potassium depletion or excess can result in numerous abnormalities, including an abnormal heart rhythm and various electrocardiographic (ECG) abnormalities (Kipping, 1951).

Since this plant is claimed to be useful in the treatment of rheumatism, it is said to possess anti-inflammatory action (Craig, 2009).

It was concluded that the selected plant possesses various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance. Continuous research and studies of potential herbs and medicinal plants are important as natural products from plant origin will continue to be in demand.

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I wish to express my sincere thanks to Dr San San Mar, Rector and Dr. Myat Nyunt, Pro-Rector, Kyaing Tong University, for their encouragement and permission to this paper. I would like to express my deepest gratitude to Dr Moe Moe Lwin, Professor and Head, Department of Botany, for her permission to carry out this study.

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NUTRITIONAL VALUE OF RED ALGAE *GRACILARIA* FROM SOUTHERN RAKHINE STATE

Tin Tin Maw[1], Ah Nge Htwe[2], Thida Oo[3], Zin Moe Moe[4], Ohn Maung[5]

Abstract

Morphological characters, nutritional value and elements composition of *Gracilaria* were studied. The red algae *Gracilaria* wre collected from Ngapali and Kyauk Phyu in the Southern Rakhine State during 2019. Algae are used as food in different parts of the world. In Rakhine State, a few kinds of marine algae have been harvested for food and algal salad is one of the traditional diet for local people. There is no scientific documents concerning with the edible algae. Edible red algae *Gracilaria* species were generally found in winter season and summer months. In the present research, morphological characters of *Gracilaria* were studied and elements composition and nutritional value were determined. Several constituents of nutritional value are present in red algae *Gracilaria* such as protein 16.57 %, carbohydrate 47.26 %, fiber 5.99 %, fat 0.23 %, ash 14.83 %, moisture 15.12 % and energy value 258 Kcal. Elements composition are Ca 19.24 %, K 23.20 %, S 27.70 %, Si 19.87 % and Fe 7.75 % respectively.

Keywords: *Gracilaria*, nutritional value, marine algae

Introduction

Seaweeds are generally classified into four main groups, largely on the basis of their structure and pigmentation: red algae (division Rhodophyta), brown algae (division Phaeophyta), green algae (division Chlorphyta) and blue green algae (division Cyanophyta). Red seaweeds show a variety of colours, from pink to purple and black. Red and brown algae are usually associated with marine environment, often rocky shores. Many species occur in temperate to tropical water, among which are several of considerable commercial interest. (Reine and Trono 2002)

Since ancient times, seaweeds are a dried food source for humans. In China and Japan, more than 70 species of marine algae are consumed. There is an almost infinite variety of health-care products available commercially lotions, shampoos and soaps, for skin production; against myocardial infarction, diabetes, rheumatism and as a source of vitamins and minerals (Wolfram Braune 2011).

In Myanmar, a total of 307 species of the tropical marine algae dominate along the costal regions i.e the Rakhine costal region, Ayeyarwaddy and the gulf of Mottama costal region and the Tanintharyi costal region (Kyaw Soe and Kyi Kyi Win 1977). Among the benthic marine algae, the Rhodophyta, with more than 42 orders, contains the greatest number of species. Currently, more than 6500 species of red algae are known, but actually may be more than 12000 species. (Norris 2014). Red algae *Gracilaria* is one of the genus in the family Gracilariaceae with more than 100 species worldwide, inhabiting temperate and tropical sea water, covering from intertidal to subtidal areas. *Gracilaria* is used as a food in Japan, Hawaii.

In Myanmar, the genus *Gracilaria* known as Kyauk-kyaw in local name, grown commonly along the Rakhine and Tanintharyi costal region and 15 species of *Gracilaria* were reported (Min Thein and Aung Myint 1977). In the present research, morphological characters, uses, elements composition and nutritional value of edible red algae *Gracilaria* were studied. The aim and

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objective in this research are to know uses and morphological characters of *Gracilaria* and to analyze elements composition and to analyze nutritional value of *Gracilaria.*

Materials and Methods

Edible red algae *Gracilaria* were studied for nutritional value, elements composition and morphological characters. *Gracilaria* were collected from Ngapali (Lat. 18°37' N, Long. 94° 33' E) and Kyauk Phyu (Lat. 19° 15' N, Long. 93° 61' E) in Southern Rakhine State of Myanmar from 2018 to 2019. Ngapali and Kyauk Phyu costal region consists of larges beach and some rocky area.

The location were measured by GPS (Global Positioning System). In collection field, temperature and p^H of collected water were measured by thermometer and p^H meter. The fresh specimens of *Gracilaria* were collected during low tide and wash with water to remove the adhering materials such as sand particles, rocks, shell, mud and other debris. Morphological characters of fresh specimens were identified and record with the photograph by digital camera. Identification of specimens were made by mainly base on the following taxonomic references; Kyaw Soe (1977), Soe Htun (1984), Kyi Win (1972), Norris (2014). Photogeography and potential uses of these algae were acknowledged from the worldwide literature records.

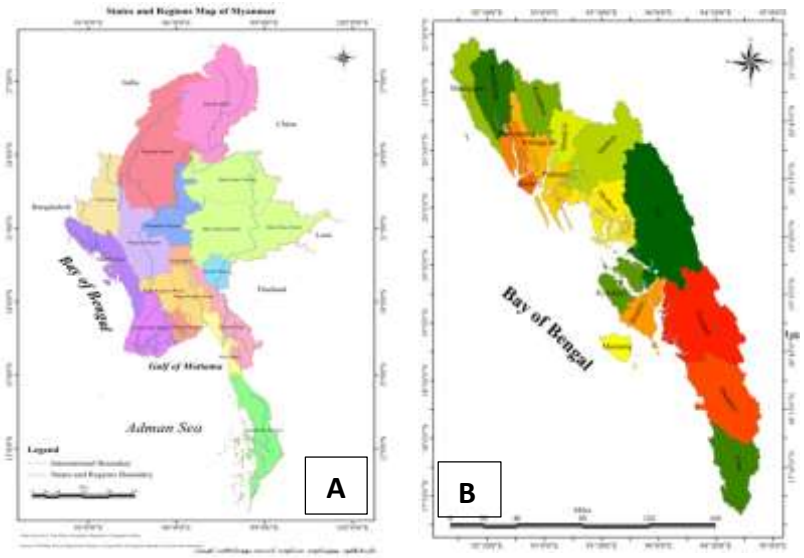


Figure 1 Map showing the collection sites of sample and study areas
A. Location map of Rakhine State in Myanmar
B. Location map of Township in Rakhine State

Determination of nutritional value and elements composition

The fresh *Gracilaria* were collected and dried in room temperature. Dried *Gracilaria* were ground into powder for determination of elements composition and nutritional value. The analysis of element composition were carried out at Department of Chemistry, Monywa University. All the determination of nutritional value were carried according to laboratory procedures at Myanmar Food Processors and Exporters Association (MFPEA), Yangon Region.

Protein content of *Gracilaria* were determined by Kjeldahl method. Ash content was obtained by burning it and were calculated combustion, Muffle Furnace method. Moisture content were estimate by Moisture Analyzer. Determination of water soluble carbohydrate by Phenol-

Sulphuric Colorimetric method was made and determination of crude fat was done by Buchi Soxhlet Extraction method.

Results

Gracilaria are red algae (sea weed). It grow abundantly on rock, coral, mangrove root and on intertidal mud flats. *Gracilaria* species were collected from Ngapali and Kyauk Phyu in southern Rakhine State. In Ngapali beach, the water temperature was 25°C and pH was 7.8 and water temperature of Kyauk Phyu was 23°C, pH was 7.4 respectively.

In this result, 3 speices of *Gracilaria* were found in Ngapali and Kyauk Phyu, namely *G. crassa* Harvey; *G. edulis* (J. Ag) Silva and *G. follifera* (Forssk.) Borgesen, *Gracilaria* species were collected by costal communities for food used either as a salad after blanching with hot water or a source of homeprepared agar. *Gracilaria edulis* (J. Ag) Silva described morphological characters.

Morphological Characters of *Gracilaria edulis* (J. Ag) Silva.

Division - Rhodophyta

Class - Florideophyceae

Order - Gracilariales

Family - Gracilariaceae

Scientific Name - *Gracilaria edulis* (J. Ag) Silva

Myanmar Name - Kyauk-Kyaw

Thalli up to 20 cm tall, brownish red, each arising from a discoid hold fast, branching dense and fastigiated, divaricate, dichotomus to trichotomous, up to 7 orders and with long branch intervals; branches 1-1.5 mm in diameter, cartilaginous, flexuous, with or without a constriction at their base or with only a slight constriction, cylindrical, ending in pointed apies.

Figure 2 Habit of *Gracilaria edulis* (J. Ag) Silva.

Morphological Characters of *Gracilaria crassa* Harvey.

Division - Rhodophyta

Class - Florideophyceae

Order - Gracilariales

Family - Gracilariaceae

Scientific Name - *Gracilaria crassa* Harvey.

Myanmar Name - Kyauk-Kyaw

Figure 2 Habit of *Gracilaria crassa* Harvey.

Thalli more than 30 cm tall, green red, coarse, cartilaginous, with disc-shaped holdfast; frond cylindrical; branching at short intervals, profusely or more scarcely irregular; branches 4-7 mm in diameter, thick, succulent, branch based of only the long branches constricted.

Morphological Characters of *Gracilaria follifera* (Forssk) Borgese.

Division - Rhodophyta
Class - Florideophyceae
Order - Gracilariales
Family - Gracilariaceae
Scientific Name - *Gracilaria follifera* (Forssk) Borgese.
Myanmar Name - Kyauk-Khat

Figure 2. Habit of *Gracilaria follifera* (Forssk) Borgese.

Thalli usually bushy, more than 5 cm tall, dark red or purple red, arising from a small discoid base, regularly dichotomously branched with entire margins; ranch 2-15 mm wide, divaricate, dictotomus to trichotomous, flattened, fleshy to cartilaginous.

Preparation of *Gracilaria* algal salad

Edible red algae *Gracilaria* (seaweed) are maintly harvested from natural habitats. Harvested plant of *G. edulis* should be cleaned and dried packed in bags and stored in dry place. Dried and fresh *Gracilaria* were sold in the local market. Dried and fresh form of *Gracilaria* were used as vegetable and home-made agar. That are eaten in the form of algal salad.

To prepare algal salad, *Gracilaria* were washed well with water and blanching with hot water. The juice from lemon were squeezed and poured into *Gracilaria* and then these *Gracilaria* with salt, pea nut oil, green onion, garlic and chilli are also mixed with it. After, that are eaten in the form of algal salad were shown in figure 3.

(a) Dry *Gracilaria*

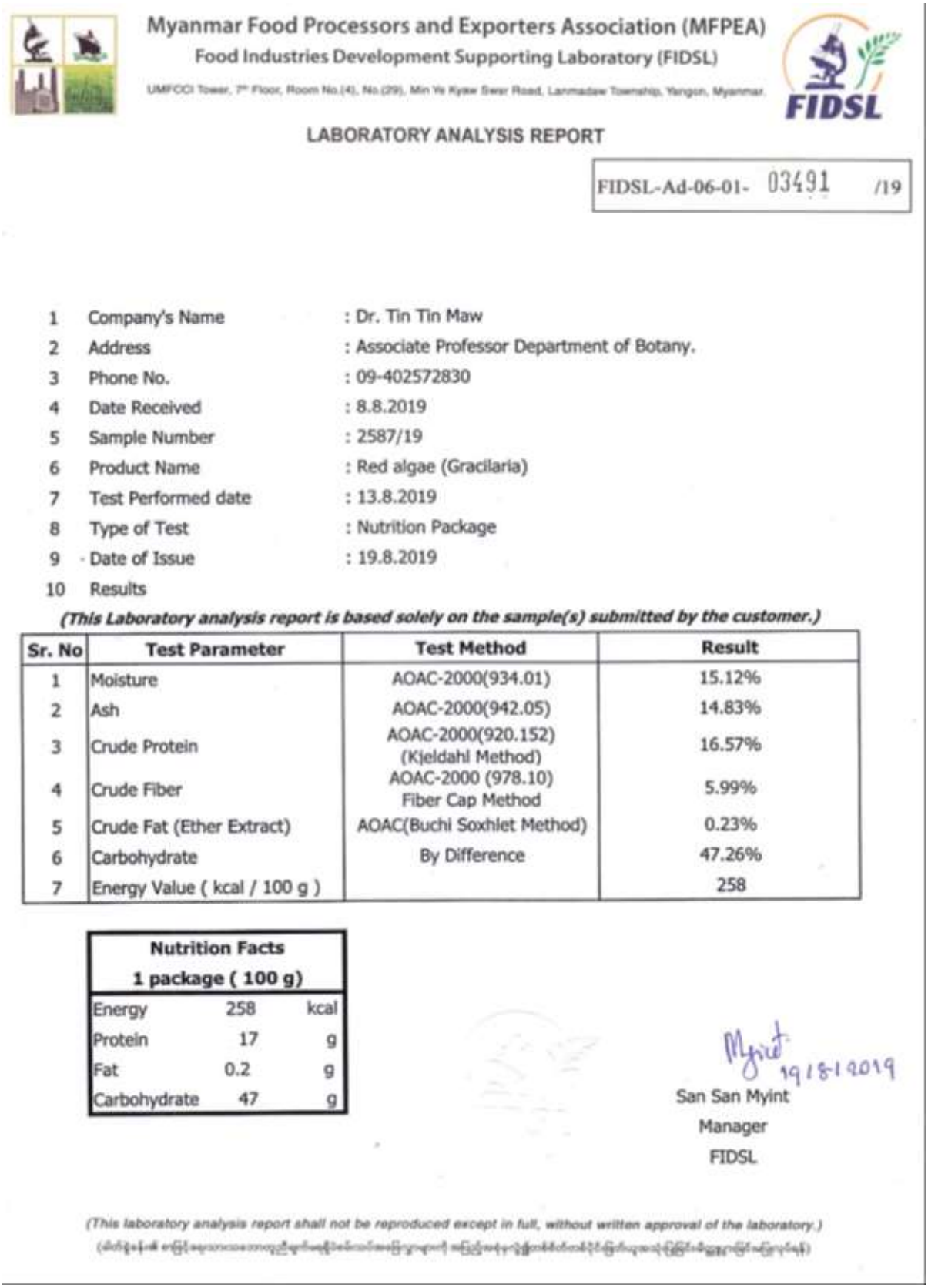
(b) Fresh *Gracilaria*

(c) *Gracilaria* algal salad

Figure 3 Preparation of *Gracilaria* algal salad

Nutritional value and elements composition of *Gracilaria*

Nutritional value analysis of *Gracilaria* include percentage of protein, carbohydrate, lipid, ash, moisture, fiber and energy value were shown in figure 4.



Myanmar Food Processors and Exporters Association (MFPEA)
Food Industries Development Supporting Laboratory (FIDSL)
UMFCCI Tower, 7th Floor, Room No.(4), No.(29), Min Ye Kyaw Swar Road, Lanmadaw Township, Yangon, Myanmar.
FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-01- 03491 /19

1	Company's Name	: Dr. Tin Tin Maw
2	Address	: Associate Professor Department of Botany.
3	Phone No.	: 09-402572830
4	Date Received	: 8.8.2019
5	Sample Number	: 2587/19
6	Product Name	: Red algae (Gracilaria)
7	Test Performed date	: 13.8.2019
8	Type of Test	: Nutrition Package
9	Date of Issue	: 19.8.2019
10	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr. No	Test Parameter	Test Method	Result
1	Moisture	AOAC-2000(934.01)	15.12%
2	Ash	AOAC-2000(942.05)	14.83%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	16.57%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	5.99%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	0.23%
6	Carbohydrate	By Difference	47.26%
7	Energy Value (kcal / 100 g)		258

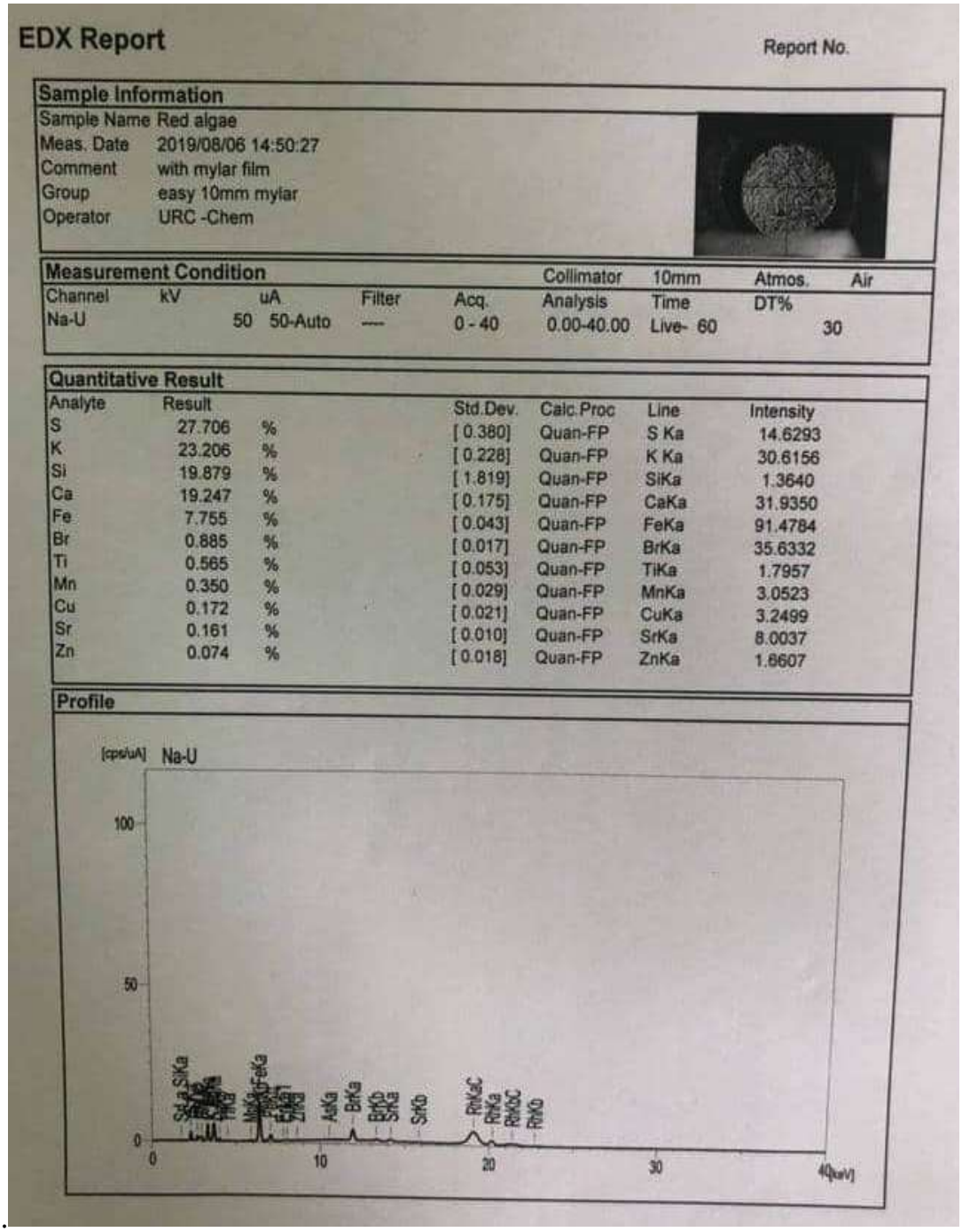
Nutrition Facts 1 package (100 g)		
Energy	258	kcal
Protein	17	g
Fat	0.2	g
Carbohydrate	47	g

19/8/2019
San San Myint
Manager
FIDSL

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)

Figure 4 Nutritional value analysis report of *Gracilaria*

Elements composition of *Gracilaria* were shown in Figure 4.



EDX Report

Report No.

Sample Information

Sample Name	Red algae
Meas. Date	2019/08/06 14:50:27
Comment	with mylar film
Group	easy 10mm mylar
Operator	URC -Chem

Measurement Condition

Channel	kV	uA	Filter	Acq.	Collimator	10mm	Atmos.	Air
Na-U	50	50-Auto	----	0 - 40	Analysis	0.00-40.00	Time	Live- 60
							DT%	30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
S	27.706	%	[0.380]	Quan-FP	S Ka	14.6293
K	23.206	%	[0.228]	Quan-FP	K Ka	30.6156
Si	19.879	%	[1.819]	Quan-FP	SiKa	1.3640
Ca	19.247	%	[0.175]	Quan-FP	CaKa	31.9350
Fe	7.755	%	[0.043]	Quan-FP	FeKa	91.4784
Br	0.885	%	[0.017]	Quan-FP	BrKa	35.6332
Ti	0.565	%	[0.053]	Quan-FP	TiKa	1.7957
Mn	0.350	%	[0.029]	Quan-FP	MnKa	3.0523
Cu	0.172	%	[0.021]	Quan-FP	CuKa	3.2499
Sr	0.161	%	[0.010]	Quan-FP	SrKa	8.0037
Zn	0.074	%	[0.018]	Quan-FP	ZnKa	1.8607

Profile

Figure 5 The EDX report of *Gracilaria*

Discussion and Conclusion

In the present study, the three species of *Gracilaria* were found in study areas namely *G. crassa* Harvey., *G. edulis* (J. Ag.) Silva. and *G. follifera* (Forssk.) Borgesene. The species of *Gracilaria* were eaten in Southern Rakhine state. Morphological characters of *Gracilaria edulis* (J.Ag) Silva reported in this research that are thalli up to 20 cm tall, brownish red, each arising from a discoid hold fast, branching dense and fastigiate, divariacate, dichotomus to trichotomous, up to 7 orders and with long branch intervals. Reine and Trono (2002) reported that taxonomic description of red algae *Gracilaria.* Norris (2014) described that traxonomy and nomenclature of

red algae species. Braune (2011) stated that morphological character, distribution and uses of *Gracilaria.*

In this results, edible *Gracilaria* species were harvested by native people as food in southern Rakhine State. *Gracilaria* are eaten in the form of algal salad and home-made agar. Nutritional value and element composition of red algae *Gracilaria* were emphasized. The nutritional value contents of *Gracilaria* are protein 16.57 %, carbohydrate 47.26 %, fiber 5.99 %, ash 14.83 %, moisture 15.12%, fat 0.23 % and energy value 258 Kcal. Moreover Ca 19.2 %, S 27.70 %, Fe 7.75 % and other elements are also present.

Than Nyunt and Hla Hla Cho (1975) reported that the extraction of agar from some species *Gracilaria.* Kyaw Soe (1977) stated that *Gracilaria* species were used as food and agar extraction. Reine and Trono (2002) described that uses, nutritional value and chemical products of marine macroalgae. They reported that algae are used as vegetable, medicinal uses, animal feed and producers of phycocolloids.

Arasaki (1983) reported nutritional value and elements composition of sea vegetables. He stated that nutritional values of red seawed *Gracilaria* sp. were protein 7.9 %, fat 0.05 %, carbohydrate 58.4 %, fiber 3.0 % and ash 17.8 % respectively. Moreover, sea vegetables contain more minerals than other kind of food. An extremely wide range of minerals accounts for from 7 to 38 % required by human beings, including calcium, sodium, magnesium, potassium, phosphorus, iodine, iron, and zinc are present in sufficient amounts. The high amount of calcium in sea vegetables, if all nutritionally effective, would make them the richest food after milk. The iron content of sea vegetables in from two to more than ten times that of egg yolks and spinach. Brown algae are very high in iodine content. Iodine 0.1 to 0.2 mg required by a normal adult or 0.2 mg needed by children and pregnant women.

