JOURNAL OF THE MYANMAR ACADEMY OF ARTS AND SCIENCE

Botany and Marine Science

Vol. XIX, No.4B, January, 2023

Myanmar Academy of Arts and Science

Journal of the Myanmar Academy of Arts and Science

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Journal of the Myanmar Academy of Arts and Science

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EXTRACTION OF ANTIFUNGAL COMPOUNDS FROM TR-2 OF MANGROVE WATER AND SLUDGE OF CHAUNG-THA AREA

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Abstract

Extraction of antifungal metabolites from selected strain *Actinomycetes* was carried out by using paper chromatography and four solvent systems. Ethyl acetate was the most suitable solvent and R_f value had 0.86. Crude ethyl acetate extract (2.0 g) was obtained from 10 L of fermented broth and purified over silica gel column chromatography. The ethyl acetate extract was carried out by employing various solvent systems on Thin Layer Chromatography (TLC). The results showed well separated spots on TLC by using the solvent system (pet ether: ethyl acetate). By silica gel column chromatographic separation, compound A (23 mg, white amorphous form) was isolated and subjected to various examinations such as chemical reagent tests, ultraviolet (UV) and FTIR (Fourier Transform Infrared). Based on the results of physicochemical properties and spectroscopic methods, compound A may be flavonoid. In the investigation of minimum inhibitory concentrations (MICs), the MIC value of compound A was 2.5 µg/mL on *Candida albicans*.

Introduction

Chromatography is a separation method based on the partitioning of a solute between a mobile phase and a stationary phase. The mobile phase may be liquid, gas or a supercritical fluid. The stationary phase may be an immobilized liquid or a solid, in either a planar or column form. Based on the physicochemical characteristics of the analyte, and the availability of instrumentation, a chromatographic system is chosen to separate, identify and quantify the analyte (Tomita, 1988).

Marine sediment is an inexhaustible resource that has not been properly exploited. It is estimated that less than 1% of potentially useful chemicals from marine environment has been screened so far, with microbial products representing approximately 1% of the total number. Exploration of microbial secondary metabolites has led to the discovery of hundreds of biologically active compounds.

Members of the actinomycetes, which live in marine environment, are poorly understood and only few reports are available pertaining to microorganism from mangroves (Vikineswari 1997; Rathana and Chandrika, 1993).

Streptomycin was one of the first antibiotics found. It is produced by *Streptomyces griseus.* Today, various *Streptomyces* species are responsible for approximately 75% of both medical and commercial antibiotics and work very well in these areas. Due to the need for new antibiotics, studies have steered towards the isolation of streptomyces and the careful screening of different habitats in which they are used. It has also been found through research that different conditions such as nutrients, culturing, and other factors may affect how *Streptomyces* develop to form antibiotics (Schatz *et al.*, 2005).

Thin layer chromatography is the most familiar and efficient technique method used for the detection, analysis and separation of the bioactive compounds, so it is probably that 60% of the analyzed compounds are performed based on TLC over international.

Thus, it is important to know the basic operation and performance of the TLC protocol (Maitland *et al.*, 2010).

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Thin layer chromatography has been important for the separation of secondary metabolites, Polyphenol, saponin, alkaloids, flavonoid, aromatic amines, amino acids, alcohols, acids, glycols, proteins, amides, antibiotics, peptides, pesticides, bile acids, vitamins and porphyrins in soft drinks (Bhawani *et al.*, 2012).

The bioautography is one of the techniques useful in direct tracing out bioactive compounds from extracts on thin layer chromatogram. Antifungal bioautographic assays system have used classically one- dimensional thin layer chromatography (TLC) to separate the chemical constituent from the extract (Wedge and Nagle, 2000).

Antifungal metabolites can be readily located on the plates by visually observing clear zones where active compound inhibit fungal growth. 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). And MICs are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials (Mirbach & Ali, 2005).

The aim and objectives of this research were to study organic compound from the ethyl acetate extract of the isolate TR-2 by using column chromatographic separation techniques, to characterize the isolated compound by physiochemical tests and spectroscopic techniques such as UV, FT IR and to determine the Minimum Inhibitory Concentrations (MICs) of metabolite on *Candida albicans*.

Materials and Methods

Paper Chromatography Method (Tomita, 1988)

To study of extraction of antibacterial metabolites of selected TR-2 was work done by using paper chromatography. The filter paper and four solvents; 20% NH4Cl, n-butanol saturated water, n-butanol-acetic acid-water (3:1:1) and ethyl acetate saturated with water, were used for preliminary characterization of compound.

The obtained fermented broth samples (100 mL) were applied on the paper and allowed to dry. The papers were chromatographed 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, then the plate were incubated for 24-36 hours. In this case, the inhibitory zone was measured yielding a R_f value for the corresponding bioactive compound.

$$R_f \text{ value} = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

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

Thin layer chromatography (TLC) was performed on ethyl acetate crude extract from the culture broth. The crude fraction was spotted on TLC plate and performed by using various solvent systems. Spots were observed under UV light or by spraying spot with iodine vapour and R_f values were recorded.

$$R_f \text{ value} = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

Silica gel column chromatography (Simon & Grey, 1998)

According to thin layer chromatographic analysis, the ethyl acetate extracted residue of TR-2 metabolite was developed to isolate the active compound by silica gel column chromatography with pet ether: ethyl acetate and ethyl acetate: methanol as eluting solvent. The

silica gel (ca. 50 g) was dissolved in chloroform and the column was packed by the wet method. EtOAc crude extract (2.0 g) was then passed through silica gel column and eluted with pet ether: ethyl acetate solvent (3:1, 2:1, 1:1 and ethyl acetate only). Fractions of each equal to 2 mL, were collected individually, the compound present were checked with TLC and examined the activity with *Candida albicans* by using agar well method.

Column size - (61 ×2 cm)
Flow rate - 1 mL/ 1 min
Eluted solvent - PE: EtOAc

Figure 1 Silica gel column chromatography

Determination of solubility of isolated compound

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

Characterization and Identification of Isolated Compounds

- Determination of R_f value
- Classification by using chemical reagents
- Identification of isolated compound by using modern spectroscopic methods such as: UV, FT IR spectroscopy as well as by comparing with reported data

Determination of Minimum Inhibitory Concentrations (MICs) (Domain, 1999; Jennifer, 2006)

Minimum inhibitory concentration (MICs) was determined by serial dilution method. The concenteations were 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL and 0.312 µg/mL respectively. The selected test organism was *Candida albicans*. After incubation for 24 hours, the MICs values were determined by selection the lowest concentration of antibacterial metabolites which caused complete inhibition of test growth.

Results

Extraction of antifungal compounds from TR-2

In this study, TR-2 was carried out by using paper chromatography and four kinds of solvent 20% NH_4Cl, n-butanol saturated water, n-butanol-acetic acid-water (3:1:1) and ethyl acetate saturated with water, were used. According to R_f value, ethyl acetate was the most suitable solvent and R_f value was (0.86) in this solvent.

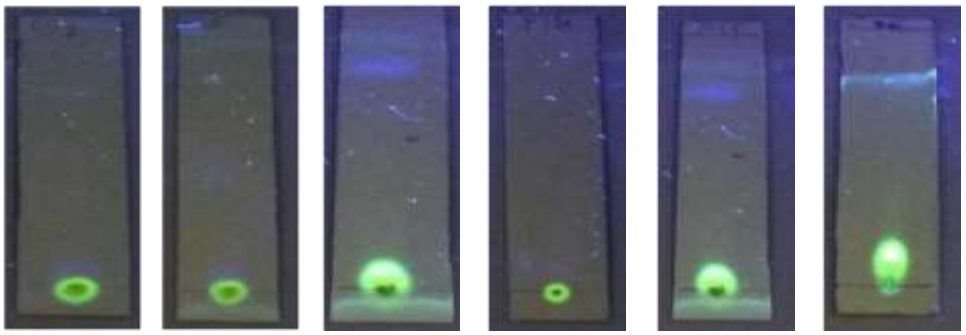
1. 20% NH_4Cl
2. n-butanol saturated with water
3. ethyl acetate-acetic acid water (3:1:1)
4. ethyl acetate saturated with water

Figure 2 Paper Chromatography bioautographic assay

Thin layer chromatographic analysis with various solvent systems

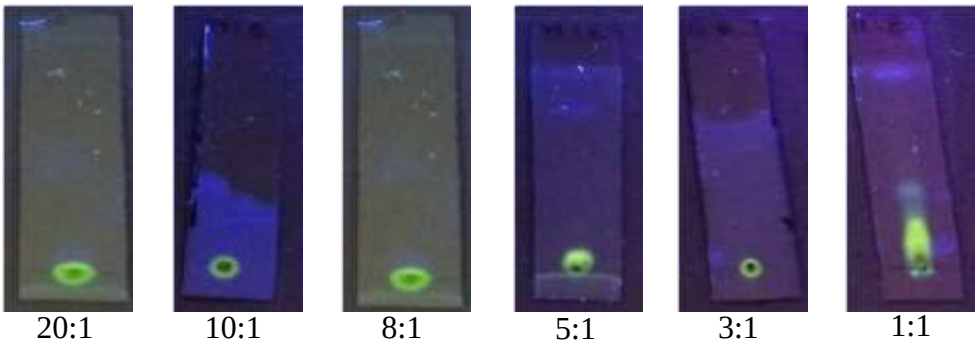
Thin Layer Chromatography was performed on ethyl acetate crude extracts by employing solvent systems: ethyl acetate only, chloroform only, pet ether only, methanol only, hexane only, pet ether: ethyl acetate (20:1, 10:1, 8:1, 5:1, 3:1 and 1:1 v/v), hexane: ethyl acetate (20:1, 10:1, 8:1, 5:1, 3:1 and 1:1 v/v), ethyl acetate: methanol(70:1, 30:1, 10:1, 8:1, 4:1 and 2:1), ethyl acetate: pet ether (20:1, 10:1, 5:1, 4:1, 2:1 and 1:1). The extract showed well- separated spots on TLC by using solvent systems. Therefore, (pet ether: ethyl acetate, ethyl acetate only and ethyl acetate: methanol) solvent systems were chosen to isolate pure compounds by silica gel column chromatography.

Figure 3 Thin layer chromatographic analysis with various solvent systems



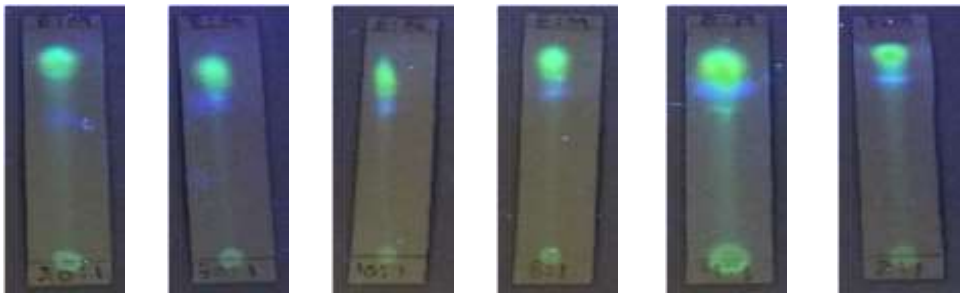
PE: EtOAc

Figure 4 Thin layer chromatographic analysis with different ratios of pet ether: ethyl acetate



Hexane: EtOAc

Figure 5 Thin layer chromatographic analysis with different ratios of Hexane: ethyl acetate



EtOAc: ME

Figure 6 Thin layer chromatographic analysis with different ratios of ethyl acetae: methanol

EtOAc: PE

Figure 7 Thin layer chromatographic analysis with different ratios of ethyl acetate: pet ether

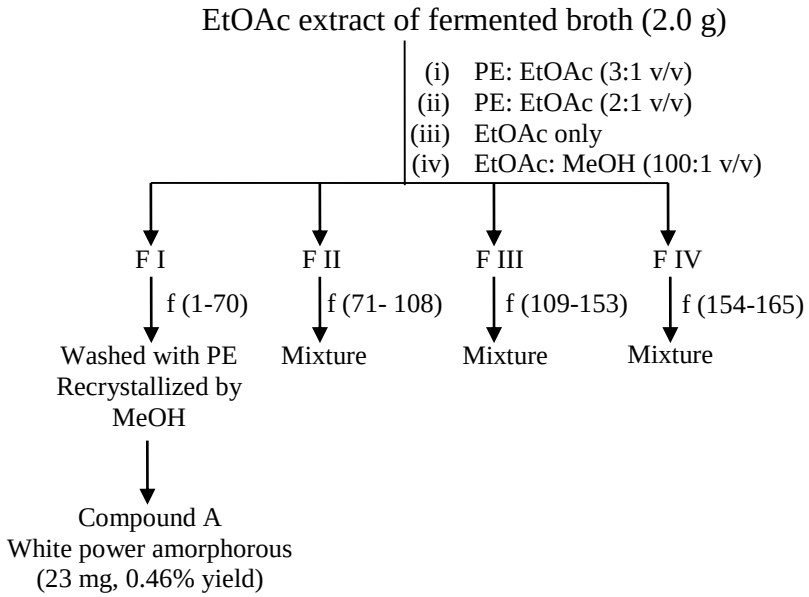


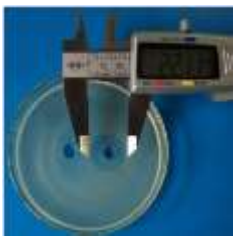
Figure 8 Isolation of organic metabolites from ethyl acetate crude extract, culture broth of isolated Streptomyces TR-2 by column chromatography

3.3.3. Isolation of some organic compounds from ethyl acetate extract of the fermented broth TR-2

2.0 g of ethyl acetate crude extract from fermented broth TR-2 was separated by column chromatography using silica gel absorbed and eluted with pet ether : ethyl acetate (3:1, 2:1,

1:1 v/v), ethyl acetate only, ethyl acetate : methanol (100:1 v/v). Fraction were collected in accordance to their TLC profiles and tended for their antifungal activity with *Candida albicans.* The TLC plates revealed that the fractions were to be pooled into four main fraction (FI to FIV). The fraction FI (f 1-70) was evaporated and washed with pet ether and recrystallized with methanol, yielded 23 mg, white amorphous powder (Fig. 3.9). Its R_f value was found to be 0.51 in (Hexane: EtOAc 15:1 v/v) solvent system and it gave brown colouration on TLC plate when spraying with 10% $FeCl_3$. The isolated compound A was active against on *Candida albicans* with inhibitory zone 22.11 mm. Thin layer chromatogram of compound A and its antifungal activity was presented in (Fig. 3.9). The remaining fractions (F II to F IV) were observed as mixture and no activity recorded against *Candida albicans.*

Compound A
Solvent system : Hexane: EtOAc (15:1)
R_f value : 0.51
Spraying agent : $FeCl_3$



Inhibitory zone-22.11 mm

Compound A from ethyl acetate extract of TR-2

Figure 9 Thin layer chromatogram of isolated compound A and its antifungal activity against *Candida albicans*

3.3.4. Characterization of isolated compound A

Some chemical properties of isolated compound from fermented broth TR-2 was characterized by spraying agents on TLC, solubility test, modern spectroscopic measurement such as UV and FT IR.

Compound A (23 mg, white amorphorous powder) was isolated from ethyl acetate extract of fermented broth TR-2. R_f value of compound A was found to be 0.51 in Hexane: EtOAc (15:1 v/v) as shown in (Fig. 3.9.). Compound A was soluble in MeOH, EtOAc and CH_2Cl_2 but insoluble in PE, Hexane and EtOH as shown in (Table 3.4).

According to the result obtained from the chemical reagent tests, compound A gave yellow spot on TLC with iodine vapour, reddish brown spot with anisaldehyde followed by heating, brown spot with 10% $FeCl_3$ and 5% H_2SO_4 followed by heating and yellow ppt with 2, 4 DNP. These result was shown in Table 3.1.

Compound A was UV active because the presence of conjugated double bond. The UV absorption spectrum showed the absorption at 223, 267, 275 nm. These bands attributed to - * transistion and showed the presence of carbonyl compound (Fig. 3.11 and Table 3.2).

The functional groups present in compound A was studied by FT IR spectroscopy. FT IR spectrum was shown in (Fig. 3.12 and the interpreted spectral data was illustrated in Table 3.3. The FT IR spectrum of compound A showed the band at 3.383 cm^{-1} due to O-H stretching vibration of

alcoholic group. Absorption band at 2924 cm^{-1} and 2654 cm^{-1} were due to C-H stretching vibration of CH_2 and CH_3 groups. Stretching band at 1729 cm^{-1} indicated the presence of C=O stretching vibration of ketone group. The C=C stretching vibration of aromatic compound as observed at 1650 cm^{-1}. The band at 1462 cm^{-1} was attributed to O-H bending vibration. The C=O stretching vibration of alcohol was shown as intense band at 1057 cm^{-1}. Absorption band at 796 cm^{-1} was due to the C-H bending vibration of aromatic compound. From the results of physicochemical properties, R_f value, UV and FT IR spectral data, the isolated compound A may be flavonoid.

Table 1 Chemical properties of isolated compound from TR-2 metabolite

No	Separating agent	Compound A
1	10% $KMnO_4$	ND
2	Iodine	Yellow
3	Anisaldehyde	Reddish Brown
4	$FeCl_3$	Brown
5	5% H_2SO_4	Brown
6	2,4 DNP	Yellow ppt

ND- not detected

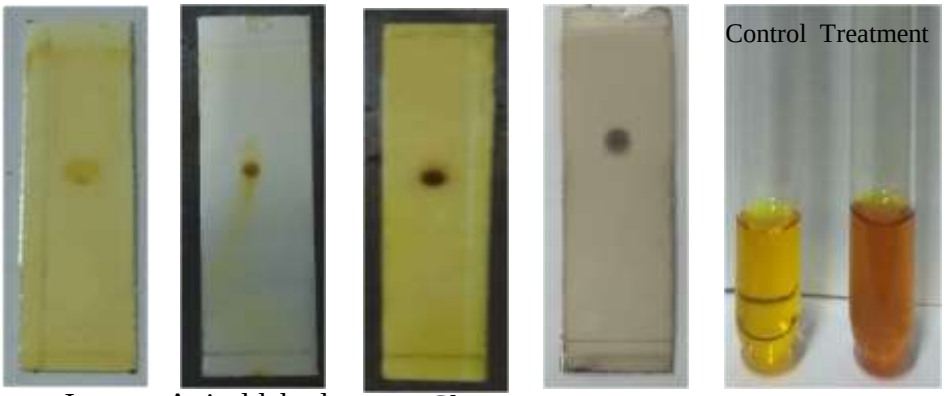


Figure 10 Chemical reagent tests of isolated compound from TR-2 metabolite

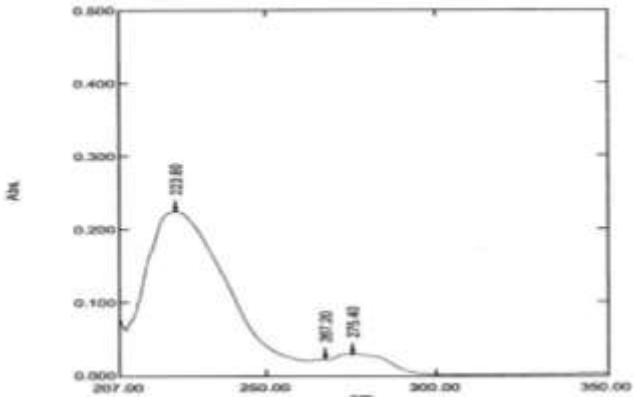


Figure 11 UV spectrum of isolated compound A

Table 2 UV spectrum of isolated compound A

Compound	λ_{max} (nm)	Remark
A	223, 267, 275	$\pi \rightarrow \pi^*$ (carbonyl compound)

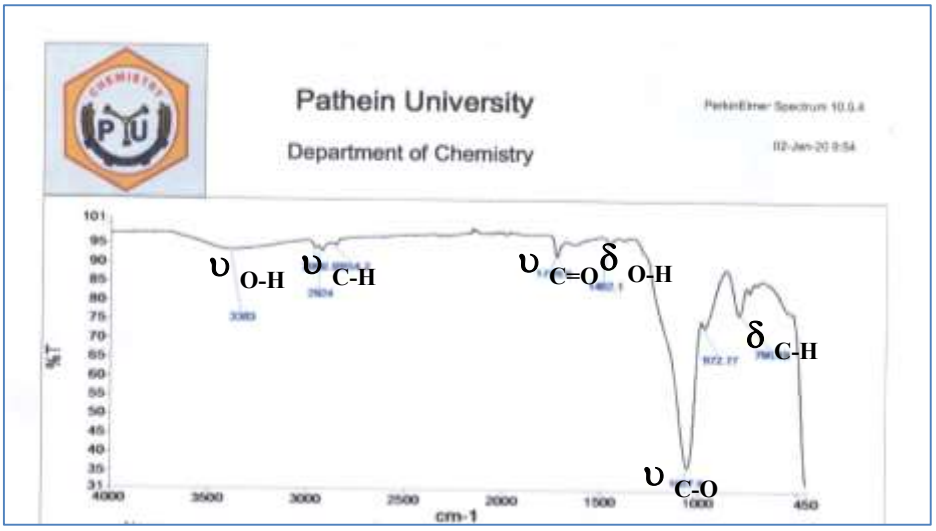


Figure 12 FT IR spectrum of isolated compound A

Table 3 FT IR spectral data of isolated compound A

Wave number (cm^{-1})	Literature* Wave number (cm^{-1})	Remark
3383	3570-2900	O-H stretching vibration
2924, 2854	2935-2845	C-H stretching vibration
1729	1740-1675	C=O stretching vibration
1650	1655-1580	C=C stretching vibration
1462	1470-1280	O-H bending vibration
1057	1270-1025	C=O stretching vibration
796	900-670	C-H bending vibration

Table 4 Solubility test for selected Streptomyces TR-2

No	Solvent	Result
1.	MeOH	+
2.	EtOAc	+
3.	PE	-
4.	CH_3Cl_2	+
5.	Hex	-
6.	Ethanol	-

\+ Soluble
\- Insoluble

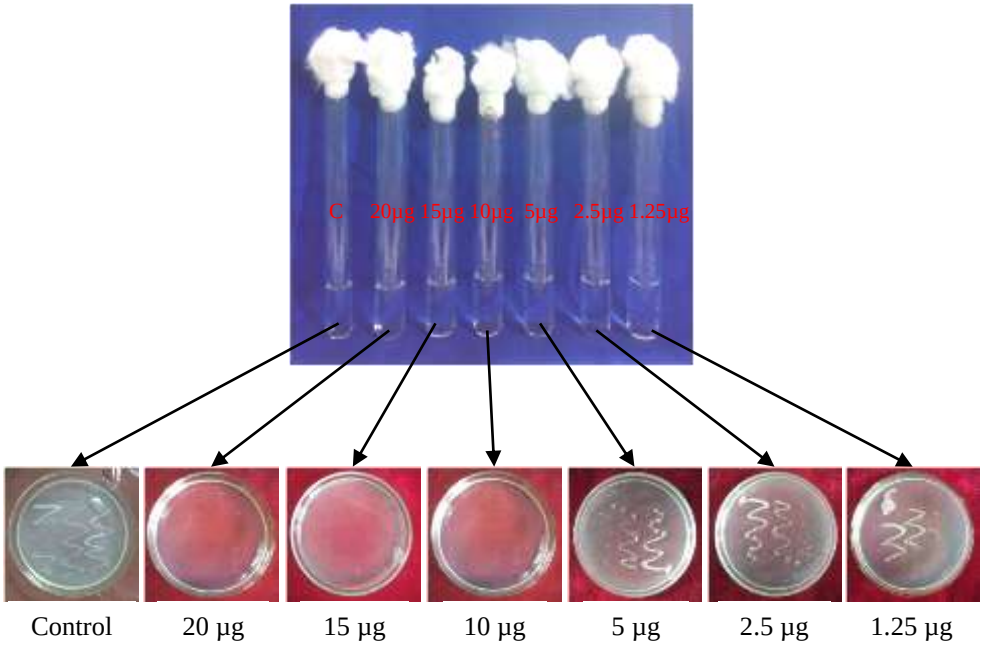


Figure 13 Minimum inhibitory concentrations of secondary metabolites from compound A on *Candida albicans* (Streak culture method)

Table 5 Minimum inhibitory concentrations of secondary metabolites from compound A on *Candida albicans*

MIC values of compound A (µg/well)	Antifungal activity (mm)
Control	-
10	10.78
5	8.91
2.5	**8.87**
1.25	-
0.625	-
0.312	-

- No activity

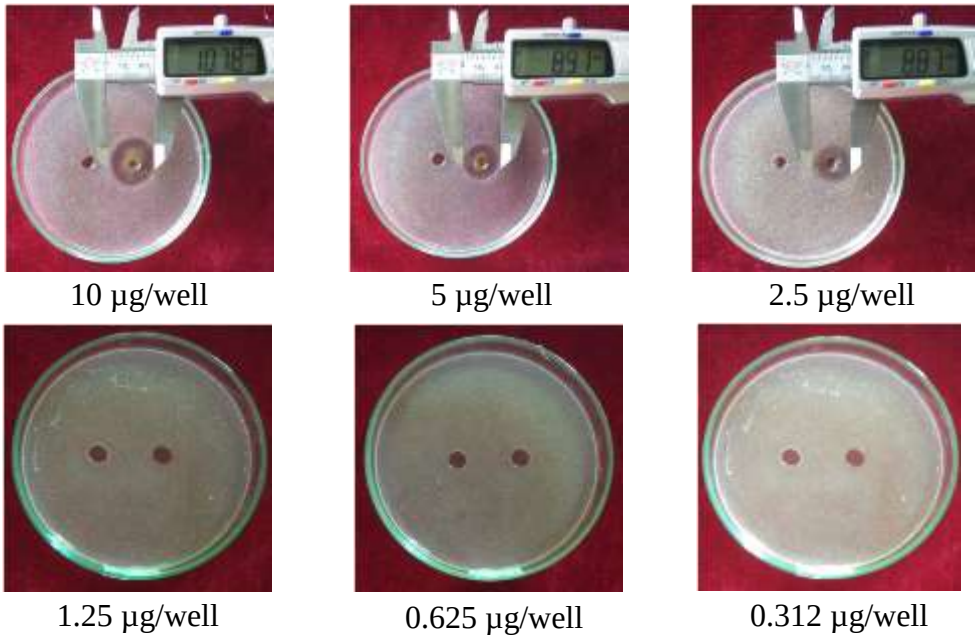


Figure 14 Minimum inhibitory concentration of secondary metabolites from compound A on *Candida albicans* (Agar well diffusion method)

Discussion and Conclusion

Abdul Wahab *et al.,* 2015, who documented that ethyl acetate provided the best solvent for the extraction of antimicrobial metabolites followed by chloroform.

Ripa *et al.,* 2010 noticed that ethyl acetate extract of *Streptomyces* sp. which showed antibacterial activity against a number of both Gram positive and Gram negative bacteria but did not have antifungal activity under in vitro conditions.

Differently, ethyl acetate extract of culture broth of selected *Streptomyces* TR-2 possessed antifungal activity on *Candida albicans*.

Thin layer chromatography (TLC) is very helpful in the identification and comparison of antibiotics, but very few results from the application of this technique have been published so far (Alfred and David, 1955).

Tirta *et al.,* 2017 reported that the metabolites from potent strain was produced by extraction of culture filtrate with ethyl acetate: methanol (4:1), it was tested for their antifungal activity by well diffusion method and it showed good antifungal activity.

In this study, thin layer chromatography (TLC) was performed on ethyl acetate crude extracted by employing solvent systems: 100% ethyl acetate, 100% chloroform, 100% pet ether, pet ether: ethyl acetate (20:1, 10:1, 8:1, 5:1, 3:1 and 1:1 v/v), 100% hexane only, hexane: ethyl acetate (20:1, 10:1, 8:1, 5:1, 3:1 and 1:1 v/v), 100% methanol only, ethyl acetate: methanol (70:1, 30:1, 10:1, 8:1, 4:1 and 2:1), ethyl acetate: pet ether (20:1, 10:1, 5:1, 4:1, 2:1 and 1:1). The extract showed well- separated spots on TLC by using pet ether: ethyl acetate solvent system (3:1 v/v).

Pandey *et al.,* 2011 suggested that the minimum inhibitory concentration is the minimum concentration of the antibacterial or antifungal agent in a given culture medium below which bacterial or fungal growth is not inhibited.

Likewise, on the studying the Minimum Inhibitory Concentrations (MICs), it was found that the MICs value of selected *Streptomyces* was 2.5 µg/ well on *Candida albicans* (8.87 mm).

Vineeta *et al.*, 2018 suggested that the compound extraction with ethyl acetate was found the maximum soluble in methanol, suggesting its polar nature.

In the study on the solubility test for compound A, it was observed that the compound was maximum soluble in methanol, ethyl acetate and ethanol and minimum soluble in petroleum ether, hexane and chloroform. Thus, compound A was the polar nature.

Oskay, 2011 also noticed that UV and Infrared spectrometry (IR) are being routinely used for the analytical estimation of various antibiotics. UV Scans of his strain dissolved in methanol were performed. Absorbance maxima were obtained at 275 and 286.5 nm, indicating the existence of a carbon- carbon double bond.

Similar to Oskay results, absorbance maximum of UV spectrum for the isolated compound A was obtained 275 nm.

According to the column chromatographic separation techniques, physiochemical tests and spectroscopic techniques such as UV and FT IR, it can be concluded that the isolate compound A may be flavonoid.

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 also extended my thank to Professor Dr Than Tun Pro-Rector, Pathein University, for their valuable instruction and guidance. I would like to record my deep thank to Professor Dr Wah Wah Lwin, Head of Botany Department, Pathein University and Professor Dr Min Min Soe, Department of Botany, Pathein University for their suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Zar Zar Yin, Associate Professor, Department of Botany, Bago University, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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FERMENTATION OPTIMIZATION AND SELECTION OF SOLVENT TO EXTRACT ANTIBACTERIAL COMPOUND FROM ISOLATED SOIL FUNGUS NLF-12

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Abstract

The present paper was studied by the fermentation optimization and selection of solvent to extract antibacterial compound from isolated soil fungus NLF-12. Twenty fungi were isolated from four different soil samples of Beikthano Ancient City, Taungdwingyi Township, Magway Region. Antimicrobial activity of all fungi was tested by agar well method on four test organisms and nine fungal strains showed the activity. Among them, soil fungus NLF-12 showed the highest antibacterial activity (30.54 mm) on *Micrococcus luteus*. Therefore, it was selected and the fermentation optimization of fungus NLF-12 were carried out by proper age, size, different carbon and nitrogen sources, fermentation medium (FM), pH, temperature, static and shaking culture. In the results of paper chromatography, ethyl acetate was the most suitable solvent for extraction of antibacterial secondary metabolites from fermented broth of fungus NLF-12 and R_f value had 0.92 on *Micrococcus luteus*. These results indicated that the selected soil fungus may be utilized by the optimal fermentation conditions for the screening of antibacterial activity.

Keywords: fermentation optimization, bioautography, metabolite

Introduction

Some microbe can perform an immense range of metabolic function. Fungi are considered as a good natural source for a production of biotic secondary metabolite that contains different bioactive agents including antibiotic, antitumor and antioxidant. Generally, the reason why they produce such metabolites is not known, but it is believed that many of these metabolites may act as chemical defense of microbes competing for substrates (Kumar *et al.*, 2012).

Paper chromatography separates dried liquid samples with a liquid solvent (mobile phase) and a paper strip (stationary phase). Separation of components depends on both their solubility in the mobile phase and their differential affinity to the mobile phase and stationary phase.

Solvent extraction is widely used during early purification of fermentation derived products and indeed, of all natural product matrices for initial and intermediate purification prior to final purification by chromatography, crystallization, or precipitation (Schugerl, 1999).

The aims and objectives of this research are to isolate the soil fungi from four different places, to observe the optimal fermentation conditions of soil fungus NLF-12 and to select the best solvent for extraction of antibacterial compound by paper chromatography.

Materials and Methods

Study Area and Collection of Soil Samples

Soil samples were collected from four different places of Beikthano Ancient City Taungdwingyi Township, Magway Region from July, 2016 to August, 2016. These samples were isolated by using two media (LCA and MEA).