Marine algae have been used as food not only in Myanmar but also in other countries. In this result, local people especially Taunggoke, Ngapali, Thandwe, and Kyauk Phyu from Southern Rakhine State are used red algae *Gracilaria* as seasonal food to prepare algal salad and home made agar. *Gracilaria* species are considered to be an important source for diet and food additive because it high content of protein, carbohydrate and minerals. These experimental results may be useful for local people who are collecting and selling the dried *Gracilaria* in the local markets. Moreover, agar can be extracted from the *Gracilaria* spp. (Than Nyunt and Hla Hla Cho 1975). Due to a lack of technology for mass production of agar form *Gracilaria* sp; there is no agar industry in Myanmar. Natural beds of *Gracilaria* remain unexploited and the only seaweed pilot-form of *Gracilaria edulis* in Maung Shwe lay Gyaing, Thandwe, in the Rakhine state, stopped successful production, due to lack of demand for an agar industry by local users (Soe Htun 1998).

To fiulfill the domestic demands for agar, collaboration between scientists and potential investors are required to establish a viable agar industry in Myanmar. However, it is expected that the abundance of natural beds of *Gracilaria* will lend support to the development of modern alginate factories in our country. The study of this research will inform the nutritional value and elements composition of *Gracilaria* for researchers. Further investigation should be made other edible marine algae for the purpose of mass production and commercial scale.

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ETHNOMEDICINAL STUDY OF SOME EDIBLE PLANTS IN KALWIN VILLAGE, MYEIK TOWNSHIP

Yadana[1], Htay Htay Lwin[2], Aye Aye Aung[3]

Abstract

In this paper conducts the ethnobotanical study of some edible plants in Kalwin village, Myeik Distric, Tanintharyi Region. In this study, twenty seven species belonging to fifteen families were collected from Kalwin village near Myeik University during during June to October 2019. Local people used these plans not only for food but also medicinal for their daily life. Ethnobotanical methods using structured interview were employed and collected data have been analyzed by using quantitative methods of data analysis. Different plants parts are used to cure different diseases as home remedy. These plants species are used in study area to cure various diseases like cough, headache, boils, fever, diarrhea and external ulcers etc. Score given to the species according to preference ranking for treatment of cough, ulcer, food poisoning and diarrhea were calculated. Used value, ranking, informant consensus factor (ICF) and fidelity level (FL) of twenty seven species were also computed.

Keywords: Quantitative methods, Ethnobotany, Used value, Informant consensus factor, Fidelity level

Introduction

Plants have traditionally been used as a source of medicine long time ago to control various ailments afflicting human. The knowledge of usage plants have been orally transferred from elder to younger. Today 80% of the world's population rely dominantly on plants and plant extract for health care.

Ethnomedicine refers to the study of traditional medicinal practice which concern valuable information and relationship between plants and people. Ethnomedicine even today plays an important role in rural areas and a lot of locally produced drugs are still used as household remedies for various types of illness. The ethnomedicinal data include the actual sources of ethnobotanical data, i.e. the interviewer and interviewee: plant used for medicine, plant part and preparation for use.

Edible plants are part of plants that are eaten by humans. Edible plants in the kitchen are not only for cooking but they also have enough medicinal properties. Woman of the household was well versed in the use of edible plants for treat illness. There is much overlap between medicinal and edible plants. Many plants used as food for local people are also used as therapeutic system. Let food be medicine and medicine be the food. Those are famous words from the ancient Greek physician Hippocrates, often called the father of Western medicine.

In this paper ethnomedicinal uses of 27 edible plant species belonging to 15 families were under taken. The aim of this paper is to know the outstanding characters of eighteen edible plant species and their ethnomedicinal uses by qualitative and quantitative approaches.

Aim and Objectives

- To investigate ethnomedicinal utilization of some edible plants in Kalwin village, Myeik township
- To know most effective medicinal plants used by the community to treat the diseases

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Materials and Methods

Data collection and identification

The data were collected from Kalwin village during June to October 2019. Ethnobotanical methods like structured interviews were used for qualitative and quantitative data analysis. The questionnaires include gender, age of respondent, local name of plant, part use, how to prepare for medicinal purpose. In this ethnobotanical study, total of 12 interviewees were selected randomly between the different ages of 50 to 75 years and they are workers, fishermen and housewife. The ethnomedicinal data include the actual sources of ethnobotanical data, i.e. the interviewer and interviewee: plant used for medicine, plant part and preparation for use.

Preference Ranking was done by using methods described by Martin (1995) to indicate most effective medicinal plants used by the community to treat the disease. Twelve key informants were selected to assess the degree of effectiveness of medicinal plants against the ailment. The medicinal plant believed to be most effective to treat the illness has got the highest value and the least effective got the lowest value. The value of each species was summed up and rank was determined based on the total score.

Used value (UV), developed by Phillips and Gentry (1994) is to provide a quantitative measurement for the relative importance of species. UV is based on the number of uses and number of people that cited a given plant, is used to indicate the species that are considered most important by a given population. UV is calculated by using the formula $UV = (\sum Ui)/n$, where Ui is the number of use-reports cited by each informant for a given species, and n is the total number of informants. UV is high when there are many use report for a plant, implying that the plant is important, and low approach to zero when there are few use reports.

Informant Consensus Factor (ICF) was used to analyzed the agreement degree of the informants' plant knowledge about each category. This quantitative method is based on the classic paper by Trotter and Logan (1986) who introduced the informant agreement ratio (IAR), which has come to be called as Informant Consensus Factor (ICF). ICF is calculated using the following formula: $ICF = (Nur – Nt)/ (nur – 1)$, where Nur is the number of use report of informants in each category, and Nt is the number of the taxa used for a particular category. High ICF values (approach to 1.00) are obtained when only one or a few plant species are reported to be used by a high proportion of informants for a particular category, whereas low ICF values indicate that informants disagree over which plant to used.

Fidelity Level (FL) is calculated by Friedman *et al*. (1986) using formula of $FL(\%) = (Np/n) \times 100$, where Np is the number of informants who suggested the use of a plant for a particular purpose and n is the total number of informants. High FL values are obtained for plants for which almost all use mentions refer to same purpose, that the plants are most preferred and low FL obtained for plants are used for many different purposes.

Study Area

Myeik is saturated in Tanintharyi Region of southern part of Myanmar. It is located 12˙ 26′ N and 98˙ 37′ E elevation 15 meter above sea level. The town is closed to the sea, so the weather is neither too hot nor too cold. The area of Myeik is 7783 square miles. As of 2014, the estimated population was over seven lakh. They are workers, government servants, traders, business men and fishermen. Most of the people are Myanmar and Buddhist. Myeik is famous for its products pearl, rubber, edible bird's nest, dried fish, dried prawn and ngapi. Kalwin is a village located near Myeik University. Most of people in the study area are Myanmar Buddhist but they speak Myanmar language with distinctive accent.

Results

Table 1 List of edible plants used by villagers in study area

No.	Scientific name	Family	Part Use	Ethnomedicinal Uses
1.	*Acacia concinna* (Wild.)DC.	Fabaceae	Fresh leaves	Fever, Constipation
2.	*Allium cepa* L.	Amaryllidaceae	Bulb	Urinary disorder, Antidote for bee, Scorpion sting, Nail fungus
3.	*Allium sativum* L	Amaryllidaceae	Bulb	Colic
4.	*Ananas comosus* (L.) Merr.	Bromeliaceae	Fresh ripe fruits, Fresh leaves	Digestion, Vermifug, Food poisoning, Fever
5.	*Carica papaya* L.	Caricaceae	Fresh ripe fruits, Latex, Fresh leaves	Mild laxative, Diabetes, Hypertension, Eczema, Antidote
6.	*Cassia tora* L.	Fabaceae	Leaves, Root	Insomnia, Food poisoning
7.	*Citrus aurantiifolia* L.	Rutaceae	Fruits	Dizzy, Fungus infection, Cough
8.	*Citrus hystrix* D.C.	Rutaceae	Fresh fruits	Antidandruff, Paralysis
9.	*Cocos nucifera* L.	Arecaceae	Oil	Hair tonic , Heat burn, Skin ulcer, Diarrhea, Sunburn,
10.	*Curcuma longa* L.	Zingiberaceae	Dried rhizome powder	Stomach ache, Colic, Astringent, Antidiarrheal drug, Knee pain, Backaches, colic, Diabetic
11.	*Colocasia esculenta* (L.) Scott.	Araceae	Leaves	Painful sore, Joints swelling pain
12.	*Dregea volubilis* Benth.	Apocynaceae	Leaves	Vermifuge,
13.	*Gnetum gnemon* L.	Gentaceae	Leaves	Antidote, Burn
14.	*Hibiscus sabdariffa* L.	Malvaceae	Leaves	Ulcer
15.	*Ipomoea aquatic* Forsk.	Convolvulaceae	Young leaves	food poisoning, Dysentery,
16.	*Morinda citrifolia* L.	Rubiaceae	Fresh ripe fruits, Fresh leaves	Cough, Asthma, Muscle pain, Cough, Constipation
17.	*Musa spp.*	Musaceae	Young Fruit, Sap	Hair tonic, Lactogenic food, Headache, Wounds,
18.	*Nipa fruticans* (Thumb.) Wurmb.	Arecaceae	Fermented Liquid	Food poisoning, Burn, Asthma, Mumps,
19.	*Ocimum basilicum* L.	Labiateae	Fresh leaves, Dry seeds	Cough, Dysentery
20.	*Piper betle* L.	Piperaceae	Fresh Leaves	Ulcers, Cough, Fever
21.	*Piper nigrum* L.	Piperaceae	Dry seeds	Milk supply, Malaria, Relief pains
22.	*Psidium guajava* L.	Myrtaceae	Fresh Bark, Fresh leaves	Dizziness, Bad breath, Toothache, Diarrhea
23.	*Sandoricum koetjape* (Burm.f.)Merr.	Meliaceae	Fresh Bark, Fruit skin	Toothache, Bedsore, Chickenpox, Prickly Heat, Wounds, Diarrhea,
24.	*Sauropus albicans* Burm.	Phyllanthaceae	Fresh leaves	Thrush, Asthma,
25.	*Terminalia catappa* L.	Combretaceae	Fresh leaves	Dysentery, Diarrhea,
26.	*Tamarindus indica* L.	Caesalpiniaceae	Fresh Bark, Fruit, Seed	Sun cover , Prickly heat, Aperient, Antidote
27.	*Zingiber officinale* L.	Zingiberaceae	Fresh rhizome	Cough, Knee pain, Motion sickness, Tincture ,Fever

Table 2 Medicinal knowledge and gender

Respondents	Male/Female	Score for Male	Score for Female
R1	F		15
R2	M	13	
R3	F		14
R4	F		13
R5	M	12	
R6	M	14	
R7	F		12
R8	M	11	
	Total Score	**50**	**54**

Comparison of knowledge of medicinal plants between men and women

Generally medicinal knowledge is a gender base practice both man and women perform in this practice. It was found that among eight respondents interviewed 50% were women and 50% of men show in Table 2. The source of medicinal plant knowledge is different between women and men. From the table total score for men is 50 and for women is 54 respectively. From this result women have more significant medicinal knowledge than men in study area of Kalwin village.

Table 3 Preference ranking values of five medicinal plants for cough treatment in study area

Respondant	R1	R2	R3	R4	R5	Total Score	Ranking
Citrus aurantiifolia L.	5	2	5	4	1	17	2nd
Morinda citrifolia L.	2	1	1	3	1	8	4th
Ocimum basilicum L.	1	3	1	1	1	7	5th
Piper betle L.	5	4	4	5	5	23	1St
Zingiber officinale L.	4	5	1	1	1	12	3rd

Rank of five medicinal plants used to treat cough

All the five popular species mention above are popular in curing cough. Simple preference ranking exercise conducted on six plants for treatment of cough in study area was listed in Table 3. According to the information *Piper betle* L. was listed as most significantly used medicinal plant that local people want to cure cough. Most local people cultivate the *Piper betle* L. as cash crop in their home garden. The second rank of plant species was *Citrus aurantiifolia* L. and *Zingiber officinale* L. got third rank and these are medicine from the kitchen. The fourth rank of plant species for curing cough is *Morinda citrifolia* L. it is used not only for medicine but also used as vegetable. The last one is *Ocimum basilicum* L. it is popular spice among local people and cultivates for their income.

Table 4 Ranking of five medicinal plants used to treat external ulcer

Plant Name	Respondents						Scores	Rank
	R1	R2	R3	R4	R5	R6		
Cocos nucifera L.	4	3	3	4	5	4	23	2nd
Colocasia esculenta Scott.	2	5	2	3	2	1	15	4th
Hibiscus sabdariffa L.	3	2	4	2	3	3	17	3rd
Musa spp.,	1	1	1	1	1	2	7	5th
Sandoricum koetjape Merr.	5	4	5	5	4	5	28	1st

Rank of five medicinal plants used to treat external ulcer

Five species are valued for their medicinal effect in healing external ulcers. According to the information, *Sandoricum koetjape* Merr. was listed as most significantly used medicinal plant that local people want to cure external ulcers. The bark is grind with rice water to treat bedsore, chickenpox, measles, prickly heat and wounds. The second rank of plant species was *Cocos nucifera* L. Coconut oil mixed with *Curcuma* powder is an effective natural remedy for healing the skin ulcer. *Hibiscus sabdariffa* L. was third rank and crush fresh leaves mixed with cooked rice and apply on the ulcer. *Colocasia esculenta* Scott. leaf was fourth rank. Ash of banana used for wound was fifth rank described in (table 4).

Table 5 Ranking of four species for food poisoning

Plant Name	Respondents						Scores	Rank
	R1	R2	R3	R4	R5	R6		
Ananas comosus Merr.	2	3	4	2	3	3	17	2nd
Cassia tora L.	1	2	2	3	1	2	11	3rd
Ipomoea aquatic Frosk.	4	4	3	4	4	4	23	1st
Nipa frutican Wurmb.	3	1	1	1	2	1	9	4th

Rank of plant species for food poisoning

Local people used four species for curing food poisoning table 4. Juice from leaves of *Ipomoea aquatic* Forsk. was first rank and *Ananas comosus* Merr. was second rank for food poisoning. Drinking root paste of *Cassia tora* L. with rice water can relieve food poisoning was third rank. Local people used fermented *Nipa frutican* Wurmb. as vomiting agent when food poisoning was fourth rank were shown in Table 5.

Table 6 Preference ranking values of herbal remedy for diarrhea

Plant Name	Respondents						Scores	Rank
	R1	R2	R3	R4	R5	R6		
*Cocos nucifera*L.	4	2	3	3	3	3	18	1st
Psidium guajava L.	1	3	2	4	1	4	15	2nd
Terminalia catappa L.	2	1	4	2	4	2	15	2nd
Sandoricum koetjape Merr.	3	4	1	1	2	1	12	3rd

Preference ranking values of herbal remedy for diarrhea

Plants species which were listed as remedy for diarrhea by the local people are listed in Table 5 in their order of preference. *Cocos nucifera* L. was first rank to help the diarrhea. Treat the diarrhea *Psidium guajava* L. fresh leaves juice mix with sugar was second rank. Herbal tea made from leaves or juice from of *Terminalia catappa* L. was also second rank. Eating dried fruit skin *Sandoricum koetjape* Merr. was third rank.

Table 7 Use value, ranking and fidelity level of plants

No.	Scientific Name	Use Value	Ranking	FL%
1.	*Acacia concinna* (Wild.)DC.	0.78	2	78.21
2.	*Allium cepa* L.	0.35	3	38.67
3.	*Allium sativum* L	0.33	3	40.21
4.	*Ananas comosus* (L.) Merr.	0.86	2	70.14
5.	*Carica papaya* L.	1.53	1	90.21
6.	*Cassia tora* L.	0.45	3	60.32
7.	*Citrus aurantiifolia* L.	1.23	1	89.00
8.	*Citrus hystrix* D.C.	1.00	1	92.00
9.	*Cocos nucifera* L.	0.69	2	75.00
10.	*Curcuma longa* L.	2.30	1	97.34
11.	*Colocasia esculenta* (L.) Scott.	0.23	3	34.21
12.	*Dregea volubilis* Benth.	0.34	3	14.12
13.	*Gnetum gnemon* L.	0.39	3	45.56
14.	*Hibiscus sabdariffa* L.	0.34	3	21.43
15.	*Ipomoea aquatic* Forsk.	0.48	3	65.23
16.	*Morinda citrifolia* L.	1.22	1	90.17
17.	*Musa spp.*	0.83	2	77.47
18.	*Nipa fruticans* (Thumb.) Wurmb.	0.76	2	75.83
19.	*Ocimum basilicum* L.	1.10	1	89.55
20.	*Piper betle* L.	3.2	1	94.78
21.	*Piper nigrum* L.	1.25	1	93.14
22.	*Psidium guajava* L.	0.62	2	70.21
23.	*Sandoricum koetjape* (Burm.f.) Merr.	0.73	2	73.44
24.	*Sauropus albicans* Burm.	0.88	2	74.80
25.	*Terminalia catappa* L.	0.03	3	67.21
26.	*Tamarindus indica* L.	1	1	94.00
27	*Zingiber officinale* L.	2.00	1	96.21

Use Value and Ranking of Plants species

The villagers used edible plants for medicinal purposes. The Uv value 1 and above was first group, between 0.5 and 0.9 was second group and less than 0.5 was third. The first group has ten species, highest UV value was observed in 2.30 *Curcuma longa* L. and followed by *Zingiber officinale* L. 2.00. The second group show eight species and Uv = 0.88 *Sauropus albicans* Burm. and *Ananas comosus* (L.) Merr. UV= 0.86 were high value.In the third group the lowest UV value was 0.03 *Terminalia catappa* L.

Fidelity Level (FL)

FL values for total of twenty seven species were shown in table. A high FL 80 to 100% can imply that the plants were most preferred as long as there is considerable number of used mention informants.

Table 8 Informant Consensus Factor (ICF)

Used categories	Number of used report (Nur)	Number of taxa	Informant Consensus Factor
cough	18	5	0.76
external ulcer	23	5	0.81
food poisoning	12	4	0.72
diarrhea	19	4	0.83

Informant Consensus Factor (ICF)

The CIF value of each of four categories was showed in table. CIF value range from 0.72 to 0.83 and the value 0.83 and 0.81 showed the informant agreed of using a single species for each category. Diarrhea and external ulcers showed the CIF value of 0.83 and 0.81 respectively. It indicated that the informants agreed of using a single species for each category. Five plant used report of eighteen was CIF value of 0.76 found in treatment of cough. Other category of CIF 0.72 for external ulcers the informants used four medicinal plants recorded twelve use report shown in table.

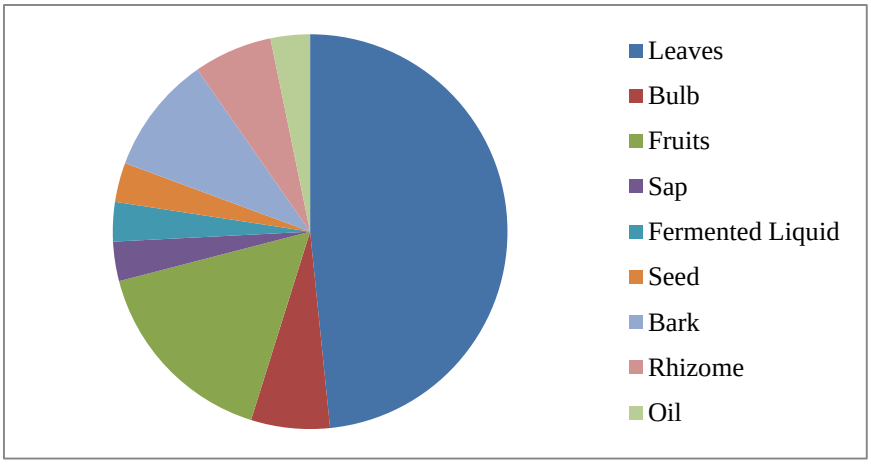

Figure 1 Medicinal plant part used by local people

Medicinal plant part used

Medicinal properties derived from plants can come from many different parts of plant including leaves, roots, bark, fruits, seeds and flowers etc. In this study 48% of edible plants leaves are used as medicine and that of 16% from the fruits. Bark 10% and other plant parts include the following: bulb and rhizome 6%, Seed, fermented liquid, and sap, 4% and oil 1% were shown in Figure 1. In study area the most frequently utilized medicinal plant parts were leaves. The reason why leaves used mostly is they are collected very easily than other parts.

Discussion

In this research, the morphological characters and ethnomedicinal uses of twenty seven species belonging to fifteen families were documented.

In this research, ethnomedicinal uses of twenty seven species belonging to fifteen families were documented. All these plants were cultivated in the home garden. As the informants mention during the study, women spend much time for garden duty. These are agreed with those of Zemede (2004). It was found that women have more significant medicinal knowledge than men in Kalwin village. Many respondents indicated that they used to preferred modern medicine primarily and

they would use herbal medicine if the modern medicine did not help. Some respondents said that they preferred herbal medicine than modern medicine because plants are safer and cause less harmful side effects. These are agreed with those described by Yu Yu Tin (2020).

Over all analysis showed that uses of plants for treatments of different ailments range from simple to fatal diseases. Five plants species were used for treatment of cough, among them *Piper betle* L. was listed as most significantly used. For the treatment of external ulcers, *Sandoricum koetjape* Merr. was most significant than other four species. Local people used four species for curing food poisoning, among them juice from leaves of *Ipomoea aquatic* Forssk. was first rank and *Ananas comosus* Merr. was second. Four species were valued for medicinal effect in diarrhea, among them *Cocos nucifera* L. was most significant.

There is no standardized measure on the dose for most of the ethnomedicine in the study area. The dose depends on the healer that prepares the herbs for medicinal purpose or it may also depend upon the disease severity. These are agreed with those reported by Mussarat *et.al.* (2014). Local people in study area used palm sugar, salt, honey, rice water, lime stone, cooked rice, indigo and sugar as medicinal ingredient for both external and oral. Most of ethnomedicines are prepared using single plant in the region while some others are prepared by mixing parts of more than one plant. These are agreed with those reported by Mussarat *et.al.* (2014).

Plants were cultivated in home gardens, so can be easily collected. Freshly harvested plant parts are used for traditional remedy preparation against various ailments. The uses of fresh plant materials for remedy preparations better than use of dried plant materials. These are agreed with those reported by Mussarat *et.al.* (2014).

Conclusion

In conclusion, edible plants are not only important for local food consumption but also income in the local community. Further investigation on nutritional value and pharmaceutical activities of edible plants will add more value to the traditional knowledge.

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EFFECTIVENESS OF ISOLATED NATIVE *AZOSPIRILLUM SPP* ON GERMINATION AND GROWTH PROPERTIES OF RICE

Zaw Lwin Oo[1], San Nyunt Nwe[2], Thanda Myat Mon[3]

Abstract

The present study deals with three *Azospirillum* strains (Azo-1, 2 and 3) were isolated from the root of *Saccharum spontaneum* L. (Kaing), *Saccharum officinarum* L. (Kyan), *Dichanthium caricosum* (L.) A. Camus. (Padaw ni) of Poaceae family during 2019. The growth responses of rice inoculation with indigenous isolated strains were studied. These experiments were carried out with 3 isolated strains from the roots of some grasses to know the germination percentage, shoot and root length. The inoculation of isolated strains increased in germination up to 4.12%, 16.25% in shoot length and 12.97% in root length over the control by isolated strain Azo-3. In pot culture, the inoculation of isolated strains increased in plant height (21.87%), leaf area (18.78%), tillering number (27.75%), panicle length (8.88%) and fertile seed per panicle (10.94%), and 1000 grain weight (5.28%) over the control by Azo-3. These results indicated that certain diazotrophs can promote vegetative growth and yield characters of rice.

Keywords: *Azospirillum*, isolate, inoculation, indigenous

Introduction

Plant nutrients, which come primarily from chemical fertilizer, are essential for crop production. In agriculture, nitrogen is an essential element for crop growth and development. Nitrogen is a basic constituent of chlorophyll, proteins and all enzymes are involved in photosynthesis, especially Rubisco which alone accounts for more than 75 % of the total leaf nitrogen (Hak *et al*., 1993).

Soil microorganisms, like *Azospirillum* spp., *Azotobacter* sp. and *Enterobacter* sp. have shown to encourage plant growth, by promoting the outbreak of secondary roots. Inoculation with indigenous *Azospirillum* is an important procedure when studying their inherent capacity to benefit crops. In some cases, indigenous strains can perform better than introduced strains in promoting the growth of crops due to their superior adaptability to the environment (Kanimozhi and Panneerselvam, 2011).

Azospirillum species are commonly found in soils and in association with roots of plants namely rice, maize, wheat and legumes. Rhizosphere colonization by *Azospirillum* species has been shown to stimulate the growth of a variety of plant species (Lopez-de-victoria. 1989).

Bio-fertilizers are substances which comprise of living microorganisms that stimulate the plant growth by increasing the supply or availability of primary nutrients to the plant and the synthesis of growth promoting substances. Hence, bio-fertilizers can be expected to reduce the use of chemical fertilizers (Keyeo *et al*., 2011).

Biofertilizer, an alternate low cost resource have gained prime importance in recent decades and play a vital role in maintaining long term soil fertility and sustainability. They are cost effective, eco-friendly and renewable sources of plant nutrients to supplement chemical fertilizers. Nitrogen fixing and P- solubilizing inoculants are important biofertilizers used in rice (Singh *et al*., 2015).

In Myanmar, a few works have been done on the effects of *Azospirillum* inoculation on growth of rice. The aims of the present study were to isolate *Azospirillum* spp. from native grasses,

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to obtain more knowledge on the useful microorganisms isolated from local area, to know the effects of *Azospirillum* spp. on germination and vegetative growth of rice plant under natural soil-climatic conditions and to develop the effective *Azospirillum* spp. inoculation and useful as biofertilizer.

Material and Methods

Plant Samples Collection

Plants and root samples of *Saccharum spontaneum* L. (Kaing), *Saccharum officinarum* L. (Kyan), *Dichanthium caricosum* (L.) A. Camus. (Padaw ni), were collected from Monywa University Campus, Monywa Township, Sagaing Region during 2019.

Culture Media and Solution

Azospirillum strains were cultured in the following nitrogen free semi-solid malate medium (NFb) and Congo Red Agar Medium (CRA) according to Dobereiner and Baldani (1980).

Isolation of *Azospirillum* strains

In the present, following procedure were used for the isolation *Azospirillum* strains from the roots of three grasses of Poaceae family. Fresh roots samples were washed in rapidly running tap water for 5 minutes to remove the soil particles adhering to the root surface. The roots were rinsed in sterile water and then these roots were cut into small pieces (3-5 mm) and placed into the test tube. Small amount of sterile water was added into the test tube. These small pieces were macerated with glass rod and 0.5 ml introduced into 10 ml semisolid NFb medium. These test tubes were incubated at 33˚C. After 72 hours, white halo pellicle formed 3-6 mm below the media surface. It was a sign of nitrogenase activity. When the cultures medium exhibited a positive nitrogenase activity, they streaked out on Congo Red Agar (CRA) plates. Typical pink, often wrinkled colonies were picked out and transferred into semi-solid NFb medium and transfer into CRA medium for purification. The isolated strains were designated as **Azo-**(***Azospirillum-***) only just for this research work.

Germination Test

The test crop rice seeds (Sin Ekari – 2) were obtained from the Myanmar Agriculture Service, Zalote Research Center, Monywa District in Sagaing Region. The seeds were surface sterilized by immersion in hydrogen peroxide (H_2O_2) for 30s. The seeds were rinsed for five times of distilled water and floating seeds were discarded. Then, the seeds were air dried over a clean filter paper. Sterilized seeds were placed into the bacterial culture medium for 1 hour and control are inoculated with only nutrient medium as shown in Figure 2 and 3. And then the seeds are placed on agar plate and germinated in dark at 30 °C for 120 hours. The percentage germination of rice on each plate was counted and then shoot and root lengths were measured and recorded. Average germination percentage, shoot and root length were calculated for each plate.

Pot culture experiment

The second set of experiments was designed to evaluate the carryout effects of seedling vigor on yield parameters at maturity during May to August, 2019, in pot culture. The pot experiment was structured following a randomized complete plot design. The isolated *Azospirillum* strains (Azo-1, Azo-2 and Azo-3) were used to treat tested crop. Potted soil was watered and puddle before planting. Representative, 22-days old similar sized seedlings of rice plants were transplanted in to pots (35 cm by 20 cm by 15 cm and two plants per pot) containing 7 kg of soil. At

transplanting, immerse rice roots into liquid innoculant for 10 - 15 min before transplanting and 25 ml of innoculant spread into each pot at regreening and flowering stages. Tap water was used to irrigate the potted plant. After harvesting, randomly 20 panicles were selected and measure the length of panicle and counted for total seed per panicle. Data were analysed by analysis of variance and the treatment mean were compared relative to control following the F-test on IRRISTAT.

Climatological Data

Climatological data (monthly mean maximum and minimum temperature, rainfall and humidity) were taken from Meteorology and Hydrology Department, Monywa Township in Sagaing Region during 2019.

Table 1 Monthly annual Temperature, rainfall and humidity of Monywa District during 2019

2019	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean
Maxi Temp: (°C)	29.5	33.4	35.9	40.2	41.9	37.3	36	34.9	34	34.3	33	29.3	34.98
Mini Temp: (°C)	14.1	16.7	19.3	24.2	26.1	26.4	25.6	25.7	25	23.4	21.3	15.5	21.94
Rainfall (mm)	3	Tr	Tr	15	39	169	35	114	99	16	26	Tr	516.00
Humidity (%)	70	66	54	52	53	70	70	78	80	80	75	71	68.25

Source: Department of Meteorology and Hydrology, Monywa District

Results

Isolated *Azospirillum* strains

All isolated strains (Azo-1, 2 and 3) were isolated from the root of *Saccharum spontaneum* L. (Kaing), *Saccharum officinarium* L. (Kyan) and *Dichanthium caricosum* (L.) A. Camus. (Padawni) of Poaceae family as shown Figure 1. They were isolated and culture on NFb and CRA nutrient medium and then sub-culture to obtain the pure culture. All strains in NFb medium showed white colour pellicle formation about 2 – 5 mm below the surface of the medium and colour changed from pale yellow to dark blue colour after 72 hours incubation. And then, the isolated stains were transferred to CRA medium. In this medium, the colonies of isolated strains were dark pink or red colour, 1.0 – 2.5 mm in diameter after 96 hours incubation as shown in Figure 2.

Figure 1 (A) Plant Habit of *Saccharum spontaneum* L.
(B) Plant Habit of *Saccharum officinarium* L.
(C) Plant Habit of *Dichanthium caricosum* (L.) A. Camus.

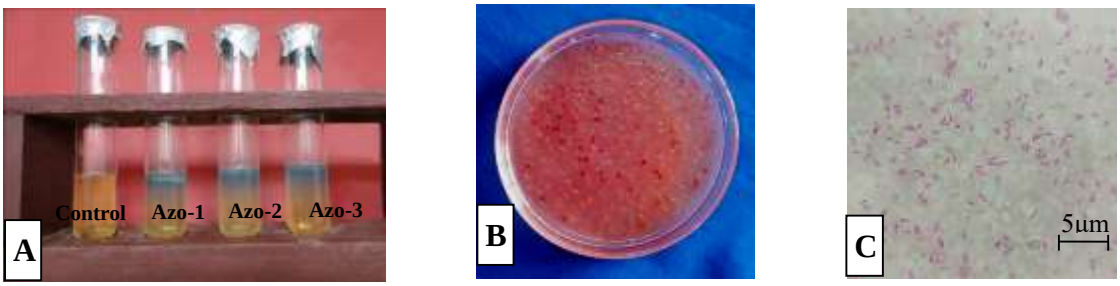

Figure 2 Isolated *Azospirillum* strain culture in semisolid NFb medium
(A) Isolated *Azospirillum* strains in NFb medium
(B) Isolated *Azospirillum* strain spread on CRA medium
(C) Photomicrograph of isolated *Azospirillum* strain

Effect of isolated strains on germination on rice

The results of the germination tests has indicated that among the three isolated strains, Azo-1, Azo-2 and Azo-3 were found to be the most effective strains which provided 96%, 94% and 97% while the control is 93% in germination percentage. Moreover, the isolated strain also gave the highest length of shoot and root length such as 6.78 cm, 6.32 cm and 7.14 cm and control has 5.94 cm and 6.64 cm, 6.18cm and 6.94 cm in root length while the control has 6.04 cm respectively as shown in Table 1 and Figure 3.

Table 1 Effect of inoculation on germination percentage, root and shoot length of rice

Treatment	Germination %	Shoot length (cm)	Root length (cm)
Control	93	5.94	6.04
Azo-1	96	6.78	6.64
Azo -2	94	6.32	6.18
Azo-3	97	7.14	6.94

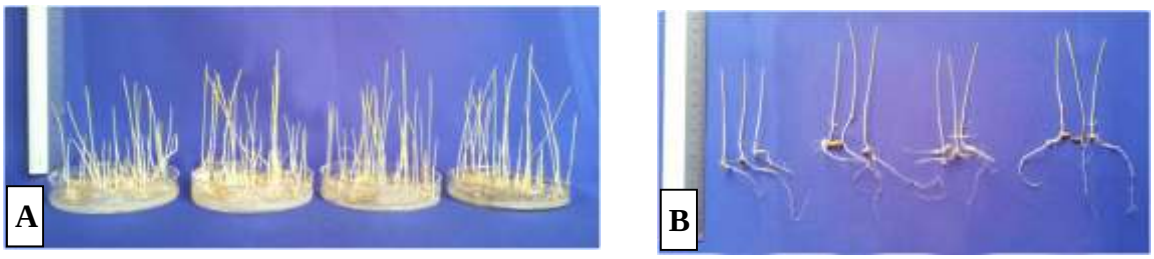

Figure 3 A. Effect of rice germination inoculated with Azo-1, 2, 3 and control
B. Shoot and root formation of rice inoculated with Azo-1, 2, 3 and control

Pot culture experiment

In the pot culture, the growth responses to inoculation varied during vegetative growth stages for rice seedling. The inoculation of rice with Azo-1, 2 and 3 were higher significant in plant height, leaf area and tillering numbers of rice at 20, 40 and 60 days after sowing when compared to control Figure 4 - 6.

Figure 4 Effect of isolated strain and control on plant height of rice after 20 days after sowing

Figure 5 Effect of rice inoculation with isolated strains and control after 40 days after sowing

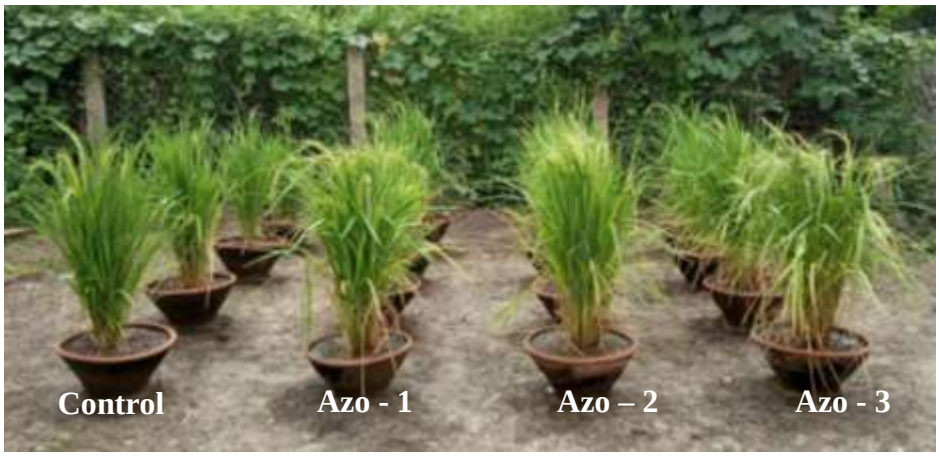

Figure 6 Effect of rice inoculation with isolated strains and control after 60 days after sowing

Effect of isolated *Azospirillum* strains on plant height

In the present study, the mean of plant height ranged from 36.33 to 46.50 cm. The highest length was found in treated plant with Azo-3 (46.50 cm) where as the lowest length was observed in control (36.33 cm) at 60 days after sowing. All treated plants possessed higher plant height than the control ones. The plant height of treated plants with Azo-3 has significantly higher than Azo-1, 2 and control (Table 2).

Table 2 Effects of isolated strains on plant height of rice after 20, 40 and 60 days after sowing

Factor	Plant Height (cm) Growing Period (DAS)			Mean(cm)
	20DAS	40DAS	60DAS	
T_1 (Azo - 1)	11.88	24.43	41.50	25.94
T_2 (Azo - 2)	11.13	23.93	38.25	24.44
T_3 (Azo - 3)	12.33	24.75	46.50	27.86
Control	11.13	23.08	36.33	23.51
F-test	*	**	**	
5%LSD	0.80	0.65	1.13	
CV%	4.5	1.8	1.8	

** = 1% level of significant, *= 5% level of significant, ns = non-significant, DAS = day after sowing

Effect of isolated *Azospirillum* strains on leaf area

The effects of isolated strains on leaf area of rice at 20, 40 and 60 days are showed in Table 3. Leaves area increased with the age in all treatments. The highest leaf area was found in treatment with Azo-3 (53.25 cm^2) and followed by Azo-1 (45.86 cm^2), Azo-2 (44.86 cm^2) and the lowest leaf area was observed in the control at 60 days after sowing.

Table 3 Effect of inoculation on leaf area of rice after 20, 40 and 60 days after sowing

Factor	Leaf Area (cm^2) Growing Period (DAS)			Mean(cm^2)
	20DAS	40DAS	60DAS	
T_1 (Azo - 1)	28.20	35.38	45.86	36.48
T_2 (Azo - 2)	26.88	33.86	44.86	35.20
T_3 (Azo - 3)	30.38	37.50	53.25	40.38
Control	25.63	32.25	43.25	33.71
F-test	**	**	**	
5%LSD	0.86	1.42	1.83	
CV%	2.0	2.6	2.5	

** = 1% level of significant, *= 5% level of significant, ns = non-significant, DAS = day after sowing

Effect of isolated *Azospirillum* strains on tillering number

The result indicated that the number of tiller per plant ranged from 47.75 with inoculation while the control has 34.50. All of the treatments were increased in number of tiller with the age in all accounting times (20, 40 and 60 days). At 60 days, comparison on the number of tillers per plant, Azo-3 showed significantly difference from the other treatments and control as shown in Table 4.

Table 4 Effect of isolated *Azospirillum* strains on tillering number of rice

Factor	Tiller Number			Mean
	Growing Period (DAS)			
	20DAS	40DAS	60DAS	
T_1 (Azo - 1)	13.33	36.50	39.25	29.69
T_2 (Azo - 2)	10.75	34.63	37.50	27.63
T_3 (Azo - 3)	14.50	40.75	47.75	34.33
Control	10.38	30.63	34.50	25.17
F-test	**	**	**	
5%LSD	1.05	1.01	0.83	
CV%	5.6	1.8	1.4	

** = 1% level of significant, *= 5% level of significant, ns = non-significant, DAS = day after sowing

Table 5 Effect of isolated *Azospirillum* strains on panicle length, fertile seeds per panicle and 1000 grains weight

Factor	Panicle length (cm)	Fertile seed/ panicle	1000 grains weight (g)
T_1 (Azo - 1)	25.95	189.82	20.50
T_2 (Azo - 2)	25.83	185.63	20.33
T_3 (Azo - 3)	27.63	200.15	21.20
Control	25.18	178.25	20.08
F-test	**	**	ns
5%LSD	0.62	1.94	0.88
CV%	1.5	0.7	2.8

** = 1% level of significant, *= 5% level of significant, ns = non-significant, DAS = day after sowing

Effect of isolated *Azospirillum* strains on panicle length

The mean of panicle length was measured from 25.83-27.63 cm in the treatment while the control has 25.18 cm. The highest panicle length was found in treated plant with Azo-3 (27.63 cm) but the lowest length was observed in control (25.18 cm). The panicle lengths of treated plants with Azo-3 have significantly more than Azo-1 and Azo-2 over the control as shown in Table 5.

Effect of isolated *Azospirillum* strains on fertile seeds per panicle

The mean values of fertile seeds per panicle were accounted from 178.25 in non inoculated plant to 200.15 for inoculated plant. In contrast, the inoculation of plant with isolated strains had positive significant effect on fertile seeds per panicle. When the plants inoculated with isolated strain were 189.82, 185.63, and 200.15. (Table 5).

Effect of isolated *Azospirillum* strains on 1000 grains weight of rice

In the present study, the inoculation of isolated *Azospirillum* strains on 1000 grains weight arranged from 20.33-21.20 g in the treatment while the control has 20.08 g. One thousand grains weight of rice was significantly improved by the strain Azo-3 expect Azo-1 and 2. The most significant strain Azo-3 which increased up to 21.20 g (5.28%) over the control (Table 5).

Discussion and Conclusion

In the present study, all isolated strains (Azo-1, 2 and 3) were isolated from the root of *Saccharum spontaneum* L. (Kaing), *Saccharum officinarium* L. (Kyan) and *Dichanthium caricosum* (L.) A. Camus. (Padawni) of Poaceae family.

The isolates made from roots of grasses forming the subsurface pellicle in N-free semisolid malate medium is often taken to be an absolute proof of the presence of *Azospirillum* spp. (Okon *et al.* 1977). Appearance of pellicle formation on Nfb semi-solid medium indicated successful isolation of *Azospirillum* (Kanimozhi and Panneerselvam, 2011).

In this study, for the isolation of *Azospirillum* spp., Nfb semisolid medium was used. After 72 h incubation, the Nfb semi-solid medium showed pale yellow to dark blue colour, white colour pellicle formed 2 - 5 mm below the surface of the medium. Then the isolated strains were culture on CRA medium. In this medium, the colonies of the isolated strain were pale to dark pink or scarlet colour, irregular form and convex, 1.0 – 2.5 mm in diameter. Therefore, the result characters are in agreement with the above mentioned literature.

Good seed germination behavior is important for horticulture and agriculture. Uneven or poor germination and subsequently inhomogeneous seedling growth can lead to great financial losses (Ghiyasi *et al*., 2008 b).

Sanzida *et al*. (2008) stated that inoculation effect of *Azospirillum* spp. on growth of wheat at 30 days up to 43.24% over the control in germination percentage. Win Naing (2009) stated that the germination percentage of isolated *Azospirillum* strain promote 18.37% over the control in the treatment of rice. Similarly, Zaw Lwin Oo (2010) and Htar Htar (2013) stated that inoculation of endophytic bacteria strains on germination increased up to 7.29% and 20.0% in rice over the control.

The inoculations of isolated *Azospirillum* strains (Azo-1, 2 and 3) were 96%, 94% and 97% while the control has 93% in germination percentage, 6.78 cm, 6.32 cm and 7.14 cm and control has 5.94 cm in shoot length and 6.64 cm, 6.18 cm and 6.94 cm and 6.04 cm in control in root length respectively. Therefore, the inoculation of isolated strains increased in germination up to 4.12%, 16.25% in shoot length and 12.97% in root length over the control by the isolated strain Azo-3.

Inoculation with indigenous *Azospirillum* is an important procedure when studying their inherent capacity to benefit crops. In some cases, indigenous strains can perform better than introduced strains in promoting the growth of crops due to their superior adaptability to the environment (Kanimozhi and Panneerselvam, 2011).

The effect of *Azospirillum* inoculation on the total yield increase of field grown plants generally ranged from 10 to 30% (Watanabe and Lin, 1984). Okon (1985) evaluated the worldwide success of *Azospirillum* inoculation and concluded that positive effects on yield were obtained in approximately 65% of all field experiments. Yield increases due to inoculation were reported in 75 % of all experiments using summer cereals and only in 50% of the experiments using spring wheat (Smith *et al*., 1984*b*). Recently, about 70-75% of all pot experiments in cotton and several vegetables resulted in yield increase (Bashan *et al*., 1989).

Zaw Lwin Oo (2010) state that the inoculation of rice with isolated *Azospirillum* strains increased plant height upto 17.69%, 33.30% in tillering number, 14.28% in leaf area and 10.50% in total seed per panicle. Similary, Htet Htet Aung (2014) has been shown that the inoculation of wheat with the isolated *Azospirillum* strains increased upto12.64% in plant height, 30.26% in tillering number and 30.10% in leaf area over the control.

Tiller yield in percent over control was ranged from 8 to 27.75% at maturity in case of Azo-1, 2 and 3. Biswas *et al*. (2000) who have reported 16% increase in number of panicle per plant of

rice and suggested that the improvement was due to increased availability of nutrients and phytohormones like indole acetic acid and ethylene.

The bacterias of the *Azospirillum* genera presents application potential in agricultural systems, around 70% of the experiments up to 30% in productivity (Dalla *et al*. 2004). In the same way, Win Naing (2009) has been shown that inoculation with endophytic microorganisms (*Azospirillum* sp.) in Ma-naw-thu-kha rice significantly increased in grain yield up to 43.15% over the control. Similarly, Kleopper *et al.* (1992) has been reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43 % with *Baccilus* inoculation. In this study, it was observed that grain yield increased between 3.98% and 10.94%. Among the selected strains, strain Azo-3 was effective and significant in grain yield up to 10.94 % over the control.

In the present study inoculation with selected strains of *Azospirillum* sp. caused significant increase in length of panicle with ranges of 2.52 – 8.88 % respectively. The best strain contributing significant increase in panicle length was Azo-3 (8.88 %) over the control. This is an agreement with the findings of Wedad *et al.* (1988).

In the present study, the total seed per panicle increase up to 10.94% over the control. This result was agreed with Hussain *et al.* (2009) who have reported 22.90% increased in number of grain per panicle. Also, an increase of 30-40% in grain yield of rice due to nitrogen fixing bacteria inoculation over control was recorded by Su San and Yan (1998). Bashan *et al*. (1989) reported that rhizobacteria and AM fungi increase plant growth but controverts results were observing by many authors where occasionally microorganisms inoculated decreasing plant growth. But, in this result work, the inoculation of *Azospirillum* sp. increase plant growth and yield of rice.

Hassamzadeh *et al.* (2007) also showed that seed priming with PGPR has caused an increase in 1000 grain weight of barley. Gholami *et al.* (2009) have been reported that seed priming with *Azotobacter brasilense* DSM 1690 increased 44% 1000 grain weight more than no priming. In the present, the inoculation of isolated *Azospirillum* strains increased 1000 grain weight up to 5.28 % by Azo-3 over the compared to control.

In the present study, comparison of the inoculated *Azospirillu*m strains among each other demonstrated that the strain of Azo-3 performed better than the other isolated strains and control. The higher plant height, number of tillers, total seed per panicle, 1,000 grain weight and grain yield respond to all inoculants compare to control clearly showed the beneficial role of these rhizobacteria. These findings can be concluded that in future valued inoculants could be developed with these organisms to use as biofertilizer for cereal crops like rice, wheat and maize etc. Therefore, further research in this area will be able to develop a sustainable biofertilizer technology for greater and environment friendly cereal production system.