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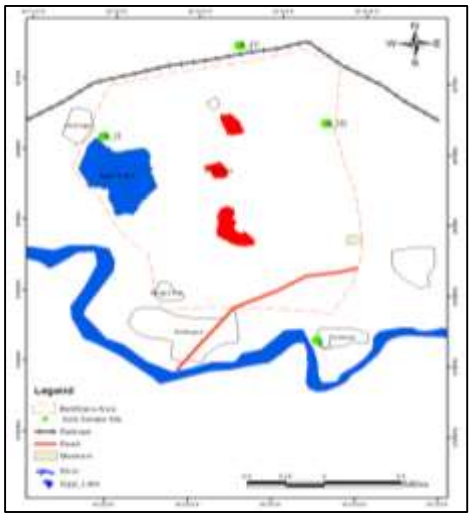

Map Sources: Beikthano Museum)

Table 1 Four different soil samples collected from Beikthano Ancient City

Samples Collected areas	pH	Soil type	Location
Gokkone village	9.54	Loam	N 19º 59.421' E 95º 24.432'
Shwe-yaung-taw compound	6.87	Sandy Loam	N 20º 0.874' E 95º 23.371'
Nan-twin-taw ya Monastery	9.04	Sandy Loam	N 20º 0.852' E 95º 23.277'
Beikthano railwaystation	6.80	Sandy Loam	N 19º 59.646' E 95º 23.353'

Figure 1 Four different sites of Beikthano Ancient City for soil sample collection

Chemical Treatment Dilution Method

The collected soil was air-dried at room temperature for 5 days. The soil sample was grounded and sieved in 2 mm screen. The sample was placed in the hot air oven at 120 ˚C for 1 hrs. The dried soil sample was suspended with 1.5 % phenol and diluted with sterile water. The dilution series were cultured on LCA medium. 30 µL of soil suspension was cultured on plates containing LCA medium and incubated for 5-10 days. Pure colonies were picked up to start containing in PGA medium (Phay and Yamamura, 2005).

Preliminary Study for Antimicrobial Activity

The isolated fungi were grown on PGA medium for 5 days. The isolated fungi were inoculated into 25 mL seed medium and incubated at room temperature for 3 days. After 3 days, 20 mL of seed culture was transferred into the 80 mL of fermentation medium and incubated at room temperature. Fermentation was carried for 3-10 days (Ando, 2004).

Screening of Antimicrobial Activity by Agar Well Diffusion Method

1 day old culture test broth (0.01 mL) was added to 25 mL of assay medium and thoroughly mixed and poured into plate. After solidification, Cork borer was used to make the wells (wells - 8 mm). The fermented broth (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 (Collins, 1965).

Test organisms

Agrobacterium tumefaciens NITE09678, *Aspergillus paraciticus* IFO5123, *Micrococcus luteus* NITE83297, *Pseudomonas fluorescens* IFO94307 were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan)

Effects of ages of inoculums on the fermentation

The selected fungus NLF-12 was grown on PGA medium for 5 days at room temperature. After 5 days incubation period, seed cultures (48, 54, 60, 66, 72, 78, 84 and 90 hours) incubation was inoculated into the flasks containing fermentation medium. Fermentation medium was carried

out from 48 to 90 hours and antibacterial activity was tested by agar well diffusion method (Crueger, 1989).

Effects of sizes of inoculums on the fermentation

The selected fungus NLF-12 was grown on PGA medium for 5 days at room temperature. After 5 days incubation period, this fungus was inoculated into 150mL seed medium. Based on the result of the ages of inoculums of NLF-12, (5%, 10%, 15%, 20%, 25%, and 30%) of 72 hours seed cultures were utilized for the fermentation. All fermentation media were carried out 5 days and antibacterial activity was investigated by agar well diffusion method (Crueger, 1989).

Effect of different carbon and nitrogen sources on fermentation medium

Optimal fermentation is very important for maximal productivity metabolites. In this study, carbon and nitrogen sources were employed in the fermentation for the production of antibacterial metabolites. Carbon sources such as dextrose, fructose, lactose, maltose, sucrose, manitol, molasses and glycerol and various nitrogen sources such as casein, gelatin, potassium nitrate, malt extract, sodium nitrate, ammonium chloride, ammonium sulphate and peptone were supplemented with the basal medium. The fermented broth containing these medium were assayed for antibacterial activity against *Micrococcus luteus* using agar well diffusion method.

Carbon sources in basal fermentation medium			**Nitrogen sources in basal fermentation medium**		
Peptone	-	0.5g	Glucose	-	1.0g
Yeast extract	-	0.5g	Sucrose	-	0.5g
K_2HPO_4	-	0.001g	K_2HPO_4	-	0.001g
$CaCO_3$	-	0.01g	$CaCO_3$	-	0.01g
$MgSO_47H_2O$	-	0.001g	$MgSO_47H_2O$	-	0.001g
DW	-	100mL	DW	-	100mL
pH	-	6.5	pH	-	6.5

Media selection in the fermentation study

Eight fermentation media (FM-1 to FM-8) were prepared for the fermentation study. In the preparation of eight fermentation media best carbon sources (molasses, glycerol and dextrose) and best nitrogen sources (peptone, NH_4Cl, and malt extract) were utilized as suitable ratios and compositions. The medium constituents were sterilized by autoclaving at 121ºC for 30 min, and were cooling thoroughly before inoculation. Fermentation was carried out 5 days and antibacterial activity was tested by agar well diffusion method.

Medium composition (gram per liter)

FM- 1		**FM- 2**		**FM- 3**		**FM- 4**	
Glycerol	10 g	Dextrose	10 g	Maltose	10 g	Molasses	0.5 g
NH_4Cl	10 g	Malt extract	10 g	$NaNO_3$	10 g	$NaNO_3$	10 g
K_2HPO_4	0.01 g	K_2HPO_4	0.01 g	K_2HPO_4	0.01 g	K_2HPO_4	0.01 g
$CaCO_3$	0.01 g	$CaCO_3$	0.01 g	$CaCO_3$	0.01g	$CaCO_3$	0.01 g
$MgSO_47H_2O$	0.01 g	$MgSO_47H_2O$	0.01 g	$MgSO_47H_2O$	0.01 g	$MgSO_47H_2O$	0.01 g
pH	6.5	pH	6.5	pH	6.5	pH	6.5

FM- 5		**FM- 6**		**FM- 7**		**FM- 8**	
Molasses	10 g	Molasses	15 g	Molasses	20 g	Molasses	25 g
Peptone	10 g	Peptone	10 g	Peptone	10 g	Peptone	10 g
K_2HPO_4	0.01 g	K_2HPO_4	0.01 g	K_2HPO_4	0.01 g	K_2HPO_4	0.01 g
$CaCO_3$	0.01 g	$CaCO_3$	0.01 g	$CaCO_3$	0.01 g	$CaCO_3$	0.01 g
$MgSO_47H_2O$	0.01 g	$MgSO_47H_2O$	0.01g	$MgSO_47H_2O$	0.01 g	$MgSO_47H_2O$	0.01 g
pH	6.5	pH	6.5	pH	6.5	pH	6.5

Effect of pH on fermentation conditions

The optimization of pH of the fermentation broth for antibacterial metabolite production was done by carrying out the fermentation at seven different pH values viz. 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. For each pH value, desired pH by using either 0.1M NaOH or 0.1 M HCl was adjusted into fermentation medium (Furtado *et al*., 2005).

Effect of incubation temperature

The optimization temperature for antibacterial metabolite production was carried out at six different incubation temperatures viz. 20, 25, 30, 35, and 40 and 45°C. The fermentation medium was carried out 5 days and antibacterial activity was studied by agar well diffusion method (Cazar*et al*., 2004).

Comparison of static culture and shaking culture

250 mL conical flask containing 100 mL of the fermentation medium was incubated on the shaker (100 rpm) for 5 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 method (Hassan *et al*., 2017).

Suitable synthetic fermentation medium

Based on the results of above fermentation parameters, composition of the suitable synthetic medium was prepared the following conditions. The effect of fermentation period for maximum production was observed up to 10 days by agar well diffusion method.

			Fermentation medium 7		
Ages of inoculum	-	72 hours seed culture	Molasses	-	20g
Size of inoculums	-	10%	Peptone	-	10g
Temperature	-	25ºC	K_2HPO_4	-	0.01g
pH	-	5	$MgSO_4$	-	0.01g
Fermentation period	-	5 days	pH	-	6.5
Agitation	-	100rpm	DW	-	1000ml

Paper chromatography (Tomita, 1998)

Paper chromatography of NLF-12 metabolite was carried out by the method of Tomita, 1998. 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 antibiotics. The fermented broth samples were 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. Each paper was placed on assay agar plates. After one hour the

paper was taken out, and then the plates were incubated for 24-36 hours. The inhibitory zone was measured yielding R_f value for the corresponding bioactive compound. R_f value was calculated in the following equation.

$$R_f \text{ value} = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

Results

Isolation of fungi from soil samples

In this study, 20 fungi were isolated from four different soil sample from Beikthano Ancient city, Taungdwingyi Township, Magway Region.

Table 2 Isolated fungi from soil samples

No.	Samples Collected areas	LCA Medium	MEA Medium	Total
1	Gokkone village	NLF-1, NLF-2	NLF-3,NLF-4, NLF-5	5
2	Shwe-yaung-taw compound	NLF-6	NLF-7, NLF-8, NLF 9,NLF-10	5
3	Nan-twin-taw ya Monastery	NLF-11, NLF-12	NLF-13, NLF-14, NLF-15	5
4	Beikthano railway station	NLF-16, NLF-17	NLF-18,NLF-19,NLF-20	5
	Total Isolated fungal strains	7	13	20

Screening on the antimicrobial activity of isolated fungi by agar well diffusion method

All isolated were tested for antimicrobial activity with four test organisms. Among them, NLF-12 showed the best activity against *Micrococcus luteus* NITE 83297 (30.54 mm) in 5 days fermentation period. According to these results, NLF-12 was selected further studies because it showed the highest antibacterial activity against *Micrococcus luteus* (30.54mm).

A B C

Figure 2 (A) Surface view of NLF-12, (B) Reverse view of NLF-12 and (C) The Antibacterial activity of selected fungus NLF-12 against *Micrococcus luteus*

Effects of ages of inoculums on the fermentation

In the effect of age of inoculums, NLF-12 was investigated by using 48, 54, 60, 66, 72, 78, 84 and 90 hours old culture age of inoculums. The results showed that 72 hours age of inoculums gave the highest activities (22.99mm) followed (22.95mm) at 66 hours and (22.92mm) at 60 hours age of inoculums.

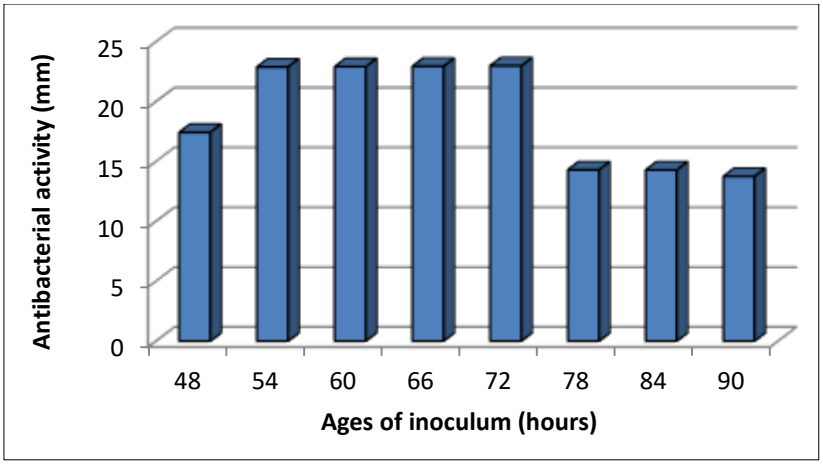

Figure 3 Effects of ages of inoculums of NLF-12 against *Micrococcus luteus* on the fermentation

Effects of sizes of inoculums on the fermentation

In this research work, the effect of size of inoculums were studied by using 5%, 10%, 15%, 20%, 25% and 30% inoculums. Using 10% inoculums showed the highest activities (26.53mm) than others, followed by 20% and 30% (25.06 mm and 25.10 mm).

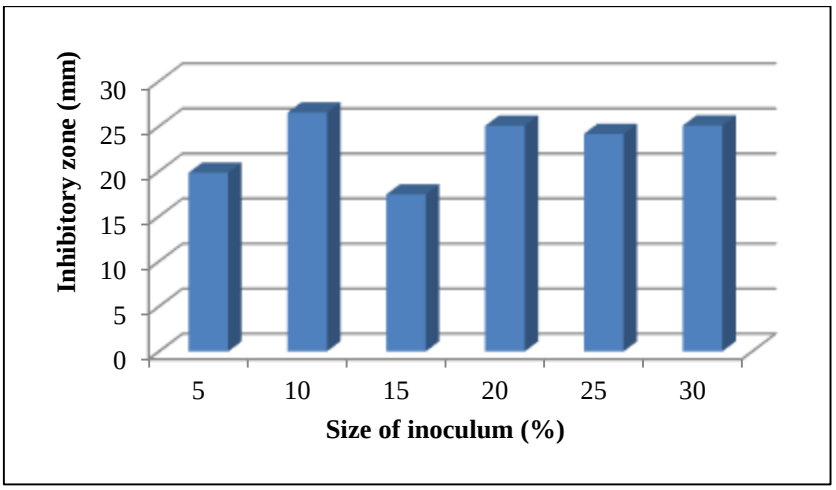

Figure 4 Effects of sizes of inoculums of NLF-12 against *Micrococcus luteus* on the fermentation

Effect of different carbon and nitrogen utilization on fermentation medium

There were variations in the level of antibacterial activity when the eight different carbon and nitrogen sources were used in the fermentation medium. The addition of the carbon sources showed the maximum antibacterial activities on molasses (31.36mm), followed by glycerol, dextrose and maltose and then fructose, lactose, sucrose, manitol were showed the minimum antibacterial activity. Similarly, when the addition of the various nitrogen sources, the maximum inhibition zone (30.94mm, 29.06mm, 28.71mm, 28.01mm) were obtained peptone, ammonium chloride, malt extract and sodium nitrate. Gelatin, potassium nitrate and ammonium sulphate showed moderate inhibition zone respectively. Casein as regarded as poor inhibition zone.

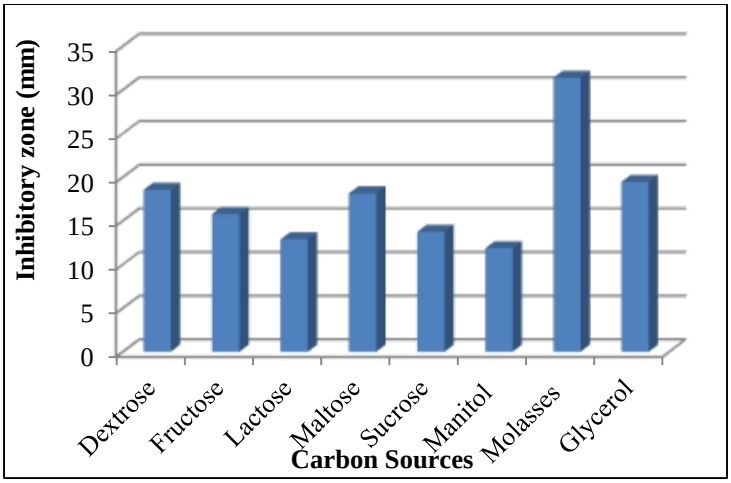

Figure 5 Effects of different carbon utilization on fermentation against *Micrococcus luteus*

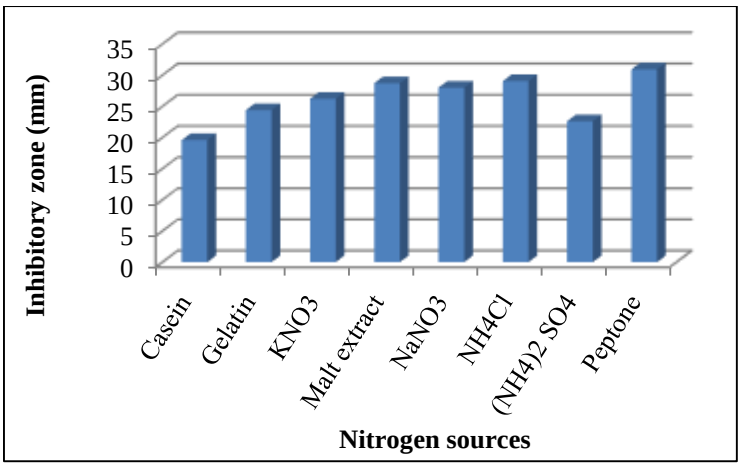

Figure 6 Effects of different nitrogen utilization on fermentation against *Micrococcus luteus*

Media optimization in the fermentation study

In the study of media optimization with eight fermentation media, FM-7 gave the highest antibacterial activity (29.33mm) followed by FM-8 (29.10mm), FM-6 (26.04mm) and FM-5 (24.35mm) respectively. It was determined that FM-7 showed the best activity on *Micrococcus luteus*, FM-7 was selected for the production of antibacterial metabolite.

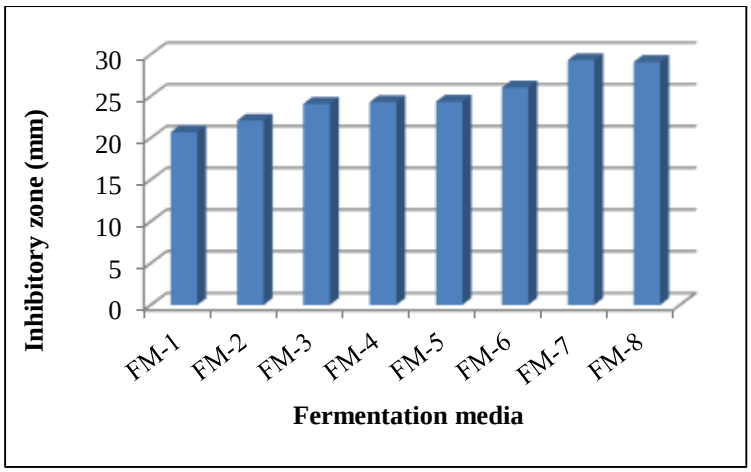

Figure 7 Antibacterial activity of selected fungus NLF-12 on the fermentation medium

Effects of different pH and temperature on bioactive metabolite production of selected fungus NLF-12 against *Micrococcus luteus*

The effect of pH and temperature were tested with different pH level (pH- 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0) and different temperature ranges (20ºC, 25ºC, 30ºC, 35ºC, 40ºC and 45ºC). The best antibacterial activity was found at pH-5.0 (24.04mm) and temperature 25ºC showed the highest antibacterial activity (29.03mm) against *Micrococcus luteus*.

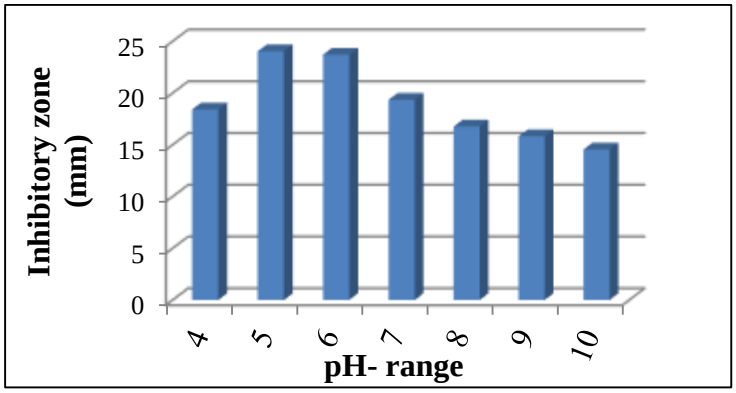

Figure 8 Effects of different pH on bioactive metabolite production of selected fungus NLF-12 against *Micrococcus luteus*

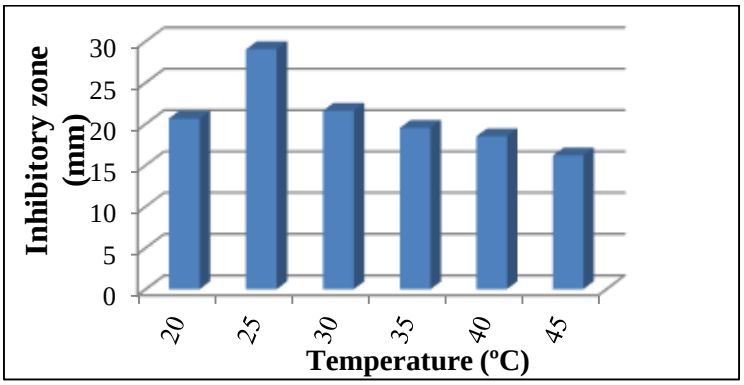

Figure 9 Effects of different temperature on bioactive metabolite production of selected fungus NLF-12 against *Micrococcus luteus*

Comparison of static culture and shaking culture

When comparing the static culture and shaking culture of fermentation medium, antibacterial activity from shaking is better than (28.19 mm) than that of static culture (26.76 mm).

Shaking culture

Static Culture

Figure 10 Comparison of static culture and shaking culture of of NLF-12 against *Micrococcus luteus*

The suitable synthetic fermentation medium

Based on the results of above fermentation parameters, such as 72 hours seed culture, 10% inoculums size, temperature 25ºC, pH 5.0 and shaking culture (100 rpm), FM-7, the maximum antibacterial activity of NLF-12 was observed by fermentation period 3 days to 10 days. In 5 days fermentation period, the NLF-12 showed the moderate antibacterial activity (29.32mm).

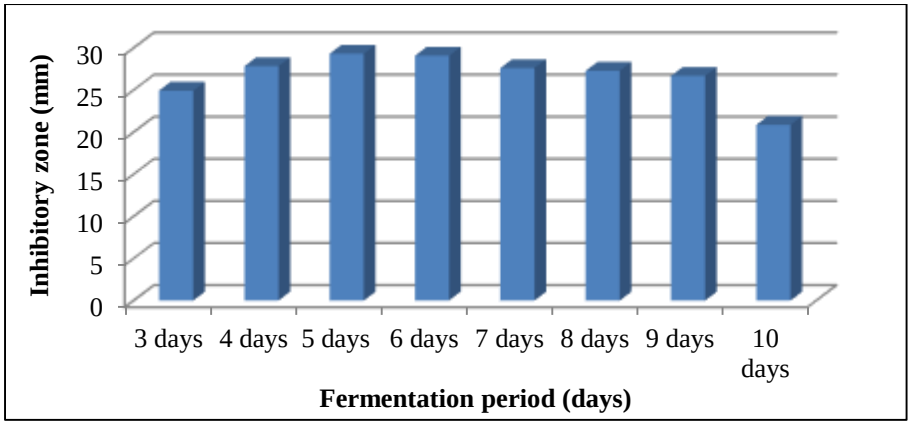

Figure 11 Antibacterial activity on suitable synthetic medium by selected fungus NLF-12 against *Micrococcus luteus*

Paper chromatography

In this study, four kinds of solvent 20%NH_4Cl, n-butanol saturated with water, n-butanol acetic acid with water (3:1:1) and ethyl acetate saturated with water were used. According to R_f value (0.92) was more extractable the antibacterial metabolites than other solvent, followed by n-butanol- acetic acid water (3;1:1)solvent (0.79), n-butanol (0.71) and the lowest R_f value at NH_4Cl (0.64).

1. 20%NH_4Cl
2. n-butanol saturated with water
3. n -butanol –acetic acid – water
4. Ethyl acetate saturated with water

Figure 12 Paper chromatography bioautographic assay

Discussion and Conclusion

Microorganism are able to synthesize secondary metabolites of various structure and hence bioactive (Demain, 1998). In the present research, fungus NLF-12 was focused by the fermentation optimization and selection of solvent systems. To study the optimization of inoculums age, incubation time (48, 54, 66, 72, 78, 84 and 90 hours) were used and the highest antibacterial activity was found at 72 hours (22.99 mm).In the inoculum size, 10% was the most suitable and gave the maximum activities (26.53 mm).

In the carbon sources, the maximum antibacterial metabolite of NLF-12 was found on molasses (31.36 mm). Kotake,(1992) described that the different carbon sources like sucrose, glycerol, starch, dextrose, lactose, and fructose to be suitable for production of secondary metabolite by different organisms. In the nitrogen sources, the maximum antibacterial metabolite

of NLF-12 was found on peptone (30.94 mm). El-Gammal, (1986) reported that peptone was the suitable nitrogen source for the production of metabolites from microorganism.

In the fermentation media (FM), eight kinds of fermentation media were used and FM-7 gave the highest antibacterial activity (29.33 mm). El-Tayeb *et al.*, (2004) described the choice of a good fermentation medium is virtually as important to the success of an industrial fermentation as is the selection of an organism to carry out of the fermentation.

In the effect of pH, the best antibacterial activity was found as pH-5.0 (24.04 mm). Jain and Pundir, (2011) reported that 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 various range of temperature were optimized by 20ºC, 25ºC, 30ºC, 40ºC and 45ºC and maximum inhibitory activity was recorded at the incubation temperature of 25ºC (29.03 mm). Temperature is one of important factors affecting microbial fermentation, due to it correlation with all biochemical enzyme catalysis processes (Zhou *et al.*, 2018). In the comparison of static culture and shaking culture, the antibacterial activity of static culture (26.76 mm) was less than that of shaking culture (28.19 mm). Mukhtar and Haq (2007) assumed that variation in the aeration level of the culture medium has specific effect on the growth of the microorganism and product formation during a submerged fermentation. The highest antibacterial activity was obtained by the suitable synthetic fermentation medium.

The rate of fermentation depends on the concentration of microorganisms, cells, cellular components as well as temperature and pH (Cruegar and Cruegar, 1989). According to the R_f value (0.92), ethyl acetate was the suitable solvent for NLF-12. Anuhya, (2017) described that the extraction of the secondary metabolite was effectively done with ethyl acetate.

Therefore in the next study, this solvent will use to extract bioactive compound and carry out purification of extracted bioactive compound from isolated soil fungus NLF-12.

Acknowledgements

I am greatly indebted to Dr Khin Maung Oo, Rector, University of Magway, for this permission to write this research paper. I would like to record many my deep thank to Professor Dr. Aye Aye Kyi, Head of Botany Department, University of Magway and Professor Dr. Thandar, Department of Botany, University of Magway for their suggestion and kind understanding during this study. I wish to special thanks to Dr. Zar Zar Yin, Associate Professor, Department of Botany, University of Bago, for her valuable guidance and kind help throughout this research work.

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EFFECT OF WATER TREATMENT ON VEGETATIVE GROWTH OF *PLUKENETIA VOLUBILIS* L.

Lae Lae Aung[1], Thanda Aye[2]

Abstract

The effect of days interval water treatment on growth of Sacha inchi (*Plukenetia volubilis* L.) was studied in this experiment. The field experiment was conducted in the field of Department of Agricultural Research Oil Crops, Research center in Magway Region (May2019-2020). This experiment with four replications in a completely randomized block design, T_1 3days interval T_2, 6days interval, T_3 9days interval and T_4 daily water treatments. The collected data such as plant height, number of branch, number of node, number of leaves, leaf area. The result of different days interval water treatment showed that the tallest plant height (90.97 cm), the maximum-number of branch (1.86), the maximum number of node (21.94), the maximum number of leaf (15.86), the largest number of leaf area (590.95 cm^2) were obtained from daily water treatment in this study.

Keywords: water treatment**,** 3days (T1) 6days (T2) 9days (T3) Daily (T4).

Introduction

Water supply is a major limitation for crop production in many areas of the world since it not only reduces cell growth rate, but also limits the crop's reproductive process. A major challenge in food production is to achieve the goal of increasing both food production and resource mainly (water and nitrogen) use efficiency. To maintain sustainable production and efficient use of the limited water resources, various type of water-saving irrigation techniques have been widely introduced, many of them taking advantage of the fact that changes in hydraulic and chemical signals induced by root zone, drying caused partial closure of stomata and inhibition of leaf expansion.

It has been identified that regulated deficit irrigation can save irrigated water up to 20%-30% and increase water–use efficiency greatly with a subtle or even positive impact on the grow, yield and quality of some grain and fruit crops, especially in arid and semiarid regions. Exposure of crops to warmer and drier environments will increase leaf-air water vapor pressure deficit, resulting in increased drought susceptibility and reduced productivity, not only in arid region but also in tropical monsoon regions with seasonal dry periods.

It still remains debatable if the water saving techniques could achieve the goal of increasing crop yield and saving water, especially for the sparsely planted woody crops. *Plukenetia volubilis* L, a tropical evergreen liana native to South America, is a promising new oilseed crop species belonging to the family Euphorbiaceae. *Plukenetia volubilis* plants grow continuously throughout the year. *Plukenetia volubilis* are highly variable and depend on environmental conditions and agricultural management practices. Irrigation in the dry season is necessary for increasing the potential because *Plukenetia volubilis* plants grown under natural drought conditions have lower numbers of female flowers and higher fruit abortion compared to the well-watered plants. (http//www researchgate.net.2017). Therefore the experiments for this study were conducted to investigate the effects of water supply on the growth of *Plukenetia volubilis* in Magway Township.

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[2] Dr, Professor, Department of Botany, University of Yangon

Materials and Methods

Experimental site

The field experiment was conducted in the field of Department of Agricultural Research Oil Crops, Research Center in Magway Region from May 2019 to March 2020.

Soil for growing of *Sacha inchi*

Preparation of Soil for growing of *Plukenetia volubilis* L. (Sacha inchi). In the study area, the wild grasses were cut and land was ploughed to clear root stock and to clean up the land one week before the experimentation.

Soil Analysis

The soil samples at the depth of 1 feet (30.48cm) were randomly collected from 10 different places of the growing area. Then the collected soil samples were thoroughly mixed and analyzed in laboratory of Land Use Division, Myanmar Agricultural Service (MAS), Insein Township, Yangon Region.

Experimental design

Forty-five days old seedling were transferred experimental field. The field was laid out using Randomized Complete Block Design (RCBD), using four replicates of each treatment. Sacha inchi plants were planted 300cm x 105cm apart in each row with uniform plant height. The rows were 300cm apart. Ten liters of water added to each plant, four water treatments were carried out at 3days (T_1), 6days (T_2), 9days (T_3), daily (T_4) interval, respectively. Total experimental area was 2700,000 cm^2 and total sown plants were 64.

Germination of *Plukenetia volubilis* L.

At first, the seeds of Sacha inchi were immersed about 24 hours in water. After immersed in water, 10 seeds were placed on the prepared tissue paper in each plastic box, total 80 seeds were used in this germinations. Plastic boxes were placed at room temperature and nature condition. Each box was regularly water with 10ml once a day.

Germination rate was completed in 15 days after sowing and germination percentage was calculated using the method of Soup (2009).

$$\text{Germination rate (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of swon seeds}} \text{ x } 100$$

At 15 days all seeds were sprouting. They were transplanted into polyethylene bags containing the 1:1:1 volume of soil, rice husk ash and sand were placed under semi shadow to protect the bags from strong sun light. After 45days, all plants height became 20cm were selected to grow in the field.

Cultural management practices

During the growing period of Sacha inchi plants, crop management practice such as watering about 10 liter for each plant, organic pesticide (Neem) was soil drenched in every week. Weed control were done whenever it was necessary. (Basal fertilization 40g of NPK) was drenched to each plant.

Data collection and statistical analysis

Starting from the 14^{th} Day after sowing (DAS) the plants were collected at intervals of two weeks. Data were collected at two weeks intervals the vegetative growth such as the plant height,

the number of leaves, number of branches, number of nodes, leaf areas, were collected in this study. The data are analyzed using CROPSTATS software.

Results

Figure 1 *Plukenetia volubilis* L

Scientific Name	:	***Plukenetia volubilis* L.**
English Name	:	Sacha Inchi, Mountain peanut
Myanmar	:	Kyal-pe
Family	:	Euphorbiaceae

Morphological characters of *Plukenetia volubilis* L.

Habit: Sacha Inchi plant is perennial woody climbing shrub. Leaves: Simple, alternate, exstipulate with long petiole. Limina brodly ovate or ovate-cordate, serrate along the margin, acute at the apex, cordate at the base. Inflorescences: axillary, spike-like racemose, about 30 staminte flowers clustered at the top, 2 pistillate flowers at the base. Staminate flowers: minute, perianth, 4-5, apotepalous, tepal yellowish green, alternate at the ovary lobe, superior. Androecium: stamen, apostemnous, exerted attached on the convex receptacle, filament filiform, anther dithecous, oblongoid, longitudinal dehiscence, dorsifixed. Gynoecium: carpel (4), syncarpous, tetralocular, style long, stigma 4 lobed, ovary superior. Fruit: capsule 4-7 lobed, green in juvenile, dark brown in mature. Seed: 4-7 seeds per fruit.

Soil analysis

Physical and chemical properties of cultivated soil. The result of the analyzed soil from growing area showed loamy sand in soil texture which contained 94.9% sand, silt 9.9% and 3.9% clay. The cultivated soil had low in total nitrogen content (0.12%). The pH of the soil was 6.79% (nearly neutral) and the moisture content was 1.26%. The contents of exchangeable cat-ions, medium content of potassium 0.34% and very high phosphorous content 31.51%. The soil is loamy soil with very low organic carbon content was resulted from analysis (Table 1).