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WILD ORCHIDS GROWING IN HAKHA EDUCATION COLLEGE CAMPUS AND THREATS TO THEIR EXISTANCE IN FUTURE

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Abstract

The campus of Hakha Education College has been explored to make inventory, identify and document the wild orchid species during flowering seasons from February to June 2020. The threats to their existence in future have been identified. A total of 27 species and one variety of wild orchids under 13 genera have been identified. Among the genera found in the campus genus *Dendrobium* is largest and comprising 12 species with showy and colorful flowers. The remaining species consist of only one species expect genus *Coelogyne* and *Vanda*. There are 3 species under genus *Coelogyne* and 2 species under genus *Vanda*. One variety is under *Thunia alba*. All orchid species growing in the campus are facing the habitat loss due to increasing development activities. The showy and colorful flowers of *Dendrobium* species make the discriminating collection of construction workers and people living in the campus and hence those species are more highly facing the habitat loss than other orchid species. To save the naturally growing orchid species in the study area, adequate attention is necessary to conduct in situ and ex situ conservation for their sustainable existence and development in the future.

Keywords: Wild orchid, habitat loss, indiscriminate collection, Hakha Education College

Introduction

Orchids belonging to family Orchidaceae are one of the most popular ornamental plants. They exhibit several peculiarities in vegetative and floral features. Their unique features and remarkable specialization make the scientists and amateurs to be highly interest. As some orchids have beautiful flowers and unique habitats they become popular resources for horticulture and man-made hybrid orchids. Although they can grow in such various habitats as on ground, decay matter and rock most orchids are epiphytes growing on trees. However, they are very sensitive to habitat change (Jalal 2012) because they are very site-specific and need optimum conditions to thrive in a given ecosystem (Jones et al 2005). The causes to be habitat change are majority due to anthropogenic activities such as indiscriminate collection and habitat destruction. The land use alternation, civilization, construction of roads and buildings imposed the habitat destruction highly influencing on rarity and e extinction of orchid species through the world including Myanmar.

Hakha Education College has opened on December, 2017 and is situated beside the road linking Hakha, capital of Chin State, to Falam. The area where the land is used to build Education College was a paradise of some species of wild orchid. Before Education College is established epiphytic orchids are plenty seen on the pine and some woody trees in this area where there is mountain pine forest under low human disturbance because epiphytic orchids are more diverse and more abundant under low human disturbance in primary forest than other region (Adhikari et al 2012). Nowadays, this area immediately changes into populated area and human disturbance become high. The orchids in this area become dramatically disappear due to the cutting of trees for building construction and indiscriminately collecting by the people living in the campus including construction workers. The cutting of the trees to be cleaning the land for construction also highly impact on the survival of epiphytic orchids owing to the loss of host and altering the relative humidity, light intensity and temperature. The wild orchids in this area are suffering from an uncertain future through indiscriminating collection and habitat destruction. It thus is urgently necessary to record and to conserve those wild orchids before totally disappearing due to their habitat loss and degradation. Although there is a little research on wild orchids growing in Chin State it is still lacking to study and survey the

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orchids growing in a particular area like Hakha Education College Campus. The present study is an attempt to provide a document concerning the native wild orchid growing in Hakha Education College campus and surrounding areas. The objectives of this study are to survey how many wild orchid species in Hakha Education College campus and surrounding areas, to identify the wild orchids species and to generate a full checklist of wild orchids in Hakha Education College campus and to provide scientific information for development of conservation research for the wild orchids.

Materials and Methods

Study Area

Hakha Education College is located beside the Hakha-Falam road between Chin state road No. 117/3 and 117/4 and about 7 miles far from center of Hakha town. It is situated between 93˚ 34' 28.5"E and 22˚ 41' 52.42"N and at altitude of 6100 feet. The total area of its campus is 97.95 acres. The dominant tree species within campus and surrounding areas is pine.

Field Survey

The field surveys were conducted during flowering seasons from February to June 2020 and some living plants were collected and planted in home garden within compound of Staff Quarter. All collected specimens were recorded by taking photographs while flowering. Description for these species was based on fresh specimens. According to comparisons of morphological characters, species was identified or keyed out by using the floristic literatures or references (Soon 1989; Rakpaibulsombat, 1992; Vaddhanaphuti, 2001 and Chen XQ, 2009). The online herbarium specimens providing from GBIF (Global Biodiversity Information Facility) and POWO (Plants of the world online) were reviewed to confirm the species. The species name was confirmed by checking in online database (www. The plantlist.org; www.ipni.org).

Results

The diverse orchid species naturally growing in Hakha Education College campus have been explored and identified. The threats facing the orchid species for their existence have been also identified. A total of 27 orchid species with one variety distributed under 13 genera have been identified from Hakha Education College campus. All species found in the campus are epiphyte and scented. Many species have fragrant odor but some have unpleasant odor. The species along with scientific name, Myanmar name, growth pattern, some outstanding morphological characters and IUCN conservation status are listed in table 1. The 13 genera found in the campus with the number of species are shown in figure 1. The habitat and habitat destruction of some orchid species are shown in Fig.2. The habit and flowers of 27 species and one variety are shown in Fig.3. The major threats facing the orchid species found in the campus is habitat loss due to anthropogenic activities including log cutting for construction and indiscriminating collection.

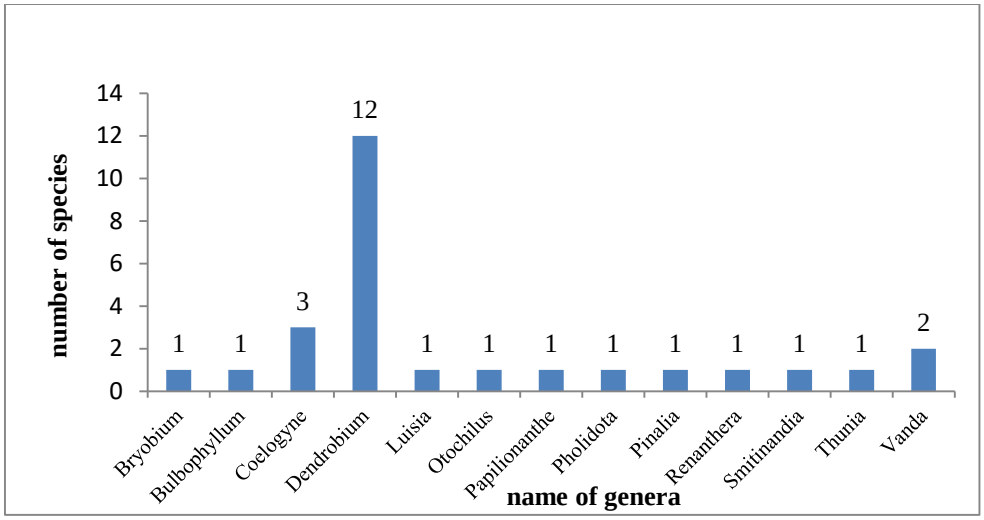

Figure 1 species composition of each genera found in Hakha Education College campus

Table 1 IUCN conservation status and some outstanding morphological characters of orchid pecies naturally growing in Hakha Education College campus

Scientific name	Myanmar common name	IUCN conservation status	growth pattern	Flowering time	Shape of pseudobulb	Leaf presence or absence on pseudobulb during flowering	Flower appearance	Flower color	Flower number per inflorescence	shape of labellum	color of labellum
Bulbophyllum odoratissimum (Sm.) Lindl. ex. Wall.	Thazin-Hmew	NE	s	June	oblong	P	I	yellowish white	many	falcate	pale yellow
Bryobium hyacinthoids (Blume) Y.P.Ng. &P.J.Cribb	-	NE	s	June	oblong elliptic	P	S	White	many	3-lobed	Yellow
Coelogyne prolifera Lindl.	Ngwe-nin-phyu (myo kwe)	NE	s	May	conical	P	I	greenish yellow	2-5	subquadrate	greenish yellow with deep yellow patch inside
Coelogyne schultesii S.K.Jain & S.Das	-	NE	s	May	conical	P	I	greenish brown	2-5	oblong	light brown to brownish white
Coelogyne stricta (D.Don)Schltr.	-	NE	s	June	conical	P	S	white	3-5	ovate	white with two reddish brown stripe on yellow patch
Dendrobium bensoniae Rchb.f.	Pale- hnit	LC	s	June	cane	A	S	white	1-3	infundibulate	white with with yellow patch and two reddish brown spot inside

Scientific name	Myanmar common name	IUCN conservation status	growth pattern	Flowering time	Shape of pseudobulb	Leaf presence or absence on pseudobulb during flowering	Flower appearance	Flower color	Flower number per inflorescence	shape of labellum	color of labellum
Dendrobium chrysotoxum Lindl.	Mauk-cham-war	NE	s	June	club	p	S	orange-yellow	8-15	Round with wavy margin	yellow with darker golden yellow inside
Dendrobium crepidatum Lindl. &Paxton	Ganaing-na-be-pauk	NE	s	March	cane	a	S	pale purple	1-3	Round	pale purple with large yellow patch inside
Dendrobium crystallinum Rchb.f	Pan-setku-thit kwa	NE	s	April	Cane	a	S	white with pale purple patches	1-2	rounded	yellow with white margin and small pale purple patch
Dendrobium dickasonii L.O. Williams	-	NE	s	June	rod	a	S	dark orange	1-2	ovate oblong	orange with reddish veins
Dendrobium falconeri Hook.	Myat-thitkwa	NE	s	May	knobby	a	S	white with deep purple patch	1-3	infundibulate	White with purple and yellow patch, dark reddish purple inside
Dendrobium fimbriatum Hook.	Arme –let-tan- shae	NE	s	May	cane	a	S	golden yellow	2-10	subobicular	Yellow with deep brown patch inside
Dendrobium heterocarpum Wall. ex Lindl.	-	NE	s	March	cane	a	S	pale yellow to creamy yellow	1-3	infundibulate	Pale yellow with reddish brown stripe
Dendrobium infundibulum Lindl.	Taung-ngwe- tu	NE	s	March	cane	a	S	pure white	1-7	infundibulate	white with bright yellow patch
Dendrobium ochreatum Lindl.	Taung-nabe-pauk	NE	s	April	cane	a	S	golden yellow to bright yellow	1-2	Funnel-shaped	golden yellow with reddish brown inside
Dendrobium parishii Rchb. f.	Thazin-Hmew	NE	s	June	cane	a	S	reddish purple	1-2	rounded	pale purple
Dendrobium pulchellum Roxb. ex Lindl.	Ngwe-nin-phyu (myo kwe)	NE	s	April	cane	a	S	pale yellow to purplish pink	3-10	ovate oblong	pale yellow to purplish pink
Luisia trichorrhiza (Hook.) Blume	-	NE	m	March	nil	P	I	White	1-3	ovate triangular	dark purple
Otochilus fuscus Lindl.	pa-tee-sint	NE	s	March	Cylindrical fusi-form	p	I	White	8-15	narrowly oblanceolate	brownish white.
Papilionanthe vandarum (Rchb.f.) Garay		NE	m	April	nil	P	S	pale purple	1-3	3-lobed	Purple
Pholidota articulata Lindl.	Sin-pa-tee	NE	s	June	cylindrical	P	I	greenish white	many	bilobed oblong	whitish brown

Scientific name	Myanmar common name	IUCN conservation status	growth pattern	Flowering time	Shape of pseudobulb	Leaf presence or absence on pseudobulb during flowering	Flower appearance	Flower color	Flower number per inflorescence	shape of labellum	color of labellum
Pinalia obesa(Lindl.) Kuntze	-	NE	s	June	oblong elliptic	P	I	yellowish white	3-6	3-lobed	light yellow
Renanthera imchootiana Rolfe	Chin-thit-kwa-ni	NE	m	June	nil	P	S	orange-red	many	3-lobed	Red
Smitinandia micrantha (Lindl.) Holttum		NE	m	April	nil	P	I	pale purple	many	Oblong	deep purple
Thunia alba (Lindl.)Rchb.f.	Kyauk-thit-khwa	NE	s	June	cane	P	S	White	2-10	rounded	yellow with white margin
Thunia alba var. *marshalliana* (Rchb.f.)B.Grant	Kyauk-thit-khwa (Myo kwe)	NE	s	June	cane	P	S	White	2-10	rounded	purple with white margin
Vanda coerulescens Griff.	Moe -lone - Hmaing – galay	NE	m	June	nil	P	S	Pale blue	10-many	oblance olate	dark blue
Vanda motesiana Choltco		NE	m	April	nil	A	S	greenish yellow	5-10	bilobed ovate	brownish yellow

s= sympodium; m= monopodial; P= present, A= absent I= indistinct; S= showy

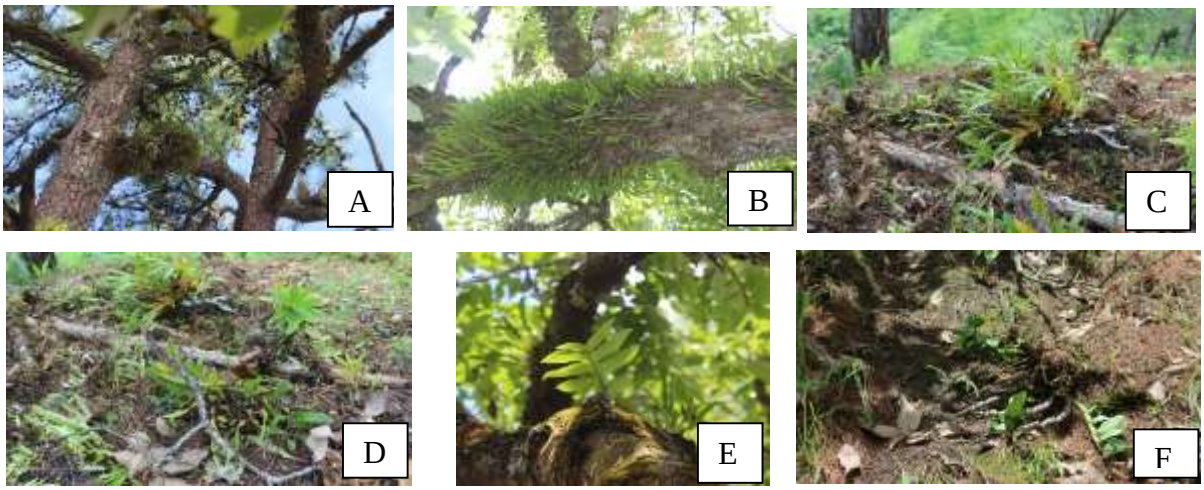

Figure 2 habitat and habitat losing status: A and B. natural habitat of epiphytic orchids, C and D. habitat losingepiphytic orchids on the cutting tree, E. *Dendrobium ochreatum* healthy surviving in natural habitat, F. *Dendrobium ochreatum* losing habitat due to tree cutting


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Figure 3 habit and flowers of all species found in Hakha Education Degree College campus: 1. *Bryobium hyacinthoids*, 2. *Bulbophyllum odoratissimum*, 3. *Coelogyne prolifera*, 4. *Coelogyne schultesii*, 5. *Coelogyne* stricta, 6. *Dendrobium bensoniae* 7. *Dendrobium chrysotoxum*, 8. *Dendrobium crepidatum*, 9. *Dendrobium crystallinum*, 10. *Dendrobium dickasonii*, 11. *Dendrobium falconeri*, 12. *Dendrobium fimbriatum*, 13. *Dendrobium heterocarpum*, 14. *Dendrobium infundibulum*, 15. *Dendrobium ochreatum*, 16. *Dendrobium parishii*, 17. *Dendrobium pulchellum*, 18. *Luisia trichorrhiza*, 19. *Otochilus fuscus*, 20. *Papilionanthe vandarum*, 21. *Pholidota articulata*, 22. *Pinalia obesa*, 23. *Renanthera imchootiana*, 24. *Smitinandia micrantha*, 25a. *Thunia alba*, 25b. *Thunia alba* var. *marshalliana*, 26. *Vanda coerulescens*, 27. *Vanda motesiana*

Discussion

A total of 27 orchid species with one variety under 13 genera have been identified from Hakha Education College. The number of species in 13 genera varies from 1 to 12. As shown in Fig. 1 the largest genus found in the campus is *Dendrobium* with 12 species and *Coelogyne* contain 3 species. The remaining genera consist of only one species except *Vanda* consisting of 2 species. One variety namely *Thunia alba* var *marshalliana* (Rchb.f.) B.Grant is a variety of *Thunia alba*.

In this study the species *Dendrobium infundibulum*, *Otochilus fuscus* and *Pholidota articulata* were very common found but the species *Dendrobium parashii* and *Renanthera imchootiana* were rarely found in the study area. In IUCN conservation status all species found in the campus were found to be listed under not evaluated (NE) category expect *Dendrobium bensoniae* listed under Least concern (LC) (IUCN 2020) as shown in table 1. However *Dendrobium bensoniae* and *Renanthera imchootiana* were found to be listed in CITES appendix I and the remaining species were found to be listed in CITES appendix II (UNEP-WCMC (Comps) 2014).

In the campus, different development activities, lack of knowledge of plant collection technique and lack of awareness on plant conservation are great threats to the existence of wild orchid species in future. The major threat is habitat loss due to tree cutting for construction and indiscriminating collection. Especially indiscriminating collection is a possibility of losing in number of orchid species which are hard to thrive outside the natural habitat such as *Dendrobium falconeri* and *Renanthera imchootiana*.

The tree cutting highly impacts the habitat destruction of the species with crowded growth as shown in Fig.2. When their habitat was destructed for some orchids with indistinct flower such as *Bulbophyllum odoratissimum*, *Coelogyne prolifera*, *Coelogyne schultesii* and *Smithinandia micrantha* the opportunity to get new habitat will be few because plant collectors are not interest to collect such species. Therefore their existence in the future is not certain if they do not receive the opportunity to get new habitat because they cannot thrive on ground. As shown in Fig.2 *Dendrobium ochreatum* is healthy growing on the tree (Fig.2.E) but the plants of this species are falling on ground due to cutting their host tree (Fig.2 C, D and F).

Conclusion

The population of all orchid species observed in the campus is dramatically declining day by day due to especially indiscriminate collection by people living and working in the Campus as well as land use changes due to increasing development activities within the campus. The habitat destruction resulted from land use alternation to construct the buildings enhance the reducing in population and species diversity of the orchids growing in the campus. To save the naturally growing orchid species in the study area, adequate attention is necessary to conduct in situ and ex situ conservation for their sustainable existence and development in the future.

Acknowledgements

First and foremost, we would like to thank to Daw Mi Nyo Nyo Shein, Principal, Hakha Education College for giving permission to do this paper. Thanks to the teachers of Hakha Education College and the individuals who always accompanied us when we went into the forest.

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GROWTH CONDITIONS AND OPTIMIZATION PARAMETERS OF FERMENTATION OF SELECTED SOIL BACTERIUM KM-39

Khin Min Min Kyaw[1], Zar Zar Yin[**]

Abstract

The present study was focused by the growth conditions and fermentation optimization of selected soil bacterium KM-39 against *Candida albicans.* In the growth of KM-39, carbon and nitrogen sources were used and the excellent growth were found on lactose and yeast extract. The highest antifungal activity of KM-39 was found by the fermentation conditions such as 2 days old culture period, 20% inoculum size, 72 hours age of inoculum, FM-5 at pH 8 and temperature 40ºC. The maximum antifungal activity was obtained by using the maltose in the carbon source and potassium nitrate in the nitrogen source. In the comparison of static culture and shaking culture of KM-39, the antifungal activity of shaking culture was more higher than the static culture on *Candida albicans*.

Keywords: Fermentation conditions, growth of bacteria, antimicrobial activity

Introduction

Bacteria are single-cell organisms and the most numerous denizens of agriculture, with populations ranging from 100 million to 3 billion in a gram.They are capable of very rapid reproduction by binary fission (diving into two) in favorable condition.One bacterium is capable of producing 16 million more in just 24 hours (Soltner *et al*, 2003).

The secondary metabolite is obtained by fermentative process.Fermentation is a complex process, it not only depends on the performance and fermentation medium, also requires the suitable environmental conditions such as inoculation volume, medium capacity, fermentation time, temperature, agitation rate and initial pH. These factors may affect the antibiotics production (Martin et al., 1980).

Thus soil is comples product of parental material, or geology, topography, climate time and biological activity on anthropogenic activity (Graffin, 1972).

The nature and concentration of nutrients in the medium such as carbon, nitrogen, phosphorus and minerals as well as the essential substances for biosynthetic pathway can promote the production of larger quantities of one or more compound inferring directly on its performance (Gupte and Pulkarni, 2002).

The *Candida albicans*is human-pathogenic fungus whose control is one of the most problems in today's medical field. It is part of the normal microflora of the mouth, vagina and gastrointestinal tract. Among the various *Candida albicans*infections that can occur under these conditions is a vaginitis (inflammation of the vagina) characterized by a thick, cheeselike discharge. Another condition caused by *Candida albicans*urethritis in both women and man (Michael, 1993).

This research work was carried out by the optimum fermentation conditions of selected bacterium (KM-39).

The aim and objectives of this research were to investigate the utilization of carbon and nitrogen sources of the selected bacterial growth and to optimize the fermentation condition of selected bacterium (KM-39).

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Materials and Methods

The effects of fermentation period, size and age of inoculum for fermentation of KM-39

The fermentation period (24, 48, 72, 96 and 120 hrs) were employed for the production of antimicrobial metabolite.

In the investigation of sizes of inoculum (5%, 10%, 15%, 20%, 25% and 30%) were used for the antimicrobial activity of KM-39. Seed culture was inoculated at room temperature. In the study of ages of inoculum, the incubation of seed culture times (24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144 and 156 hrs) were used and transferred into the fermentation media. Fermentation were carried out for 7 days and antimicrobial activity was tested by agar well diffusion method.

Preparation of Agar Well Method

Isolated strains were subjected with antimicrobial activities by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Well impregnated with 24, 48 and 72 hours fermented broth (20 µL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured.

Therefore, the diameter of clear zones has observed as potent activity shown by respective strain. Clear zones surrounding the test wells were indication of the presence of antimicrobial activities which inhibit the growth of the test organisms selectively (Collins, 1965).

Test organisms

Table 1 Test Organism Used for Antifungal Activity

Test No	Test Organism	Diseases
1	NITE 09542	Alimentary tract, skin infection

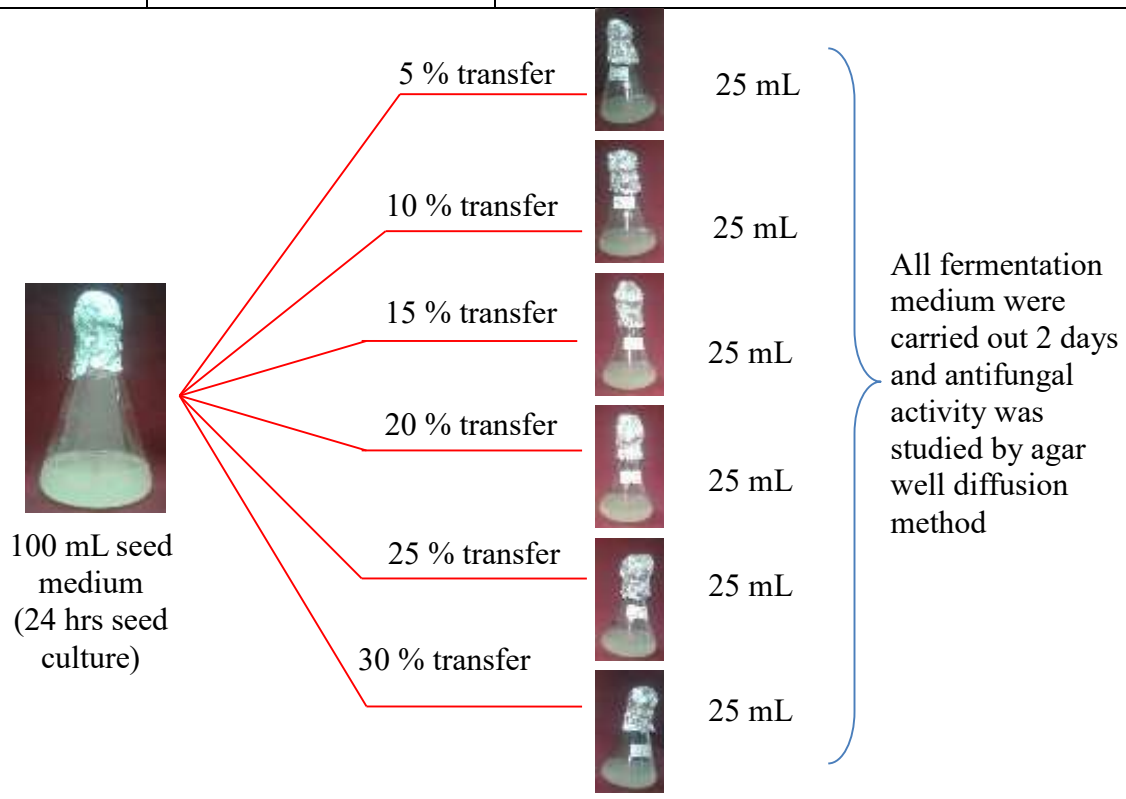


Figure 1 Study on the effects of sizes of inoculum in the fermentation

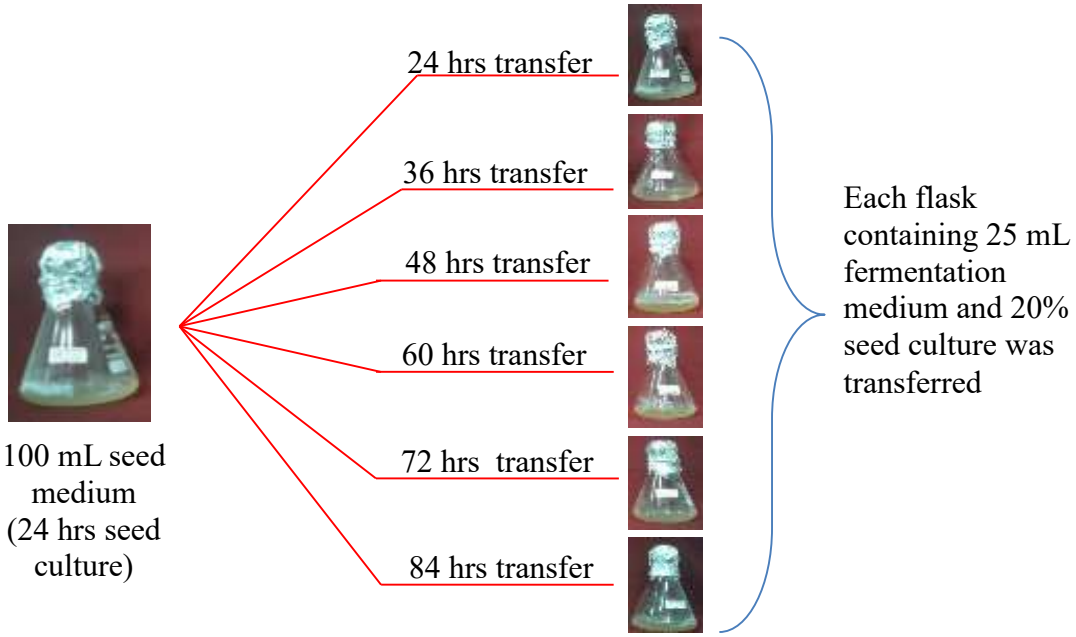


Figure 2 Study on the effects of ages of inoculum in the fermentation

Carbon and Nitrogen Utilization

Optimal fermentation conditions are very important for maximal productivity of metabolites. In this study, carbon and nitrogen sources were employed in the fermentation for the production of antimicrobial metabolites. Carbon sources such as arabinose, dextrose, fructose, galactose, lactose, maltose, sucrose, xylose, glycerol, mannitol and soluble starch were used. Nitrogen sources such as asparagine, casein, gelatin, peptone, urea, yeast extract, ammonium chloride, ammonium sulphate, ammonium nitrate, potassium nitrate, sodium nitrate and malt extract were also used.

Media used in fermentation study (NITE, 2005)

Fermentation was undertaken with suitable conditions of 20% sizes and 48 hrs ages of inoculum with twelve different media. Fermentation was carried out 5 days and antimicrobial activity test was carried out every 24 hrs.

Effect of incubation pH and temperature on KM-39

Effects of different pH were used for antimicrobial activity of pH 4, 5, 6, 7, 8, 9 and 10. These different pH were adjusted by NaOH and HCl. The selected bacterium KM-39 was inoculated and incubated at five different temperatures by using 25ºC, 30ºC, 35ºC, 40ºC and 45ºC.

Effect of aeration upon the secondary metabolite

100 mL conical flask containing 50 mL of the best fermentation medium was incubated on the shaker (100 rpm) for 2 days. At the same time, another those fermentation medium was incubated under static condition without shaking. These shaking culture and static culture were compared by using agar well diffusion assay method.

Results

The effects of carbon sources for the growth of soil bacterium KM-39

In the present study, the effects of different carbon sources were observed for the growth rate of KM-39. KM-39 showed the excellent growth by the addition of arabinose, dextrose, lactose, maltose, sucrose, xylose, glycerol and mannitol and good results on fructose, galactose and soluble starch.

Table 2 Growth morphology of selected bacterium KM-39 on various carbon sources

No.	Carbon sources	Growth result	Growth mm
1	Arabinose	Excellent	11.78
2	Dextrose	Excellent	10.51
3	Fructose	Good	5.10
4	Galactose	Good	6.23
5	Lactose	Excellent	21.17
6	Maltose	Excellent	7.81
7	Sucrose	Excellent	8.79
8	Xylose	Excellent	9.21
9	Glycerol	Excellent	7.91
10	Mannitol	Excellent	9.35
11	Soluble starch	Good	5.94

1-2 mm = poor 3-4 mm = moderate 5-6 mm = good 7> = excelle

Figure 3 Growth morphology of selected bacterium KM-39 on carbon sources (2 days old culture)

The effects of nitrogen sources for the growth of soil bacterium KM-39

In this study, excellent growth of KM-39 was found on asparagine, gelatin, urea, yeast extract, ammonium chloride, ammonium sulphate and sodium nitrate, moderate growth on casein and showed good results on other sources peptone, ammonium nitrate, potassium nitrate and malt extract.

Table 3 Growth morphology of selected bacterium KM-39 on various nitrogen sources

No.	Nitrogen sources	Growth result	Growth mm
1	Asparagine	Excellent	7.99
2	Casein	Moderate	4.97
3	Gelatin	Excellent	7.40
4	Peptone	Good	6.57
5	Urea	Excellent	7.12
6	Yeast extract	Excellent	16.78
7	Ammonium chloride	Excellent	7.34
8	Ammonium sulphate	Excellent	7.10
9	Ammonium nitrate	Good	6.08
10	Potassium nitrate	Good	6.93
11	Sodium nitrate	Excellent	7.06
12	Malt extract	Good	5.65

1-2 mm = poor 3-4 mm = moderate 5-6 mm = good 7> = excellent

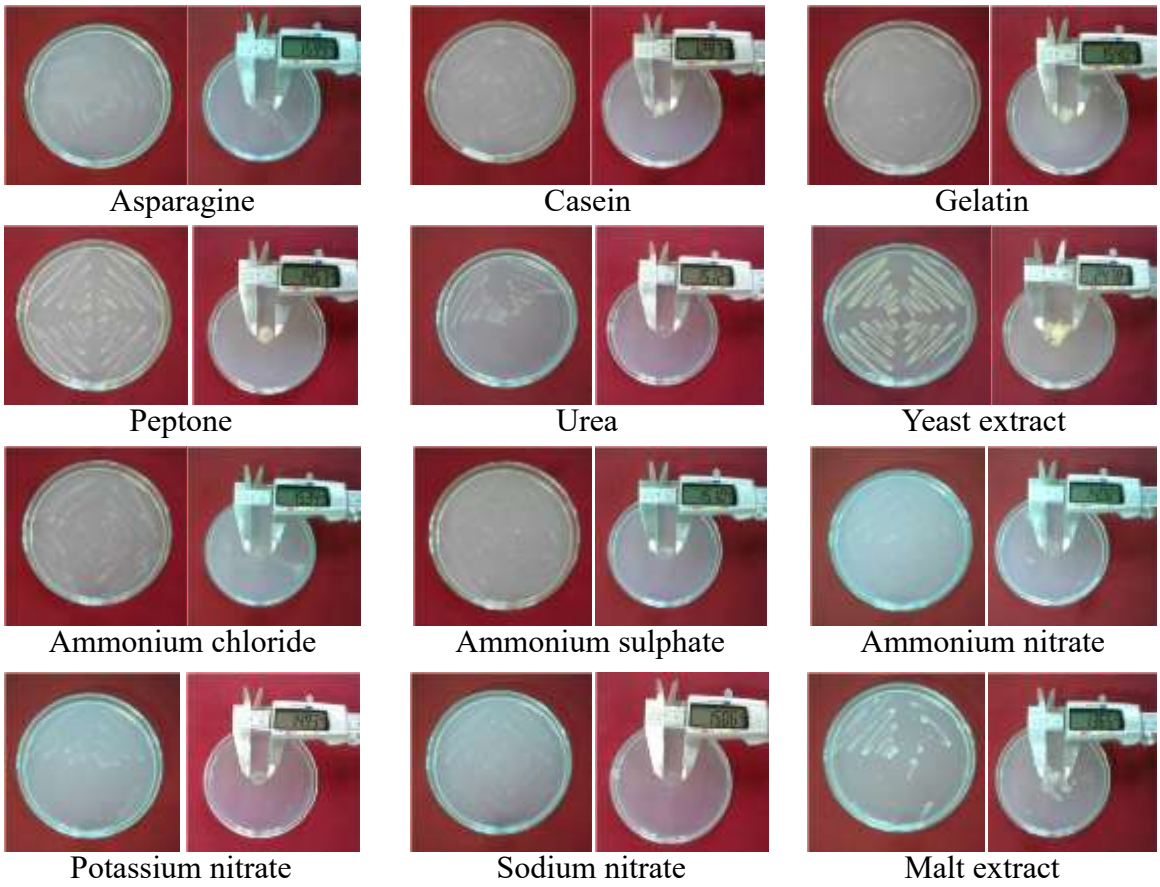


Figure 4 Growth morphology of selected bacterium KM-39 on nitrogen sources (2 days old culture)

Table 4 Antifungal activity on fermentation period of selected bacterium KM-39 against *C. albicans*

Fermentation period (day)	Antimicrobial activity (mm)
1 day	28.74
2 days	**32.35**
3 days	24.79
4 days	22.12
5 days	20.96

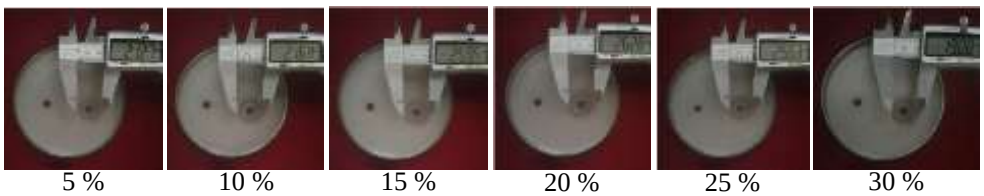
1 day 2 days 3 days 4 days 5 days

Figure 5 Antifungal activity of selected bacterium KM-39 against *C. albicans*

Table 5 Effect of size of inoculum for selected bacterium KM-39

Size (%)	Inhibitory zone (mm)
5%	21.27
10%	21.64
15%	25.95
20%	**26.28**
25%	25.53
30%	19.08

Test organism was *C. albicans*(fermentation 2 days)



5 % 10 % 15 % 20 % 25 % 30 %

Figure 6 The effects of sizes of inoculum on *C. albicans*for KM-39

Table 6 The effect of ages of inoculum of the fermentation against *C. albicans*

Age of Inoculum (hrs)	Antifungal activity inhibitory zone (mm)
24 hrs	22.79
36 hrs	25.98
48 hrs	29.53
60 hrs	29.61
72 hrs	**31.93**
84 hrs	28.69
96 hrs	27.37
108 hrs	27.29
120 hrs	25.88
132 hrs	25.80
144 hrs	25.49
156 hrs	21.75

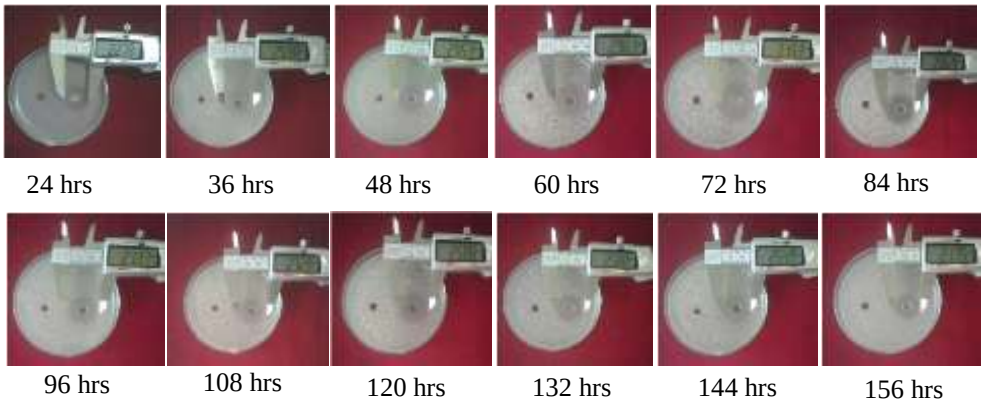


Figure 7 The effects of age of inoculum on *C. albicans*for KM-39

Investigation of carbon sources utilization

The effects of different carbon sources were observed for the growth rate and maximum antimicrobial metabolites production. The addition of arabinose, dextrose, lactose, maltose, sucrose, xylose, glycerol and mannitol were excellent growth, fructose, galactose and soluble starch showed good results. In the antifungal activity, the highest activity of KM-39 was obtained by using the maltose (23.08 mm) on *C. albicans.*

Table 7 Antifungal activity on different carbon sources of selected bacterium KM-39

No.	Carbon sources	Inhibitory zone (mm)
1	Arabinose	21.18
2	Dextrose	20.34
3	Fructose	20.87
4	Lactose	21.78
5	Galactose	20.34
6	Xylose	21.50
7	Sucrose	21.28
8	**Maltose**	**23.08**
9	Glycerol	19.76
10	Mannitol	19.64
11	Soluble starch	20.93

Agar well = 8 mm

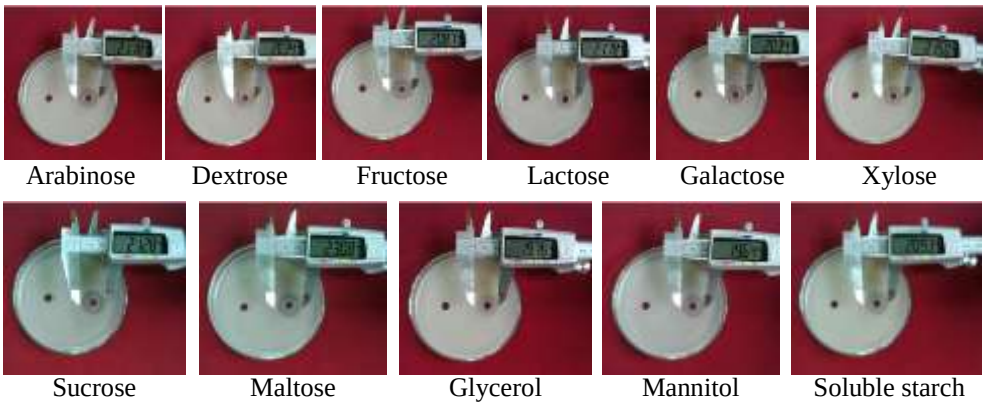


Figure 8 Effects of carbon utilization of fermentation against *C. albicans*

Investigation of nitrogen sources utilization

In this study, excellent growth of KM-39 was found on asparagine, gelatin, urea, yeast extract, ammonium chloride, ammonium sulphate and sodium nitrate, moderate growth on casein

and other peptone, ammonium nitrate, potassium nitrate and malt extract showed good results. The highest antifungal activity was obtained by using potassium nitrate (38.04mm) on *C. albicans.*

Table 8 Antifungal activity on different nitrogen sources of selected bacterium KM-39

No.	Nitrogen sources	Inhibitory zone (mm)
1	Asparagine	20.36
2	Casein	18.61
3	Gelatin	18.54
4	Peptone	19.32
5	Urea	17.61
6	Yeast extract	21.67
7	Ammonium chloride	17.64
8	Ammonium sulphate	12.63
9	Ammonium nitrate	27.35
10	**Potassium nitrate**	**38.04**
11	Sodium nitrate	31.66
12	Malt extract	19.21

Agar well = 8 mm

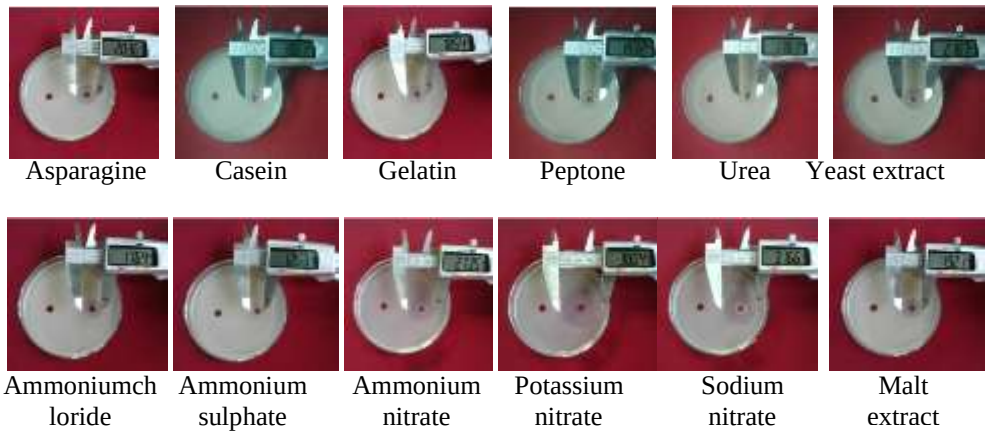


Figure 9 Antifungal activity on different nitrogen sources of selected bacterium KM-39

Table 9 Effects on pH utilization of KM-39 against *C. albicans*

pH range	Inhibitory zone (mm)
4	29.84
5	30.11
6	30.25
7	31.66
8	**32.00**
9	30.48
10	29.15

Figure 10 Effects of pH on the fermentation of KM-39 on *C. albicans*

Table 10 Effect of different temperature utilization of fermentation against *C. albicans*

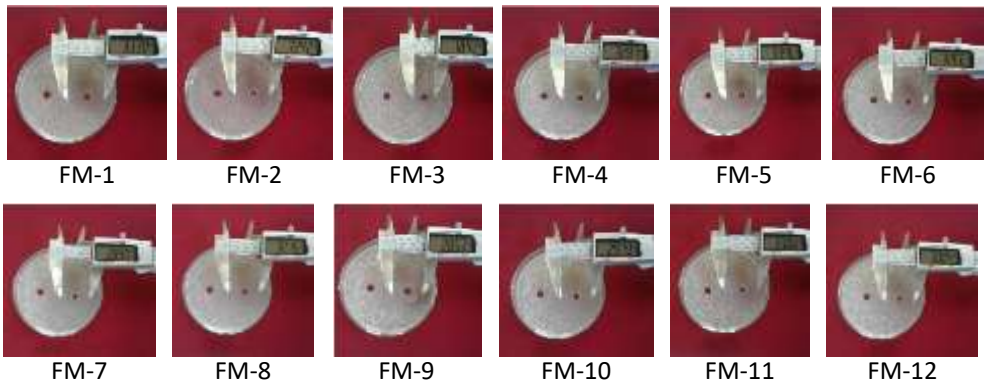
Temperature range	Inhibitory zone (mm)
25ºC	16.69
30ºC	21.41
35ºC	22.07
40ºC	**23.17**
45ºC	-

25ºC 30ºC 35ºC 40ºC

Figure 11 Effects of different temperature utilization of fermentation against *C. albicans*

Table 11 Selection of fermentation medium based on the results of antifungal activity of KM-39

Fermentation media	Antifungal activity (mm)
FM-1	33.30
FM-2	32.42
FM-3	18.42
FM-4	32.87
FM-5	**33.46**
FM-6	32.66
FM-7	28.40
FM-8	32.16
FM-9	28.53
FM-10	29.19
FM-11	33.06
FM-12	30.04



FM-1 FM-2 FM-3 FM-4 FM-5 FM-6

FM-7 FM-8 FM-9 FM-10 FM-11 FM-12

Figure 12 Selection of fermentation medium based on the results of antifungal activity of KM-39

Comparison between shaking culture and static culture

In the comparison between shaking culture and static culture, the shaking culture of KM-39 showed the inhibitory zone (25.22 mm) and the static culture showed (22.00 mm) for 2 days fermentation against *C. albicans.*

Shaking culture on *C. albicans*

Static culture on *C. albicans*

Figure 13 Comparison of shaking culture and static culture against*C. albicans*

Discussion and Conclusion

In the present study, the fermentation period of selected bacterium KM-39 showed the highest antifungal activity(32.35mm) on *C.albicans.*In the investigation to optimize the fermentation, it was found that 72 hrs age of inoculum and 20% of size of inoculum were suitable for fermentation.

Mansi and Charlie, 2003 reported that 72 hrs ages of inoculum was suitable for fermentation.The addition of lactose as a carbon source resulted the excellent growth(21.17mm) and yeast extract as nitrogen source also showed the excellent growth of (16.78mm) in KM-39.

Moreover maltose as the carbon source, KM-39 showed the highest activity(23.08mm) while potassium nitrate (38.04mm) showed the best results in nitrogen sources.The carbon substrate has a dual role in biosynthesis and energy generation, with carbohydrates being the usual carbon source for microbial fermentation processes(ward 1991; Stanbury *et al*. 1995).

In the comparison of shaking culture and static culture, the diameter of inhibitory zone was more higher activity (25.22 mm) on *C. albicans*than the static culture.

Mansi and Charlie, 2003 also reported that shake-flask cultures are better in an incubator shaker to produce large volumes of culture for analysis or for use as an inoculum of a fermenter.

It was concluded that the present study, the optimal fermentation conditions are very important for maximum productivity metabolites and further work will be studied detail characterization of bioactive compounds.