Table 1 Physical and chemical properties of soil samples

Parameter	Results	Remarks
Moisture (%)	**1.26**	**Nearly Neutral**
PH (soil : water) 1:2.5	**6.79**	
Soil Texture		
Sand (%)	**84.9**	
Silt (%)	**9.9**	
Clay (%)	**3.9**	
Total (%)	**98.7**	**Loamy sand**
Organic Carbon (%)	**0.82**	**Very low**
Humus (%)	**1.42**	
Total N_2 (%)	**0.12**	**Low**
Exchangeable Cations		
K^+	**0.34**	**Medium**
Available Nutrients		
P (ppm) (Olsen)	**31.51**	**Very High**
K_2O (mg 100gm)	**15.8**	**Medium**

Weather condition

The maximum temperature (41.7ºC) was obtained on May and the minimum temperature (10.2ºC) in November 2017. The maximum temperature (42.4ºC) was obtained on April and the minimum temperature (10.3ºC) in June 2018. The maximum rainfall (11.73 inch) was obtained in August and the minimum rainfall (0.08 inch) in December 2017. The maximum rainfall (6.42 inch) was obtained in May and the minimum rainfall (zero inch) on Feb, March, April in 2018 (Table 2). The average temperature during the cultivation period was 26.7 ºC and the average rainfall was 5.3 inches (Table 2).

Table 2. Monthly meteorological data of Magway area during growing of Sacha inchi

Meteorological Parameters	2019						
	May	Jun	Jul	Aug	Sep	Oct	Mean
Mean Temperature (˚C)	31.25	29.35	30.00	29.30	36.90	16.90	28.95
Mean Rainfall (mm)	163.06	390.65	77.97	192.27	8.63	163.06	165.94
Mean Relative Humidity	90.50	79.50	78.00	82.00	75.00	81.00	81.00

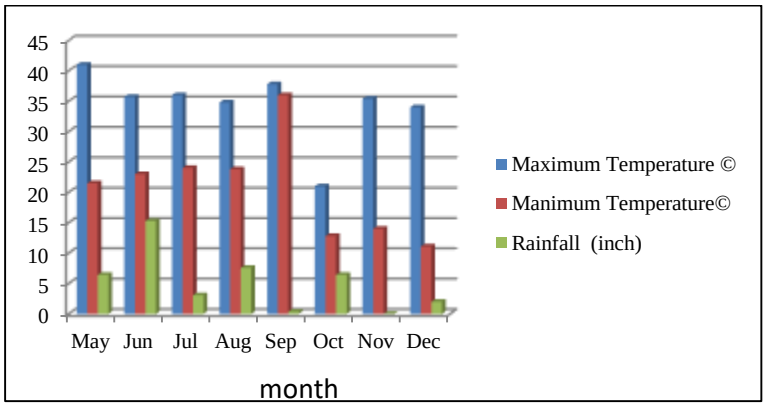

Figure 2 Monthly meteorological data of Magway area during growing of Sacha inchi

Table 3 Effect of days interval water treatment on plant height of Sacha inchi.

Treatment	Plant Height								
	15Days	30Days	45Days	60Days	75Days	90Days	105Days	120Days	mean
T1 (3 days interval)	36.75	39.25	48.5	67.75	91.38	109.88	116.38	120	78.74
T2 (6 days interval)	35	38.75	41.88	60.25	81.88	107.25	115.63	120	75.08
T3 (9 days interval)	37.13	42	46.25	60.88	74.13	91.13	102.38	120	71.74
T4 Daily	55.63	61.75	67.88	81.88	103.88	116.75	120	120	90.97
f-test	*	**	*	**	**	**	**	ns	
5%LSD	12.97	12.02	16.17	8.43	7.31	6.8	4.86	0	
cv%	19.7	16.5	19.8	7.8	5.2	4	2.7	0	

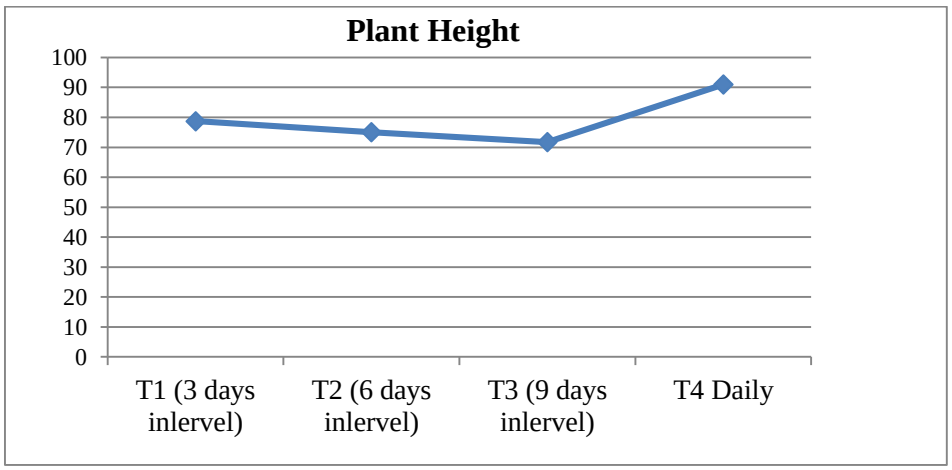

Figure 3 Effect of Days Interval Water Treatment on Plant Height

Plant Height

The growth of plant height were gradually increased in all treatments during 120 days of growing period. The differences in plant height were significant in Sacha inchi plant among treatment up to 15 days to 105 days. In 120 days is non significant. In different days interval water treatment, the tallest plant height (90.97cm) was obtained from daily water supply. The shortest plant height. (71.74cm) was obtained from 9days interval water treatment.

Table 4 Effect of Days interval water Treatment on Branch of Sacha inchi

Treatment	Number of Branch								
	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS	Mean
T1 3days interval	0	0	1.88	2	2.38	2.5	3.38	4.88	2.13
T2 6days interval	0	0	1.63	1.88	2.38	2.5	3.13	3.8	1.92
T3 9days interval	0	0	1.25	1.63	2	2	2.5	3.5	1.61
T4 (Daily)	0	0	1.88	2.8	2.8	2.88	4.5	6.38	1.86
F-test	ns	ns	**	**	**	**	**	**	
5%LSD	0	0	0.27	0.48	0.26	0.19	0.53	0.65	
cv%	0	0	10.4	14.6	7	9.9	9.9	8.8	

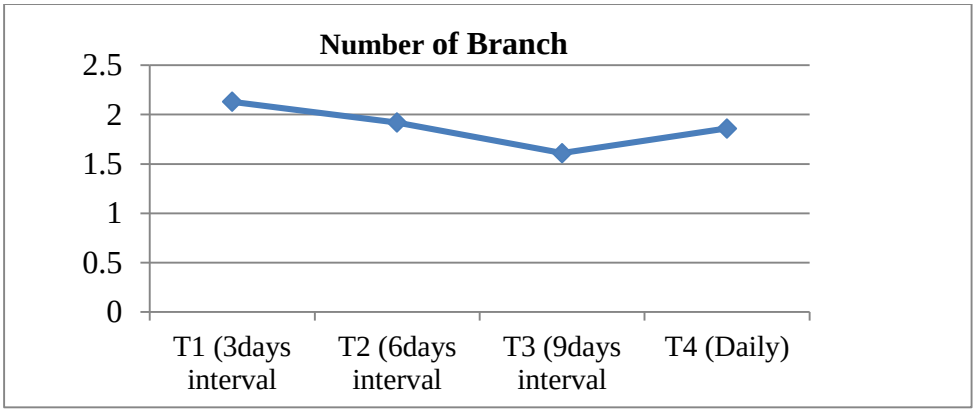

Figure 4 Effect of Days interval water Treatment on Number of Branch

Number of Branch

In all treatment, the statistical result showed that, the highest number of branches was obtained (2.13) from 3 days intervals. The lowest number of branches (1.61) was obtained from 9 days intervals.15 days and 30 days were non-significant.

Table 5 Effect of Days interval water Treatment on Number of node of Sacha inchi

Treatment	Number of Node								
	15 Days	30 Days	45 Days	60 Days	75 Days	90 Days	105 Days	120 Days	Mean
T1(3Daysinterval)	10.88	13	15.13	20.13	24.63	27.5	27.75	28.38	20.92
T2(6Daysinterval)	10.75	12.75	14.75	18.63	22.13	23.75	24.13	25.5	19.05
T3(9Daysinterval)	8.75	10.88	12.5	16.75	21.13	24.5	25	25.63	18.14
T4(Daily)	10.5	12.5	15.75	23.5	27.63	27.63	29	29	21.94
f-test	ns	ns	ns	ns	*	*	**	ns	
5 %LSD	3.29	3.25	3.05	5.54	4.34	2.95	2.48	3.47	
cv%	20.2	16.6	13.1	17.5	11.4	7.2	5.9	8	

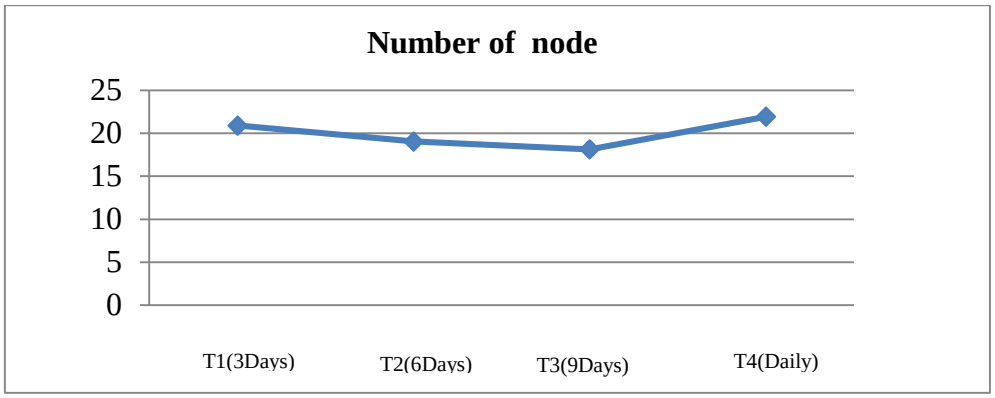

Figure 5 Effect of Days interval water Treatment on Number of Node

Number of Node

The statistical result showed that the days interval water treatments, the number of note (21.94) were obtained from water daily supply, (20.92) 3 days interval water supply, (19.05) from 6 days interval water supply, and number of node (18.14) were obtained from 9 days interval water supply. 75, 90 days are significant and 105 days is highly significant.

Table 6 Effect of days interval water treatment on number of leaves of Sacha inchi

Treatment	Number of Leaves								
	15Days	30Days	45Days	60Days	75Days	90Days	105Days	120Days	Mean
T1 (3Daysinterval)	6.5	9	10.63	15.25	17.75	19.5	17.5	15.38	13.93
T2 (6Daysinterval)	6.38	7.75	9.5	12.75	15.75	18.38	16.88	15.75	12.89
T3 (9Daysinterval)	5.75	6.38	7.63	13.25	14.25	17.13	17.88	16	12.28
T4(Daily)	7.75	10.38	12.13	16.75	18.63	20.25	20.5	20.5	15.86
f- test	ns	*	*	ns	*	ns	ns	**	
5% LSD	1.73	2.44	2.89	3.91	3.14	3.65	3.8	3.12	
cv%	16.5	18.2	18.2	16.9	11.9	12.2	13.1	11.6	

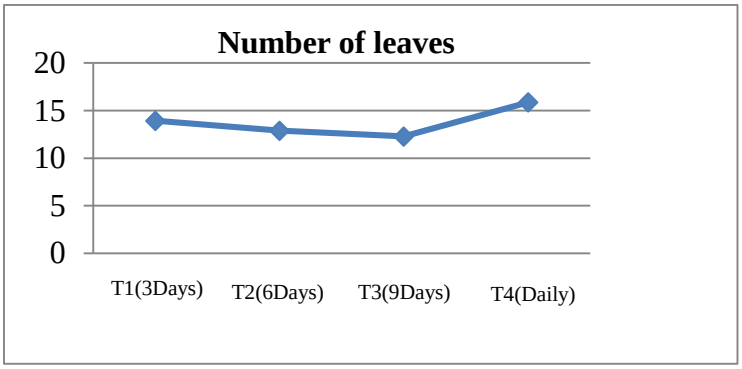

Figure 6 Effect of days interval water treatment on number of leaves

Number of Leaves

The statistical results showed that the number of leaves of all treatments are increased days by days. In different days interval water treatments, the largest number of leaves on main stem was obtained (15.86) from daily water supply. The smallest number of leaves (12.28) was obtained from 9 days interval water treatment. 30, 45, 75days are significant and 120 days is highly significant.

Table 7 Effect of Days interval water Treatment on Leaf Area of Sacha inchi

Treatment	Leaf Area (cm^2)								
	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS	Mean
T1 (3days interval)	**231.88**	**245.66**	**258.88**	**487.18**	**624.36**	**763.45**	**866.37**	**921.83**	**549.95**
T2 (6days interval)	**231.91**	**241.65**	**252.4**	**483.68**	**598.54**	**743.42**	**842.53**	**897.39**	**536.44**
T3 (9days interval)	**207.45**	**231.47**	**218.83**	**496.58**	**585.82**	**670.76**	**738.2**	**825.21**	**494.54**
T4 (Daily)	**287.92**	**301.35**	**317.24**	**492.07**	**683.99**	**791.87**	**878.6**	**974.52**	**590.95**
F-test	*	**	**	ns	ns	ns	ns	*	
5%LSD	**41.87**	**39.4**	**43.53**	**74.46**	**130.28**	**152.29**	**139.91**	**98.45**	
cv%	**10.9**	**9.8**	**10.4**	**9.6**	**13.1**	**12.8**	**10.5**	**6.8**	

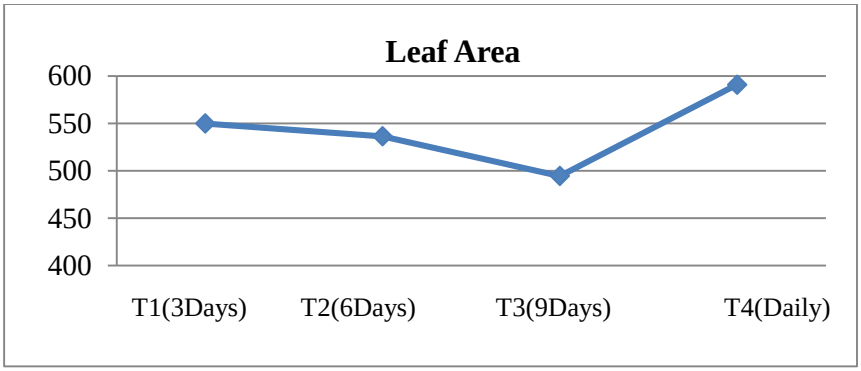

Figure 7 Effect of Days interval water Treatment on Leaf Area

Leaf Area (cm^2)

The statistical results showed that the leaf areas of all treatments 15,30,45,120 days were significant. In days interval water treatment, the largest leaf areas (590.95cm^2) was obtained from daily water supply. The second largest leaf areas (549.95cm^2) was obtained from 3days interval water supply and the smallest leaf areas (494.54cm^2) was obtained from 9days interval water supply.

Discussion and Conclusion

The result of the analyzed soil from growing area showed Loamy sand in soil texture which contained 84.9% sand, silt 9.9% and 3.9% clay. It had low in total nitrogen content (0.12%). The pH of the soil was 6.79 (nearly neutral) and the moisture content was 1.26%. The contents of exchangeable cations, from this experiment showed that the potassium content was 0.34% and phosphorous contents 31.51% very high. Sacha Inchi plant tolerate the acidic soil with a pH as low as 4.5 that contains aluminum (http://www.tandfonline.com). The maximum temperature (36.9)°C was obtained in September 2019 while the minimum temperature of (16.9)°C in October. The maximum rainfall (390.65) mm was obtained in June and there no rainfall in November. Sacha Inchi plants prefer to grow in tropical climates at 5577 feet (1, 700m) above sea level and the best annual rain fall is within 850-1000 mm. Irrigation is important during dry months at the temperature of 36°C. (www.nature.com/ scientificreports, 2018). It is difficult to match the detailed morphological characteristics of Sacha Inchi with any other references because there was no study on this plant. However, most of the characters of this plant could match with the characters mentioned for Euphorbiaceae by Lawrence (1964), Brandi (1906) and (www.nature.com/ scientificreports, 2018). The results of water treatment showed that the tallest plant height (90.97cm) the highest number of branches (2.13) and the maximum number of node (31.94) were obtained from daily water treatment.

This observation agreed with Zareian (2004) who revealed that Sacha Inchi plant requires daily watering for its optimum growth. The height of plants under adequate water supply is higher than that of moderate water stress. These agree with Zareian(2004). In many species, the vegetative growth is more likely to be retarded by soil water deficiency than the period of the reproductive growth. Deficiency of water during stem elongation or shooting period with notation of a marked retarding effect upon the height of plant (Zareian, 2004). Kramer (1983) also stated that vegetative growth, in general and leaf expansion, in particular are several inhibited by relatively moderate water stress. Moreover, the largest number of leaves on main stem was (15.86), the largest leaf area (590.95cm^2) were obtained from the control, daily water treatment. The numbers of leaves of water stress are generally less than that of under well water plants. This observation were in accordance with Marc and Palmer (1976); Yegappan *et al.*, (1980). The area of leaf of well watered

plants were greater than that of moderated water stress plants. This finding is in agreement with the finding of Zhang *et al*., (2016) mentioned that the leaf area of well watered plants were greater than that of stressed plants and it was increased with time.

This study agreed with Zhang *et al*., (2016). The decreased a single leaf area of Sacha Inchi plants are important to maintain leaf function and allow to conserve water under the drought conditions (Jiao *et al*., 2012). Although water stress induced abscisic acid accumulation is generally regarded as an inhibitor of shoot growth (Zhang *et al*., 2016). Leaves and plant growth of Sacha Inchi plants were generally influenced by deficit irrigation (www.nature.com/ scientificreports, 2018). In this study, the vegetative growth of Sacha Inchi plants were well developed from daily water treatment. Zhang *et al*., (2016) reported that even natural drought conditions decreased the plant growth. In Sacha Inchi plant, along other managements practices such as concentrating irrigation on the developmental stages is the most sensitive factor for plant growth (Zhang *et al*., 2016 and http://dx.doi.j.indcrop. 2017.09.002). In many dry regions irrigation is an important way in which water is supplied to crop plants Bernard S. Meyer, (1963).

It is therefore concluded that the experimental results showed that water has an influence on the plant growth. The plants grown under sufficient water supply condition produced highest plant height, larger leaf area than the plants grown under water deficit plants.

Acknowledgements

I would like to express my heartfelt thanks of my Professor Dr. Aye Pye, Head of Botany Department, Yangon University, for allowed me to conduct this research for my phD degree. I wish to express my deepest gratitude to my supervisor, Dr. Thanda Aye, Professor Departments of Botany, University of Yangon, for her invaluable advices, supervisions, guidance and supports for the experiment.

I would to my gratitude to Daw Hla Hmwe Kyu, Assistant research officer, Magway Central farm Oil Seed Crop Research Center in Magway Regions, for her kind helps during my research work.

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IN VITRO SCREENING OF ANTIMICROBIAL ACTIVITY OF SELECTED MYANMAR MEDICINAL PLANTS

Swe Swe Aye[1], Khin Thin Thin[2], Thet Thet Mar Win[3]

Abstract

The global burden of microbial infection is very high and antibiotic resistance leads to failed treatment of infections, which can ultimately lead to death. To overcome antibiotic resistance, it is necessary to find new antimicrobial agents. In this study, twenty medicinal plants which are traditionally used for the treatment of various diseases in Kalonehtar village tract, Yebyu Township were selected to examine their antimicrobial potential against five pathogenic microorganisms such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans*. This research was performed at Department of Botany at University of Yangon in 2019. The extraction of phytopharmaceuticals from twenty medicinal plants was performed by using Ultrasound assisted solvent extraction technique. The polar solvent, methanol (80%) was employed for extraction process. The result showed that phytopharmaceuticals from *Symphorema involucratum* (20.6%) and *Portulaca oleracea* (20.2%) were the most soluble in methanol where as *Peperomia pellucida* (3.2%) was the least soluble in methanol. The antimicrobial activity of selected medicinal plants was conducted by paper disc diffusion method. It was revealed that the methanol extract of selected medicinal plants such as *Piper cubeba, Amomum subulatum* showed the higher antimicrobial activity on *Escherichia coli* (20 mm), *Dracaena angustifolia*, *Emilia sonchifolia*, *Pittosporum glabratum* showed the highest inhibitions (22mm-32mm) on *Pseudomonas aeruginosa* whereas *Melastoma malabathricum* exhibited the significant activity (32mm) on *Staphylococcus aureus ,the plants Amomum subulatum , Glycosmis pentaphylla , Portulaca oleracea , Homonoia riparia* showed the activity (20 mm-28 mm) on *Bacillus subtilis.* Moreover, the result showed that *Homonoia riparia Lour.* posses antifungal activity (30mm) on *Candida albicans.* The present findings indicated that the active chemical compound responsible for the antimicrobial action must be a polar soluble compound.

Keywords: antimicrobial activity, methanol extract, *Pittosporum glabratum* Lindl.

Introduction

Medicinal plants have long been used to treat many infectious diseases (Mohanta *et al*., 2014). The use of medicinal plants as complementary and alternative medicine has increased dramatically in the last 20-25 years (Rios & Recio, 2005). According to a WHO report, 80% of the world's inhabitants depend on traditional medicines as their main source of health care (Ballabhet *et al*., 2007).

Worldwide research for antimicrobial agents continued to focus on flowering plants, non-flowering plants, fungi and bacteria (Fabry *et al*., 1998). Myanmar is one of richest countries in the world with regards to the genetic resource of medicinal plants. The country has a wide range of topography and climate which influences its vegetation and floristic composition (DeFlipps, 2018). Although a great potential of the plants to be studies and developed into commercial products there are many species are unknown and their useful functions are still undiscovered (Thet Thet Mar Win, *et al*., 2019).

The secondary metabolites can be identified through random, ethno (including ethnobotanical, ethnomedical and ethnopharmacological) and ecological searches. (Fabricant *et al.*, 2001). The random collection of plant samples from certain habitats (eg. Tropical rain forest) can be very useful for identification of novel chemical entities. However, this method is time consuming and labor intensive (Vuorela *et al*., 2004).

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Previous studies have provided evidence that antimicrobial compounds isolated from different solvent extracts never provided the expected final output based on the activity of crude extracts and fractions (Eloff *et al*., 2008). This is probably because different plant metabolites often work in combination with other compounds to regulate microbial infections and may therefore not be effective alone (Lewis *et al*., 2006). The solvent (extraction agent) used to prepare phytopharmaceuticals must be able to dissolve all key phytoconstituents (Pauw *et al*., 2014).

In this study, methanol (80%) extract was used in the preliminary screening (paper disc diffusion method). It is believed that the organic solvent methanol could efficiently penetrate the cell membranes, permitting the extraction of high amounts of endocellular components in contrast to low polarity solvents which can only extract extracellular material. Methanol (methyl alcohol) primarily dissolves polar constituents together with median and low polarity compounds extracted by cosolubilization. Therefore, the present investigation was conducted to evaluate methanol (80%) extracts of selected plants belonging to a wide range of families based on random sampling.

Materials and Methods

Study area

Ye-Byu Township is located in the district of Dawei. The region is extended over 2447.0 sq.km between 97° 50′ 00″ E to 98° 20′ 00″ E and 14° 15′0″ N to 15° 0′ 0″N. The region is known to have a rich floral diversity with many of them having medicinal values Fig. (1). The tribal communities residing around the study area are Htar wai, Kayen, and Mon. This place offers unique opportunities to study indigenous medicinal plants used by populations. It is estimated that more than hundred plant species are collected from that area during the flowering period of 2017-2018. In this research, twenty medicinal plants were randomly selected for antimicrobial activity screening. The authors samples mostly leaf materials, unless ethnomedicinal information was available regarding other parts in Table (1), because leaves are renewable and resource and it is easier to recollect leaves from the same plant for follow-up work.

Figure 1 Location Map of Study Area in Yebyu Township in Dawei District

Processing

The leaves, roots the whole plant were collected separately during field trips to different places of Ye-Phyu Township. Healthy leaves were plucked from the large plant and the herbs were washed with distilled water. Following collection, the sample were dried at room temperature for three weeks. Then, the dried sample were powdered separately and stored in airtight containers to prevent from moisture and air-borne contamination.

Preparation of plant extract

A hundred gram of each powdered sample were added to s round bottom flask (1000ml) then moistened with 80% methanol (600 ml) and the sample was sonicated in an ultrasonic bath (Power Sonic 410) for 30 min at room temperature. The obtained extract was reduced by Rotary evaporator (at 50°C, 40 rpm, for 45 min) to get methanol soluble matter. Yield of extract was calculated in mg/g and converted into percentage (Azwanida, 2015).

Yield (%) of extract = Weight of dry extract ×100 / Weight of dry powdered sample

Antimicrobial assay

The antimicrobial activity was assessed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis Candida albicans.* using the paper disc diffusion assay (Cruickshank *et al*., 1975; Madigan *et al*., 2005). In brief, the isolated microbial strains grown on nutrient agar were inoculated into conical flasks containing 10 ml of sterile growth medium. They were incubated at 30°C for 72 hours on a reciprocal shaker at 200 rpm. Then, test organisms (0.3ml) was added to assay medium, then poured into plates. The solutions of 50mg/ml plant extract were prepared by dissolving 80% methanol. Sterile 6mm paper discs (Whatman) were impregnated with 100µL of the plant extract solutions, dried at 40°C. Then, all disc was added to the agar surface. After 48-hour incubation at 42°C, the diameters of the zones of inhibition were measured. The tests were conducted at Microbiology Laboratory, Department of Botany, University of Yangon.

Table 2 List of plant species screened for antimicrobial activity

Scientific Name	Family	Local name	Part of use
Hydrolea zeylanica (L.)Vahl	Hydroleaceae	Le-hgin tha	the whole plant
Bulbophyllum careyanum Spreng.	Orchidaceae	Kaung-say-thi	the whole plant
Cryptocoryne retrospiralis (Roxb.) Kunth	Araceae	Unknown	The whole plant
Portulaca oleracea L.	Portulacaceae	Mya-Pyit	the whole plant
Glycosmis pentaphylla (Retz.)DC.	Rutaceae	Taw-shauk	leaves
Sympheorema involucratum Roxb.	Lamiaceae	Daung-ttalaung	leaves
Peperomia pellucida (L.)Kunth	Piperaceae	Thit-yay-kyi	the whole plant
Dracaena angustifolia (Medik) Roxb.	Asparagaceae	Ein-lone-chan-thar	leaves
Melastoma malabathricum L.	Melastomataceae	Say-oh-pok	leaves
Pterospermum semisagittatum Buch-Ham	Malvaceae	Nwa-labyin	leaves
Buchanania arborescens (Blume) Blume	Anacardiaceae	Che-ti	leave
Piper cubeba L. f.	Piperaceae	Peik-chin	leaves
Hellenia speciosa (J. Koeing) SR	Costaceae	Phalan-taung-hmwe	the whole plant
Homonoia riparia (Lour.)	Euphorbiaceae	Yeta - kyi	the whole plant
Amomum subulatum Roxb.	Zingiberaceae	Chin-baung-phar-lar	the whole plant
Emilia sonchifolia (L.) DC. ex Wight	Asteraceae	Unknown	the whole plant
Getonia floribunda Roxb.	Combretaceae	Bu-nwe	leaves
Syzygium lineatum (DC).Merr & L. M. Perry	Myrtaceae	Tha-bye	leaves
Ochna integerrima (Lour.) Merr.	Ochnaceae	Indaing – say-ni	leaves
Pittosporum glabratum Lindl.	Pittosporaceae	Hin- cho- pin	leaves

Results

. In this research, twenty medicinal plants were randomly selected for antimicrobial screening. The scientific name, local name, family, plant parts used and its medicinal values are mentioned precisely in Table (1), (2) and Figure (2).

Figure 2 Twenty selected medicinal plants from Ye-phyu Township namely (A) *Hydrolea zeylanica*(L.) Vahl. (B) *Bulbophyllum careyanum* Spreng. (C) *Cryptocoryne retrospiralis* (Roxb.)Kunth (D) *Portulaca oleracea* L. (E) *Glysosmis pentaphylla (Retz.) DC.* (F) *Symphorema involucratum Roxb.* (G) *Peperomia pellucida (L.)Kunth* (H) *Dracaena angustifolia (Medik) Roxb.* (I) *Melastoma malabathricum* L.(J) *Pterospermum semisagittatum Buch-Ham.* (K) *Buchanania arborescens* (Blume) Blume (L) *Piper cubeba* L. f. (M) *Hellenia speciosa* (J. Koeing) SR (N) *Homonoia riparia* (Lour.) (O) *Amomum subulatum* Roxb. (P) *Emilia sonchifolia* (Q) *Getonia floribunda* Roxb. (R) *Syzygium lineatum* (DC).Merr & L.M.Perry (S) *Ochna integerrima* (Lour.) Merr. (T) *Pittosporum glabratum* Lindl.

Extraction yield of selected medicinal plants

In this study, Table (2) represents the results for 80% methanol soluble matter content of selected medicinal plants. It was observed that phytoconstituents in *Peperomia pellucida (L.)Kunth* were least soluble in 80 % methanol as succulent herbs.

Table 3 Yield of the methanol (80%) of twenty selected medicinal plants using Sonication method

Scientific Name	Part of use	Yield (%)
Hydrolea zeylanica (L.)Vahl	the whole plant	10.3
Bulbophyllum careyanum Spreng.	the whole plant	7.5
Cryptocoryne retrospiralis (Roxb.)Kunth	the whole plant	12.5
Portulaca oleracea L.	the whole plant	20.2
Glysosmis pentaphylla (Retz.)DC.	leaves	9.4
Symphorema involucratum Roxb.	leaves	20.6
Peperomia pellucida (L.)Kunth	the whole plant	3.2
Dracaena angustifolia (Medik) Roxb.	leaves	8.5
Melastoma malabathricum L.	leaves	15.4
Pterospermum semisagittatum Buch-Ham	leaves	8.1
Buchanania arborescens (Blume) Blume	leave	6.4
Piper cubeba L. f.	leaves	10.2
Hellenia speciosa (J. Koeing) SR	the whole plant	18.6
Homonia riparia (Lour.)	the whole plant	10.3
Amomum subulatum Roxb.	the whole plant	15.6
Emilia sonchifolia (L.) DC. ex Wight	the whole plant	7.4
Getonia floribunda Roxb.	leaves	17.5
Syzygium lineatum (DC).Merr &L.M.Perry	leaves	18.3
Ochna integerrima (Lour.) Merr.	leaves	8.4
Pittosporum glabratum Lindl.	leaves	10.1

Antimicrobial activity of twenty selected medicinal plants

The inhibitory activities of methanol extract of twenty medicinal plants obtained using paper disc diffusion assay against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans*. It was found that the methanol extract of selected medicinal plants such as., *Piper cubeba* L.f.,, *Ammomum subulatum* Roxb., showed the higher antibacterial activity on *Escherichia coli* (20 mm), *Dracaena angustifolia* (Medik.) Roxb, *Emilia sonchifolia* (L.)DC., *Pittosporum glabratum* Lindl showed the highest inhibitions (zone of inhibition between 22mm-32mm) on *Pseudomonas aeruginosa* whereas *Melastoma malabathricum* exhibited the significant activity (32mm) on *Staphyllococcus aureus, the plants Amomum subulatum* Roxb., *Glycosmis pentaphylla* (Retz.) DC., *Portulaca oleracea L., Homonia riparia* (Lour.) showed the activity (zone of inhibition between 20mm-28mm) on *Bacillus subtilis*. Moreover, the result indicated that *Homonoia riparia (Lour.)* possess antifungal activity (30 mm) on *Candida albicans* The results are shown in Table (3) and Fig. (3), (4) and (5).

Table 4 Antimicrobial activity of methanol (80%) extract of selected plants

Scientific Name	Part Use	Zone of Inhibition in mm				
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*	*Candida albicans*
Hydrolea zeylanica (L.)Vahl	WP	-	14	8	14	16
Bulbophyllum careyanum Spreng.	WP	8	10	8	16	8
Cryptocoryne retrospiralis (Roxb.)Kunth	WP	12	8	10	18	12
Portulaca oleracea L.	WP	10	16	14	26	16
Glysosmis pentaphylla (Retz.)DC.	L	8	10	10	22	18
Symphorema involucratum Roxb.	L	8	10	8	16	10
Peperomia pellucida (L.)Kunth	WP	-	10	8	20	14
Dracaena angustifolia (Medik) Roxb.	L	-	22	12	18	12
Melastoma malabathricum L.	L	-	18	32	16	8
Pterospermum semisagittatum Buch-Ham	L	-	8	16	12	8
Buchanania arborescens (Blume) Blume	L	8	8	-	14	14
Piper cubeba L. f.	L	20	8	8	14	12
Hellenia speciosa (J. Koeing) SR	WP	10	12	8	20	8
Homonia riparia (Lour.)	WP	8	12	-	28	30
Amomum subulatum Roxb.	WP	20	8	8	20	12
Emilia sonchifolia (L.) DC. ex Wight	WP	8	22	8	16	8
Getonia floribunda Roxb.	L	8	8	-	14	12
Syzygium lineatum (DC).Merr &L.M.Perry	L	12	12	8	18	10
Ochna integerrima (Lour.) Merr.	L	-	8	-	8	12
Pittosporum glabratum Lindl.	L	-	32	-	-	8

Paper disc - 6mm
WP - The Whole Plan
L - Leaves

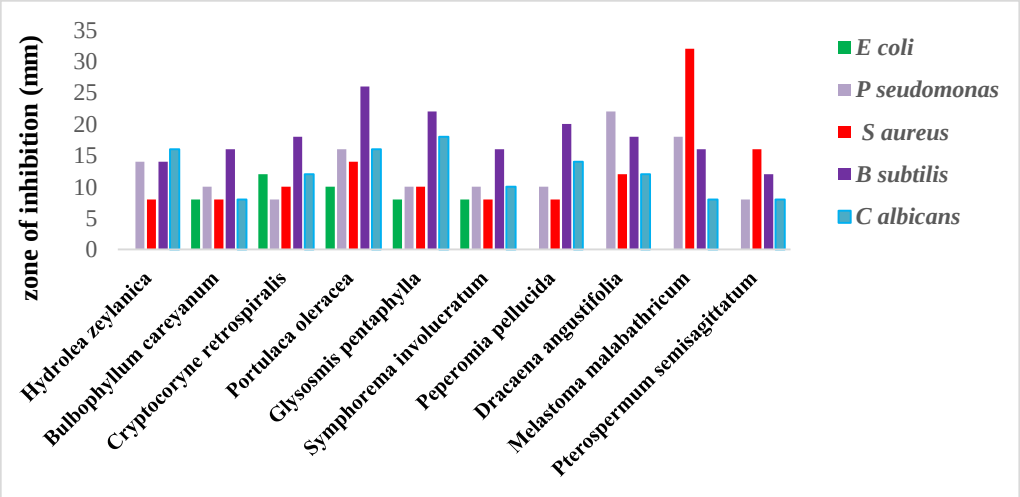


Figure 3 Effects of selected plants 80%methanol extract on Microbial growth

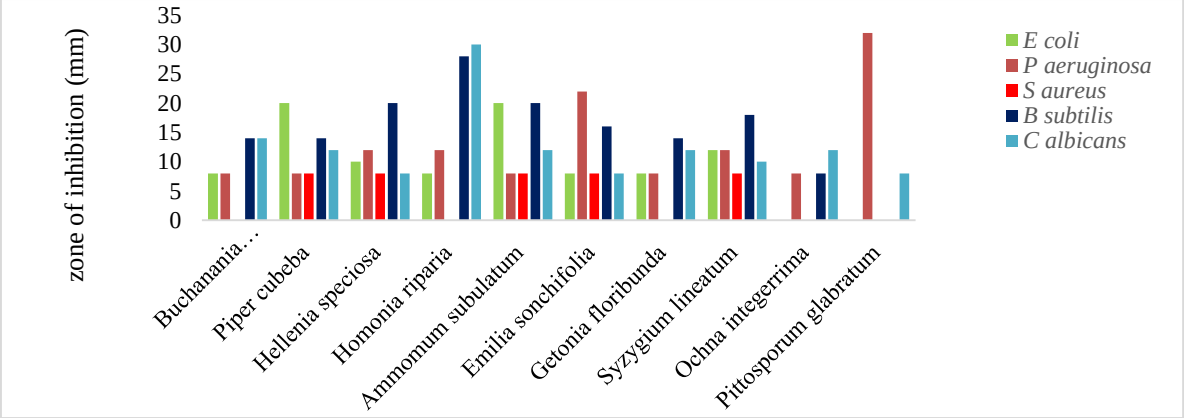
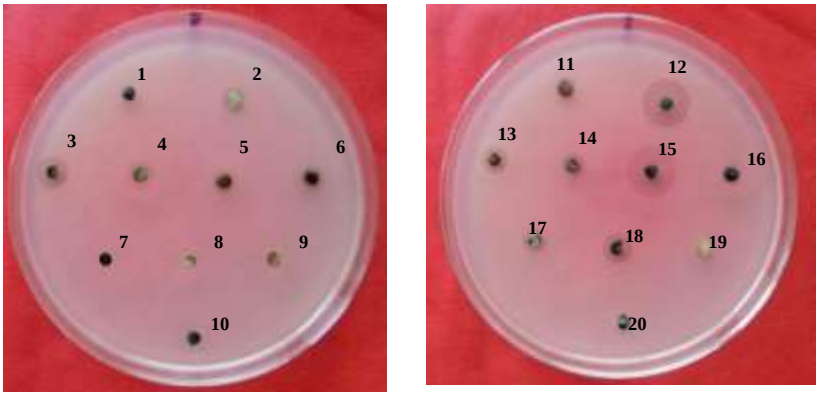
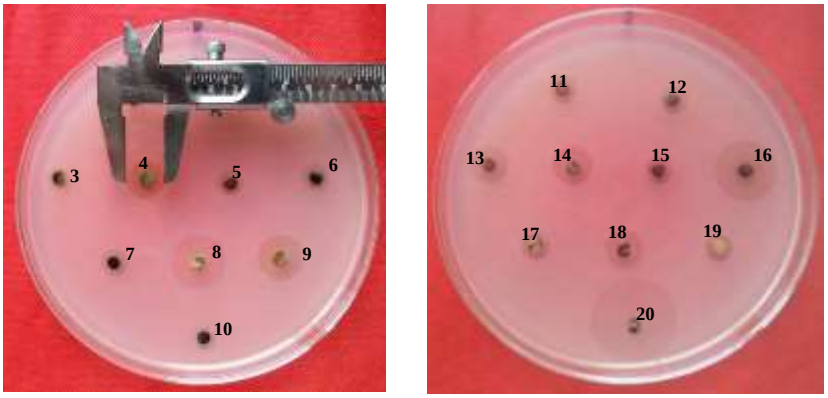


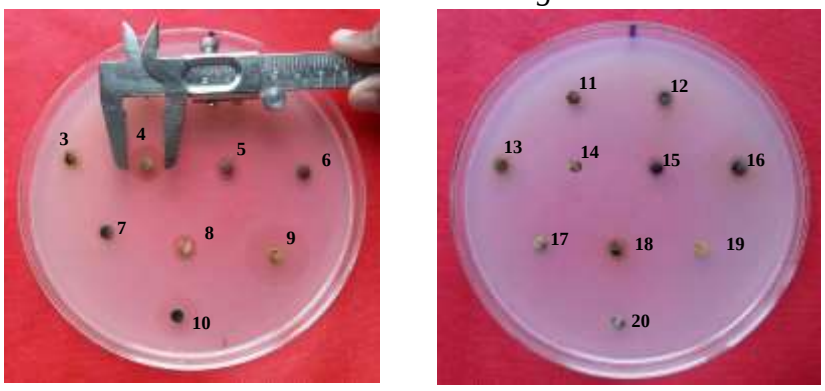
Figure 4 Effects of selected plants 80% methanol extract on Microbial growth



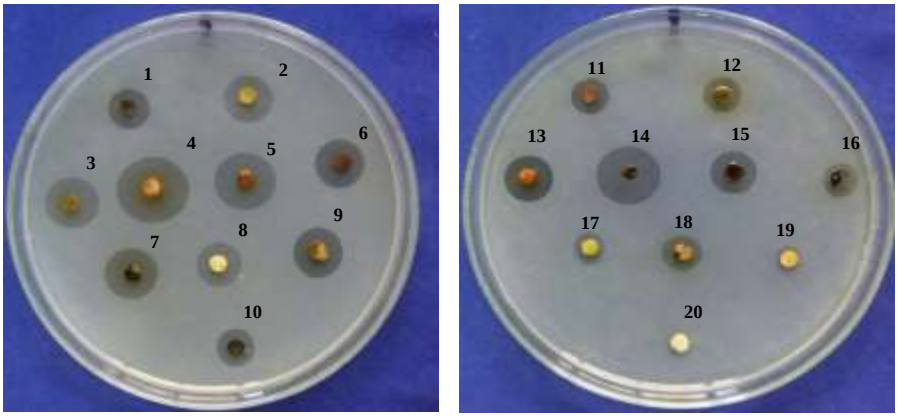
Escherichia coli



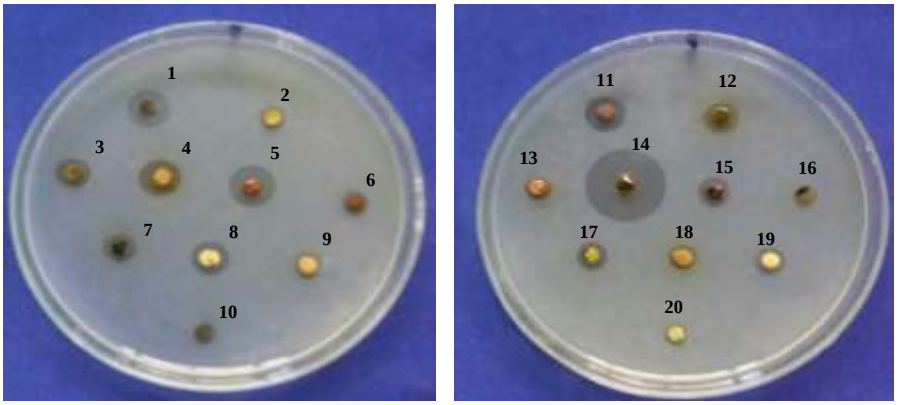
Pseudomonus aeruginosa



Scaphylococcus aureus



Bacillus subtilis



Candida albicans

1. *Hydrolea zeylanica* (L.)Vahl.
2. *Bulbophyllum careyanum* Spreng.
3. *Cryptocoryne retrospiralis* (Roxb.)Kunth.
4. *Portulaca oleracea* L.
5. *Glycosmis pentaphylla (Retz.)DC.*
6. *Symphorema involucratum Roxb.*
7. *Peperomia pellucida (L.)Kunth.*
8. *Dracaena angustifolia (Medik) Roxb.*
9. *Melastoma malabathricum* L.
10. *Pterospermum semisagittatum Buch-Ham*
11. *Buchanania arborescens* (Blume) Blume
12. *Piper cubeba* L. f.
13. *Hellenia speciosa* (J. Koeing) SR
14. *Homonoia riparia* (Lour.)
15. *Amomum subulatum* Roxb.
16. *Emilia sonchifolia* (L.) DC. ex Wight
17. *Getonia floribunda* Roxb.
18. *Syzygium lineatum* (DC). Merr & L. M. Perry
19. *Ochna integerrima* (Lour.) Merr.
20. *Pittosporum glabratum* Lindl

Figure 5 Effect of Antimicrobial activity of twenty selected medicinal plants on six pathogenic microorganisms

Discussion

The total of twenty species (twenty genera, twenty families) were tested on four species of bacteria and one species of fungus mostly involved in common infections such as gastroenteritis, diarrhea, dysentery, skin diseases, food and water contamination.

In total of seven plant species were unable to inhibit the tested organism (*Escheria coli*) namely *Hydrolea zeylanica* (L.) Vahl, *Peperomia pellucida (L.) Kunth, Dracaena angustifolia (Medik) Roxb., Melastoma malabathricum* L., *Pterospermum semisagittatum Buch-Ham, Ochna integerrima (Lour.) Merr., Pittosporum glabratum* Lindl. whereas four species namely *Buchanania arborescens*, *Homonia riparia* (Lour.), *Getonia floribunda* Roxb, *Ochna integerrima*

(Lour.) Merr., did not show any activity on *Staphyllococcus aureus* and the plant, *Pittosporum glabratum* Lindl did not exhibit on *S aureus* as well as on *Bacillus subtalis*.

However, nine methanol extracts of selected medicinal plants showed strong inhibition on different test organisms (zones of inhibition ≥ 20 mm). The methanol extract of other plants also showed weakly inhibitions (zone of inhibition in between 8-18 mm).The different results concerning with antimicrobial activity might be due to different secondary metabolites of each plant (Cowan,1999)

The antimicrobial activities of some of these plants such as *Hydrolea zeylanica*(L.), Vahl. (L.)Vah, *Bulbophyllum careyanum* Spreng., *Cryptocoryne retrospiralis* (Roxb.)Kunth, *Portulaca oleracea*, *Glysosmis pentaphylla (Retz,)DC.* , *Symphorema involucratum Roxb.*, *Peperomia pellucida (L.)Kunth*, *Dracaena angustifolia (Medik) Roxb.*, *Melastoma malabathricum* L., *Pterospermum semisagittatum Buch-Ham*, *Buchanania arborescens* (Blume) Blume, *Piper cubeba* Lf. , *Hellenia speciosa* J Koenig) SR , *Homonoia riparia* (Lour.), *Ammomum subulatum* Roxb., *Emilia sonchifolia*, *Getonia floribunda* Roxb., *Syzygium lineatum* (DC).Merr &L.M.Perry, *Ochna integerrima* (Lour.) Merr., were previously described by other researchers (Qureshi *et al*., 2017; Manna *et al*.,2017; Oraibi *et al.*, 2017; Khan *et al*., 2012; Idris *et al*.; Bapat *et al*., 2014; Abbas Ali *et al*., 2011; Saeed *et al*., 2013; Wadkar *et al*., 2017; Roy *et al*., 2019; Pavithra *et al*., 2009). However, the antimicrobial activity of selected plants such as *Buchanania arborescens* (Blume) Blume and *Pittosporum glabratum* Lindl leaves methanol extract have not mentioned in previous studies.

Conclusion

The twenty selected medicinal plants from Kalonehtar village tract, Ye-Pyu Township showed antimicrobial activity. These plants contained active antimicrobial agents which may use as a good source for antibiotics. Further study is required to identify the active compounds, synergetic affects, toxicity and safety of these plants and eventually clinical evaluations.

Acknowledgments

Authors are thankful to Korea Research Institute of Bioscience and Biotechnology (KRIBB) for providing project funding. We are very grateful to Dr Aye Pe, Professor & Head, Department of Botany, University of Yangon for his valuable suggestions.

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APPENDIX I

Table 1 List of plants and their medicinal uses by indigenous communities in Ye–Phyu Township, Dawei District

Scientific Name	Family	Local name	Part of use	Ethno Medicinal uses
Hydrolea zeylanica (L.)Vahl	Hydroleaceae	Le-hgin tha	leaves	callous ulcers, skin diseases
Bulbophyllum careyanum Spreng.	Orchidaceae	Kaung-say-thi	pseudobulb	tuberculosis, tonic
Cryptocoryne retrospiralis (Roxb.)Kunth	Araceae	Unknown	rhizome	diarrhea, jaundice,
Portulaca oleracea L.	Portulacaceae	Portulacaceae	Whole plant	use as vegetable
Glycosmis pentaphylla (Retz,)DC.	Rutaceae	Taw shauk	leaves	leaves extract possess a healing effect
Symphorema involucratum Roxb.	Lamiaceae	Daung-ttalaung	Roots	ear diseases , wounds and burns
Peperomia pellucida (L.)Kunth	Piperaceae	Thit-yay-kyi	the whole plant	urinary troubles
Dracaena angustifolia (Medik) Roxb.	Asparagaceae	Ein-lone-chan-thar	leaves	cure for swelling of joints
Melastoma malabathricum L.	Melastomataceae	Say-oh-pok	leaves	skin diseases
Pterospermum semisagittatum Buch-Ham.	Malvaceae	Nwa-labyin	barks	used as a replacement for Areca nut
Buchanania arborescens (Blume) Blume.	Anarcardiaceae	Che-ti	leave	to treat headache
Piper cubeba L.f.	Piperaceae	Peik-chin	leaves	antiseptic, expectorant
Hellenia speciosa (.J Koeing) SR	Costaceae	Phalan-taung-hmwe	shoot	Used as vegetable
Homonoia riparia (Lour.)	Euphorbiaceae	Yeta - kyi	the whole plant	cure for pile
Amomum subulatum Roxb.	Zingiberaceae	Chin-baung-phar-lar	seeds	digestive disorders
Emilia sonchifolia (L.)DC.ex Wight	Asteraceae	Unknown	the whole plant	Used for febrifuge, anthelmintic
Getonia floribunda Roxb.	Combretaceae	Bu-nwe	flowers	Cure for abdominal menstruation
Syzygium lineatum (DC).Merr & L.M.Perry	Myrtaceae	Tha-bye	fruits	edible for tonic
Ochna integerrima (Lour.) Merr.	Ochnaceae	Indaing – say-ni	bark	Used for blood dysentery
Pittosporum glabratum Lindl.	Pittosporaceae	Hin- cho- pin	roots	Cure for arthritis, insomnia

Thet Thet Mar Win *et al*., 2019

PHYTOCHEMICAL SCREENING AND EVALUATION OF VARIOUS EXTRACTS OF *CASSIA FISTULA* L. FRUIT PULP FOR ANTIMICROBIAL ACTIVITY

Ni Ni Htun[1], Sandar Sann[2], Khin Thuzar[3]

Abstract

Cassia fistula L., belonging to family Fabaceae, is known for its beautiful inflorescences and medicinal properties of various plant parts. This plant is called Ngu-shwe-war in Myanmar. It is widely cultivated and used in folk medicine. The present study was designed to evaluate the preliminary phytochemical constituents and antimicrobial activity of fruits of *Cassia fistula* L. The specimen were collected from Banmaw Township, Kachin State. The morphological characters of this plant have been studied in detail and identified by the literatures (Backer, 1968; Burkill, 1935; Hooker, 1881; Dassanayake, 1981 and Hu-Qi-ming, 2009). The diagnostic characters of dried fruit pulp powder of *Cassia fistula* L. were investigated for standardization of powdered drugs. Then the powdered sample was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. From this, it is known that it contained alkaloids, glycoside, reducing sugar, saponin, steroid, terpenoids, carbohydrate, tannin, phenolic compound, flavonoid, starch, protein and amino acid. For antimicrobial activity, the fruit pulp powder of *Cassia fistula* L. was extracted with six different solvents to carryout antimicrobial screening in vitro on six different types of microorganisms by paper disc diffusion method. It was found that the watery extract showed most significant antimicrobial activity on *Aspergillus flavous* and *Candida albicans* whereas ethanol extract also gave highest activity on *Escherichia coli*. The phytochemical investigations and antimicrobial activity of fruit of *Cassia fistula* L. prove its importance as a valuable medicinal plant.

Keywords: *Cassia fistula* L., Morphology, Phytochemistry, Antimicrobial activity

Introduction

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Researchers have aimed at identifying and validating plant-derived substances for the treatment of various diseases. Interestingly it is estimated that more than 25% of the modern medicines are directly or indirectly derived from plants (Danish, 2011). Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. The derivatives of medicinal plants are non-narcotic with little or no side effects (Banjare Paul, 2014).

Cassia fistula L. (family Fabaceae) is known as not only the medicinal plant but also ornamental plant. It is also commonly known as golden or yellow shower because of its characteristics yellow flowers in pendulous raceme and with typical branches. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil. It has been extensively used in Ayurvedic system of medicine for various ailments (Joshi, 2004).

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According to Hartwell, *Cassia fistula* plants are used in folk remedies for tumors of the abdomen, glands, liver, stomach and throat cancer, carcinomata and impostumes of the uterus. Ayurvedic medicine recognizes the seed as antibilious, aperitif carminative and laxative the root for adenopathy, burning sensations, leprosy, skin diseases, syphilis and tubercular glands the leaves for erysipelas, malaria, rheumatism and ulcers the buds for biliousness constipation, fever, leprosy and skin disease the fruit for abdominal pain, constipation, fever, heart disease and leprosy. Unani use the leaves for inflammation the flowers for a purgative the fruit as anti-inflammatory, antipyretic, abortifacient, demulcent, purgative, refrigerant good for chest complaints eye ailments, flu, heart and liver ailments, and rheumatism. In the West Indies, the pulp and leaves are poulticed onto inflamed viscera, e.g., the liver (Anitha & Miruthula, 2014).

This research aims to provide a comprehensive review on the phytochemical and antimicrobial aspects of fruit of *Cassia fistula* L.

Materials and Methods

Collection of plant materials

In this study, fruit pulp of *Cassia fistula* L. were collected in the month of April-May, 2019 from local area of Banmaw Township. Then fruit pulp were dried and finely powered and used for the study.

Diagnostic characters of the fruit pulp powder

The sensory characters of fruit pulp powder were observed in sight and the microscopical characters of powder sample were examined under the light microscope. Chloral hydrate solution was used as cleaning reagent.

Preliminary phytochemical investigation

For the phytochemical study, fruit pulp powder of *Cassia fistula* L. were used and carried out at the Department of Botany, University of Yangon according to the methods of British Pharmacopoeia (1968) and Trease and Evans (2002).

Antimicrobial activity of *Cassia fistula* L. fruit

Antimicrobial activity of different solvent extracts from *Cassia fistula* L. fruit were carried out on six microorganisms by paper disc diffusion method at the Department of Botany, University of Yangon.

Preparation of the crude extracts

About 5 g of the powder sample was extracted with 20 ml of each solvent (ethanol, methanol, pet-ether, acetone, ethyl acetate and water) respectively. The crude extracts were then filtered. After filtration, the extracts were concentrated to dryness and the residues were transferred to a pre-weighed bottle and were stored in desiccators for further studies.

Test organisms

The test organisms used in this study were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albican*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*.

Antimicrobial screening

Isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10 ml of sterile growth medium. Then, they were incubated at 30°C for 72 hours on a reciprocal shaker at 200 rpm. 0.3 ml of test organisms was added to assay medium, then poured into plates. After solidification, paper discs impregnated with different solvent extracts were applied on the test plates and these plates were incubated for 24-36 hours at 30°C. After incubation

for 24-36 hours, the inhibition zone which appeared around the paper discs indicated the presence of bioactive compounds which inhibit the growth of test organisms. Then, the zones of inhibition diameter including 6 mm paper disc were measured with the aid of a transparent ruler.

Results

Scientific Name : *Cassia fistula* L.
Commons Name : Golden shower, purging cassia
Myanmar Name : Ngu-shwe-war
Family : Fabaceae
Flowering and fruiting Period : March to September

Taxonomic description

A medium-sized deciduous tree, 6-9 meters tall with a straight trunk and spreading branches. Young stem is pale grey in colour and smooth, while the mature stem is dark brown in colour and rough. Leaves: alternate, paripinnately compound, 20-40 cm long, petiolate, long stalked stipulate. Leaflets: 4-5 pairs, opposite, ovate, acute or acuminate, base usually rounded. Inflorescences: axillary, drooping raceme. Flower: yellow, very showy, complete, bisexual, irregular, zygomorphic, hypogynous. Calyx: sepals 5, synsepalous, lanceolate, pubescent, yellowish green. Corolla: petals 5, apopetalous, obovate, unequal, shortly clawed, yellow. Androecium: stamens 10, free, vary in length; filaments long and curved; anther dithecous, introrse, dorsifixed and basifixed. Gynoecium: carpel 1, linear, pubescent, unilocular, many ovules in each locule, marginal placentation; style long; stigma reduced and curved. Fruit: elongated and rounded pod, 30-60 cm long with 60-75 seeds. Seeds are pointed at end and blunt at the other (as shown in Figure.1).

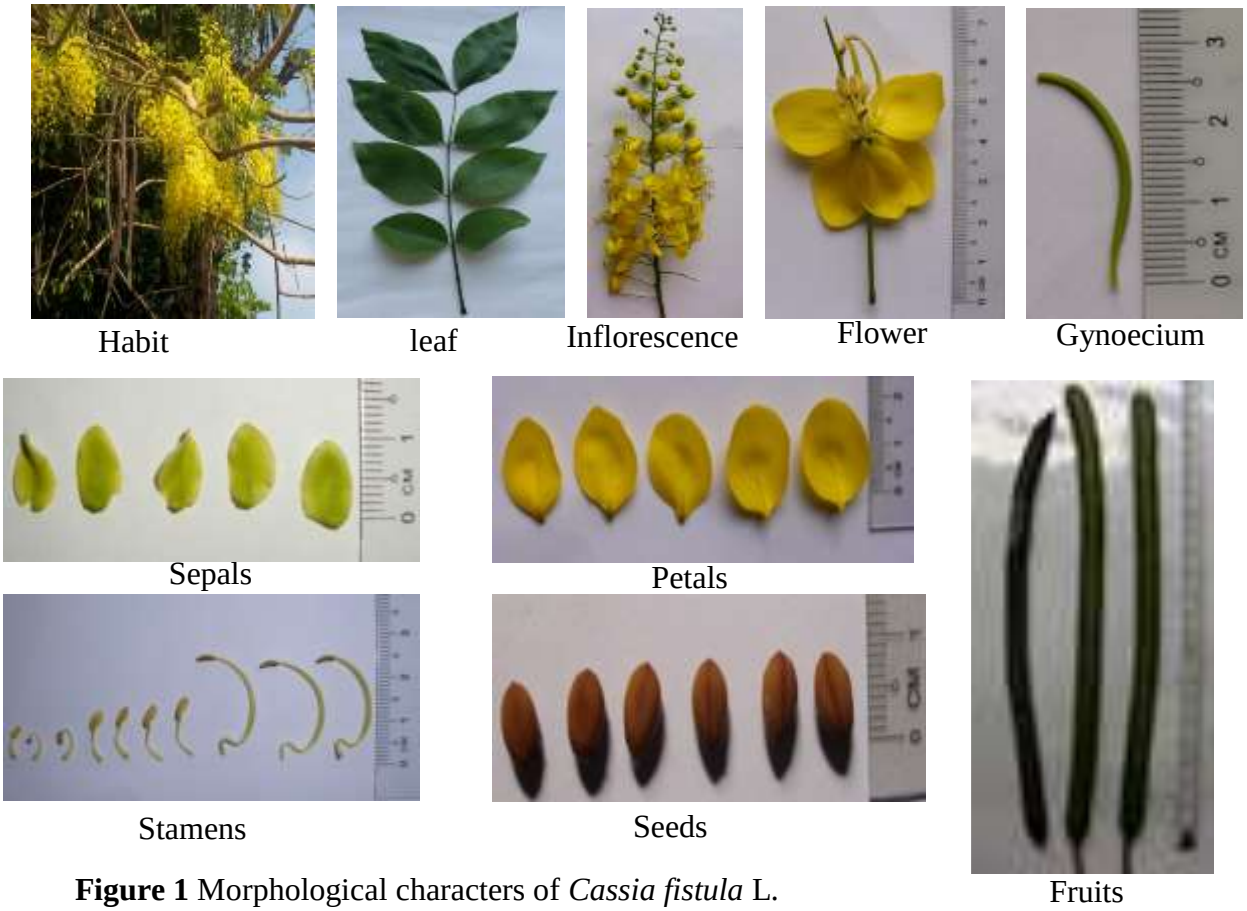


Figure 1 Morphological characters of *Cassia fistula* L.

Diagnostic characters of fruit pulp powder

Sensory characters of fruit pulp powder of *Cassia fistula* L.

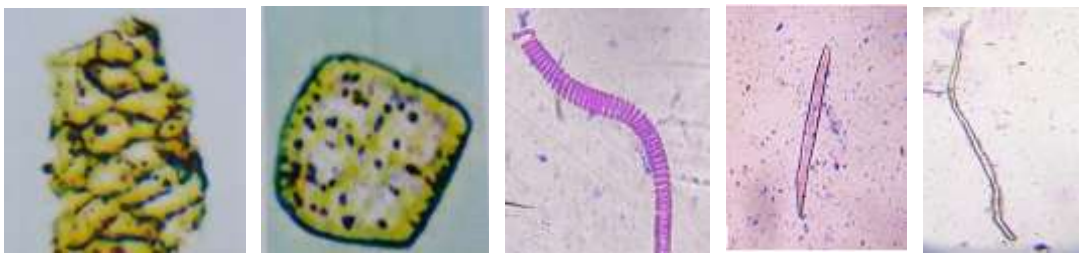
Colour = dark brown

Odour = characteristic

Taste = sweet and mucilaginous

Texture = sticky

Figure 2 Fruit pulp powder



Epidermal cells Stone cells Spiral vessel Treached Fibre

Figure 3 Microscopical characters of fruit pulp powder of *Cassia fistula* L.

Preliminary phytochemical investigation

Preliminary phytochemical test on the fruit pulp of *Cassia fistula* L. was investigated and the presence or absence of phytochemical constituents in this plant were presented in Table 1 and Fig. 4.

According to preliminary phytochemical study, it is found that the fruit pulp of Cassia fistula L. contained alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, α-amino acid, starch, reducing sugar, saponin, tannin, carbohydrate and protein. These tests were shown in Figure 4.

Figure 4 Preliminary phytochemical test of the fruit pulp of Cassia fistula L.

Table 1 Phytochemical test on the fruit pulp of *Cassia fistula* L.

No	Constituents	Extract	Test Reagent	Observation	Result
1.	Alkaloid	Methanol	1% HCL + Mayer's reagent 1% HCL + Hager reagent 1% HCL + Wagner's reagent.	White ppt. Yellow ppt. Reddish Brown ppt.	+
2.	Glycoside	Methanol	1 ml H_2O + NaOH	Yellow colour	+
3.	Phenolic compound	Methanol	2 ml H_2O + 10% $FeCL_3$	green colour	+
4.	Flavonoid	Methanol	HCL (dil) + Mg coil	Pink colour	+
5.	Steroid	Methanol	$CHCL_3$ + conc. H_2SO_4	Green colour	+
6.	Terpenoid	Methanol	$CHCL_3$ + conc. H_2SO_4	Reddish brown colour	+
7.	α- amino acid	Water	Ninhydrin reagent	Pink spot	+
8.	Starch	Water	I_2 solution	blue-black ppt.	+
9.	Reducing sugar	Water	1 ml H_2O + mixture equal part Fehling's A and B	Brick red ppt.	+
10.	Saponin	Water	Shaken with 2 ml H_2O	Frothing	+
11.	Tannin	Water	5% $FeCL_3$ + dil H_2SO_4	Yellowish-brown ppt.	+
12.	Carbohydrate	Water	1 ml benedict's reagent and boil for few minute	Brick red ppt.	+
13.	Protein	Water	Million's reagent (heated)	White ppt. turned red when heated	+

+ = Present – = Absent ppt = Precipitate

Antimicrobial activity

Screening of antimicrobial activity of fruit of *Cassia fistula* L. was carried out by using different solvents namely, acetone, ethyl acetate, ethanol, methanol, pet-ether and water. The diameter of inhibition zones that appeared were given in Table 2.

Table 2 Inhibition zone exhibited by different extracts of *Cassia fistula* L. Fruit pulp

No.	Extract	Microorganisms					
		A. flavous	***B. subtilis***	***C. albican***	***E. coli***	***P. fluorescens***	***X. oryzae***
1.	Pet-ether	16 mm	8 mm	12mm	8 mm	8 mm	10 mm
2.	Acetone	14 mm	8 mm	8 mm	14 mm	8 mm	8 mm
3.	Ethyl acetate	12 mm	10 mm	12 mm	12 mm	8 mm	8 mm
4.	Ethanol	10 mm	16 mm	12 mm	18 mm	8 mm	16 mm
5.	Methanol	14 mm	14 mm	16 mm	12 mm	14 mm	14 mm
6.	water	18 mm	14 mm	18 mm	16 mm	12 mm	12 mm
7.	Control	-	-	-	-	-	-

Paper disc = 6 mm - = no activity

In this experiment, acetone extract was found to be highest activity on *Aspergillus flavous* and *Escherichia coli*. Ethyl acetate shows significantly the antimicrobial activity on *Aspergillus flavous, Candida albican* and *Escherichia coli*. Ethanol extract showed the highest activity against *Escherichia coli* (inhibition zone 18 mm). Methanol extract and pet-ether extract are more sensitive against *Candida albican* and *Aspergillus flavous* respectively. Water extract showed highest

activity on *Aspergillus flavous* and *Candida albican* (inhibition zone 18 mm). The antimicrobial test were showed in Figure 5.