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OPTIMIZATION OF FERMENTATION CONDITIONS, EXTRACTION OF CRUDE EXTRACT AND IDENTIFICATION OF SOIL FUNGUS MM-34

Mya Min Min Myo[1], Swe Swe Myat[2] and Myo Htaik Aung[3]

Abstract

In the present study, twenty different soil samples were collected from twenty different places of Belu-Gyun, Chaung-Zon Township, Mon State. Totally 37 fungi were isolated from 20 soil samples. Among these 37 isolates, MM-34 was found to show maximum antibacterial activity, in compare to other isolates. Thus, soil fungus MM-34 was selected for the study of effects of pH on fermentation. Initial pH of 5.0 was the best for fermentation conditions. Maximum antibacterial activity (31.01 mm inhibitory zone) against *Micrococcus luteus* was observed in pH 5.0. Different temperature such as 20°C, 25°C, 30°C, 35°C and 40°C was studied for maximum production of antibacterial activity by soil fungus MM-34. Maximum antibacterial activity by fungal isolate MM-34 was recorded at temperature 30°C (30.52 mm inhibitory zone) against *Micrococcus luteus.* In the study of static and shaking culture, static culture was optimal condition for MM-34 fermentation (30.68 mm inhibitory zone). Based on the all optimum fermentation conditions, the selected soil fungus MM-34 was carried out by fermentation period. It was observed that the culture which were incubated for 6 days shows maximum antibacterial activity (34.84 mm inhibitory zone) against *Micrococcus luteus.* According to the R_f values, ethyl acetate is suitable for the extraction of crude extract from the fermented broth. Crude extract of ethyl acetate was observed antibacterial activity (26.19 mm inhibitory zone) against *Micrococcus luteus*. The TLC plates were developed in the solvent of chloroform and chloroform-methanol mixture (9:1, 8:2, 7:3, 6:4, 4:6 and 3:7) and Hexane only and Hexane-ethyl acetate mixture (9:1, 8:2, 7:3, 6:4, 5:5, 4:6 and 3:7). Based on the morphological and microscopical characters, soil fungus MM-34 was identified as *Penicillium* sp.

Keywords: Antibacterial activity, Optimization, *Penicillium* sp.

Introduction

Soil is a naturally occurring loose mixture of mineral and organic particles, considered as one of the most suitable environments for microbial growth (Nejad *et al.*, 2013). The dominant microorganism in all soils is fungi. Fungi are an abundant group in acid soil because acidic condition is not promoting the growth of bacteria and actinomycetes. Many new and interesting bioactive metabolites such as antibiotics, antiviral, anticancer and antioxidant compounds having pharmaceutical, industrial and agricultural importance are isolated characterized from soil fungi (Stobel and Daisy, 2003).

Antimicrobial substances or antibiotics are now referred to compound produced by microorganisms, or to a similar compound which inhibits other microorganisms at low concentration. The most well-known antibiotics produced by fungi are penicillins, cephalosporins and fusidic acid (Denyer *et al.*, 2004). In 1928, penicillin, the first antibiotic was incidentally discovered by Sir Alexander Fleming who isolated *Penicillium notatum*, which produced Gram positive bacteria killing compound. This discovery was the starting of the attention of secondary metabolites produced by microorganisms (Taylor *et al.*, 2003) and fungi became the interesting source of bioactive compounds since then.

Penicillium is a genus of ascomycetous fungi and has an important role in various natural processes. The wide and ubiquitous presence of the *Penicillium* species has been researched in several studies. According to a comprehensive literature analysis *Penicillium* is one of the most

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common fungi occurring in various environments such as soil, air and extreme environments (temperature, salinity, water deficiency and pH) and is also associated with plants and specific food products. Due to its huge diversity and existence in extreme environments there is great potential in using it for various environmental, biotechnological and industrial applications (Yadav *et al.*, 2018).

The aim and objectives of this research were to study the optimum fermentation conditions viz., pH, temperature, static and shaking conditions of fermentation and fermentation period, to select the suitable solvent for the extraction of crude extract and to identify the selected soil fungus MM-34.

Materials and Methods

Isolation of fungi

The isolation of microorganisms was taken by feeding method (Hayakawa, and Kobayashi, 2005). To prepare the isolation medium, glucose 1.0 g, yeast extract 0.7 g, K_2HPO_4 0.01 g, KNO_3 0.02 g, agar 1.8 g were placed in a 250 mL conical flask. The transfer medium was glucose 1.2 g, yeast extract 0.8 g, K_2HPO_4 0.01 g, $MgSO_4$ 0.01 g, agar 1.8 g.

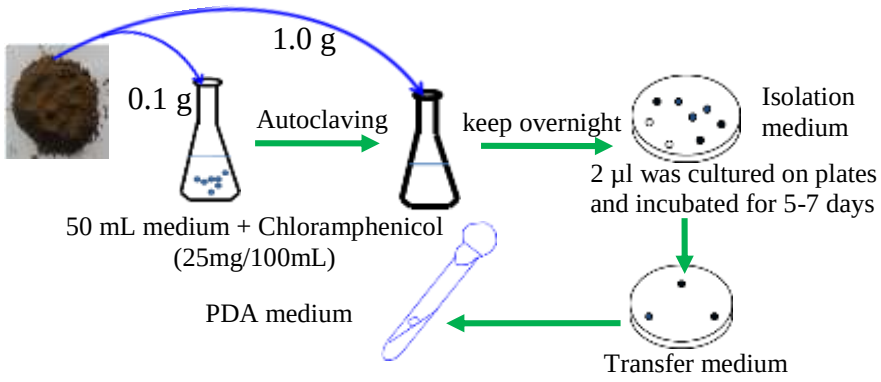


Figure 1 Feeding method (Hayakawa, and Kobayashi, 2005)

Effect of pH on antibacterial activity of soil fungus MM-34

Effect of different pH viz. 3, 4, 5, 6, 7, 8 and 9 was examined on the antibacterial activity of selected fungal isolates MM-34. The fermentation broth was adjusted to the desired pH by adding 1 N HCl or 1 N NaOH. The antibacterial activity was checked by paper disc diffusion assay method.

Effect of temperature on antibacterial activity of soil fungus MM-34

The optimum temperature for antibacterial activity was measured by incubating the fermentation broth at temperature 20ºC, 25ºC, 30ºC, 35ºC and 40ºC. The antibacterial activity was checked by paper disc diffusion assay method.

Effect of Static and Shaking Condition

To determine the effect of static and shaking on antibacterial activity of fungus MM-34, culture flasks (250 mL flask containing 100 mL fermentation medium) were incubated as static cultures as well as incubated in a rotatory shaker at 150 rpm.

Figure 2 Comparison with static and shaking cultures of MM-34 fermented broth

Paper chromatography to extract the crude extract of soil fungus MM-34

Paper chromatography was carried out to extract the crude extract from the fermented broth. The purpose of paper chromatography is to extract the crude extract using suitable solvent systems: 100% hexane, n-butanol saturated with water, 100% toluene and ethyl acetate saturated with water were used as solvents. The paper was chromatographed in each solvent. Then, bioautography was done to check the antibacterial activity of each. Each paper was placed on assay agar plates. After one hour, they were peel off and kept at over one night. Finally based on R_f value, optimum solvent will be chosen.

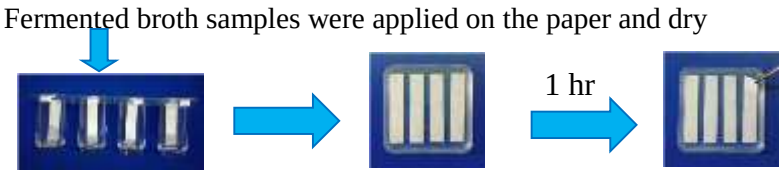


Figure 3 Preparation of paper chromatography

Extraction of crude extract from fermented broth of soil fungus MM-34

Based on PPC result, the fermented broth was carried out by extraction. The broth culture was filtered to separate the mycelia and the filtrate. To the filtrate added to extraction with ethyl acetate in the same volume, shaked well for half an hour and kept for 5 minutes until the two clear immiscible layers was formed. The upper layer of ethyl acetate containing the bioactive component was separated using a separating funnel. The antibacterial activity of the concentrated crude extract of ethyl acetate against *Micrococcus luteus* was checked by paper disc diffusion assay method.

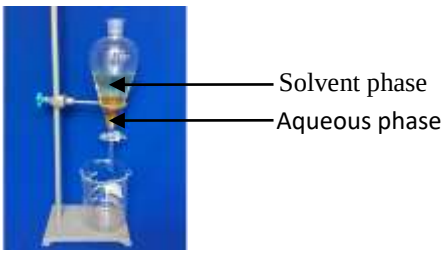


Figure 4 Extraction of crude extract from fermented broth of soil fungus MM-34 with ethyl acetate

Thin layer Chromatography and Bioautographic Assay

The obtained crude extract of ethyl acetate (20 µL) were applied on the TLC plate and allowed to dry. The TLC plates were developed in the solvents of Hexane and Hexane-Ethyl Acetate mixture (9:1, 8:2, 7:3, 6:4, 5:5, 4:6 and 3:7) and chloroform and chloroform-methanol mixture (9:1, 8:2, 7:3, 6:4, 4:6 and 3:7). Then, bioautography was done to check the antibacterial activity of each. Each TLC plate was placed on assay agar plates, then the plates were incubated for 24 hours.

Identification of soil fungus MM-34

Soil fungus MM-34 was identified up to the genus level on the basis of their morphological characteristics such as colony morphology, color and growth pattern. The morphological character of the soil fungus MM-34 was investigated by direct microscopic examination of water-agar medium. The cultures were examined microscopically after 5-9 days incubation, using light microscopy.

The fungal strain was identified by Fungi Imperfecti, Barnett, 1969.

Results

Morphology and its antibacterial activity of isolated soil fungus MM-34

Front view Reverse view

Figure 5 Morphology of isolated soil fungus MM-34

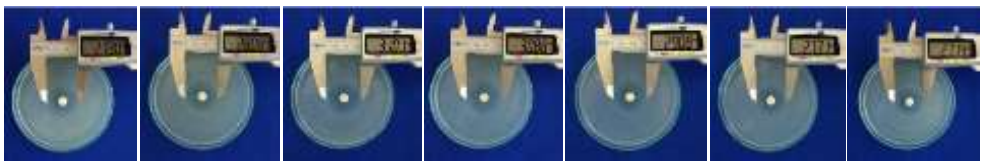
Figure 6 Antibacterial activity of isolated soil fungus MM-34

Study on the effect of pH on the fermentation

The effect of pH on antibacterial activity of the fungal isolate MM-34 was tested using liquid culture at different pH levels (pH 3 to 9). Maximum antibacterial activity was obtained at pH 5.0 (31.01 mm clear zone) (Table 1, Figure 7).

Table 1 The effect of pH on the fermentation

pH	Activity (Clear zone, mm)
3	27.30
4	28.08
5	**31.01**
6	30.88
7	29.08
8	27.31
9	27.14



pH-3 pH-4 pH-5 pH-6 pH-7 pH-8 pH-9

Figure 7 The effect of pH on the antibacterial activity of soil fungus MM-34

Study on the effect of temperature on the fermentation

In this study, the effect of temperature on the antibacterial activity of fermentation was done by carrying out the fermentation at five different temperature 20ºC, 25ºC, 30ºC, 35ºC and

40ºC. The maximum antibacterial activity was observed at 30ºC (30.52 mm clear zone) (Table 2 and Figure 8).

Table 2 The effect of temperature on the fermentation

Temperature	Activity (Clear zone, mm)
20ºC	14.63
25ºC	24.14
30ºC	**30.52**
35ºC	20.64
40ºC	16.50

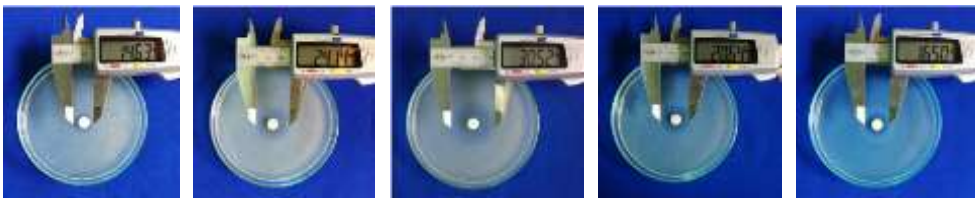


Figure 8 The effect of temperature on the antibacterial activity of soil fungus MM-34

Effect of Static and Shaking Condition

In this investigation, it was found that the antibacterial activity of static culture (30.68 mm) was higher than that of shaking culture (26.65 mm) as shown in Figure 9 and Table 3.

Table 3 Comparison with Static and Shaking Culture of MM-34 against *Micrococcus luteus*

Fermentation condition	Activity (Clear zone, mm)
Static culture	**30.68**
Shaking culture	26.65

Figure 9 Comparison with static and shaking culture of MM-34 against *Micrococcus luteus*

Effect of fermentation period on the antibacterial activity of soil fungus MM-34

Based on the result of the optimum fermentation conditions, fermentation period was carried out. Soil fungus MM-34 showed the highest antibacterial activity (34.84 mm, inhibitory zone) against *Micrococcus luteus* at 6 days fermentation period (Table 4, Figure 10).

Optimum fermentation conditions for antibacterial activity

Ages of inoculum = 84 hr seed culture
Sizes of inoculum = 15%
pH = 5.0
Temperature = 30ºC
Suitable fermentation condition = static

Fermentation medium FM-3

Potato	2.0 g
Yeast extract	1.0 g
$MgSO_4$	0.001 g
DW	100 mL

Table 4 Effect of fermentation period on antibacterial activity of soil fungus MM-34

Fermentation period	Activity (Clear zone, mm)
3	19.80
4	26.21
5	29.09
6	**34.84**
7	30.00
8	26.90
9	25.53
10	20.58

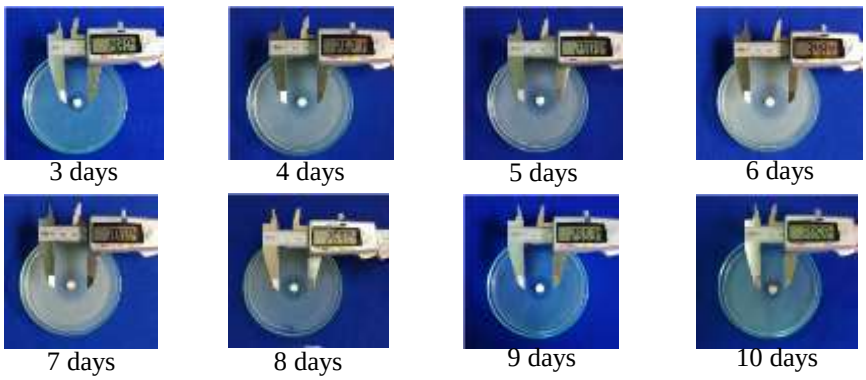


Figure 10 Effect of fermentation period of antibacterial activities against *icrococcus luteus*

Paper chromatography to extract the crude extract from fermented broth of soil fungus MM-34

In the present study, it was observed that R_f values are 0.00 in 100% hexane, 0.86 in n-butanol, 0.06 in 100% toluene and 0.96 in ethyl acetate. According to the R_f value, solvent ethyl acetate is more suitable for the extraction of crude extract than the other solvent (Figure 11).

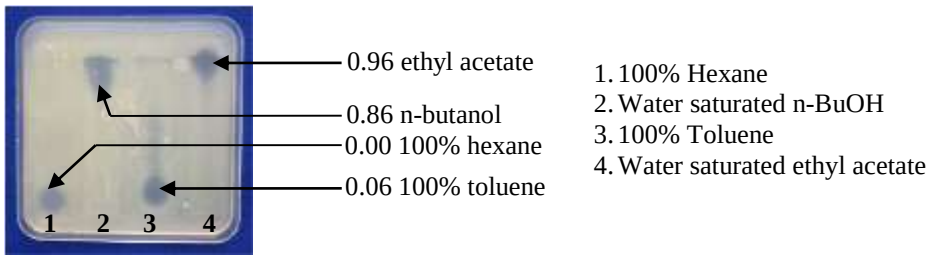
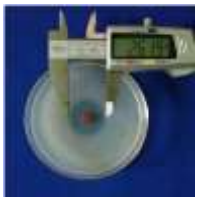


Figure 11 Paper Chromatography of antibacterial activity against *Micrococcus luteus*

Extraction of crude extract from fermented broth of soil fungus MM-34

According to the results, crude extract could be extracted with ethyl acetate. Therefore, crude extract from fermented broth was extracted with ethyl acetate in the equal volume and ethyl acetate was concentrated. Antibacterial activity of ethyl acetate extract was 26.19 mm against *Micrococcus luteus* (Figure 12).

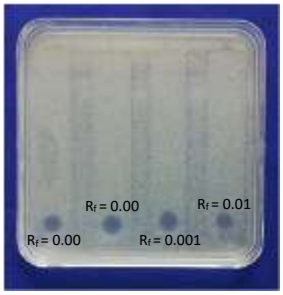


Extract

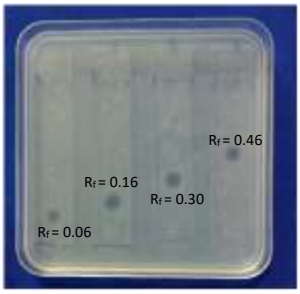
Figure 12 Antibacterial activity of crude extract of ethyl acetate against *Micrococcus luteus*

Thin layer chromatography and bioautographic overlay assay

Based on the TLC results (R_f values) (Figure 13) it was found that hexane-ethyl acetate solvent system was suitable for the separation of compound by silica gel column chromatography.



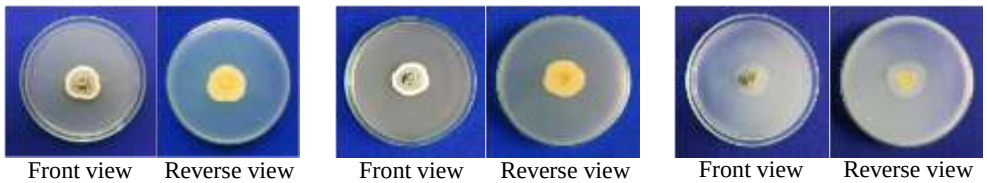
1. Hexaneonly
2. Hexane-ethyl acetate mixture (9:1)
3. Hexane-ethyl acetate mixture (8:2)
4. Hexane-ethyl acetate mixture (7:3)



1. Hexane-ethyl acetate mixture (6:4)
2. Hexane-ethyl acetate mixture (5:5)
3. Hexane-ethyl acetate mixture (4:6)
4. Hexane-ethyl acetate mixture (3:7)

Figure 13 Thin layer chromatography with Hexane-ethyl acetate mixture

Identification of soil fungus MM-34



Front view Reverse view on PDA medium

Front view Reverse view on MEA medium

Front view Reverse view on CA medium

Figure 14 Colony morphology of soil fungus MM-34 (7 days old cultures)

Figure 15 Colony morphology of soil fungus MM-34 (7 days old cultures)

Table 5. Colony morphological colour of MM-34 (7 days old cultures)

No.	Medium	Upper surface	Reverse surface
1	PDA	bluish-gray green at center and white at periphery, exudates present (2.7 × 2.7 cm)	pale yellow at center and white at periphery
2	MEA	dull green at center and white at periphery (3.0 cm × 2.6 cm)	pale yellowish green at center and pale yellow at periphery
3	CA	thin, white at center and thick, white at periphery (2.7 cm × 2.5 cm)	white
4	OA	light green at center and 3-concentric white at periphery, exudates present (2.5 cm × 2.3 cm)	pale yellow at center and white at periphery
5	YEA	pale red at center and white at periphery, exudates present (3.0 cm × 2.4 cm)	pale yellow

PDA - Potato Dextrose Agar, MEA - Malt Extract Agar
CA - Corn Agar, OA - Oatmeal Agar,
YEA - Yeast Extract Agar

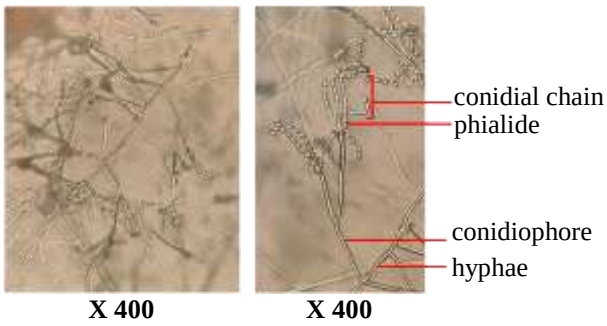


Figure 16 Photomicrograph of soil fungus MM-34

Morphological Characters of MM-34

Colonies are slow growing, produces filamentous, flat, radially sulcate colonies. These colonies are bluish-gray-green at center and white at the periphery. Reverse surface are pale yellow at center and white at periphery. Production of exudates observed at the center of the front surface.

Microscopical Characters of MM-34

Hyphae septate, conidiophores are hyaline, erect, branched, terminated by flask-shaped phialides, chains of single-celled conidia are produced in basipetal succession from a specialized conidiogenous cell called a phialide, 2-3. Conidium elliptical, catenulate.

Key to the genus *Penicillium* (Barnett, 1969)

A1 Mycelium coenocytic, septa infrequent or absent; conidia present ---------- (conidial PHYCOMYCETES)

* A2 Mycelium not coenocytic, with frequent septa; conidia normally present, except in a few genera ------- (FUNGI IMPERFECTI) -- B1

* B1 Conidia and conidiophores not produced within a pycnidium or acervulus ----- (MONILIALES)---------- C2

B2 Not parasitic on small, soil-inhibiting animals ----------(MUCORALES)

C1 Conidia more or less coiled or spirally curved, hyaline or dark ---------- (parts of Moniliaceae, Demaliaeae and Tuberculariaceae)

* C2 Conidia not coiled ---------- D1

* D1 Both conidia and conidiophores (if present) hyaline or brightly colored; conidiophores not united into sporodochia or synnemata (Moniliaceae) ----------E1

D2 Conidiophores forming a sporodochium

* E1 Conidia 1 celled, globose to short cylindrical ---------- F2

E2 Conidia more or less globoid, aquatic

F1 Conidiophores absent or reduced to phialides or peg-like sterigmata

* F2 Conidiophores present, although sometimes short ----------G2

G1 Cells of conidiophore not differing greatly from the catenulate conidia

* G2 Conidiophore and its branches distinct from conidia ----------H1

* H1 Conidiophores simple or sparingly branched; phialides, if present, not tightly clustered ---------- I1

H2 Conidiophores branched; conidia formed acropetally ----------Morilia

* I1 Conidia catenulate ---------- J2

I2 Conidia not catenulate

J1 Phialides or conidia borne on swollen portion of conidiophore

* J2 Swollen fertile cells not present ---------- K1

* K1 Conidia borne on phialides, in basipetal chains ---------- L2

K2 Conidia chains not on definite phialides

L1 Conidiophores more or less in a layer; conidia in compact columns ---------- Melarrhizium ----------Myrothecium

* L2 Conidiophores not in layer; conidia usually in loose chains ----------M1

* M1 Phialides in brush-like group, not divergent, not tapering ---------- N2

M2 Phialides divergent, loose, tapering to a tube ---------- Paecilomyces ----------5---------- Spicaria

N1 Conidia truncate at base ----------Scopulariopsis

* N2 Conidia globose to ellipsoid, not truncate at base ---------- *Penicillium*

In the literature references, the characters of soil fungus MM-34 was identified as *Penicillium* sp.

Kingdom	Fungi
Phylum	Deuteuromycota
Class	Hyphomycetes
Order	Moniliales
Family	Moniliaceae
Genus	*Penicillium*

Discussion and Conclusion

In the course of investigation, 37 fungi were isolated from twenty different soil samples which were collected from Belu-Gyun, Chaung-Zon Township, Mon State. Feeding method was used in the isolation of soil fungi. Among 37 soil fungi, MM-34 showed the maximum antibacterial activity (36.35 mm) against *Micrococcus luteus.* Therefore, MM-34 was selected for further investigation. The present investigation was carried out to determine the optimum culture conditions required for the maximum antibacterial activity of soil fungus MM-34.