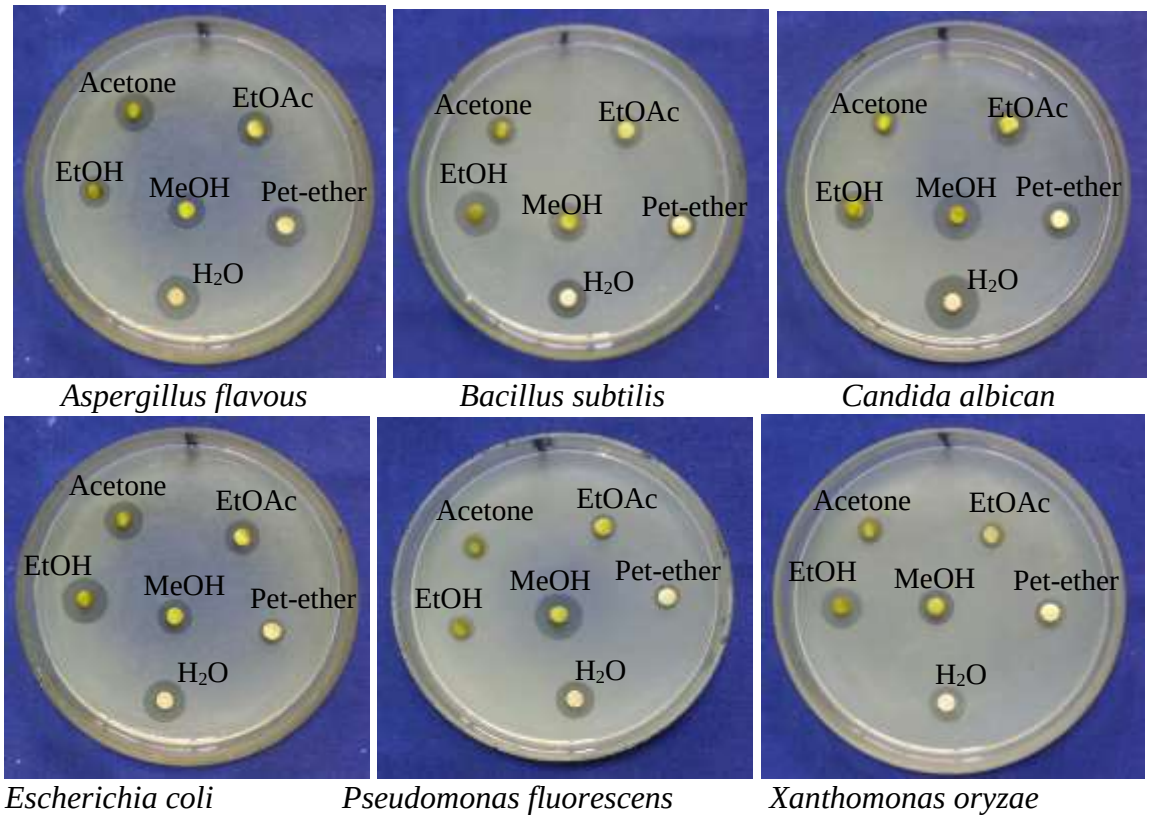


Figure 5 Antimicrobial test of different extracts from fruit pulp of *Cassia fistula* L.

Discussion and Conclusion

Research on *Cassia fistula* L. was made from two aspects such as phytochemical study and antimicrobial study. The specimens were collected from Banmaw Township, Kachin State. It is a medium tree and commonly found in many places as ornamental plant. It belongs to the family Fabaceae. The Myanmar name is Ngu-shwe-wa. This plant has been widely used as traditional medicine. Entire parts of the plant have medicinal values. The roots, leaves, fruits and seeds are the parts of the plant used as medicine.

Crude drugs are usually obtained from wild sources and are mostly collected by illiterate and unskilled people unaware of their botanical information, authentication and standardization parameters. This usually affects the safety of the final product. For safe and efficacious herbal medicine production, appropriate control of starting material is extremely crucial (Kumar, 2014).

In the present study, *Cassia fistula* L. is a medium-sized deciduous tree with a straight trunk and spreading branches. The leaves are pinnately compound, petiolate and long stalked stipulate. Leaflets: 4-5 pairs, opposite, ovate. Inflorescences are axillary and drooping raceme. Flower are yellow, very showy, bisexual, zygomorphic, hypogynous. Calyx contained sepals 5, synsepalous. Corolla consisted of petals 5, apopetalous, unequal, shortly clawed. Stamens are 10, free, vary in length. Carpel are 1, marginal placentation; style long; stigma reduced and curved. Fruit are elongated and rounded pod. These morphological characters of *Cassia fistula* L. were in accordance with those described by Backer (1968), Burkill (1935), Hooker (1881), Dassanayake (1981) and Hu-Qi-ming (2009).

In the microscopic characters of fruit pulp powder of *Cassia fistula* L., fragment of parenchyma cells, stone cells, vessels, treachid and fiber were found.

Indian medicinal plants reported that pulp of the pod contains anthraquinone glycosides, pectin and tannin. In compendium of Indian medicinal plants, Rastogi and Mehrotra revealed that fruit pulp of *Cassia fistula* L. Contained proteins and carbohydrates. The research of Anitha and Miruthula showed to be contained flavonoid, glycoside, amino acid, tannin, saponin, anthraquinone, steroid, terpenoid and reducing sugar in the fruit of *Cassia fistula* L.

In this research, the preliminary phytochemical test revealed that alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, α- amino acid, starch, reducing sugar, saponin, tannin, carbohydrate and protein are present in fruit pulp of *Cassia fistula* L. The result of this study indicated that the leaves of this plant contain some major bioactive compounds needed for organisms. So, this plant proved to be very active.

In the antimicrobial activity, the fruit pulp of *Cassia fistula* L. was extracted with different solvents. The extracts were used to carry out antimicrobial screening on *Aspergillus flavous, Bacillus subtilis, Candida albican, Escherichia coli, Pseudomonas fluorescens and Xanthomonas oryzae*. The result showed that the highest activity (zone of inhibition in diameter is about 18 mm) was demonstrated by the ethanol extract against *Escherichia coli* and water extract against *Aspergillus flavous* and *Candida albican.* According to result, watery extract of fruit of *Cassia fistula* L. is effective in treatment bronchitis disease caused by *Aspergillus flavous* and skin infection, vaginal candidiasis, cardiac infection, sinus irritation, sores and ringworm caused by *Candida albican* may also be affected. Whereas diarrhea, dysentery, abscess, septic wounds, bed sores caused by *Escherichia coli* can be protected by ethanol extract of fruit. Therefore, it is recommended that the different components detected in fruit of this plant should be isolated and tested against the susceptible microorganism in order to arrive at the most potent structure. Further in-depth research has to be carried out to use the phytochemicals in pharmaceutical industry as a substitute for medicine.

Acknowledgements

I would like to express to my gratitute to Professor Dr. Aye Pe, Head of Botany Department, University of Yangon, for providing all departmental facilities and valuable suggestions. My special thanks are Professor Dr. Myint Aung, Dr. Baydar and Dr. Thandar Aye, Department of Botany, University of Yangon for their encouragement toward the successful completion of this research.

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EVALUATION OF ADULTERATION IN FOUR SEASONING POWDER

Wai Wai Thein[1], Ko Tin[2], Pan Myat Nge[3]

Abstract

Adulteration is the act of making something impure or altering its original form by adding materials or elements that aren't usually part of it, especially inferior ones. It was aimed to find the adulterants in four seasoning powder such as turmeric, galangal, chili and black pepper. This study was carried out from January to August, 2020. Both raw materials and powders of turmeric, galangal, chili and black pepper were bought from the market and herbal shop. The macroscopic, organoleptic and microscopic characters were observed. As a result of macroscopic characters**,** some bulb turmeric rhizomes bought from herbal shop are found to be similar to rhizomes of *Curcuma zedoaria* Roscoe**.** In an organoleptic inspection, taste of homemade and readymade turmeric powder has slightly bitter because of mixing with *Curcuma zedoaria* Roscoe. Yellow food coloring matter, metanil yellow in readymade turmeric powder, can be found when it was tested with both concentrated Hydrochloric acid (HCL) and water. Adulteration in readymade chili powder changed the color of water into red due to the presence of food coloring matter. In the iodide test**,** yellowish brown precipitates in aqueous extract of homemade chili. Adulteration in readymade galangal powder showed cloudy water because of mixing with other condiment containing starch. According to the powder microscopic analysis, the starch grains were found abundantly in three seasoning powder, except chili powder. Altered starch grains and unaltered starch grains were found in both homemade and readymade turmeric powder, but the colour of altered starch grains was deep yellow in readymade turmeric powder. Starch grains were oval in shape that found in readymade chili powder. Readymade galangal powder can be mixed with the powder of *Alpinia galangal*. As the powdered spices available in the market are often contaminated with the artificial colors and other condiments containing starch, consumers should select right products. Furthermore, it is necessary to maintain its quality and purity for the commercial market.

Keywords: Adulteration, seasoning powder, organoleptic, condiments, commercial market

Introduction

Spices are the most important aspect of Indian cuisine and our food relies heavily on them for a flavor, aroma and appearance. However, due to their high demand, manufacturers may add certain adulterants that can lower overall quality of the product (Gaurang, 2019). Dried seasonings are mostly made through drying the root, stem, foliage, fruit and other parts of the plants, such as pepper, chili, anise, fennel, mustard, cinnamon, ginger slice, ginger and *Amomum tsaoko* fruit, which have a special spicy or pungent taste. Most of the powdered seasonings from plant origins belong to this category, such as chili powder and pepper powder. They are made through grinding the plant tissues and are widely applied in the food industry of China, especially the traditional food (Zhichun, *et. al*., 2011 and Ying, 2010). The commonly used adulterations have been found to be wheat bran, corn flour, rice bran, rosin powder, or traditional Chinese medicine residue. The reported identification techniques of the potential adulterants are sensory recognition, microscopic recognition (Xu & Li, 2004), and iodide test for starchy material identification (Li, 2008).

As *Curcuma longa* L. belonging to the family Zingiberaceae called Nanwin in Myanmar name or turmeric in English name is a herbaceous root commonly used for food seasoning as well as for medicinal purposes (Tayyem, *et. al*., 2006; Li, *et. al*., 2011). Turmeric is cultivated in India, China, Java, and other tropical countries. The rhizome has long been employed both as a spice and as a colouring agent. Studies have reported by the mixing of *Curcuma zedoaria* Roscoe**,** a wild relative of turmeric, into turmeric powder due to its close resemblance with turmeric (Sasikumar, *et. al*., 2004; Sen, *et. al*., 1974). *Curcuma zedoaria* cultivated in India has circular slices of rhizome

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resembling bulb turmeric. Turmeric rhizomes are short knob-like branched, longitudinally wrinkled or marked with large circular scars (Wallis, 1967); and a characteristic aromatic odour and taste (Wallis, 1967; Kraemer, 1907). Stone cells were not present; tracheae, altered starch grains and unaltered starch grains present (Kraemer, 1907).

Alpinia officinarum Hance belonging to family Zingiberaceae is called Lesser Galangal in English and Padegaw-gale in Myanmar name. Galangal rhizome is a native of and cultivated on the island of Hainan and the neighbouring south-east coast of China. It is not much used in England, but is still employed in some countries both as a medicine and as a spice. Galangal rhizomes may be distinguished into branched, frilled remains of cataphyllary leaves, longitudinally striated or shrivelled, hard and tough. Galangal rhizome has spicy odour and a strongly pungent spicy taste (Wallis, 1967), and aromatic and pungent taste (Kraemer, 1907). *Alpinia galanga* consisted of plenty of starch grains which were simple starch grains, oval in shape; and fibres (Silvy Mathew, *et. al*., 2013).

Piper nigrum L. belonging to the family Piperaceae named Black pepper in English or Ngayok-kaung in Myanmar has a certain hot taste that can be associated with it. Peppercorns and the ground pepper derived from them may be described simply as pepper, or more precisely as black pepper (cooked and dried unripe fruit), green pepper (dried unripe fruit), or white pepper (ripe fruit seeds) (*Harrison, 2016).* Black pepper is native to present-day South India (Harrison, 2016). It is usually dried and used as a spice and seasoning (Hajeski & Nancy, 2016). Odour of blackpepper has aromatic, slightly empyreumatic; taste aromatic and pungent (Kraemer, 1907). Papaya seeds are a very common adulterant present in a packet of black peppercorns (Gaurang, 2019). Stone cells of the endocarp, pericarp pigment with yellowish-brown and reddish-brown tannin masses; and parenchyma cells containing numerous starch grains **(**Kraemer, 1907).

Red pepper or Chili peppers (*Capsicum annuum* L. var. *longum*) consists of dried ripe fruits known commercially as chilies that are the third important crop of the family *Solanaceae* after tomato and potato (Naz, *et. al*., 2006). Chili peppers originated in Mexico. They are widely used in many cuisines as a spice to add heat to dishes (Kraft, *et. al*., 2013). India is the largest producer of chilies in the world followed by China. Other important chili producing countries include Pakistan, Ethiopia, Myanmar, Mexico, Vietnam, Peru, Ghana and Bangladesh. Pakistan contributes around 6% to the world's total chili production (Khan, *et. al*., 2012). *Capsicum* fruits have a characteristic but not powerful odour, and as extremely fiery, pungent taste (Wallis, 1967)**,** and odour distinct and pungent taste (Kraemer, 1907). Aleurone grains are found abundantly in the endosperm of the *Capsicum* seed treated with iodine solution that is stained yellowish brown (Shah & Seth, 2010). Starch grains somewhat spherical and 1.5 per cent of starch present; stone cells present (Kraemer, 1907). Each epidermis is developed as a secretion epithelium having thin-walled polygonal-tabular cells; inner epidermis, the endocarp consisting of cells with slightly wavy walls. The epidermis cuticle is raised in large bladdery patches. The seeds contain traces of starch (Wallis, 1967). Seed shell epidermis with mesenteric cells, cell wall very undulated, side wall markedly thickened and stratified, slightly greenish; inner epidermis of the fruit with marked pitted rosulate cell; oily droplets (Nidal and Samah Al-Jabi, 2005).

Powder analysis plays a significant role in identification of crude drug. These characters will help in the identification of right variety and search for adulterants. Powder microscopy is one of the simplest and cheapest methods for establishing the correct identity of the source materials (Sandhya, 2015). As the current detection methods used to identify the adulterated powdered seasonings are deficient, organoleptic inspection is the most common method, or sometimes supplemented by physical and chemical inspection (Zhichun, *et. al*., 2011 and Ying, 2010). This study aims to find the adulterants in four seasoning powder such as turmeric, galangal, chili and black pepper and to observe their macroscopic, organoleptic and microscopic characters.

Materials and Methods

Both raw materials and powders of turmeric, galangal, chilli and black pepper were bought from the market and herbal shop. Raw materials were washed, dried and grinded into a fine powder. Each seasoning powders were taken and put it on the microscope slides to observe and photograph the microscopic characters of the constituents of the seasoning powders and identify the adulterating substances. To find whether the adulterants present in seasoning powders, it was chemically and naturally tested by following ways according to literature of Gaurang (2019) and Marini-Bettalo, (1981).

Test for Checking Adulteration in Turmeric Powder

(1) Add a spoonful of turmeric powder in a glass of water. Natural turmeric powder leaves a certain light yellow color after settling down, whereas, an adulterated turmeric powder leaves a strong yellow color in the water while settling down.

(2) Take one teaspoon of turmeric powder in a test tube. Then add a few drops of concentrated Hydrochloric acid (HCL) in it. If pink, violet or purple color appears instantly but then disappears after adding some water turmeric does not have the artificial color. But if the color remains, it has artificial color –metanil yellow.

Test for Checking Adulteration in Chilli Powder

Sprinkle chilli powder on the surface of water taken in a glass of water. The artificial colourants will immediately start descending in colour streaks.

Test for Checking Adulteration in Black pepper Powder

Add some amount of black pepper to a glass of water, pure black pepper settles at the bottom. In the adulterated black pepper, papaya seeds float on the surface of water.

Test for Checking Adulteration in Galangal Powder

Add a teaspoon of galangal to the glass of water. Do not stir it and leave it still for a while. Check after about 20 minutes or so. If powder settles down at the bottom of glass with clear water above, the galangal is pure. The cloudy water indicates possible adulteration.

Test for starch

Powdered sample (2 g) was boiled with distilled water for about 20 minutes and then filtered. The filtrate was treated with two drops of iodine solution. Bluish-black precipitate was formed which indicated the presence of starch (Marini-Bettalo, 1981).

Results

Macroscopic characters of Turmeric

Primary rhizomes were vertically growing condensed swollen, shorter and thicker; short pieces known as bulb or round turmeric, ovate-oblong, conical to pear-shaped, 3 to 7 cm long, 2 to 3 cm wide; secondary lateral branches arising from the primary rhizomes known as finger or long turmeric were cylindrical curved or nearly straight pieces, bluntly tapering at eachend, occasionally short knob-like branched, 4 to 10 cm in length and 1 to 1.5 cm in diameter, longitudinally wrinkled or marked with large circular scars; both the rhizomes were hard and heavy, with short fracture; outer surface being a deep yellowish-brown color, marked with transverse rings (leaf-scars); internally having a uniform dull brownish-yellow, tough horny and waxy in appearance.

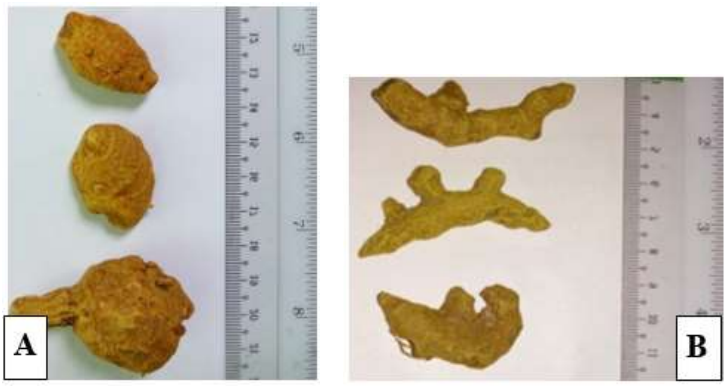

Figure 1 Turmeric rhizome (A) Bulb turmeric (B) Long turmeric

Macroscopic characters of Galangal

A branched rhizome was about 12 mm thick, in pieces about 5 to 10 cm long, frequently cylindrical; tapering or enlarged, and often branched, hard and tough; longitudinally striated or shrivelled, at interval of about 5mm pale, encircling, sinuous or frilled remains of cataphyllary leaves; reddish-brown color internally.

Figure 2 Galangal rhizome

Macroscopic characters of Chilies

The fruits of *Capsicum annuum* L. were a narrowly ovoid pod, about 7 to 12 cm long and 5 to 12 mm wide; inferior calyx and pedicel remain attached, about 20 to 40 mm long; calyx about 5 mm long, cup-shaped; pericarp glabrous, shrunken, thin, leathery and red in colour; seeds 5 to 20, disc-shaped, about 3 to 4 mm long and 2.5 to 3 mm wide.

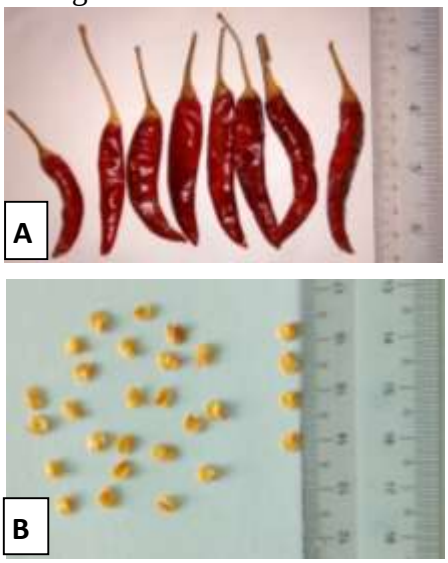

Figure 3 Fruits of Chilies (A) Fruits (B) Seeds

Macroscopic characters of Black pepper

The fruits of black pepper were dark red, 4-6 mm in diameter and contained a single seed, like all drupes; globose or ovoid; epicarp very thin; externally brownish to black with wrinkled surface, sarcocarp and endocarp dark brown.

Figure 4 Fruits of Black pepper

Galangal powder

Black pepper powder

Figure 5 Brand of readymade seasoning powder sold in the market

Table 1 Organoleptic characters of seasoning powder

Organoleptic characters	Seasoning Powder							
	Turmeric		Galangal		Chilies		Black pepper	
	Home made	Ready made	Home made	Ready made	Home made	Ready made	Home made	Ready made
Color	Deep orange-yellow	yellow	light brown	light brown	orange-red	red	grayish-black	grayish-black
Odor	aromatic	aromatic and pungent	spicy	aromatic	very pungent	very pungent	aromatic	aromatic
Flavor	Slightly bitter	Slightly bitter	Strongly pungent and spicy	sweet and pungent	very pungent	very pungent	very pungent	very pungent
Texture	Slightly coarse	fine	coarse	fine	coarse	coarse	coarse	coarse

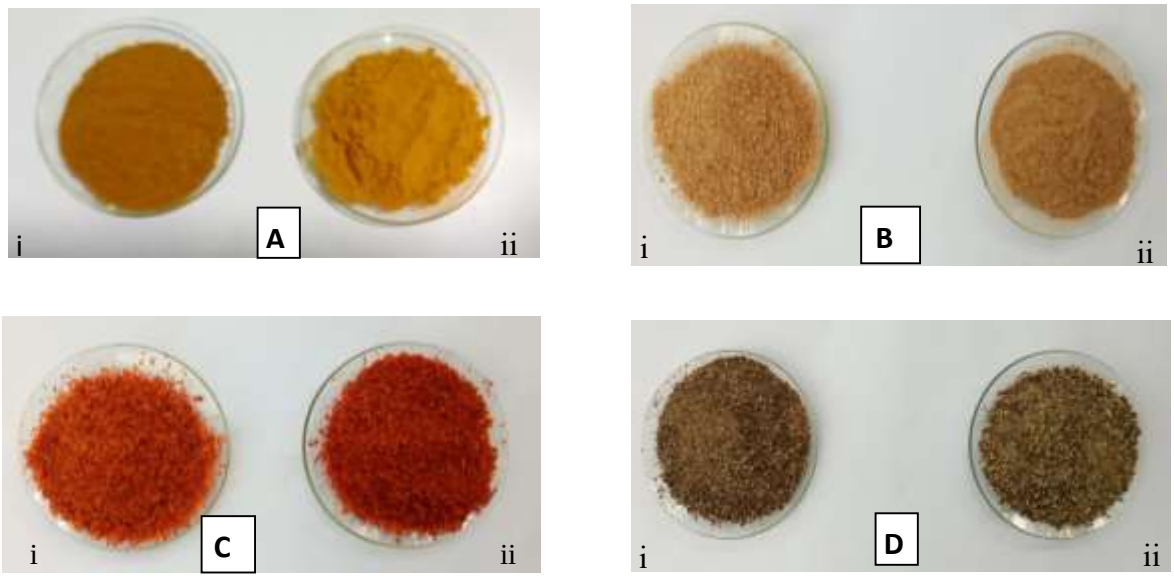

Figure 6 Comparative macroscopic characters of seasoning powder between (i) homemade and (ii) readymade, (A)Turmeric (B) Galangal (C) Chillie (D)Black pepper

Table 2 Test for checking adulteration in seasoning powder

Test Solution	Seasoning Powder							
	Turmeric		Galangal		Chillies		Black pepper	
		Ready made	Home made	Ready made	Home made	Readymade	Home made	Readymade
Water	light yellow	deep yellow	white	light brown	orange	red	brown	brown
Iodine	bluish – black ppt	bluish – black ppt	bluish – black ppt	bluish – black ppt	yellowish brown ppt	bluish –black ppt	bluish –black ppt	bluish –black ppt

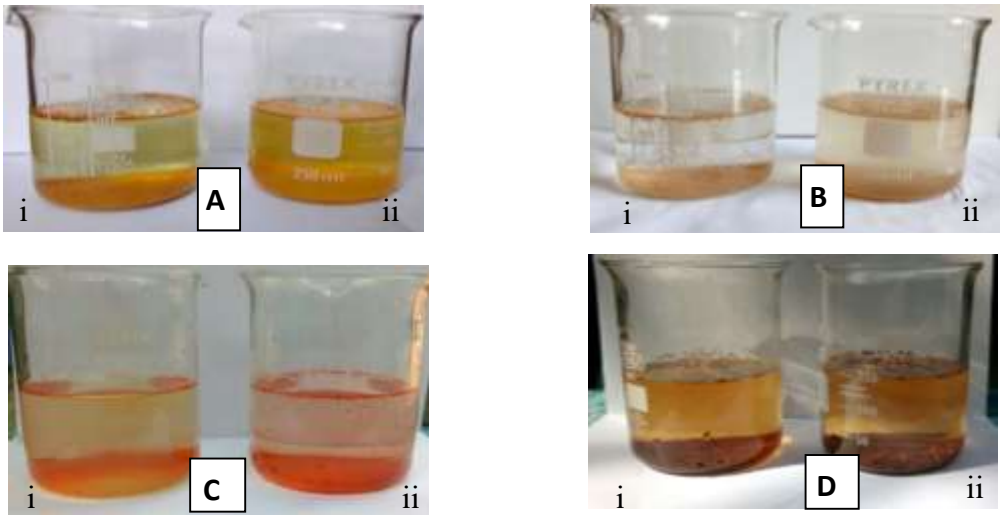

Figure 7 Comparative seasoning powder between (i) homemade and (ii) ready made to check adulteration using water (A) Turmeric (B) Galangal (C) Chilies (D) Black pepper

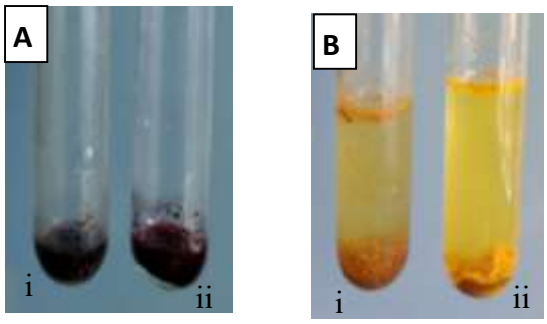

Figure 8 Comparative seasoning Turmeric powder between (i) homemade and (ii) readymade to check adulteration using (A) concentrated Hydrochloric acid (B) water

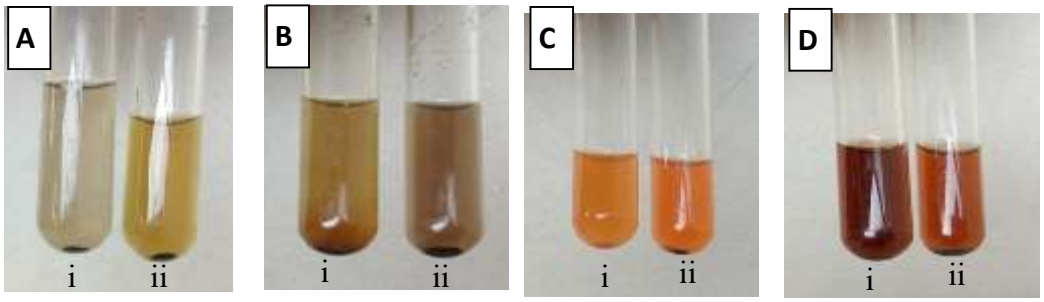

Figure 9 Comparative seasoning powder between (i) homemade and (ii) readymade to check adulteration using iodine solution (A) Turmeric (B) Galangal (C) Chilies (D) Black pepper

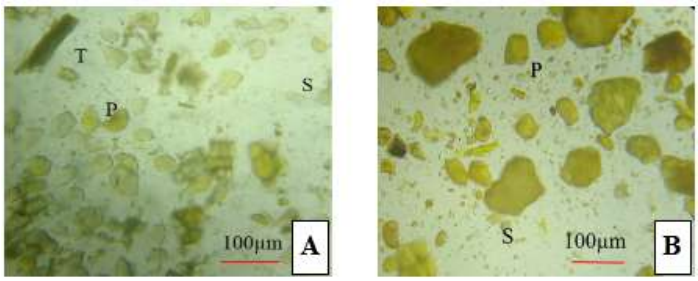

Figure 10 Microscopic characters of Tumeric powder
(A)Homemade; T, tracheae; P, fragments of paranchyma containing swollen and altered starch grains; S, unaltered starch grains
(B)Readymade; P, fragments of paranchyma containing swollen and altered starch grains; S, unaltered starchgrains

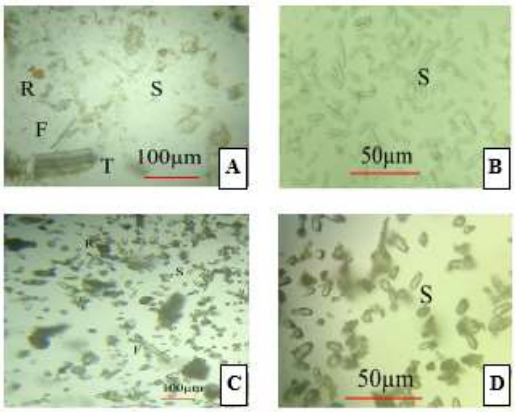

Figure 11 Microscopic characters of Galangal powder
(A) & (B) Homemade; R, cell with reddish brown pigment; F, fiber; T, tracheae; S, unaltered starch grains (C) & (D) Readymade; R, cell with reddish brown pigment; F, fiber; T, tracheae; S, unaltered starch grains

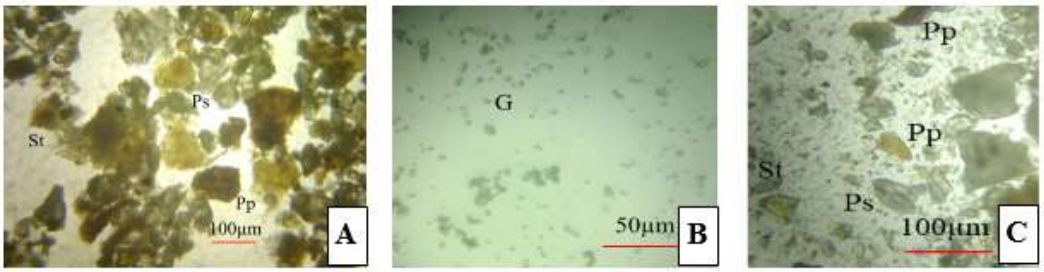

Figure 12 Microscopic characters of Black pepper powder

(A) Homemade; Ps, parenchyma cells of perisperm containing starch grains; Pp, pericarp pigment with yellowish-brown and reddish-brown tannin masses; St, stone cells of the epicarp

(B) G, starch grains

(C) Readymade;Ps, parenchyma cells of perisperm containing starch grains;Pp, pericarp pigment with yellowish-brown and reddish-brown tannin masses; St, stone cells of the endocarp

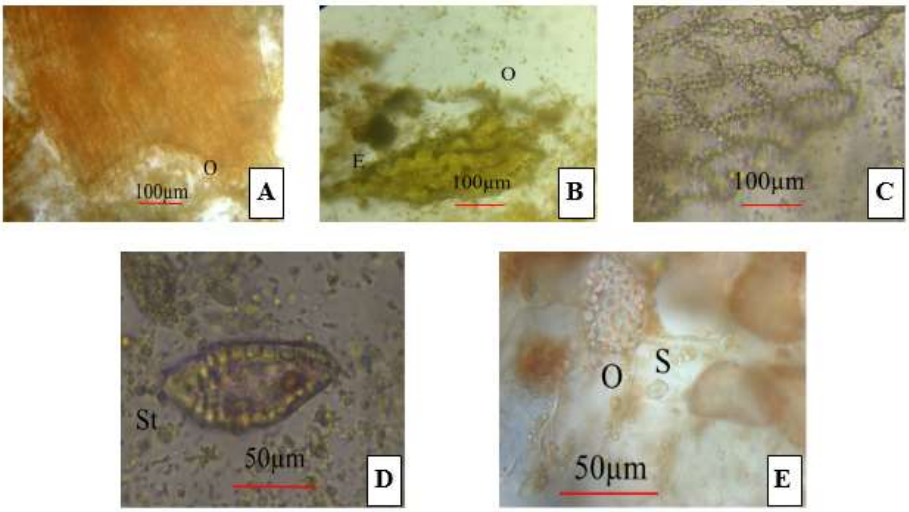

Figure 13 Microscopic characters of Chilli powder

(A) secretion epithelium having thin-walled polygonal-tabular cells; O, red oil globule

(B) E, large wavy yellow cells of the testa epidermis; O, red oil globules

(C) inner epidermis of pericarp with marked pitted rosulate cell

(D) St, stone cell

(E) Readymade; S, starch grains; O, red oil globules

Discussions and Conclusion

Adulteration is dangerous as it fails the quality of food, making it sub-standard for human consumption. The adulteration of seasonings is a long-standing problem. Pharmacognostic evaluation helps to screen the commercial varieties, substitutes, adulterants, and quality of the drugs. As a result of macroscopic characters, some bulb turmeric rhizomes bought from herbal shop are found to be similar to rhizomes of *Curcuma zedoaria* Roscoe**.**

Zhichun, *et. al*., (2011) and Ying (2010) stated that an organoleptic inspection was the most common method. As a result of organoleptic inspection, taste of homemade and readymade turmeric powder has slightly bitter because of mixing with *Curcuma zedoaria* Roscoe that are not agreed with Wallis (1967) and Kraemer (1907), but are agreed with Sasikumar, *et. al*., (2004) and Sen, *et. al*., (1974). Colour of readymade turmeric and chili powder is different from homemade powder due to the presence of the food colouring matter. Depending on the grinding machine,

texture of turmeric and galangal powder may be different. Odour and flavor of homemade and readymade galangal, chilies and black pepper powder are agreed with Wallis (1967) and Kraemer (1907). Thus, sensory recognition is able to distinguish the adulterations according to their color, smell, and taste, but this method depends on experience and is hard to quantify.

The readymade turmeric powder treated with concentrated Hydrochloric acid (HCL), and then after adding some water, colour remains. Thus, yellow food coloring matter, metanil yellow in readymade turmeric powder can be present.

The iodide test is only effective in identifying starchy adulterations. As a result, yellowish brown precipitates in aqueous extract of homemade chili indicated that was agreed with Shah and Seth (2010). Bluish–black precipitates in aqueous extract of readymade chili indicated that content of starch was slightly high. Thus, this experiment was agreed with Xu & Li (2004) that a mixture of other condiments contained starch in chili powder. Thus, physical and chemical inspection methods are more sensitive, but a plenty of time and chemical reagent will cost.

The generally applicable method which can be used to identify the adulterated seasoning powder quickly and intuitively is the process of separating them by using water. As a result, the adulteration in readymade turmeric powder was yellow food coloring matter when it was tested with the water as well as chili powder adulterated with food coloring matter changed the color of water into red. Adulteration in readymade galangal powder showed cloudy water due to the presence of other condiment containing starch. Above the statement of findings were agreed with literature of Gaurang (2019).

As a result of microscopic examination of turmeric powder, tracheae, altered starch grains and unaltered starch grains were found in both homemade and readymade turmeric powder, but the colour of altered starch grains was deep yellow in readymade turmeric powder. This finding was agreed with Kraemer (1907).

As a result of powder microscopy of galangal powder, it was found unaltered starch grains, tracheae, cell with reddish brown pigment; sclerenchymatous fiber or bast fiber in both homemade and readymade galangal powder. The shape of starch grains in readymade galangal powder was not only rod-shaped but also round- shaped and oval shaped that were agreed with Silvy Mathew, *et. al*., (2013). Thus, readymade galangal powder can be mixed with the powder of *Alpinia galangal*.

According to the microscopic characters of black pepper powder, stone cells of the epicarp and endocarp, parenchyma cells of perisperm containing starch grains**;** pericarp pigment with yellowish-brown and reddish-brown tannin masses were found that were agreed with the statement of Kraemer (1907).

As it was a combination of different powdered spices, chili powder from different manufacturers can have different tastes, which makes it quite tough to find adulterants present in it. In this study, secretion epithelium having thin-walled polygonal-tabular cells red oil globule; large wavy yellow cells of the testa epidermis; inner epidermis of pericarp with marked pitted rosulate cell; stone cell were found under the microscope that was agreed with Wallis (1967) and Nidal & Samah Al-Jabi (2005). Starch grains were oval in shape that found in readymade chili powder that was not agreed with Kraemer (1907).

According to Sandhya (2015), powder analysis plays a significant role in identification of crude drug. These characters will help in the identification of right variety and search for adulterants. Moreover, powder microscopy is one of the simplest and cheapest methods for establishing the correct identity of the source materials.

As a conclusion, before using of any seasoning powder, detection of adulteration requires. As the adulterant was indicated in the seasoning powders, it would determine that they are unsafe to consume. As the powdered spices available in the market are often contaminated with the artificial colors and other condiments containing starch, consumers should select right products. Furthermore, it is necessary to maintain its quality and purity for the commercial market.

Acknowledgements

I would like to thank Dr. Aye Pe, Professor and Head,Department of Botany, University of Yangon, for his permission. I also wish to express my deepest gratitude to Dr. Myint Aung (Professor), Dr. Baydar (Professor), Dr. Thandar Aye (Professor), Department of Botany, University of Yangon, for their encouragement and guidance in this research.

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ISOLATION OF ENDOPHYTIC BACTERIA FROM *GARCINIA MANGOSTANA* L. AND STUDY ON ITS ANTIBACTERIAL ACTIVITY

Yee Yee Nwe[1], Khin Myo Thwe[2], Thet Phoo Wai[3], Myat Myat Moe[4]

Abstract

Endophytes are ubiquitous organisms that live within the host plants without causing any apparent symptoms of disease. In the present study the endophytic microorganisms were isolated from plants *Garcinia mangostana* L. and 12 endophytic bacteria were successfully screened from different parts of leaves, bark and fruit. Characterization of endophytic bacteria was performed based on the morphology, biochemical characteristic and the antimicrobial properties. Screening of potential antibacterial metabolites was done by using paper disc diffusion method and bioautography. The antibacterial activity was tested against eight test organisms. Cell morphology and colony characters of isolated bacteria were investigated and three bacteria which show antimicrobial activity (B-2, B-7 and B-9) were selected and applied in the fermentation was media using nutrient broth for two days. After separate fermentation, the crude compounds were extracted by using ethyl acetate and butanol and partially purified by TLC. The results of TLC showed the presence of Quercetine, Tannin and Xanthone in each extract of B-2, B-7 and B-9.

Keywords- Endophytic bacteria, antimicrobial activity, biochemical characterization of endophytes.

Introduction

Garcinia belongs to the family Clusiaceae, which is native to Asia, Australia, Southern Africa, and Polynesia, and that produces edible fruits. This plant is known as "queen of the tropical fruit" in Malaysia and its scientific name is *Garcinia mangostana* L. (Mangosteen), which is famous for its sweet, creamy, and fragrant edible flesh (Phongpaichit *et al*., 2006). Different parts of *Garciniamangostana* L., such as the hull, bark, and leaf, have been used as traditional medicine to treat various diseases for hundreds of years. Applications of the mangosteen include leaves infusion to the circumcision wound to prevent infection and root decoction for the regulation of female menstruation (Moongkarndi *et al*., 2004; Nakatani *et al*., 2002). Studies based on *Garcinia* species has been greatly reviewed its biological potential activity, but the analysis based on the endophytic fungi that coexist within the fruit tree is very limited (Phongpaichit *et al*., 2007; Phongpaichit *et al*., 2006).

Endophyte simply means the location of an organism, with "endo" means "inside" and "phyte" means "plants". Therefore, endophyte refers to organisms that live within plants (Wilson, 1995). Bacteria are the most common organisms associated with the term endophyte. Endophytic bacteria are defined as bacteria that colonize healthy plant tissue without causing obvious symptoms or producing obvious injury to the host. Endophytic bacteria colonize a large number of plants, which include plant growth-promoting bacteria. Endophytic bacteria form associations with plants, at least in one phase in their life cycle. Endophytic bacteria normally live on intercellular spaces that contain carbohydrates, amino acids, and high amounts of inorganic nutrient.

The presence of fungal endophytes can cause higher rates of water loss in leaves. However, certain microbial endophytes may also help plants to tolerate biotic stress. The wide range of compounds produced by endophytes have been shown to combat pathogens and even cancers in animals including humans. Endophytes are also being investigated for roles in

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biofuels production. Some groups of endophytic microorganisms have been believed to be mutualists that protect plants against biotic stresses. Co-evolution may exist between endophytes and their host in resist to environmental stresses. (Tadych & White, 2001). Endophytic bacteria produce a wide range of phytohormones.The bioactive natural products from endophytes are promising resources for medicine kinds of Alkaloids are contributed to plant by endophytes. Some of these alkaloids raise plants‟ resistance to environmental stress, and some are growth-promoting compounds., agriculture and industry (Guo *et al*., 2008).

Many of those metabolites act as potential therapeutic agents against cancer and infectious diseases (Al *et al*., 2010). Endophytes are well known for the production of various classes of natural products and have been reported to exhibit a broad range of biological activity and are grouped into various categories, which include alkaloids, terpenoids, steroids, lactones, phenolic compounds, quinones and lignans. (Tan and Zou 2001 : Strobel and Daisy. 2003).Endophytes can be a promising source of bioactive compounds, and should be continuously isolated, characterized, and investigated for the discovery of lead bioactive compounds which can be employed in agriculture, medicine, and industries (Tiwari, *et, al,* 2013).

In this investigation, altogether twelve bacteria were isolated into pure culture by using nutrient agar medium. Twelve isolated strains are subjected into the physiochemical examinations concerning enzyme and other bioactive substances. This study was initiated to characterize the endophytes, in different parts of *Garcinia mangostana* L. This study was aimed to evaluate several isolated bacteria from *Garcinia mangostana* L. and to select best endophytic bacteria for further extraction partial identification of its metabolites.

Materials and Methods

Collection of Plant Materials

In the present research, the healthy leaf, bark and fruit of *Garcinia mangostana* L. Samples were collected from Mawlamyin Township.Samples were collected into clear plastic bags and brought to Botanical Lab of Dagon University .The identification of plant samples were done with the help of available literature as well as revised Handbook written by Wadhwa and Weerasooriya (1996) .The screening and isolation of endophytes have been performed in the Lab of Microbiology, Dagon University since July, 2006.

Pre-treatment and Surface Sterilization of Plant Parts

The leaf, bark and fruit of each plant *Garcinia mangostana* L. were washed separately under tap water to remove adhering soil particles and the majority of microbial surface epiphytes are a part of pre-treatment.

Surface sterilization

Freshly collected leaf, bark and fruit of *Garcinia mangostana* L. were washed under slow running tap water for 15 minutes followed by washing in Tween 20 (1 drop in 200 ml sterile distilled water) for 1 minute .Then they were rinsed three times with sterile distilled water in the chamber. Commonly used sterilizing agents are ethanol: 70-95% for 30 seconds. After drying, each sample segment was cut into approximately 0.5 cm and placed on Petri plates containing nutrient agar medium (NA). Petri plates were incubated at room temperature. They were monitored every day for growth of endophytic bacteria colonies. Bacterial growing out from the samples was subsequently transferred onto fresh NA media plates to isolate pure colonies. All selected isolates were subculture in NA slants and finally, all the purified endophytes were maintained at 4°C till further used.

Media for Isolating Endophytic Bacteria

The choice of the growth medium is crucial as it directly affects the number and type of endophytic bacteria that can be isolated from the leaves, bark and fruit. Nutrient Agar medium (NA) was used for the isolation of endophytic bacteria. Since there is no component in NA which can suppress the growth of endophytic fungi, so the media used for the isolation of endophytic bacteria were supplemented with antifungal agent, nystatin at a concentration of 100 μg/ml of each to suppress fungi growth.

Nutrient Agar Medium (Atlas, 1993)

Peptone	-	5.0 g
Beef extract	-	1.0 g
Yeast extract	-	2.0 g
Sodium chloride	-	5.0 g
Agar	-	20.0 g
Distilled water (DW)	-	1000 ml
pH	-	7.0

Nystatin was added to the medium after autoclaving

Biochemical Characteristic of Isolated Endophytic Bacteria

The biochemical tests of isolates were conducted according to Bergey's Manual of Determinative Bacteriology. For each strains , test include Gram Staining, Oxygen Requirement (Aerobic/Anaerobic), Hydrogen Sulfide Production Test, Salt Tolerant, Nitrate Reduction, Citrate Utilization Test, Methyl Red Test, Voges Prokaur Reaction, Urea Hydrolysis, Starch Hydrolysis and Utilization of Carbohydrate .(Prescott, 2002).

Test Organisms

All endophytic bacteria isolates were screened for antimicrobial activities. The test bacteria included *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans*, *Escherichia coli, Micrococcus* sp., *Pseudomonas aeruginosa, Saccharomyces cerevisiae* and *Staphylococcus aureus*.

Antimicrobial activity Estimation

The study of antimicrobial activity was performed by paper-disc diffusion method. Nutrient agar was prepared according to the method described by Cruickshank. 1975.

Extraction and Isolation of Crude Ethyl Acetate Extracts and Butanol Extract From Bacterial Fermentation Broths

Organic solvents used extraction and isolation of compounds in two days fermentation broths using three isolated bacteria. At the twelve isolated bacteria,B-2,B-7 and B-9 were selected for further investigation of antimicrobial activity based on the results of paper disc diffusion methods .They were subjected in the fermentation using nutrient broth as basal fermentation media , for two days at room temperature. After fermentations butanol and ethyl acetate were used in the extraction and isolation of crude compound from individual fermented broths.

Isolation Procedure of Crude Extract from Selected Bacteria

Isolation of useful metabolites in the crude extracts was conducted at the Laboratory of Chemical Engineering Department, Yangon Technological University. The screening of crude extracts using two solvents such as Butanol and ethyl acetate obtained after extraction were conducted according to the method reported by Harbone, (1973) and extraction and purification of crude extracts were performed by applying TLC plates coated with silica gel.

Results

Morphological Characteristics of Collected Plant Sample

Scientific Name - *Garcinia mangostana* L.

Myanmar Name - Mingut

Family - Guttiferae

In evergreen tree; younger stems cylindrical, glabrous, latex yellow. Leaves opposite, distichous, simple, exstipulate; petiolate; laminae elliptic-oblong, the margins entire. Inflorescences terminal cymes, bisexual flowers solitary. Flowers ebracteate, ebracteolate, peticellate, actinomorphic, tetramerous, hypogynous. Calyx aposepalous, the sepals 4, concave, decussate, yellowish persistent. Corolla apopetalous, the petals 4, imbricate, rosy pink, glabrous. Androecium polyandrous, stamens numerous, the filaments short, basifixed, dehiscence longitudinal. Pistil 1, ovary globose, 4 carpelled, syncarpous, the ovule solitary in each locule, the axile placentation, the stigma sessile, yellow.

Figure 1 Habit of the Plants, Leaves, Fruit and Bark

Table 1 Isolation of Endophytic Bacteria from *Garcinia mangostana* L

Used Basal Medium	Designated of bacteria		
	From leaves	From bark	From fruit
Nutrient Agar Medium (NA)	B-2, 4, 5, 10	B-1, 3, 6,7, 8	B-9,11,12

In the present works, twelve isolated bacteria designated as B-1 to B-12 were maintained into the pure culture for further studies.

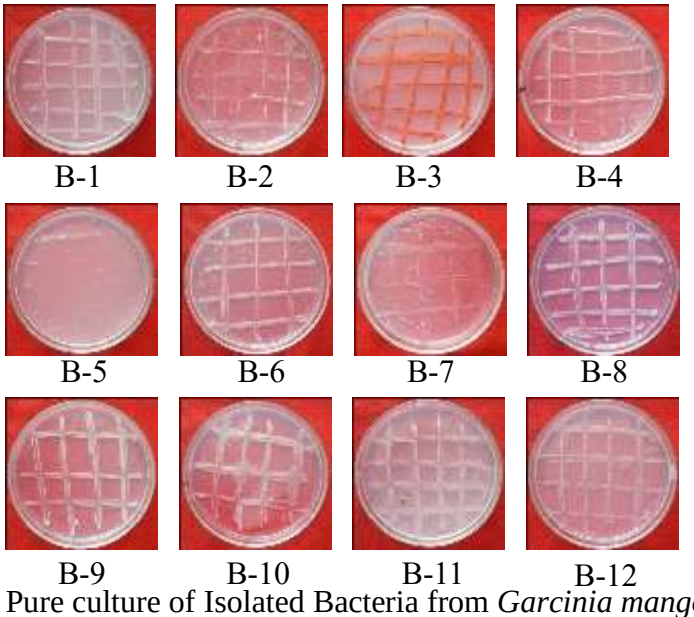
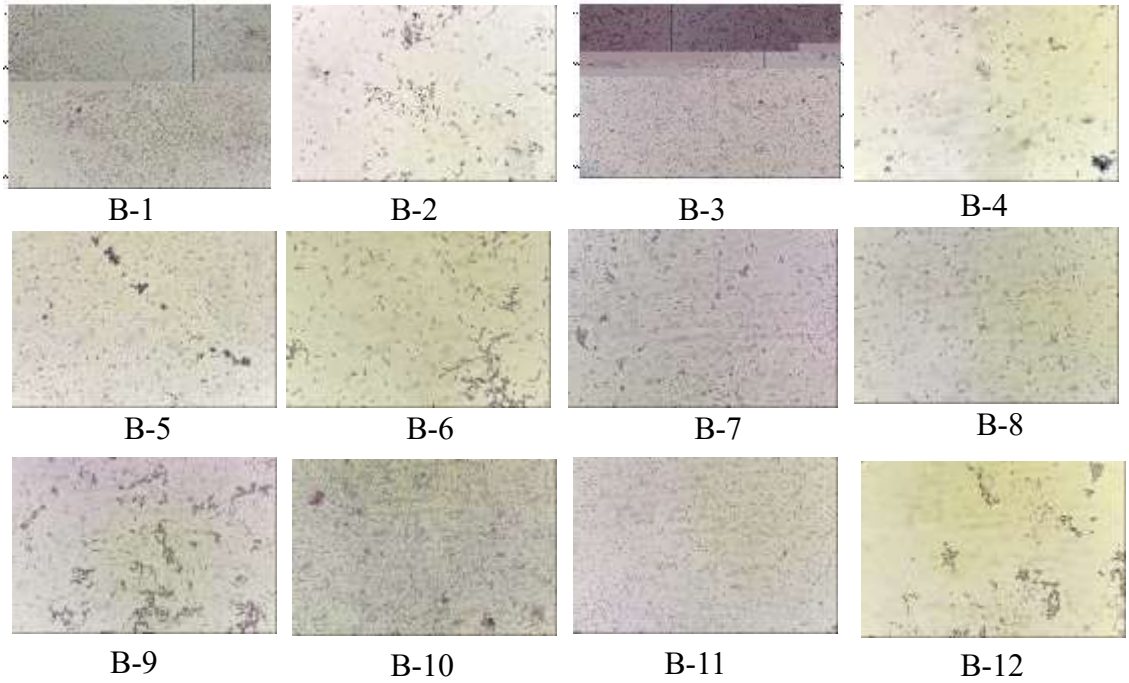


Figure 2 Pure culture of Isolated Bacteria from *Garcinia mangostana* L.



All isolated strains are small rod to rod. The scale bar of all photomicrographs is 50 µm.

Figure 3 Morphology Characters of Isolated Bacteria From *Garcinia mangostana* L.

Identification of isolates to possible Genus Level According to Bergey's Manual of Determinative Bacteriology Eighth Edition (1974)

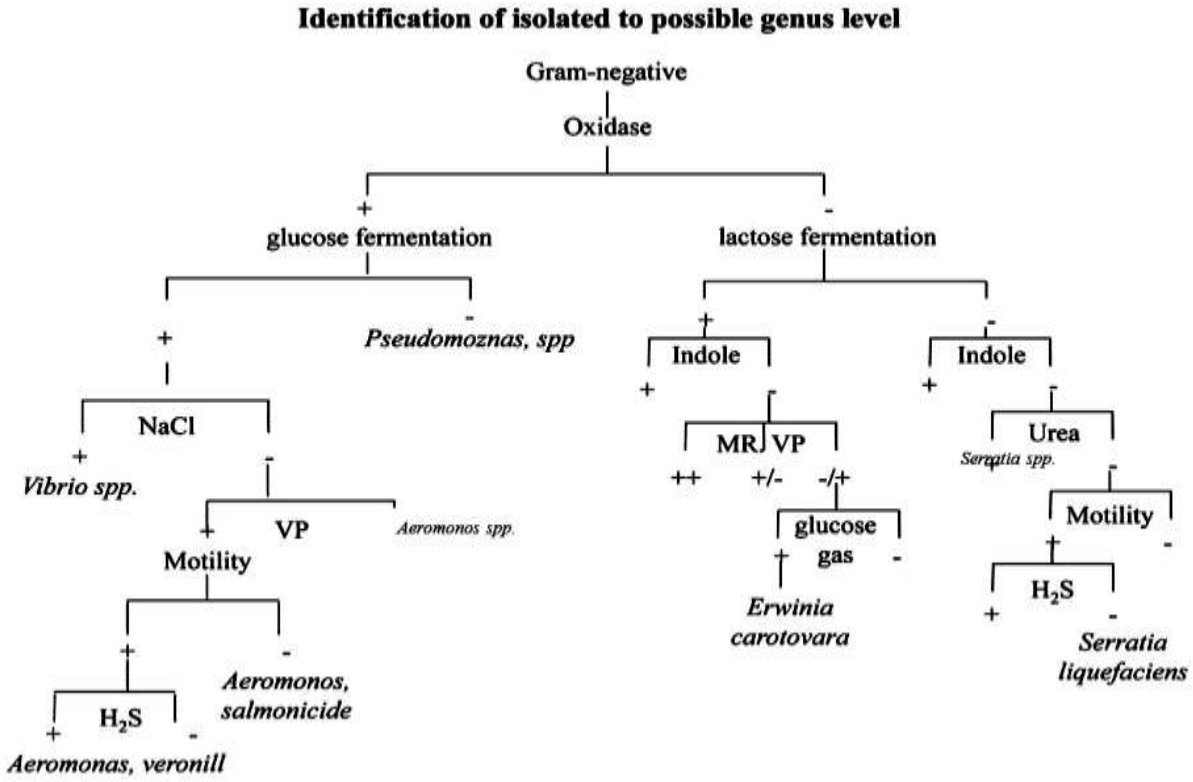


Figure 4 Flow Diagram of Identification of Isolated Bacteria to Possible Genus Level

Table 2 Results of Biochemical Tests of Isolated Bacteria from *Garcinia mangostana* L.

Test		B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
Cell Morphology		rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
Glucose	fer	-	++	++	++	++	++	+	+	++	++	+	++
	gas	-	-	+	-	+	-	-	+	-	-	-	+
Lactose	fer	-	-	-	-	-	-	-	+	-	-	+	-
	gas	-	-	-	+	+	++	-	++	-	++	++	+
Ribose	fer	-	-	++	-	++	-	-	++	++	++	-	++
	gas	-	-	+	-	+	-	-	+	+	+	-	-
Hydrogen sulphide		-	-	-	-	-	+	-	-	-	-	-	-
Aerobic / Anaerobic		A	A	A	A	A	A	A	A	A	An	A	A
Moile / Non motile		nm	m	m	m	m	m	nm	m	m	m	m	m
Soluble Starch Hydrolysis		+	+	-	-	-	+	-	+	+	-	+	-
Tapioca Hydrolysis		+	+	+	-	+	+	-	+	+	-	+	-
Sticky Rice Hydrolysis		+	+	-	-	-	+	-	+	+	-	-	-
Wheat hydrolysis		-	+	-	+	-	+	+	+	+	-	+	-
Urease Test		-	+	-	-	+	-	-	-	+	-	+	-
Citrate utilization		++	++	++	+	++	+	++	++	+	++	++	++
Methyl red (MR)		-	-	-	-	-	-	-	-	-	-	-	+
Voges-Proskaver (VP)		-	-	+	+	+	+	+	+	+	+	+	+
Nitrate Test		+	-	+	-	-	+	+	-	-	+	-	-
Gram Stain		-	-	-	-	-	-	-	-	-	-	-	-
Catalase		+	+	+	+	+	+	+	+	+	+	+	+
NaCl 6%		+	+	-	+	+	+	-	+	+	+	+	-
NaCl 1%		+	+	+	+	+	+	+	+	+	+	+	+
Oxidase Test		++	+	++	++	++	++	+	++	++	-	-	+
Indole Test		-	-	-	-	-	-	-	-	-	-	-	-
Growth on Potato		+	+	+	+	+	+	-	+	+	-	+	+

A= aerobic A n = anaerobic m= motile nm= non-motile + = positive reaction - = negative reaction

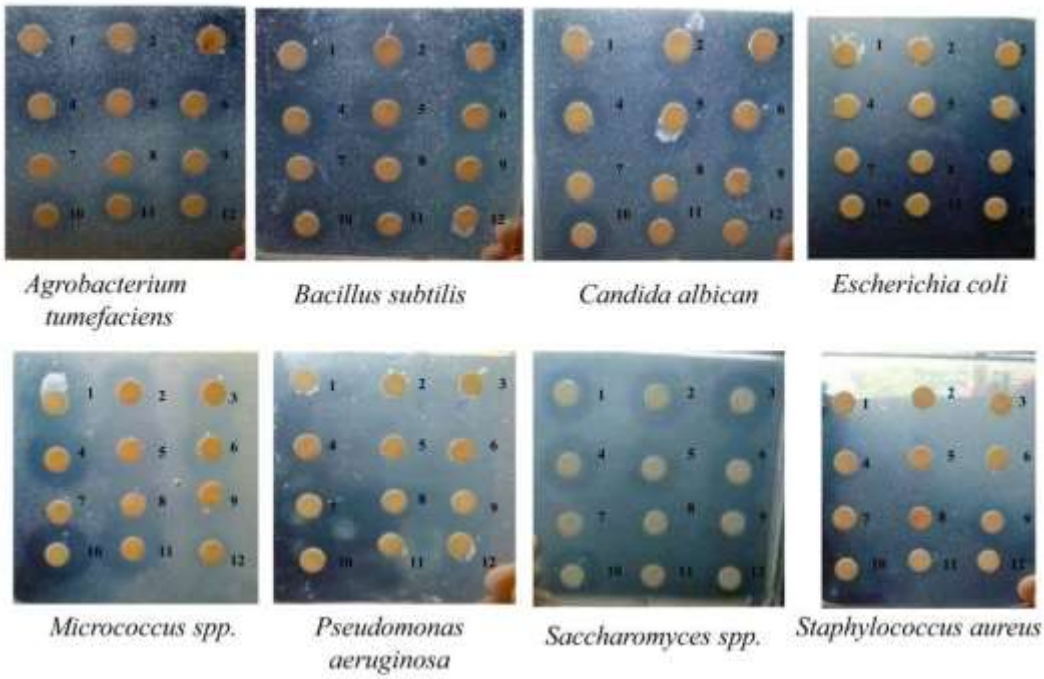


Figure 5 Antimicrobial Activity of Isolated Endophytes B-1 to 12 Against Eight Test Organisms

Screening of endophytic bacteria from different parts of *Garcinia mangostana* was done by using nutrient broth as basal isolation medium. To isolate the bacteria, the Nystatin 100 µg/ml was put into the nutrient medium for the bacteria only. Twelve isolated bacteria were obtained in the present screening. The isolated bacteria from different parts of Mangosteen were designated as B1 to 12. The colonies of isolated strains were shown in Fig. 2. The results of morphological cultural and biochemical test were shown in Table 2 and Fig. 3. One of the most important biochemical character the procedure of starch hydrolyzing activity was also experimented by using soluble starch, tapioca, wheat, sticky rice. The possible genus of isolated bacteria was estimated according to the Bergeys Manual of Determination Biology (1974). Based on the results of morphological, cultural and biochemical tests, all isolated bacteria may be possible geneus level *Pseudomonas spp,(*B- 1*)Vibrio spp,(*B- 2,4,5,6,8,9*), Aeromonas spp.* (B-3,7) , *Erwinia spp.(*B-11) and *Serratia* (10) . The antimicrobial activity of all isolates against eight test organisms was indicated by size of clear zone was shown in Table 3.