In this study, the various effects of pH utilization to the culture broth strongly influenced the growth and biosynthesis of active component by MM-34. The pH of the medium determines the rate and amount of growth and other life processes (Lilly and Barnett, 1951). The pH level of the growth medium has a marked effect on secondary metabolite production with synthesis falling rapidly either side of an optimal level. The hydrogen or hydroxyl ion concentration may have a direct effect on cell, or it may act indirectly by varying the degree of dissociation of substances in the medium.

Therefore, the change of pH is also important for the enzyme activity of microorganisms, for the intermediate products, their dissociation and solubility (Rizk *et al.,* 2007). In the present investigation, pH 5.0 is the best for the maximum antibacterial activity by MM-34 fungus suggesting the acidophilic characteristics of the isolate. Similar result had been reported earlier by Jiicheng *et al.,* (2008). It was shown that maximum antibiotics production was obtained at acidic pH.

Parameter optimization is one of the key parts of microbial production system. Temperature variation was also studied as one of the parameters for antibacterial metabolite production, a temperature range of 20ºC-40ºC was studied for maximum antibacterial activity of soil fungus MM-34. In this investigation, the highest antibacterial activity of soil fungus MM-34 was observed at 30ºC (30.52 mm clear zone). Fungi grown at different temperatures revealed 30ºC to be the optimum for maximum antibacterial activity of the selected soil fungus MM-34.

Incubation temperature is known to influence directly the overall growth and development of any organisms. It affects the physiology and subsequently the synthesis of various metabolites (Pandey *et al.,* 2005). Gunasekaran and Poorniammal (2008) have reported highest secondary metabolite production at a temperature of 30ºC in their study. Bhattacharyya and Jha (2011) and Kok and Papert (2002) also obtained maximum production of antimicrobial metabolite at 30ºC. In the present results were in agreement with those mentioned by Gunasekaran and Poorniammal (2008); Bhattacharyya and Jha (2011); Kok and Papert (2002).

In the course of investigation, the isolated soil fungus MM-34 exhibited the antibacterial activity of static culture (30.68 mm) was higher than that of shaking culture (26.65 mm). The fungi behaved in a different manner under static and shaking conditions. The analysis of the results for

antibacterial potential of soil fungi assay demonstrated static culture conditions to be more suitable for soil fungi MM-34 as compared to shaking cultures whereas shaking conditions exhibit different growth pattern in static and shaking condition varying from compact pellets to dispersed mycelium which strongly affect the production of secondary metabolites. The pellets were observed under shaking conditions while mycelial mat was seen under static conditions.

The low antibacterial activity (26.65 mm) produced by pellets under shaking conditions. This supports the earlier contention of various researchers who have used static conditions. Some group of fungi, static conditions were found to be better as reported by earlier researchers (Khadoor *et al.,* 2007; Nicoletti *et al.,* 2007; Rubini *et al.,* 2005). The result of the present work agree with Khadoor *et al.,* 2007 Nicoletti *et al.,* 2007 and Rubini *et al.,* 2005. In the present study, the isolated soil fungus MM-34 was observed on static culture was optimal condition for fermentation.

The optimum period of fermentation for antibacterial activity of soil fungus MM-34 was found to be 6 days and subsequent decline in antibacterial activity after 10^{th} days could be due to exhaustion of nutrients available for the fungi to produce such bioactive compounds. In consonance with earlier studies where the time course for the production of antimicrobial agent differs according to the strain and cultivation conditions (Miao and Qian, 2005; Khaddor *et al.,* 2007).

And then, preliminary studies of paper chromatography was required to extract the crude extract from the fermented broth. Four kinds of different solvents were used to observe the optimum extraction ability of secondary metabolites. Hexane and Toluene, non-polar solvents, were utilized in this paper chromatography. The Rf values of hexane and Toluene were 0.00 and 0.06 respectively.

The crude extract could be extracted with ethyl acetate according to the R_f value of paper chromatography bioassay. Therefore, solvent ethyl acetate is suitable for the extraction of crude extract from the fermented broth. For the purification of active compounds from crude extract of ethyl acetate will be subjected to column chromatography.

Penicillium colonies are initially white, later to green or bluish green color. The colony surface appears flat. They are commonly called the blue or green moulds because they produce enormous quantities of greenish, bluish or yellowish spores which give them their characteristic colours. The penicillia produce conidia from conidiophores that branch near the apex, forming a brush-like structure. Conidia are globose to subglobose, smooth-walled and are produced in basipetal succession from the phialides (Royer and Lobuglio, 2004); fungus MM-34 has 2-3 phialides. Similary *Penicillium pimiteouiense* has 2-3 phialides (Peterson *et al.,* 1999), based on these Characters fungus MM-34 was assumed as genus *Penicillium.*

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IDENTIFICATION AND EXTRACTION OF ANTIBACTERIAL METABOLITES OF SELECTED ENDOPHYTIC FUNGUS SL-37 AGAINST *ESCHERICHIA COLI*

Su Su Latt[1], Zar Zar Yin[2], Thandar Aung[3] and Hlaing Myint Thu[4]

Abstract

The present work was focused on identification of selected endophytic fungus SL-37 from *Prunus persica* (L.) BATSCH., Met-mon leaves, (Mogok Township, Mandalay Region in 2017) and extraction of antibacterial metabolites. According to the result of macroscopic and microscopic characters, SL-37 was identified as *Aspergillus* sp. According to the paper chromatography values, *Aspergillus* sp was produced antibacterial metabolites by using ethyl acetate solvents. The equal ratio (1:1 v/v) ethyl acetate extract was showed higher inhibitory effect (23.91 mm) than (1:1 v/v) n-butanol extract (20.15 mm) against *E coli*. Crude ethyl acetate (5g) was obtained from 20 Liter of fermented broth SL-37. Then the crude extract of SL-37 was adjusted to pH 4, 5, 6, 7, 8, 9 and 10 and pH 6.0 was showed the maximum inhibitory antibacterial activity (23.18 mm) than other pH against *E coli*. These results suggested that the selected endophytic fungus may be utilized for screening the antibacterial metabolites.

Keywords: Endophytic fungi**,** identification, extraction

Introduction

Endophytic fungi inhabit a biotype that is not well studied (Nithya and Muthumary, 2011). This means the opportunity to find new and targeting natural products from interesting endophytic microorganisms, among the myriad of plants in different niches and ecosystems, is great. Endophytes are the chemical synthesizers inside plants (Owen and Hundley, 2004). Endophytic organism especially fungi have enormous potential to produce large range of bioactive secondary metabolites in order to protect their host plant against pathogens (Strobe, 2003). Thus, the search for novel secondary metabolites should concentrate on organisms that inhabit novel biotypes.

These microorganisms are recognized as potential sources of novel natural products for exploitation in medicine, agriculture, and industry. These microorganisms are recognized as potential sources of novel natural products for exploitation in medicine, agriculture, and industry.

The morphological characterization of the fungal isolates were observed and described based on the method of Photita *et al,* 2004. Further identification of fungal isolates was based on the standard taxonomic key included colony diameter, texture, colour, morphology of hyphae and conidia Hyde *et al,* 2000. Traditionally, clinical microbiology laboratories have relied heavily on morphology-based identification methods to differentiate *Aspergillus* species. However many species, especially members of the section *Fumigati* have overlapping morphological characteristics, which has allowed several genetically distinct species to be misidentified (Balajee *et al,* 2005, 2007). This has led to the clustering of species with overlapping morphologies into "species complexes", so that laboratories may report more accurately morphology-based identifications.

Paper Chromatography is a technique that is used to separate and to identify components of a mixture. Paper chromatography is one of the most important and simple chromatographic methods. Paper chromatography has proved to be very successful in the analysis of chemical

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compound and lipid sample in particular. In this chromatography, it uses paper as the stationary phase and a liquid solvent as the mobile phase the sample mixture is placed on a piece of paper, the edge of the paper is carefully immersed in a solvent, after that the solvent moves up the paper due to capillary action. Components of the mixture are carried along with the solvent up the paper to varying degrees, in other words the components of the mixture rise up at different degrees and thus are separated from one another depending on the compound's preference to be adsorbed onto the paper versus being carried along with the solvent. In order to obtain the extent of movement of a component in a paper chromatography, we can calculate retention factor "R_f value" for each separated component in the developed chromatogram. The R_f value is a number that is defined as the ratio of the distance traveled by the solute to the distance traveled by the solvent.

In this present study, *Aspergillus* was isolated from *Prunus persica* (L.) BATSCH, Metmon plant and extraction of antibacterial compounds were studied. Especially, the large number of study have carried out on the antimicrobial compounds produced from endophytic plants. In Myanmar, *Aspergillus* and its antibacterial compounds from *Prunus persica* (L.) BATSCH., (Metmon) leaves has not been carried out yet. Therefore, identification and extraction of antibacterial metabolites produced by *Aspergillus* against *E coli* was mainly studied in this research. In this study, the aim and objectives of present research were to identify the selected endophytic fungus and extraction of antibacterial compounds from this fungus.

Materials and Methods

Identification of selected fungus SL-37

The selected fungus was cultured on eight differential media ie. Blakeslee's Malt Extract Agar (BMEA) medium, Czapek-Dox Agar (CZA) medium, Malt Extract Agar (MEA) medium, Glucose Ammonium Nitrate Agar (GAN) medium, Dichloran-Rose Bengal- Chloramphenicol Agar (DRBC) medium, Potato Dextrose Agar (PDA) medium, low carbon Agar (LCA) medium and Water Agar (WA) medium (after five days of incubation) were observed for macroscopic characteristics such as colony diameter, colony colour and microscopic characteristics including conidiophore, vesicles, phialides and conidia. For microscopic characteristics slides were stained with cotton blue and mounted in lactophenol cotton blue.

Microscopic examination with lactophenol cotton blue

The drop of LPCB was placed on a clean glass slide. With a bent dissection needle, a small portion of the colony was removed from the agar surface and it was placed in the drop LPCB with two dissection needles, apart the mycelial mass of the colony gently teased on slide, with a coverslip was covered, and under the light microscope was observed with low power (X40) magnification.

Paper Chromatography (Tomita, 1988)

The filter paper and four solvents (20% NH_4Cl n-Butanol saturated with water, n-Butanol-Acetic- Water (3:1:1) and ethyl acetate saturated with water) were used for preliminary characterization of compounds. The obtained fermentated broth sample (100 µL) was applied on the paper and allowed to dry. The papers were chromatographed in each solvent. Then, bioautography was done to check the antibacterial activity of each paper. Each paper was placed on assay agar plate. After one hour the paper was taken out, the plates were incubated for 24-36 hours. In this case, the inhibitory zone was measured yielding the R_f value for the corresponding bioactive compound.

$$R_f \text{ value} = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

Extraction of antibacterial metabolite (Natarajan *et al*, 2010)

The fungus was cultivated on BMEA by inoculating selected endophyte culture in 500 mL conical flask containing 250 mL of the medium. The flask was incubated at 30˚C for 5 days with static. After incubation period, fermentation broth of the fungus was filtered with filter paper. The filtrate was extracted with equal ratio of ethyl acetate. Then the mixture was shaken in separating funnel. The organic layer was separated and collected.

The effect of pH on extraction with ethyl acetate (Vasconcelos *et al,* 2015)

The collected organic layer (ethyl acetate layer) was tested adjusting at pH 4, 5.0, 6.0, 7.0, 8.0, 9.0 and 8.0 with desired) 0.1 M NaOH or 0.1 M HCL. Then each adjusted pH sample was tested by using agar well diffusion assay.

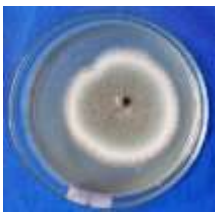
Results

Identification of selected fungus SL-37

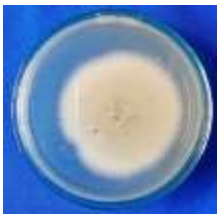
Table 1 Morphological character and colony size of selected fungus SL-37

Sr. No	Culture media	Surface colour	Reverse colour	Colony size (cm)
1	BMEA	Greenish white	Gray	6.5 - 6.4
2	CZA	Gray	Cream	4.7 - 4.7
3	MEA	Greenish white	Yellow	5.5 - 3.1
4	GAN	Pale gray	Cream	1.5 - 1.2
5	PDA	Greenish white	Yellow	7.4 - 7.0
6	LCA	Pale gray	Cream	4.6 - 4.0
7	WA	White	White	2.0 -1.6

A



Surface colour on BMEA



Reverse colour on BMEA

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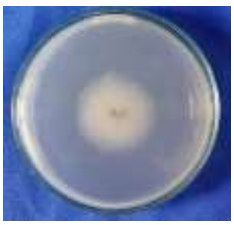
B

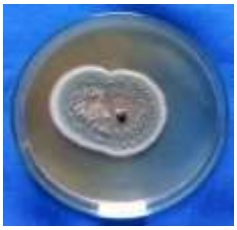
Surface colour on CZA



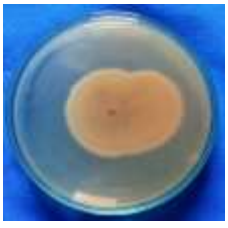
Reverse colour on CZA

X40

C



Surface colour on MEA



Reverse colour on MEA

X40

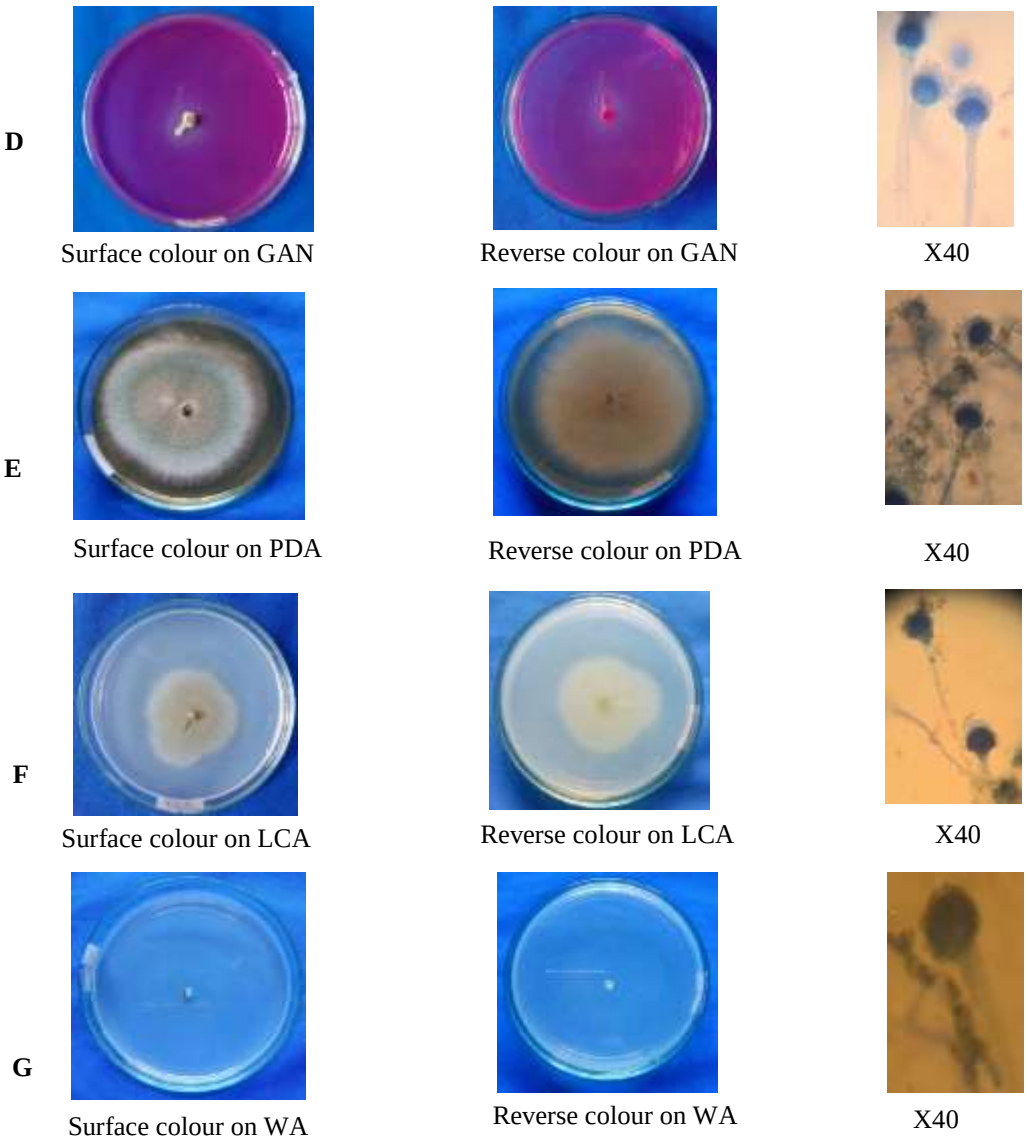


Figure 1 Morphological, colony size and microscopical character of isolated fungus SL-37 on (A) BMEA and (B) CZA media (C) MEA (D) GAN (E) PDA(F) LCA and (G) WA media

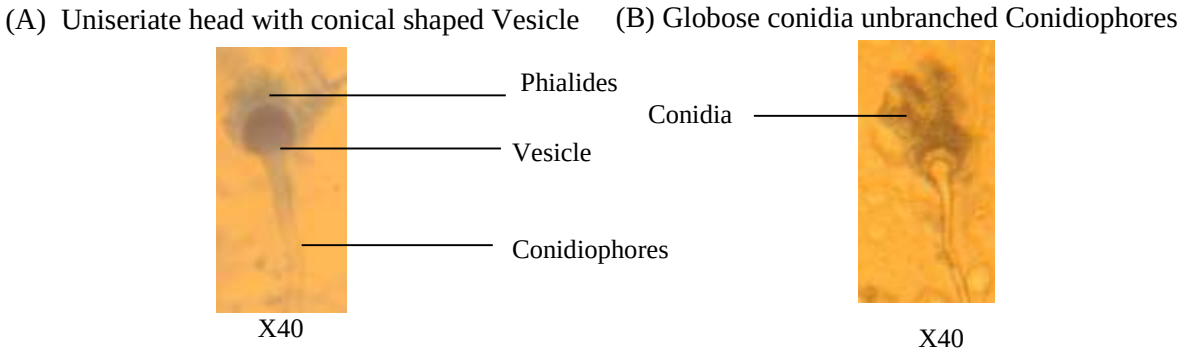


Figure 2 Microscopical character of SL-37

Description of microscopic characters of selected fungus SL-37

Isolated from *Prunus persica* (L.) Batsch. Mogok Townshop, Mandalay Region on seven medium.

Culture medium – BMEA, CZA, MEA, GAN, PDA, LCA and WA

1. Hyphae - aseptate
2. Conidium
   (a) Shape - simple, globose
   (b) Septum - amerospore
   (c) Production - drop
   (d) Colour - hyaline
   (e) Surface - texture
   (f) Size - 29.45 µm
3. Conidiophore - with aseptate, with simple and unbranch
4. Conidiophore development - elongate along with conidium production
5. Conidium development
6. Conidiogenous cell

   Conidium locus - multi loci

According to the distinct characters, selected fungus SL-37 may be identified as the genus *Aspergillus.*

Scientific classification

Kingdom : Fungi
Division : Ascomycota
Class : Eurotiomycetes
Order : Eurotiales
Family Trichocomaceae
Genus : *Aspergillus sp.*

Identification key of selected fungus SL-37

1. Isolated was predominantly uniseriate ……..….....….....……..... 2
1. They had uniserate conidia heads ……..….....….....……..... 2
   2. Conidial heads on MEA, columnar…….....….....……..... 3
   2. Conidia globose to subglobose ……..….....….....…….… 3
3. The colony color was greenish white, reverse gray on BMEA …….....…..... 4
3. The colonies were gray on CZA, reverse cream …….....….....….....4
   4. On DRBC, colonies were greenish white, reverse color gray ….....….....…...5
   4. On GAN and LCA, colony color were pale gray and reverse pale yellow ……..……..……..…….....….....……..…….. 5

5. Colonies on MEA, the colonies were gray and reverse yellow…….... 6

5. Colony diameter after incubation for 7 days, 6.5cm on BMEA, 4.7cm on CZA, 5.5cm on MEA, 1.5cm on GAN, 7.4cm on PDA, 4.6cm on LCA and 2.0cm on WA …….…... 6

    6. Conidiophore stipes are short and hyaline ……..…..…….. 7

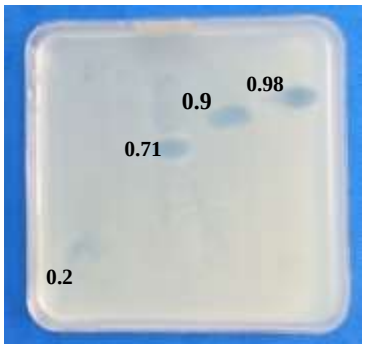
    6. Conidiophores have conical-shaped terminal vesicles ……..….7

7. Vesicles 163 µm, diameter and clavate shape ……*Aspergillus sp.*

7. Vesicles have a single row of phialides on the upper two thirds of the vesicles ……...……..……..…….. *Aspergillus sp.*

Paper chromatography

In this study, four kinds of solvents (20% NH_4CL, n-Butanol, ethyl acetate -acetic-acid-water (3:1:1), n-Butanol saturated with waterand ethyl acetate saturated with water were used. According to the R_f value, 0.98, ethyl acetate was more extractable the antibacterial metabolites than other solvent, follow by n-Butanol solvent (0.9), Ethyl acetic- Water 3:1:1 (0.71) and the lower R_f value, but 20% NH4CL was not showed and R_f value.



1. 20%NH4Cl
2. ethylacetate,n-Butanol-acetic-water (3:1:1)
3. n-Butanol saturated with water,
4. Ethyl acetate saturated with water

Figure 3 Paper chromatography bioautography assay

Comparison of antibacterial activity of metabolite in SL-37 extracted with different volume of EtOAc and n- BuOH against *E. coli*

Using ethyl acetate extract (1:1) resulted in higher inhibition zone 23.91mm, followed by 21.90mm and 20.73mm in ethyl acetate extract (2:1) and (3:1) respectively as well as inhibitory zone 20.15mm was found in n-butanol extract (1:1), 17.57mm in n-butanol extract (2:1) and 16.66mm in n-butanol extract (3:1). Therefore, ethyl acetate extract (1:1) of SL-37 displayed higher inhibition zone than n-butanol extract (1:1). There was no antibacterial activity at all of lower layer. These results were shown in table 2 and figure 4.