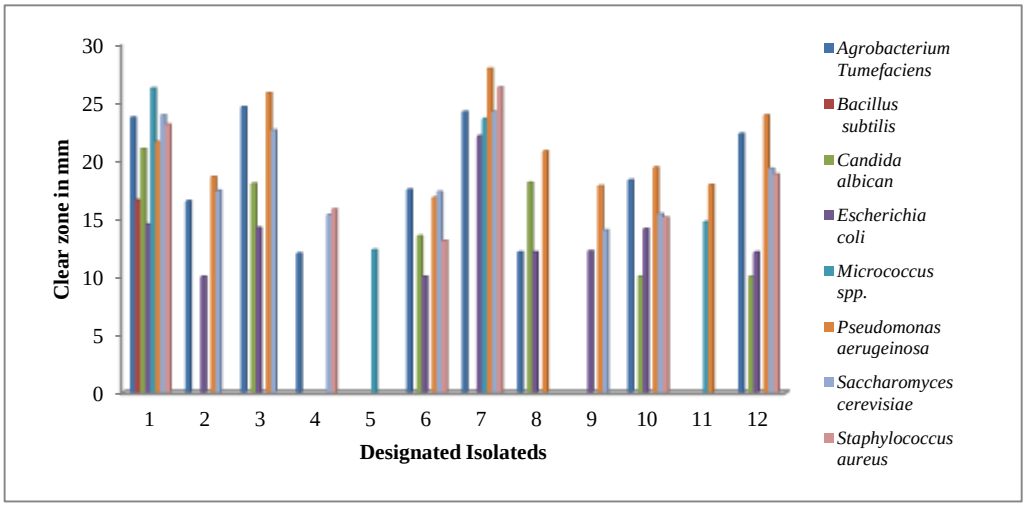


Figure 6 Antimicrobial activity of all Isolated Endophytic Bacteria Strains (B 1 to 12)

Table 3 Antimicrobial activity of All Isolated Endophytic Bacteria Strains (B-1 to 12)

Paper Disc Size = 10 mm

Strain No.	*Agro-bacterium Tumefaciens*	*Bacillus subtilis*	*Candida albican*	*Escherichia coli*	*Micrococcus spp.*	*Pseudomonas aeruginosa*	*Saccharomyces cerevisiae*	*Staphylo-coccus aureus*
B-1	12.3 mm	18.3 mm	-	18 mm	23.4 mm	18 mm	20.1 mm	-
B-2	-	19.2 mm	19.8 mm	17 mm	22.5 mm	18.2 mm	22.2 mm	-
B-3	-	19.3 mm	18.2 mm	17 mm	22.5 mm	18.1 mm	22.2 mm	-
B-4	13.5 mm	21.1 mm	18.3 mm	20 mm	22.6 mm	20 mm	21 mm	18 mm
B-5	-	22.3 mm	19.2 mm	20 mm	24.5 mm	23 mm	20 mm	19 mm
B-6	-	20.1 mm	18.3 mm	20 mm	22 mm	20 mm	20 mm	-
B-7	-	-	-	22 mm	-	20 mm	-	-
B-8	-	18.3 mm	-	21 mm	18 mm	20 mm	-	-
B-9	12 mm	18.3 mm	18.3 mm	20 mm	20 mm	20 mm	22.2 mm	20 mm
B-10	13 mm	18 mm	19 mm	12 mm	22.7 mm	20.1 mm	20 mm	-
B-11	12 mm	19.8 mm	18.1 mm	20 mm	22.7 mm	20.2 mm	-	-
B-12	12 mm	-	-	19 mm	13 mm	20 mm	19.8 mm	-

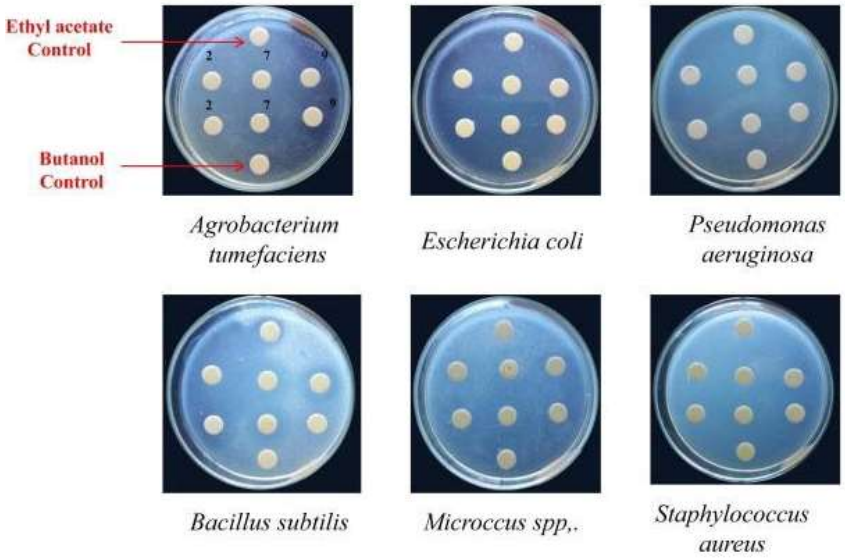


Figure 7 Antimicrobial Activity of Ethyl acetate and Butanol Extract of B -2,7 and 9

Table 4 Antibacterial activity of three crude extract from Isolated Bacteria Strains

Extracted solvents / Test organisms	Ethyl acetate			Butanol		
	B2	**B7**	**B9**	**B2**	**B7**	**B9**
Agrobacterium tumefaciens	25 mm	22 mm	23 mm	20 mm	20 mm	19.2 mm
Bacillus subtilis	32.3 mm	23 mm	20 mm	30 mm	23 mm	-
Escherichia coli	-	-	-	-	-	-
Micrococcus spp.	33 mm	25 mm	25 mm	35 mm	25 mm	23 mm
Pseudomonas aerugeinosa	33 mm	20 mm	20 mm	-	18 mm	18 mm
Staphylococcus aureus	18 mm	18 mm	18 mm	18 mm	20 mm	20 mm

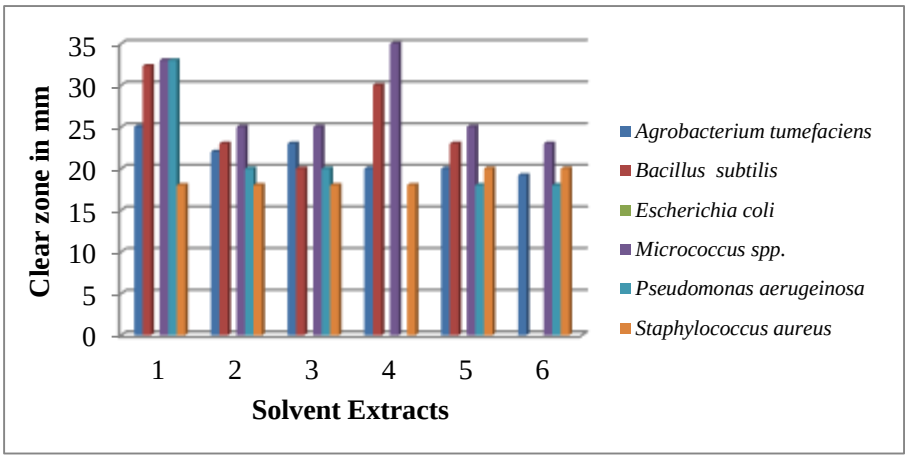


Figure 8 Antibacterial activity of three crude extract from Isolated Bacteria Strains

Antibacterial Activity of Three Crude Extract Bacteria B-2, 7and 9 Against Six Test Organisms

The second part of the present work mainly depends on extraction of metabolites by using ethyl acetate and butanol. According to the results of antimicrobial activity the isolated bacteria strains B 2, 7 and 9 were selectively used in extraction of metabolites. The isolated bacteria were grown in the 200 ml of nutrient broths. In the case of isolated bacteria the fermentation periods were checked up to three days and the best antimicrobial activity was detected in the 2 days. After each fermentation period, crude extract from fermented broth by butanol was also applied in the clear zone tests. In the metabolite extraction by bacteria, the isolate B-2 which found to give best antibacterial activity on *Micrococcus spp.* It was recorded that the ethyl acetate provide 33mm clear zone against *Micrococcus spp.* and *Pseudomonas aureginosa*, 32.3 mm against *Bacillus subtilis*. Similarly ethyl acetate extract of B-7 also showed 25 mm clear zone on *Micrococcu spp.*. The butanol extracts of B-9 also provided equally high antimicrobial activity, such as 19.2 mm on *Agrobacterium tumefaciens*, 23 mm on *Micrococcus spp.* and 1 8 mm on *Pseudomonas aeruginosa*. The results were shown in Table 4

Thin layer Chromatography of Crude Extract from Bacteria

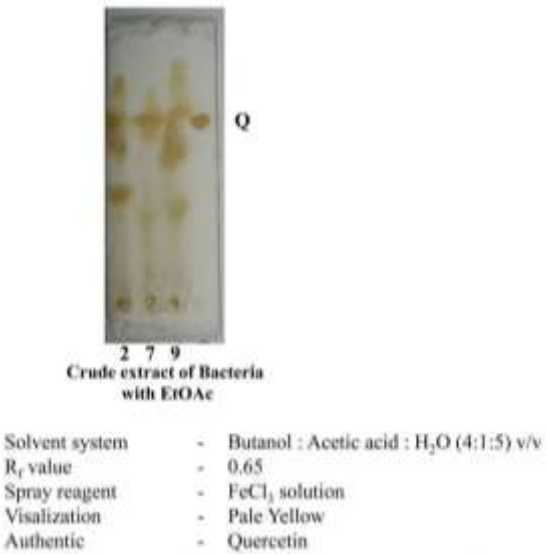
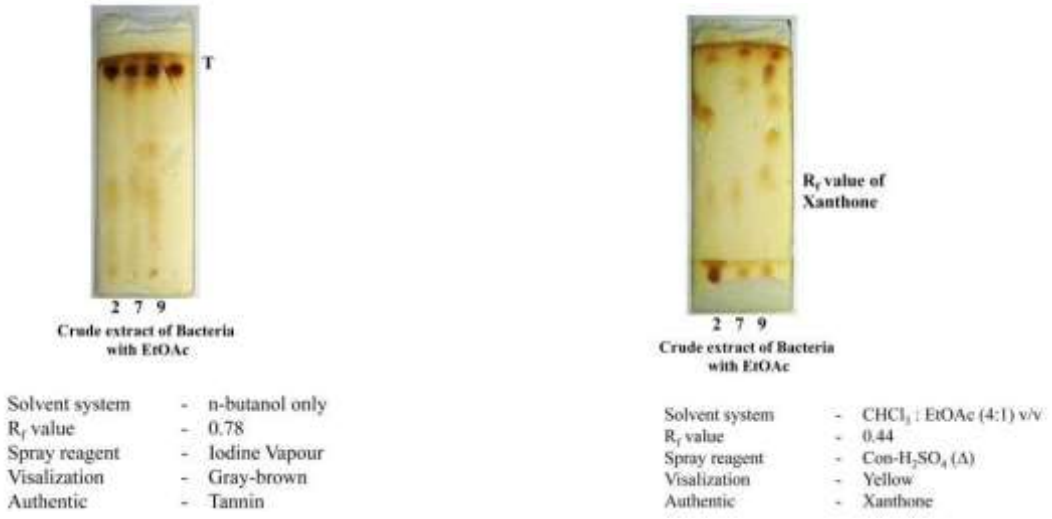


Figure 9 Thin layer Chromatography of Crude Extract by Ethyl acetate from Isolated Bacteria B-2, B-7 and B-9

Thin layer Chromatography of Crude Extract From Bacteria



Crude extract with Butanol

Figure 10 Thin layer Chromatography of Crude Extract by Ethyl acetate from Isolated Bacteria, B-2, B-7 and B-9

The third part of the present work concerned with identification of antimicrobial metabolites extracted with EtOAc using TLC. In the TLC techniques silica gel plates (3x7 cm) and butanol: acetic acid: H_2O (4:1:5) solvent system was used in the ascending methods as shown in Fig.9.After the treatment with butanol: acetic acid: water (4:1:5), the TLC plates were sprayed with $FeCl_3$ solution and pale yellow spots were recorded. The TLC of metabolic extract with butanol and developed with the solvent system of butanol: acetic acid: water ,it was observed that pale yellow spots with R_f values 0.65 was detected as shown in Fig.9. The R_f values were compared and suggest being Quercetin. When the fermented broth of bacteria extracted with EtoAc and developed the TLC with n-butanol, it was observed that tannin was contained after placing in the iodine vapor. The grey-brown spots with 0.78 R_f value were visualized Fig.10 compared with authentic tannin spot. Finally, the above extracts were subjected in the TLC with different solvent system of Chloroform and ethyl acetate (4:1 v/v). In TLC of bacteria extract using chloroform: EtOAc the spot of R_f0.44 were only detected in B-2,B-7 and B-9 it was observed pale yellow spots with R_f values 0.44 was observed as shown in Fig.10. According to the literature with same solvent system, it may be the spot of Xanthone.

Discussion and Conclusion

In the present work, the presence of entophytic microorganisms in the surfaces of leaves, bark and fruits of a famous nutraceutical fruit plant was verified by Lee (1997). Morphological, cultural, biochemical characteristics of 12 isolated bacteria were studied aiming to provide some valuate information concerning the further study of *Garcinia mangostana l.* After investigation the antimicrobial activity of 12 isolated bacteria B-2, B-7 and B-9 were selected for further research work, based on their clear zone conditions. The selected bacteria were subjected in preliminary fermentation using nutrient broth as basal medium. After fermentation at room temperature for two days the fermented broths were extracted with butanol and ethyl acetate. TLC analysis after extraction the results tentatively indicated the presences of quarcetin and tannin in all the fermented broths of bioactive crude extracted. But xanthone was estimated on the broth of B-2, B-7. Strobel *et.al* 2004 suggest that bioactive natural compounds assemble by endophytes have been promising potential usefulness in safety and human health concerns. Tan and Zou (2001) were proved that

endophytes presuming a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and others .Such bioactive metabolites find wide-ranging user as agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants, and anticancer agents by Gunatilaka (2006).

Acknowledgements

We are grateful to Dr. Nu Nu Yee and Dr Nay Thwe Kyi Pro- Rector, Dagon University for the use of research facilities related to the research work. We would like to express our deepest gratitude to Dr Myat Myat Moe Professor and Head, Department of Botany, Dagon University, for her invaluable guidance, continuous advice and for providing information and reference books. We want to express our gratitude to Rector (Retired) Dr. U Win, for his advice and encouragement. Our thanks go Dr. Sander Hlaing, Professors, Department of Botany, Dagon University, for her kind advice and suggestions. We also give our thanks to all of our teachers from whom we have learnt since our childhood and to all our colleagues for their valuable assistance.

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PHYTOCHEMICAL, PHYSICOCHEMICAL AND ANTIMICROBIAL ACTIVITIES ON FLOWERS OF *CAREYA ARBOREA* ROXB.

Yi Lay Myint[1], Khin Mar Kyu[2], Than Than Sint[3]

Abstract

Careya arborea Roxb. belongs to the family Lecythidaceae is found in many places of the world. It is also known as Bumbwe in Myanmar and Wild guava in English. The plants were collected from West Yangon University campus during the flowering period of May - November, 2019. The flowers are traditionally used to treat cough and cold, fever and tonic. In this study, the plant is deciduous trees, alternate and broadly obovate simple leaves, terminal spike with yellowish-white flowers, stamens with three whorls, inferior cup shaped ovary and large berry fruit with persistent style and calyx. In phytochemical screening, the presence of alkaloid, glycoside, saponin, phenolic compound, flavonoid, carbohydrate, steroid, terpenoid, tannin and reducing sugar were detected in flowers. However, cyanogenic glycoside and amino acid were absent. In the physicochemical properties, the powdered flowers are the most soluble in aqueous extract. The elemental analysis of powdered sample was determined by using Energy Dispersive X-ray Fluorescence Spectrophotometer. It was observed that the potassium was principal element. Antimicrobial activities of plant extracts are tested with six types of microorganisms by using agar well diffusion method. Ethanol extract of flowers exhibited effective against on *Pseudomonas aeruginosa*.

Keywords: Antimicrobial Study

Introduction

Careya arborea Roxb. is have been used for timber and ornamental plants and several parts of these species use as medicines. This plant is planted in gardens and roadsides for its large conspicuous leaves and showy flowers and fruits (Kumar *et al*., 2010). These species occur from Afghanistan through India to Ceylon and east to Thailand (Dassanayake, 1981). Range of these plants is Myanmar to the Malay Peninsula (Perry, 1980). In Myanmar, these plants occur in Western Thayetmyo, Katha, Bhamo, Mogok and Northern Tanintharyi of moist teak forest. It is also found in Pyinma forest on the plains and alluvial flats bordering the Bago Yoma forests (Kress *et al*., 2003).

Ecologically, the plant prefers a well-drained, sandy or even rocky soil and requires a sunny position. The tree is highly fire resistant and coppies well (Dassanayake, 1981). The vernacular names of this plant are Ka Li Yu Rui in Chinese, Kradonbok in Thailand, Kumbhi in Hindi and Katabhis in Sanskrit (Prabhakaran *et al*., 2014). The flowers of *Careya arborea* Roxb. have triterpenoid, steroid and tannin (Shantha *et al*., 1987).

The flowers and fruits are also used for relieving cold and cough (Ambardar and Aeri, 2013). Paste of flower of *Careya arborea* Roxb. prepared by macerating in ghee is taken orally in empty stomach to treat infertility (Mahishi *et al*., 2005). Aerial parts of this plant also exhibit antioxidant activity, antimicrobial activity against the bacteria like *Bacillus subtilis*, *Staphylococus aureus*, *Pseudamonas aeruginosa*, *Bacillus pumilus*, *Escherichia coli* and *Candida albican* (Navya and Anitha, 2018).

Therefore, the plant was chosen for this study to inform the medicinal value. To achieve this aim, the objectives were to detect the phytochemical tests for the presence or absence of

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chemical constituents in the flowers and to test the antimicrobial activities of the crude extracts by using agar-well diffusion method.

Materials and Methods

1. Morphological study of *Careya arborea* Roxb.

The plants materials were collected from West Yangon University campus, during the flowering period. The collected specimens were photographed to record the data. The plants were classified and identified by using Hooker (1879) and Dassanayake (1981).

The 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.

2. Chemical study of *Careya arborea* Roxb.

Preliminary phytochemical investigation on flower of *Careya arborea* Roxb.

In this investigation, the powdered flowers of *Careya arborea* Roxb. were tested to find out the presence or absence of chemical constituents such as alkaloids, glycoside, saponin, cyanogenic glycoside, phenolic compounds, flavonoid, carbohydrate, steroids, terpenoid, tannins, amino acid and reducing sugars. Preliminary phytochemical tests of flowers were carried out at the Chemistry Department,West Yangon University according to the methods of British Pharmacopoeia (1968) and Central Council for Research in Unani Medicine (1987). The results were shown in Table (1).

Physicochemical analysis on flower of *Careya arborea* Roxb.

Physicochemical properties which include moisture content, total ash, acid insoluble ash, water soluble ash and solubility of nonpolar and polar solvents such as pet-ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and water soluble matter contents of *Careya arborea* Roxb. flowers powered were carried out by the methods of British Pharmacopoeia (1968). The results were shown in Table (2).

Elemental analysis on flowers of *Careya arborea* Roxb. by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF)

Element analysis was performed by EDXRF (Energy Dispersive X-ray Fluorescence Spectrophotometer) at Chemistry Department, West Yangon University. The EDX-700 spectrophotometer SHIMADZU Co.Ltd., Japan is used for determination of elements. The results were shown in Table (3) and Figure (10).

3. Antimicrobial screening of different solvent extracts from flowers of *Careya arborea* Roxb.

Extraction

The powdered samples of flowers (five gram) were soaked in petroleum ether (60-80˚ C), chloroform, methanol, acetone, ethyl acetate, 95% ethanol and distilled water for about 3 weeks and then filtered. (British Pharmacopoeia 1968). The solvents were then evaporated by using water bath to obtain a paste.

Test Organisms

The different solvent extracts were tested against six tested microorganisms by using agar well diffusion method. The six microorganisms were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Escherichia coli and Candida albicans* obtained from **Central Results Development Centre**. (N.C.T.C-8236), (N.C.P.C-6371), (6749), (N.C.I.B-8982), (N.C.I.B – 8134)

The extracts of antimicrobial activity were measured from the diameter zone of inhibition. The results were shown in Table (4) and Figure (11-16).

Procedure

Nutrient agar was prepared according to the method of Cruickshank, 1975. Nutrient agar was boiled and 20 - 25 ml of the medium was poured into a conical flask and plugged with cotton wool and autoclaved at 121° C for 15 minutes. Then the tubes were cooled down to 30 - 35° C and poured into sterilized petridishes and 0.1 - 0.2 ml of test organisms were also added into the dishes. The agar was allowed to set for 2 - 3 hours. After that, 10 mm plate agar-well made with the help of sterilized agar well cutter. About 0.2 ml of sample was introduced into the agar-well and incubated at 37° C for 24 - 48 hours. The inhibition zone appeared around the agar well, indicated that the presence of antibacterial activity. This antimicrobial activity test was conducted in CRDC (Central Research Development Centre).

Results

1. Morphological study of *Careya arborea* Roxb Pl. Coromandel 3(1): 14, t. 218 (1811).

Deciduous trees, stem erect, cylindrical, pubescent; bark greyish-brown cracked and flakeing in thin stripes. Leaves alternate, crowded towards the end of the branches, simple, lamina broadly abovate, the base tapering cunneate, the margin serrate, the tips rounded with the pointed, dull-green glabrous, on the both surfaces, petiolate; the petioles cylindrical, glabrous, exstipulate. Inflorescences terminal spike, cymes and wide, sub pedunculate. Flower yellowish-white, bractcate, the bracts 3, unequal, linear- to lanceolate, green, glabrous, ebracteolate, sessile, bisexual, actinomorphic, 4-merous, epigynous. Calyx (4) synsepalous, campanulate, the lobe oval, green, imbricate, glabrous. Corolla 4-5, free petals, oblong, elliptic, light yellowish, glabrous, very fragile and soon falling. Stamens numerous twice as long as the petals in 3 whorls; outer whorl longest, without anther, reddish-white; middle whorl medium, fertile; the innermost whorl short without anther, all united at the base into a thick fleshy ring, the filaments, slender, the anther dark-brown in age, dithecous, oblongoid, basifixed, longitudinal dehiscence. Ovary inferior, cup shaped disc at the top, ovoid, tetra-carpellary, syncarpous, axile placentation, 4 - α ovules in each locule in T.S; the style long, slender long, glabrous; the stigma capitate. Fruit berry globose, bright-green pericarp in young, brownish in mature, fibrous crowned with the sepals and style, with fleshy pulp; seed, many, white, oblong embedded in fleshy pulp of the fruits.

Figure 1 Natural Habit

Figure 2 Inflorescence

Figure 3 Flower as seen

Figure 4 Stamens

Figure 5 Calyx & Style

Figure 6 L.S of flower

Figure 7 T.S of ovary

Figure 8 Fruit

Figure 9 Seeds

2. Chemical study of *Careya arborea* Roxb

Table 1 Preliminary Phytochemical Investigation on Flowers of *Careya arborea* Roxb.

No	Chemical Constituents	Extract	Test Reagent	Observation	Results
1	Alkaloid	1%HCL	1 Mayer's reagent 2 Dragendorff's reagent 3 Wagner reagent	White ppts Orange ppts Yellow ppts	Present
2	Glycoside	H_2O	10% Lead acetate solution	White ppts	Present
3	Saponin glycoside	H_2O	Distilled water	Frothing	Present
4	Cyanogenic glycoside	H_2O	Conc H_2SO_4 acid+Sodium Picrate solution	No Colour Change	Absent
5	Phenolic Compound	H_2O	$FeCl_3$ Solution	Brown colour	Present
6	Flavonoid	MEOH	Mg turing + Conc HCl	Pink Colour	Present
7	Carbohydrate	H_2O	10% α naphathol+ Conc H_2SO_4	Pink Ring	Present
8	Steroid	PE	Acetic Anhydride+Conc H_2SO_4 acid	Pink Colour	Present
9	Terpenoid	EtOH $CHCl_3$	Conc H_2SO_4acid	Reddish brown	Present
10	Tannin	$CHCl_3$	10% Gelatin solution	White ppts	Present
11	Amino acid	H_2O	Ninhydrin	No change in colour	Absent
12	Acid/Base/Nutral	H_2O	Bromocresol green	Green	Nutral
13	Reducing Sugar	H_2O	1. Benedicts Solution 2. Fehling Solution	Red ppts	Present

Table 2 Physicochemical Analysis on Flowers of *Careya arborea* Roxb.

No	Physics chemical properties	Yield percent (%)
1	Moisture content	6.65%
2	Total ash	1.03%
3	Acid insoluble ash	5.10%
4	Water soluble ash	28.47%
5	Ethanol soluble matter content	5.75%
6	Methanol soluble matter content	6.42%
7	Petroleum ether soluble matter content	2.07%
8	Ethyl acetate soluble matter content	2.41%
9	Chloroform soluble matter content	2.43%
10	Acetone soluble matter content	0.68%
11	Aqueous soluble matter content	14.17%

Table 3 Elemental Analysis on Flowers of *Careya arborea* Roxb. by using EDXRF

Elements	**Average %**
Potassium (K)	1.986
Sodium (Na)	0.206
Calcium (Ca)	0.121
Iron (Fe)	0.095
Titanian (Ti)	0.014
Manganese (Mn)	0.005
Zince (Zn)	0.002
Rubidium (Rb)	0.002
Chromium (Cr)	0.001
Copper (Cu)	0.001
Strontium (Sr)	0.001
Zirconium (Zr)	0.000
COH balance	97.566

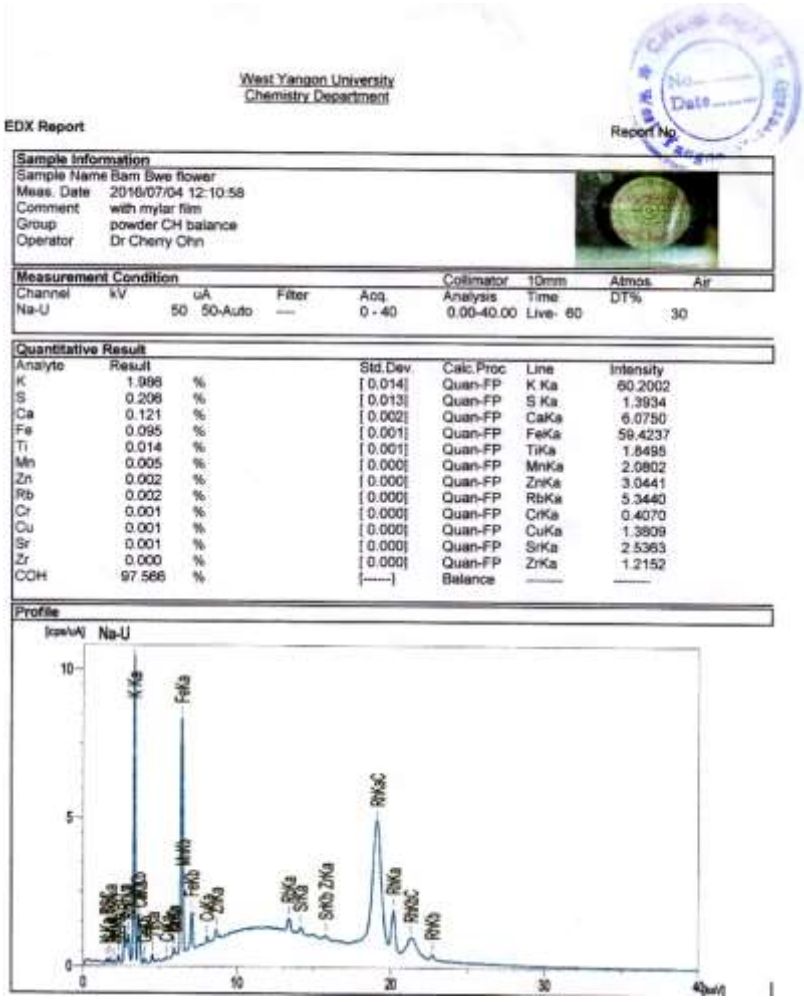

Figure 10 Elemental analysis on flowers of *Careya arborea* Roxb. by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF)

3. Antimicrobial screening of different solvent extracts from flowers of *Careya arborea* Roxb.

Table 4 Antimicrobial Activity from the Different Solvent Extracts on Flowers of *Careya arborea* Roxb.

Test Organisms	Solvents							
	Control	Pet-ether	Chloroform	Methanol	Acetone	Ethyl acetate	Ethanol	Aqueous
Bacillus subtilis	-	-	-	20 mm (+++)	14 mm (+)	13 mm (+)	16 mm (++)	14 mm (+)
Staphylococcus aureus	-	-	-	16 mm (++)	14 mm (+)	12 mm (+)	21 mm (+++)	14 mm (+)
Pseudomonas aeruginosa	-	-	-	16 mm (++)	13 mm (+)	-	24 mm (+++)	13 mm (+)
Bacillus pumilus	-	12 ml (+)	12 mm (++)	18 mm (++)	17 mm (++)	11 mm (+)	18 mm (++)	15 mm (++)
Candida albicans	-	11 ml (+)	12 mm (++)	18 mm (++)	16 mm (++)	12 mm (+)	19 mm (++)	17 mm (++)
Escherichia coli	-	11 ml (+)	12 mm (++)	17 mm (++)	15 mm (++)	12 mm (+)	20 mm (+++)	15 mm (++)

Agar well – 10 mm

10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++)

20 mm above (+++)

Organisms
(1) *Bacillus subtilis* (N.C.T.C.-8236)
(2) *Staphylococcus aureus* (N.C.P.C-6371)
(3) *Pseudomonas aeruginosa* (6749)
(4) *Bacillus pumilus* (N.C.I.B-8982)
(5) *Candida albicans*
(6) *E-coli* (N.C.I.B-8134)

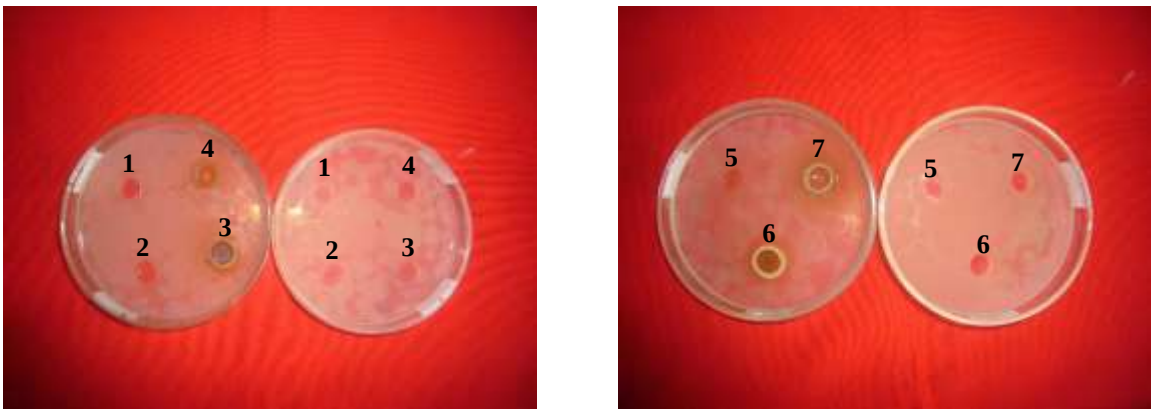

Figure 11 Antibacterial activity of the flowers extracts on *Bacillus subtilis*

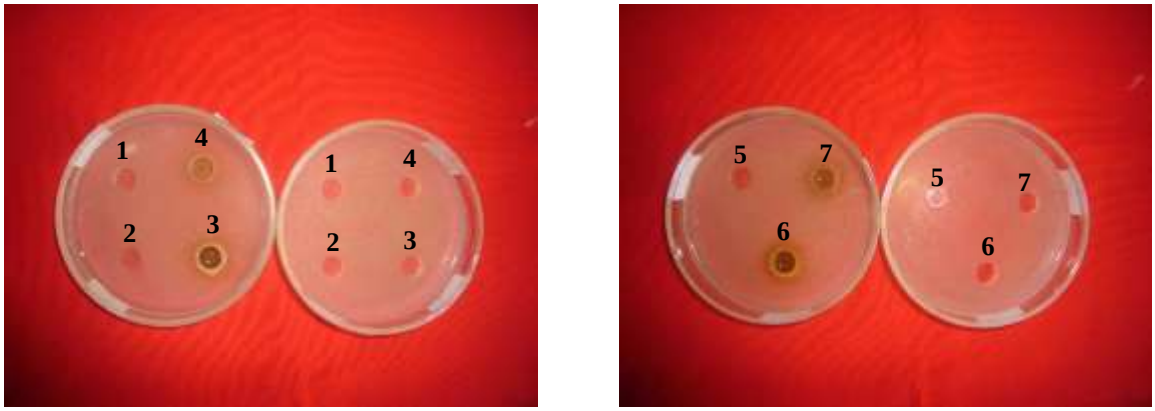

Figure 12 Antibacterial activity of the flowers extracts on *Staphylococcus aureus*

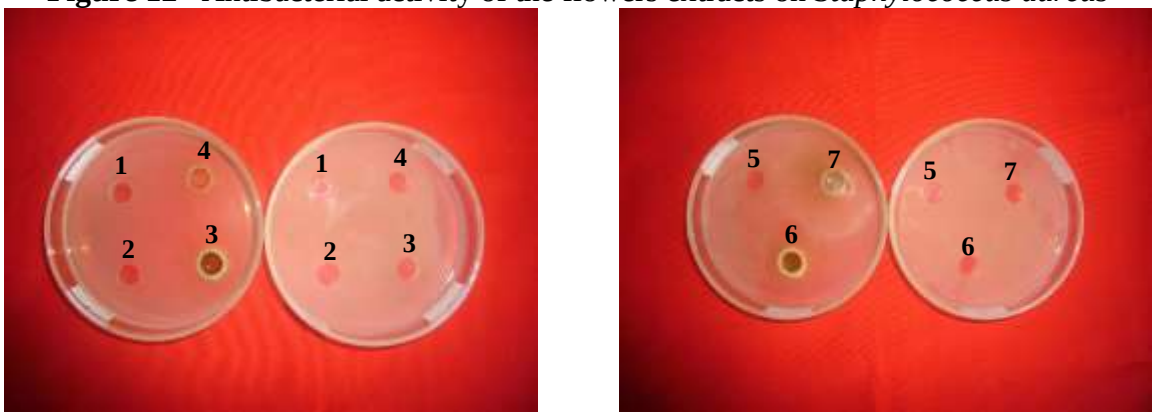

Figure 13 Antibacterial activity of the flowers extracts on *Pseudomonas aeruginosa*

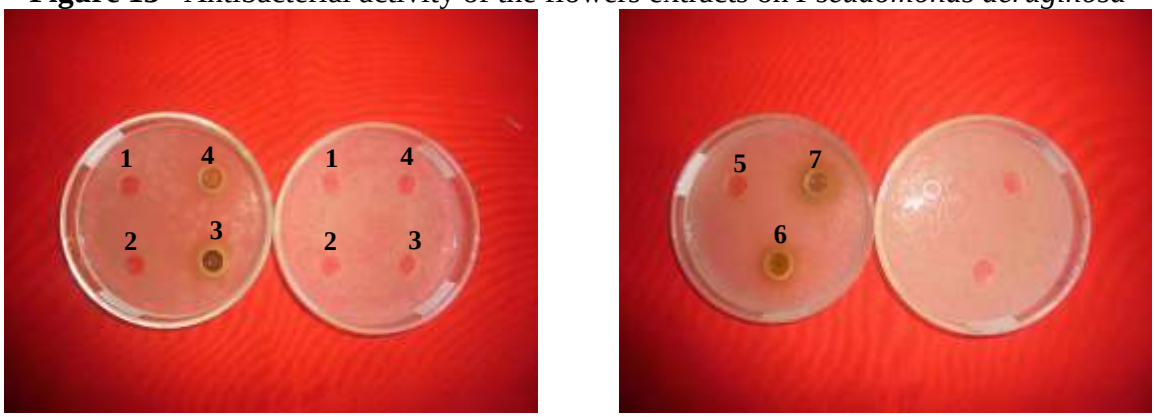

Figure 14 Antibacterial activity of the flowers extracts on *Bacillus pumilus*

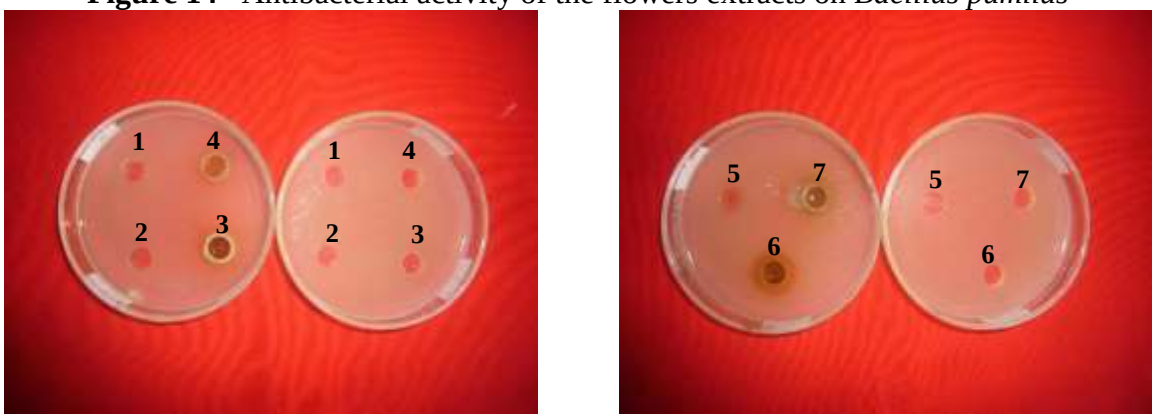

Figure 15 Antifungal activity of the flowers extracts on *Candida albicans*

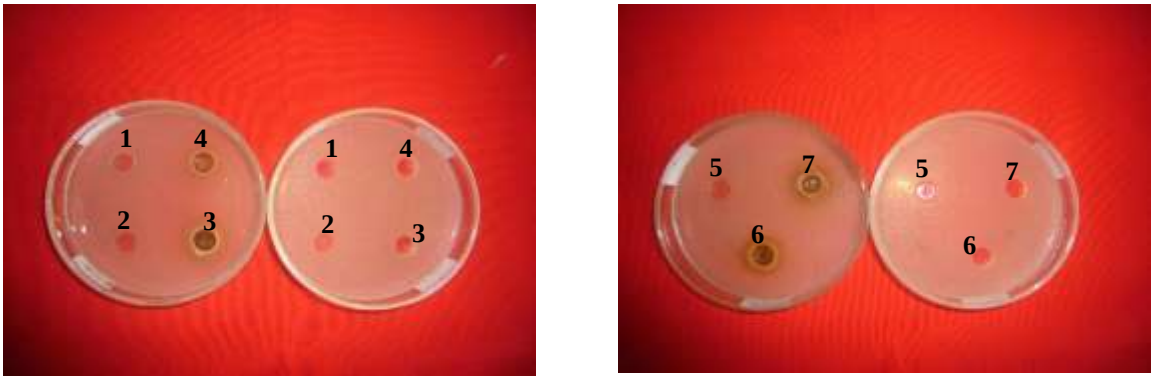

Figure 16 Antibacterial activity of the flowers extracts on *Escherichia coli*

1= pet-ether 2 = chloroform 3 = methanol 4 = acetone 5 = ethyl acetate 6 = ethanol 7 = aqueous

Discussion and Conclusion

In the morphological study, *Careya arborea* Roxb. are deciduous trees with alternate leaves, crowded to wards the end of branches; large and yellowish white flowers in crowded erect spikes; campanulate tube of calyx and oblong elliptic petals; numerous stamens in 3 whorls and connate at the base; inferior ovary with 4 – numerous ovulesin each locule in T.S; style long and simple; large and globose fruit with crowned calyx and numerous seeds immersed in the fleshy pulp. These characters are in agreement with those mentioned by Hooker (1879) and Dassanayake (1981) and Khaliq (2016).