Table 2 Comparasion of antibacterial activity of metabolite in SL-37 extracted with different volume of EtOAc and n- BuOH against *E. coli*

Different ratio of solvent	Inhibition diameter zone (mm)	
	EtOAc extract	n- BuOH extract
1:1	23.91mm	20.15mm
2:1	21.90mm	17.57mm
3:1	20.73mm	16.66mm

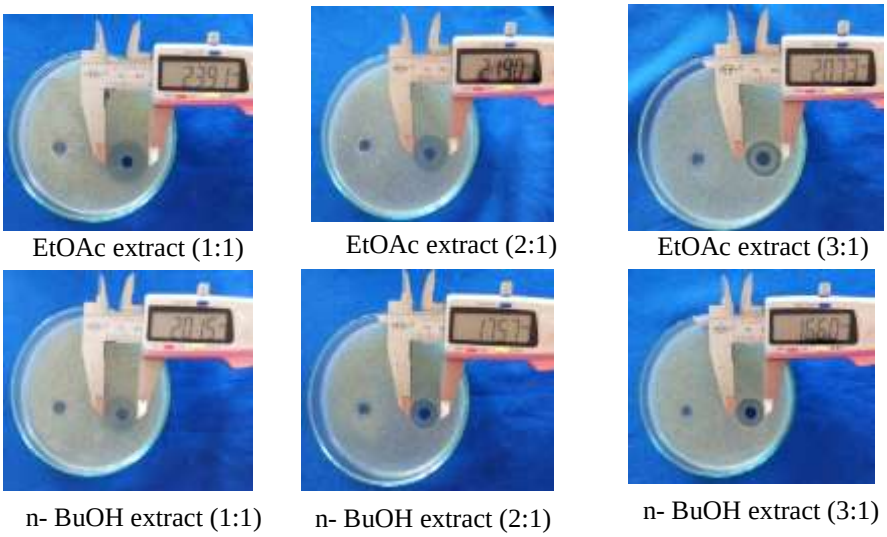


Figure 4 Comparison of antibacterial activity of SL-37 extracted with different volume of EtOAc and n- BuOH against *E. coli*

The antibacterial activity on extracted pH of SL-37

The collected organic layer (ethyl acetate layer) was tested adjustind at pH 4, 5, 6, 7, 8, 9, and 10. The minimum inhibitory zone was found at pH-5 (20.56mm) while maximum inhibitory zone occured in pH 6 in (23.18mm), followed by pH-4 and 5 (19.57mm, 20.56mm) and pH 7 and 8 (20.39mm and 19.68mm) respectively. The negative results were found as pH 9 and 10 (Table 3 and Figure 5).

Table 3 The antibacterial activity on extracted pH of SL-37

pH range	Inhibition zone (mm)
4	19.57
5	20.56
6	23.18
7	20.39
8	19.68
9	17.47
10	-

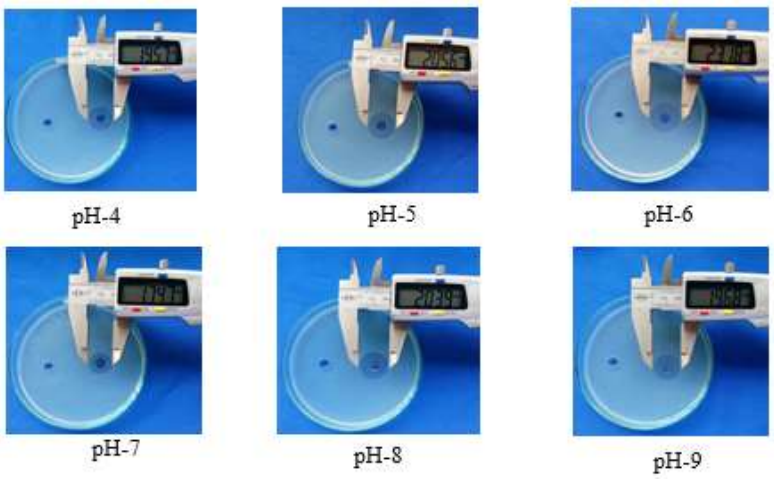


Figure 5 Antibacterial activity on extracted pH of SL-37

Discussion and Conclusion

Endophytes have been intensively studied in several unexplored environments around the world. Endophytes were distributed in each and every plant species and were investigated for endophytic microbial components (Carroll, 2004). Endophytes are chemical synthesizers inside plants (Owen, 2004).

In the identification of selected fungus SL-37, macro and micro characteristics have been studied on seven differential media. Colony diameter after incubation for 7 days, 7.4cm on PDA, 6.5cm on BMEA, 5.5cm on MEA, 4.7cm on CZA, 2.0cm on WA,4.6cm on LCA, and 1.5cm on GAN. It had short columnar and uniseriate conidia head; vesicle 163-239 µm diameter and conical shaped; majority had phialides covering half to three quarter of the vesicles; the conidiophore stipe measured 350 µm long and 45 µm wide, globose and smooth. Diagnostic features; of conidiophore stipe are short, smooth walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two third. The genus has been classified in to section based seriation either uniseriate, the shape of conidia head; globose, radiate, columnar or clavate.

According to the results, the selected fungus SL-37 was identified as genus *Aspergillus sp*. These results were agreement with Ando (2016), Larone and Davise (1995) and Barnette (1969).

Paper chromatography was performed by using four kinds of different solvent were applied to observe the optimum extraction ability of secondary metabolites. According to the R_f value, ethyl acetate was the excellent solvent for SL-37. Furtado *et al,* 2005 recorded antimicrobial activity of metabolite extracted from *Aspergillus fumigatus* within R_f values of 0.33 to 0.91. The antibacterial activity of SL-37 extracted with different ratio of ethyl acetate -and n-butanol (1:1, 2:1, 3:1) were used. The equal ratio of ethyl acetate extract was showed the highest activity of inhibition zone (23.91 mm).

Jain and Pundri 2011 reported that fermentation broth and ethyl acetate solvent (1:1) was applied and the mixture antimicrobial metabolite was obtained by using this ratio. Similarly, Anuhya *et al,* 2017 described that the extraction of the secondary metabolite was effectively done with ethyl acetate and broth culture in ratio (1:1). Garcia *et al,* 2012. reported that the ethyl acetate solvent system was most efficient method to extract endophytic fungi principle compound. In the extraction of antibacterial compounds, endophytic fungus SL-37 (20 liters) were fermented on suitable synthetic fermentation medium and extracted with equal ratio of EtOAc (1:1) to yield 5 g. Then the resultant extract of ethyl acetate solvent was adjusted to 4, 5, 6, 7, 8, 9 and 10. The maximum antibacterial activity was observed at pH 6.0 (23.18 mm). Vasconcelos *et al,* 2015 described that ethyl acetate extract (pH 7) showed antimicrobial activity of with zones of 20 mm and 22 mm.

The identification and extraction of antibacterial metabolites required for further research plan, the purification and identification of isolated compounds and minimum inhibitory concentration (MIC).

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GENE EXPRESSION OF *DLNPGR1* IN DIFFERENT TISSUES, DURING SE AND SEED GERMINATION IN *DIMOCARPUS LONGAN* LOUR.

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Abstract

Two important structural units, tetratricopeptide repeat (TPR) motifs and calmodulin-binding domain, are known for their involvement in protein-protein interaction and binding to calmodulin (CaM), respectively. Although many TPR-containing proteins, CaM-binding proteins (CBPs) and their respective encoding genes have been studied, further detail studies are still needed for more understanding of those important proteins and their respective genes. In this study, expression of a gene encoding CBP with TPRs, *NPGR1*, was analysed in different tissues, during SE and germination in *Dimocarpus longan* Lour. By using RT- PCR analysis, the expression levels of *DlNPGR1* in 12 different tissues, in different developmental stages during longan somatic embryogenesis (SE), and during seed germination in different days after sowing. According to our results, among 12 different tissues, the relatively highest expression level was detected in young fruit, followed by floral bud and female flower while mature root, pollen and mature leave exhibited the lowest expression. During SE, the highest expression of *DlNPGR1* was detected in NEC followed by CE while the lowest expression level was detected in EC stage. During seed germination, the highest expression of was detected in day 0 then the expression was significantly lower in day 4 followed by day 8 where the expression level was the lowest. In conclusion, being a gene encoding CBP with TPRs, *DlNPGR1* might be involved in cell division.

Keywords: *No pollen germination related 1*, gene expression, *Dimocarpus longan*, somatic embryogenesis, TPR motifs, CBD domain

Introduction

A sub-tropical and tropical tree *Dimocarpus longan* Lour. is economically and medicinally important. Somatic embryogenesis (SE) of longan was used as a model system for better understanding of the early development of the woody trees (Lai & Chen, 1997; Lai et al., 1997; Lai et al., 2000; Lai et al., 2010; Lai & Lin, 2013).

Embryogenesis is a critical process in the development of higher plants. Somatic embryogenesis (SE) is an in vitro developmental process of plant. SE is a module system that can be used to study of plant embryogenesis (Zimmerman, 1993). It can be used as an alternative experimental system for zygotic embryo development since SE showed close similarities to the development stages to zygotic embryogenesis (Dodeman et al., 1997; Willemsen & Scheres, 2004). Although many studies have been conducted to understand molecular basis during SE, molecular mechanism at early SE is still largely unknown (Elhiti et al., 2013).

There are various calmodulin-binding proteins (CBPs) which are involved in numerous cellular activities such as in metabolism regulation, transport of ions, etc. (Snedden & Fromm, 2001) and pollen germination (Safadi et al., 2000; Golovkin & Reddy, 2003; Zhang et al., 2012; Shin et al., 2014). Tetratricopeptide repeat (TPR) motif has been reported that it is involved in protein-protein interactions including cell cycle control, transcription repression, stress response, protein kinase inhibition, mitochondrial and peroxisomal protein transport and neurogenesis (Lamb et al., 1955).

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Many TPR protein encoding genes (D'Andrea, & Regan, 2003; Rosado et al., 2006; Zeytuni & Zarivach, 2012; Cerveny et al., 2013) and CBP encoding genes (Safadi et al., 2000; Golovkin & Reddy, 2003; Zhang et al., 2012; Shin et al., 2014) have been studied until now. However, further detail studies are still needed for better understanding of the involvement of those important genes. In this work, expression of a gene encoding CBP with TPRs, *DlNPGR1*, was analyzed by using RT-PCR techniques in different tissues, during SE and germination in *D. longan* Lour. cultivar 'Honghezi'. This study was conducted in Fujian Agriculture and Forestry University, China from January, 2015 to January, 2017. This work was conducted to determine the expression profile of *DlNPGR1* in some tissues of longan.

Materials and Methods

Embryogenic Cultures, Different Tissues and Seedlings

By following the protocols reported of Lai and Chen (1997), and Lai et al. (2000), different developmental stages of *Dimocarpus longan* embryogenic cultures i.e., embryogenic callus (EC), incomplete compact proembryogenic cultures (ICpEC), globular embryos (GE), cotyledon embryos (CE) and non-embryogenic callus (NEC) were obtained. Then those tissues were stored at -80 °C or subcultured to use in further study.

A total of 12 different tissues or organs of longan (mature root, pollen, mature leaf, pulp, young stem, vegetative bud, ripe seed, inflorescence, ripe fruit, female flower, floral bud and young fruit) were used to detect the expression pattern of *DlNPGR1* using qPCR analysis. Germinated seeds of longan in different days after sowing (das) (0d, 4d, 8d, 12d, 16d, 20d) were also used to detect the expression pattern of *DlNPGR1* during germination.

Sequence Alignment of *DlNPGR1* and *DlNPG1*

The opening reading frame (ORF) sequence of *DlNPGR1* used in this study was retrieved from NCBI (GenBank accession no. KP402183.1) published by Thu, et al. (2020a). In order to determine the sequence identity of *DlNPGR1* with downloaded previously published *DlNPG1* (GenBank: KP402181.1) (Thu et al., 2020b) we used DNAMAN (v.6.0, Lynnon Corporation, Quebec).

Quantitative Real-time PCR (qRT-PCR) Analysis

In order to determine the expression levels of the *DlNPGR1* gene during development of longan SE and in 12 different tissues, qRT-PCR analysis was performed on the LightCycler 480 (Roche Applied Science, Switzerland) after following the previous methods (Lin & Lai, 2010, 2013). The gene specific primers (GSPs) for qRT-PCR analysis were designed based on previously cloned, analyzed and published sequence of *DlNPGR1* cDNA on NCBI (GenBank: KP402183.1). The GSPs were *DlNPGR1*-qF (5′-TGTCTGCCAGTGTATTGTCA-3′) and *DlNPGR1*-qR (5′-TGCTCAAGTTCTTCCAGTGA-3′). For normalization of the expression of *DlNPGR1*, *DlFSD1a, DlEF-1a* and *DlelF-4a* were used as reference genes.

We examined and verified annealing specificity of primers by melting curve analysis. Then PCR efficiency was determined by four-point standard curve of a fivefold dilution series (1:5, 1:25, 1:375 and 1:625) from pooled cDNA. Each reaction well contained a total reaction volume of 20 µL (7.4 µL of ddH_2O, 10 µL of 2 x SYBR Premix ExTaq II (Takara, Japan), 0.8 µL of each primer (100 nM) and 1 µL of cDNA template (1:5 dilution)). The PCR conditions were as follow – preincubation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 10 s. The relative gene expression of *DlNPGR1* was evaluated using the method described by Lin & Lai (2010, 2013).

Analysis of Data

Data were analyzed using SPSS v.19 (SPSS Inc., Chicago, USA). Means were separated by Tukey HSD test at $p < 0.05$.

Results

Sequence Identity

We determined the sequence identity between ORF sequence of *DlNPGR1* and that of *DlNPG1* (Min Kyaw Thu et al., 2020b) by using DNAMAN (v.6.0). The result showed that there was 53.26% identity between the two sequences (Figure 1).

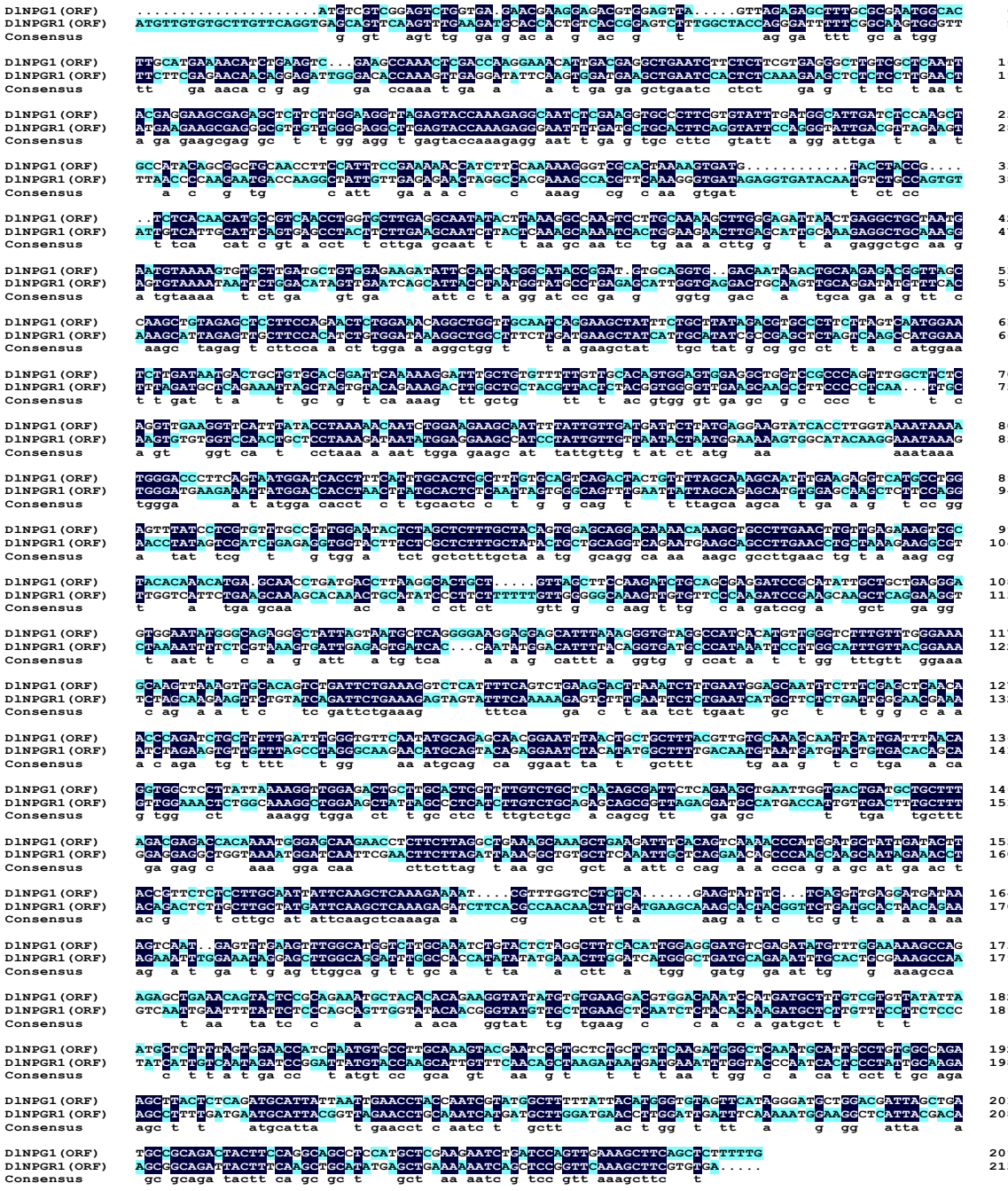

Figure 1 Multiple alignment of *DlNPGR1* with *DlNPG1* using DNAMAN.

Expression of *DlNPGR1* in Different Tissues

qRT-PCR analysis was used to determine the expression pattern of *DlNPGR1* gene in 12 different tissues of longan. Our results showed that the relatively highest expression level was detected in young fruit, followed by floral bud, female flower and so on (Figure 2). Mature root, pollen and mature leave exhibited the lowest expression.

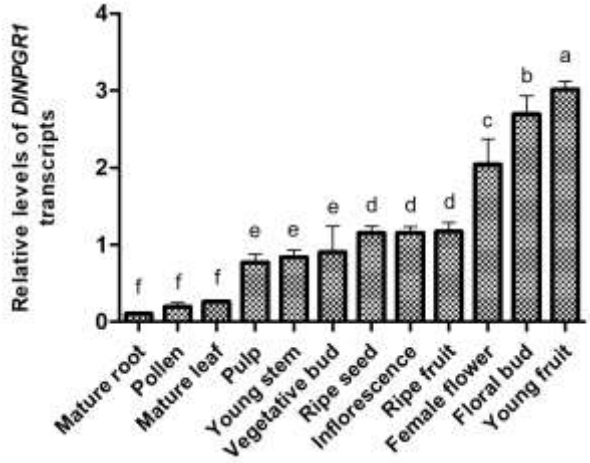

Figure 2 Expression levels of *DlNPGR1* in 12 different tissues of longan. Data are shown as means ± SD (n = 3). Different letters above each bar represent significant differences at $p < 0.05$ by Tukey HSD test.

Expression of *DlNPGR1* during Longan SE

In order to know the transcriptional regulation of *DlNPGR1* in prominent stages during longan SE, we analysed the expression levels of the gene. The highest expression of *DlNPGR1* was detected in NEC followed by CE (Figure 3). Our results showed that the lowest expression level was detected in EC stage. Then the expression level was increased in ICpEC and GE.

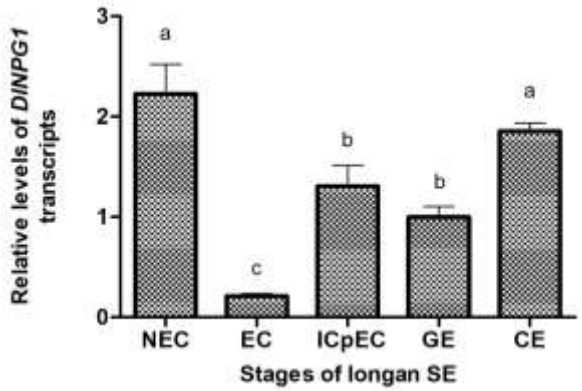

Figure 3 Expression pattern of *DlNPGR1* during longan somatic embryogenesis (SE). The tested stages were non-embryogenic callus (NEC), embryogenic callus (EC), incomplete compact proembryogenic cultures (ICpEC), globular embryo (GE), and cotyledon embryo (CE). Data are shown as means ± SD (n = 3). Different letters above each bar represent significant differences at $p < 0.05$ by Tukey HSD test.

Expression of *DlNPGR1* during Seed Germination

We determined the expression pattern of the *DlNPGR1* during seed germination from day 0 to day 20 after sowing. The highest expression of *DlNPGR1* was detected in day 0 (Figure 4).

Then expression level was lower in day 4 followed by day 8 where the expression level was the lowest. After that, the expression level was significantly higher and higher until day 20 at which we stopped the study.

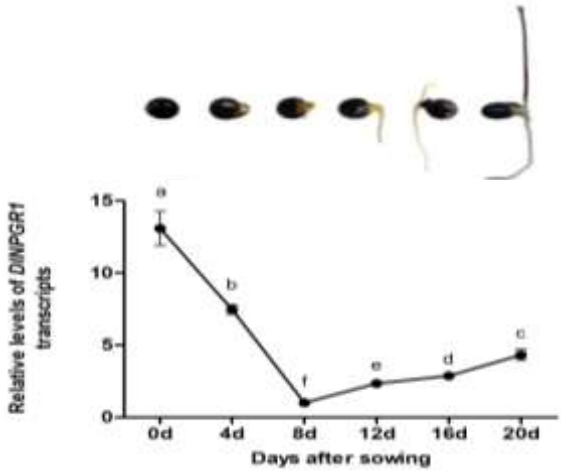

Figure 4 Expression levels of *DlNPGR1* during longan seed germination. Data are shown as means ± SD (n = 3). Different letters above each bar represent significant differences at $p < 0.05$ by Tukey HSD test.

Discussion

Expression Pattern of *DlNPGR1* in Different Tissues

The expression pattern of *DlNPGR1* might differ from that of *DlNPG1* since the similarity between them were low (53.26%). Although the high expression of MPCBP (Safadi et al., 2000), *AtNPG1* (Golovkin & Reddy, 2003) and *OsPCBP* (Zhang et al., 2006) was found in pollen, *DlNPGR1* was not highly expressed in pollen. Instead, the highest expression of this gene was found in young fruit. It can be clearly seen that *DlNPGR1* was actively expressed in developing young tissues such as in young fruit and floral bud.

It has been already reported that *DlNPGR1* gene contains eight TPRs and one CBD (Thu et al., 2020a). TPR-containing proteins are involved in many cellular processes such as cell cycle control, transcription repression, stress response, protein kinase inhibition, mitochondrial, peroxisomal protein transport etc. (Goebl & Yanagida, 1991). As a protein possessing eight TPRs and a CBD, DlNPGR1 might be involved in several cellular activities. *DlNPGR1* might be involved in developmental processes such as tip extension, cell proliferation and cell elongation in young and growing tissues.

Expression of *DlNPGR1* during Longan SE

The expression pattern of *DlNPGR1* suggests that it might be actively involved in NEC and CE followed by ICpEC and GE stages. As cytosolic free calcium is elevated during cell division (Trewavas et al., 1998), CaM and CaM-binding protein NPGR1 might be active at CE stage during longan SE. Moreover, being a TPR-containing protein, it might be involved in cell division and polarized growth.

Expression Pattern of *DlNPGR1* during Seed Germination

We can see that the highest expression of *DlNPGR1* was detected in das 0, then the expression level went down steeply until das 8. Then the expression level rose again after das 8 significantly but gradually until das 20. According to our results, there might be relatively higher

accumulation of the transcripts of *DlNPGR1* in mature seed in order to be used in subsequent germination. After using most of the *DlNPGR1* transcripts for starting germination, it might be necessary for the growth of young root and young shoot. That might be the reason we could detect higher expression after das 8. According to Lamb et al., (1955) and our finding suggests that as a TPR-containing CaM-binding protein DlNPGR1 might be involved in cell division.

Conclusion

In conclusion, *DlNPGR1*, TPR-containing CBP-encoding gene, might be involved in cell division and polarized growth during somatic embryogenesis and seed germination, and during the growth and development of young fruit and floral bud.

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