Phytochemical analysis of air dried flowers of *Careya arborea* Roxb. showed the presence of many secondary metabolites of phytoconstituents like alkaloid, glycoside, saponin, phenolic compound, flavonoid, carbohydrate, steroid, terpenoid, tannin and reducing sugar. Cyanogenic glycoside and amino acid are absent. These characters were similar to those of Kapoor *et al*., (1972), Atal (1978) and Shantha (1987).

The results of the physicochemical investigation, the most soluble in aqueous soluble was 14.17%. It was higher than those of other solvents and the least soluble in acetone was 0.68%. Elemental analysis was to determine the amount of elements in *Careya arborea* Roxb. flowers. Potassium is found as principal element.

The antimicrobial activities were tested with six types of microorganisms. Ethanolic extracts of flowers against *Pseudomonas aeruginosa* and showed higher activity as reported early by Ambardar and Aeri (2013).

Plants or plant products use as the therapeutic agents in treating various ailments by virtue of their phytoconstituents (Chalia *et al*., 2009). At present, most of the people including Myanmar have to rely on the herbal medicines as the remedies for various illness. Since ancient times plants have served producer beings as a nutral source of treatments and therapies, among them medicinal herbs have gained attention because of its wide use and less side effects. Although uses of herbal medicines reduce side effects need to assurance of the safety, quality and efficacy of medicinal for plants and herbal products.

In this work, the flowers of the *Careya arborea* Roxb. showed antimicrobial activities. Phytochemical screening of the extracts revealed the presence of phyto-compound such as triterperoids, steroids, flavonoids and tannins as major phyto-constituents with known antimicrobial agents. These phyto-constituents may be responsible for the antimicrobial activity of *Careya arborea* Roxb. (Mahadev and Shailaja, 2015). Therefore, this research will be highlighting the flowers of *Careya arborea* Roxb. for the medicinal uses in order to know the scientifically knowledge for the local people.

Acknowledgements

We would like to express deep sense of gratitude to Rector Dr. Tin Maung Tun and Prorector Dr. Soe Soe Aye, West Yangon University for their kind provision of the research facilities. We also wish to express our deep gratitude to Dr Yee Yee Than Professor, Botany Department, West Yangon University for her encouragement and good advices.

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MORPHOLOGICAL, HISTOLOGICAL AND PHYTOCHEMICAL STUDY OF FLORAL LEAVES OF *DELONIX REGIA* (BOJER EX. HOOK.) RAF. AND THEIR ANTIOXIDANT ACTIVITIES

Nwe Oo[1]

Abstract

The morphological, histological and phytochemical studies were carried out at Botany Department, University of Yangon in 2019. According to morphological study, it is a tree with bipinnately compound leaves, pulvinus and leaf-like stipules present. Flowers are slightly zygomorphic and predominantly orange-red in color. Lower surface of sepals is green and upper is red. Four smaller petals are orange-red and one larger petal is white and yellow with reddish spots. In histological study (Trease and Evans, 2009), stomata present on the lower surface of sepals. Anthocyanin containing epidermal cells present only on the upper surface of sepals. In petals, anthocyanin containing cells present on both epidermis. Bundle sheath and starch sheath present in petals. In phytochemical study (Harborne, 1973, Sofowora, 1993 and Trease and Evans, 2009), reducing sugars, alkaloids and saponins were not detected in petals. Antioxidant activities were determined by DPPH assay (Blois, 1958 and Brand-Williams *et al*., 1995) at Botany Department, University of Mandalay in 2020. The IC_{50} value of sepals was 6.44 μg/ml and petals was 20.04 μg/ml.

Keywords: morphology, histology, phytochemistry, antioxidant activities

Introduction

Delonix regia (Bojer ex. Hook.) Raf. is a tree and belongs to the family Fabaceae. It is grown as shady and ornamental plant because of its beautiful and bright coloured flowers throughout Myanmar.

Its flowers are used as natural color and as an acid-base indicator. Chemical constituents of different classes such as; flavonoid, tannins, glycosides, phenolics, sterol (phytosterol) and terpenoids (triterpenoids) were reported from its flowers and leaves. Ethanolic extracts of flower and bark were investigated to anti- inflammatory activity in rats. Leaves and flowers are reported to possess antimalarial, anti-bacterial, anthelmintic, hepato-protective, diuretic and anti-oxidant (Hussain, *et al*., 2014).

It originated from Madagascar, where it is now almost extinct but widespread in most tropical and subtropical areas of the world. The bark yields thick mucilage of water – soluble gum. The seeds contain gum and the tree could provide timber and the large pods and wood are used for fuel (Igwe and Louis, 2014).

In traditional medicines, the flowers were used for curing chronic fever in Southwestern Bangladesh. In Nigeria, the flowers were used for antibacterial activity. Some tribes of India have used the seed for curing pyorrhea. The roasted and crushed leaves were wrapped in a cloth and inhaled just after scorpion bite; infusion of flowers was used in bronchitis, asthma and malarial fever. In several African countries, the water extracts of flowers were also used in traditional healthy beverages. It is a part of local medicine and traditional byproducts (Sharma and Saroj, 2015).

Its flowers especially sepals and petals have been used as vegetables and salad in some area. The aim of this study is to know the pharmacognostic properties of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.. The objectives are to study the morphological and histological characters of floral leaves and to determine their phytochemical constituents and antioxidant activity.

[1] Dr, Lecturer, Department of Botany, University of Mandalay

Materials and Methods

Collection of plants

Delonix regia (Bojer ex. Hook.) Raf. was collected from University of Yangon campus in 2019. The morphological characters of collected plant specimens were photographed and recorded their measurements in detail. The plant was identified with the help of literatures such as Hooker (1875) and Backer (1963).

Cleaning, drying and powdering

The collected samples were thoroughly cleaned to remove impurities and separated as sepals and petals. Then they were air dried to avoid direct loss of phytoconstituents from sunlight. The dried samples were coarsely powdered to study the preliminary phytochemical analysis and DPPH scavenging assays.

Histological study

The fresh samples were sectioned by free hand method according to Trease and Evans (2009). The following reagents were used for histological purposes;

1. Chloral hydrate solution as the clearing reagent
2. Saffranin solution as the stain for lignified and cutinized cell walls
3. Iodine solution used for starch.

Preliminary phytochemical study

Phytochemical screening was performed using standard procedures such as Harborne (1973), Trease and Evans (2009) and Sofowora (1993).

Test for glycosides

Two ml of aqueous extract was mixed with a few drops of aqueous NaOH. A yellow coloration will indicate the presence glycosides.

Test for reducing sugar

Two ml of the aqueous extract was treated with mixture of Fehling A and B solutions and heated gently. Orange red precipitates show reducing sugars.

Test for alkaloids (Wagner's reagent)

Two mL of aqueous extract was mixed with 0.2 mL dilute HCl, followed by 1 mL of Wagner's reagent. If the brown precipitation appears, alkaloid is presence.

Test for flavonoids

Two ml of the aqueous extract was mixed with a few drops of NaOH solution. If the mixture shows the yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, it indicates the presence of flavonoids.

Test for phenolic compound

Two mL of the aqueous extract was mixed with 1 to 2 drops of Iron III chloride ($FeCl_3$) was added. A blue, green, red or purple color is a positive test.

Test for tannins

Aqueous extract (2 ml) was mixed with a few drops of 5% $FeCl_3$ solution and concentrated sulphuric acid. The yellow brown precipitate is the presence of tannins.

Test for saponins

Aqueous extract (2 ml) was mixed with 2 ml of distilled water and shaken vigorously. The formation of stable foam indicates the presence of saponins.

Test for proteins

Aqueous extract (2 ml) was mixed with a few drops of Millon's reagent. The white precipitates turn red when heated. This appearance shows the protein presence.

Test for α-amino acids

A few drops of aqueous extract were spotted on a filter paper, allowed to dry and spray with ninhydrin reagent. It was dried at room temperature and then kept in an oven at 110˚C for a few minutes. The violet spot indicate α-amino acids presence.

Test for steroids/terpenoids

Ethanol extract (3 ml) was dissolved with 2 ml of chloroform. Then mixture was treated with concentrated sulphuric acid. The red color showed the steroids presence. Reddish brown color at interface showed the presence of terpenoids.

Antioxidant activity by using DPPH free radical scavenging assay

DPPH (2, 2-diphenyl- 1-picryl-hydrazyl) radical scavenging assay was carried out according to the methods of Blois (1958) and Brand-Williams *et al*. (1995). The experiment was performed at Department of Botany, University of Mandalay.

Preparation of 60 µM DPPH solution

DPPH powder (2.364 mg) was mixed with 95% ethanol (100 ml) and stirred thoroughly. This freshly prepared solution was kept in brown color flask and stored in the refrigerator at - 2˚C for no longer than 24 hours.

Preparation of test sample solution

Ethanolic extracts (0.1g) was dissolved with 95% ethanol (100 ml) to get 1000 µg/ml stock solution. Different concentrations, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml solutions were prepared from stock solution by the half dilution with 95% ethanol.

Preparation of standard ascorbic acid solution

Ascorbic acid (0.01 g) was mixed with 95% ethanol (100 ml) to get 100 µg/ml stock solutions. Different concentrations, 0.3906 µg/ml, 0.7813 µg/ml, 1.5625 µg/ml, 3.125 µg/ml, 6.25 µg/ml and 12.5 µg/ml solutions were prepared by the half dilution.

Procedure

Antioxidant activity of ethanolic floral leaves extracts was determined by DPPH scavenging assay. Blank solution was prepared 3 ml of 95% ethanol. 60 µM DPPH solution (1.5 ml) and 95% ethanol (1.5 ml) were used as control solution. The different concentrations of

test sample solution (1.5 ml) was mixed with 60 µM DPPH solution (1.5 ml) and allowed to stand in the dark for 30 minutes.

The different concentrations of standard ascorbic acid solution (1.5 ml) was mixed with 60 µM DPPH solution (1.5 ml) and allowed to stand in the dark for 30 minutes. The absorbance of these solutions was measured at 517 nm by using UV spectrophotometer (UV - Vis 2550). Lower absorbance showed the higher free radical scavenging activity. The measurements were carried out triplicates for each solution and calculated the percent inhibition by the following formula;

$$\% \text{ inhibition} = A_0 - A_1/A_0 \times 100$$

Where A_0 = the absorbance of control

A_1 = the absorbance of sample

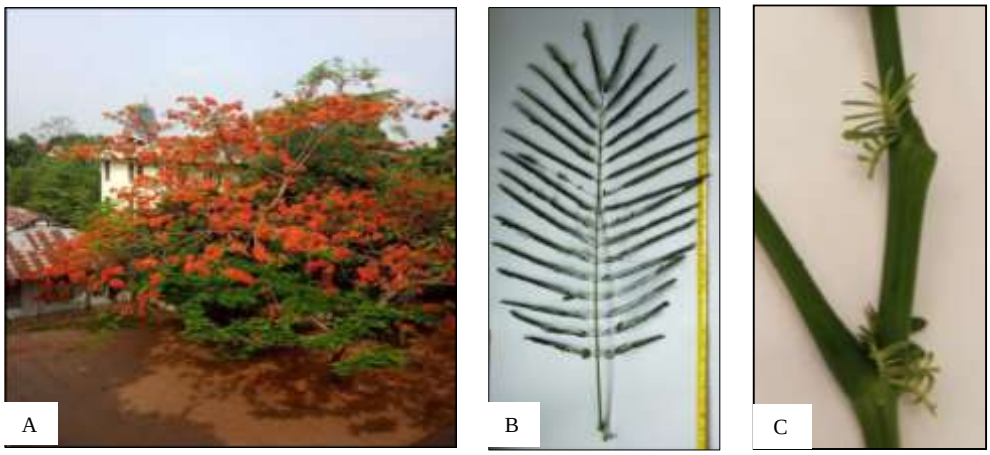
The 50% inhibitory concentration (IC_{50}) of test samples and standard ascorbic acid was calculated by nonlinear regression method of GraphPad Prism software.

Results

Morphological characters of *Delonix regia* (Bojer ex. Hook.) Raf.

Scientific Name	:	*Delonix regia* (Bojer ex. Hook.) Raf.
Family	:	Fabaceae
Common Name	:	Royal Poinciana, Flame tree, Flamboyant
Myanmar Name	:	Sein-pan

Tree, up to 15 meters height. **Leaves** bipinnately compound. **Leaflets** sessile, oblong, glabrous. **Stipules** leaf-like and caducous. Inflorescences racemose. **Flowers** bisexual, complete, irregular, slightly zygomorphic, pedicellate. **Calyx**; sepals 5, aposepalous, oblong-acuminate, reddish color inside, green color outside. **Corolla**; petals 5, apopetalous, smaller 4 petals orange-red, claw and lobe present, larger one petal white with reddish spot at upper portion, yellow with reddish spots at lower portion of lobe, claw reddish, margin of lobe reddish. **Androecium**; stamens 10, apostemonous, filament red at upper portion, white at lower portion. **Gynoeium;** carpels 1, monocarpellary, style filiform, stigma capitate, one ovule in the locule in T.S, marginal placentation. **Pods** blackish, style persistent. **Seeds** oblong-elliptic.



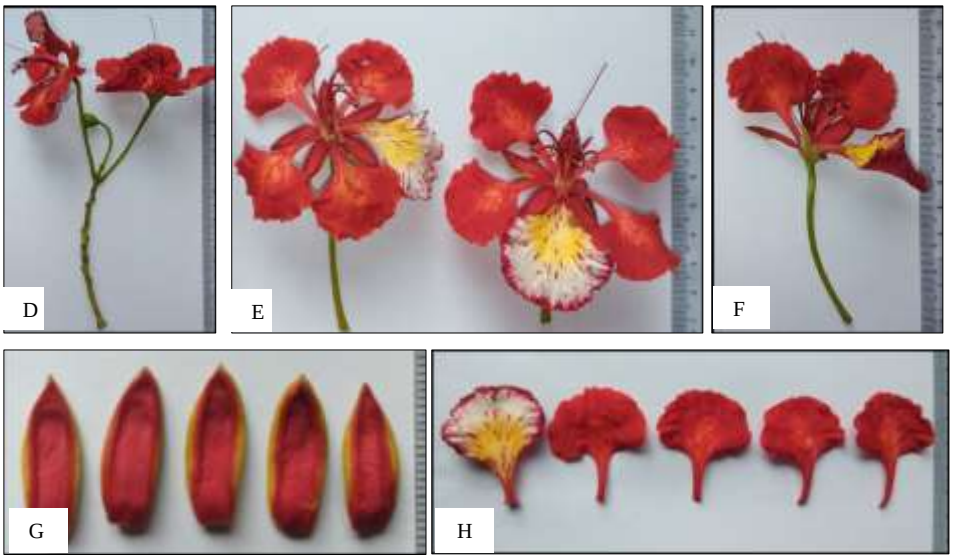


Figure 1 Morphological characters of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Habit
B. Leaf
C. Leaf-like stipules
D. Inflorescence
E. Flowers
F. L.S of flower
G. Sepals
H. Petals

Figure 2 Morphological characters of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Androecium
B. Pistils
C. T.S of ovary
D. L.S of ovary
E. Fruit
F. Seeds

Histological characters of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

In upper surface of sepals, polygonal-shaped parenchyma cells with red anthocyanin are present. Stomata are absent. In lower surface view, epidermal cells are polygonal in shaped and anomocytic stomata are present. No anthocyanin pigments found in the lower epidermal cells of sepals. In T.S of sepals, unicellular and uniseriate trichomes are found on the upper epidermis. In mesophyll, small vascular bundles arrange into ring. Numerous aerenchyma cell layers are found in the inner portion of the sepals. Mesophyll cells are not differentiated.

In both surface views of petals, striations and wavy epidermal cells with anthocyanin are abundantly present. In T.S of lobed petals, epidermal cells are larger than that of other mesophyll cells. Papillae are present on both epidermises. Mesophyll cells are aerenchymatous and vascular bundles are small with bundle sheath cells. There are 4 – 5 layers of mesophyll layers are found. The outline of clawed petal is crescent-shaped in T.S. Starch sheath is present in clawed petal.

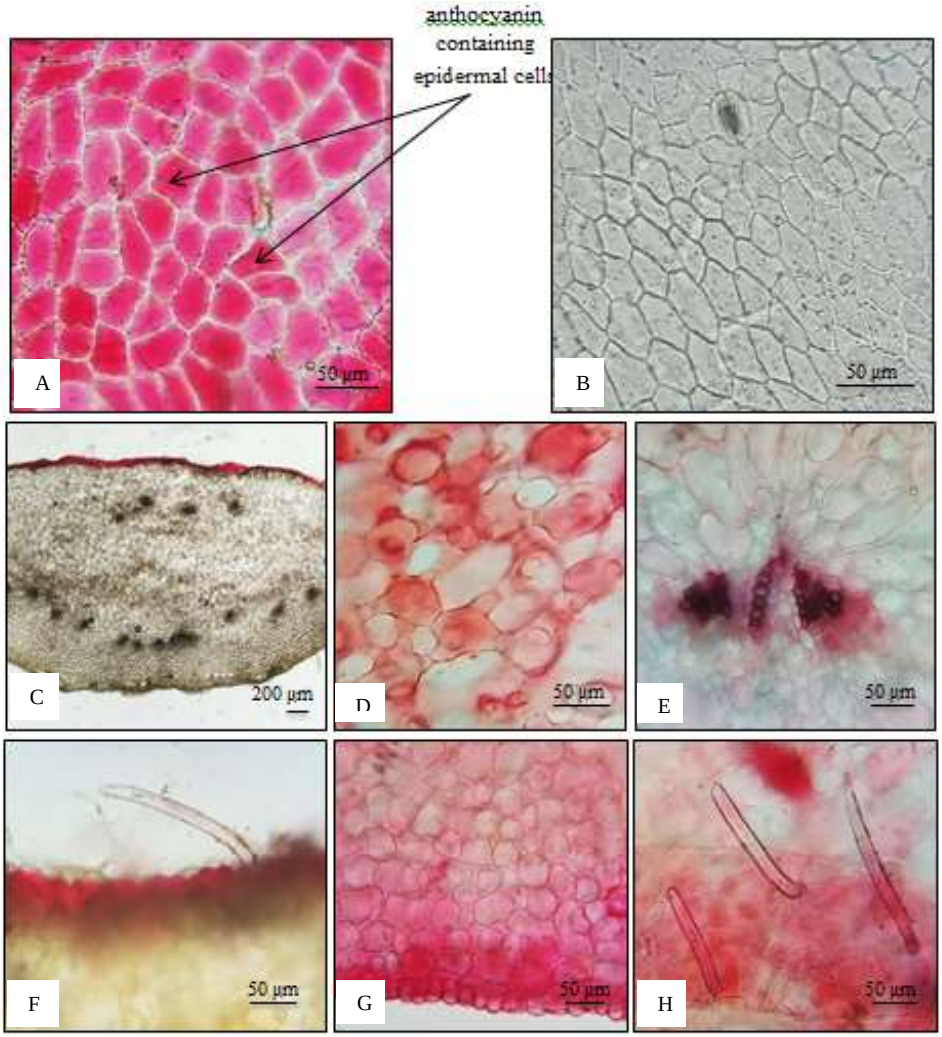


Figure 3 Histological characters of sepals of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Upper surface B. Lower surface
C. Transverse section D. Spongy cells
E. Vascular bundle F. Upper portion
G. Lower portion H. Unicellular and uniseriate **trichomes**

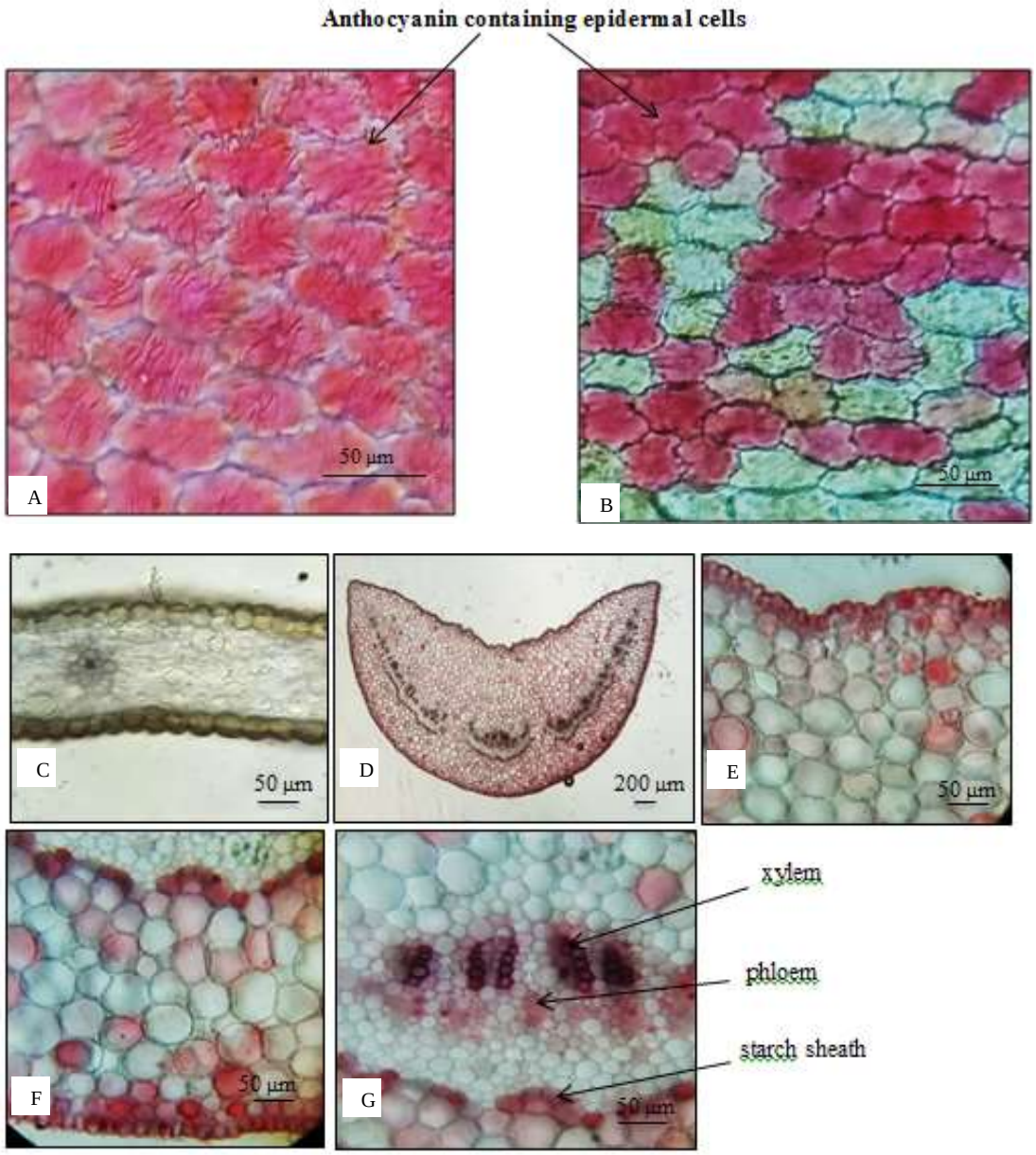


Figure 4 Histological characters of petals of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Upper surface

B. Lower surface

C. Transverse section of lobed petals

D. Transverse section of clawed petals

E. Upper portion of clawed petals

F. Lower portion of clawed petals

G. Vascular bundles of clawed petals

Samples of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

Figure 5 Fresh sepals samples

Figure 6 Dried and powdered sepals

Figure 7 Fresh petals samples

Figure 8 Dried and powdered petals

Preliminary phytochemical study of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

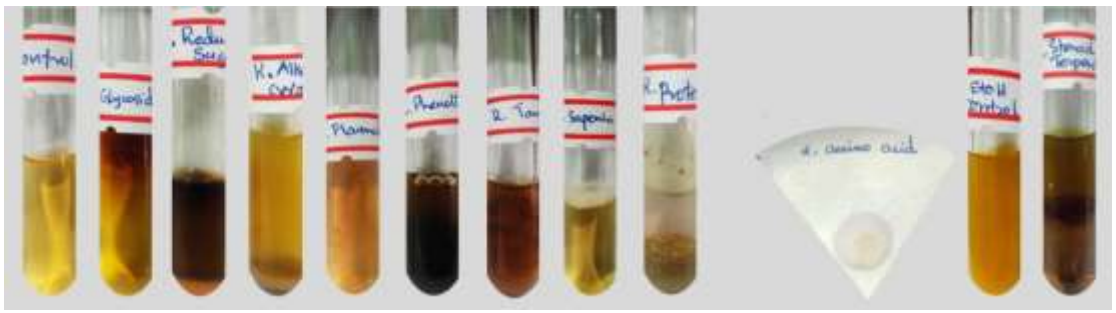


Figure 9 Phytochemical constituents of sepals

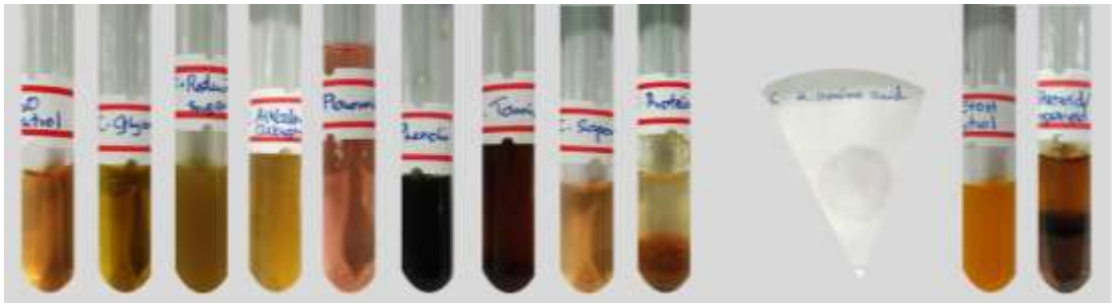


Figure 10 Phytochemical constituents of petals

Table 1 Preliminary phytochemical constituents of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

No.	Tests	Observations		Results	
		Sepals	Petals	Sepals	Petals
1.	Glycosides	Yellow color	Yellow color	+	+
2.	Reducing sugars	Orange ppt.	No ppt.	+	–
3.	Alkaloids	Brown ppt.	No ppt.	+	–
4.	Flavonoids	Colorless	Colorless	+	+
5.	Phenolic compounds	Blackish ppt.	Blackish ppt.	+	+
6.	Tannins	Yellow brown ppt.	Yellow brown ppt.	+	+
7.	Saponins	Foaming	No foaming	+	–
8.	Proteins	White ppt. turned red when heated.	White ppt. turned red when heated.	+	+
9.	α-amino acids	Pink spot	Pink spot	+	+
10.	Steroids/ Terpenoids	Red color/ Reddish brown interface	Red color/ Reddish brown interface	+	+

\+ = present
– = absent

Antioxidant activity of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf. by DPPH free radical scavenging assay

Table 2 Percent inhibition of ethanolic floral leaves extracts of *Delonix regia* (Bojer ex. Hook.) Raf.

Concentrations (µg/ml)	Absorbance			Inhibition (%)		IC_{50} value (µg/ml)	
	Sepals	Petals	Control	Sepals	Petals	Sepals	Petals
6.25	0.130	0.176	0.243	46.50	27.57	6.44	20.04
12.5	0.089	0.172	0.243	63.37	29.22		
25	0.046	0.092	0.243	81.06	62.14		
50	0.039	0.067	0.243	83.95	72.43		
100	0.032	0.047	0.243	86.83	80.66		
200	0.026	0.020	0.243	89.30	91.77		

Table 3 Percent inhibition of standard ascorbic acid

Concentrations (µg/ml)	Absorbance		Inhibition (%)	IC_{50} value (µg/ml)
	Ascorbic acid	Control		
0.3906	0.198	0.243	18.52	2.79
0.7813	0.196	0.243	19.34	
1.5625	0.175	0.243	27.98	
3.125	0.116	0.243	52.26	
6.25	0.076	0.243	68.72	
12.5	0.010	0.243	95.88	

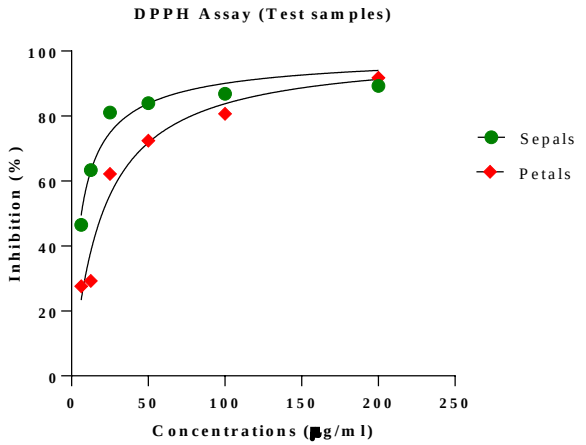


Figure 11 Line graph showing percent inhibition of ethanolic floral leaves extracts of *Delonix regia* (Bojer ex. Hook.) Raf.

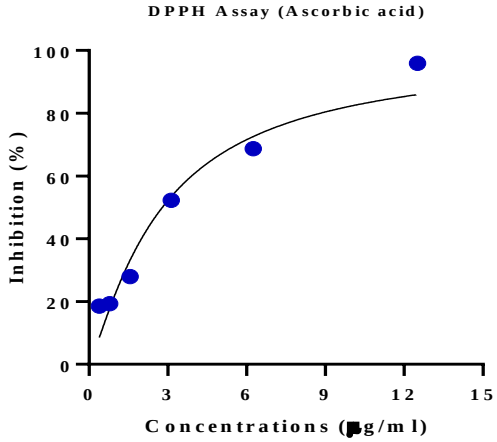


Figure 12 Line graph showing percent inhibition of standard ascorbic acid

Discussion and Conclusion

In this paper, morphological and histological character of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf. were studied and the determination of their phytochemical constituents and antioxidant activities were performed. Some distinct morphological characters were the presence of bipinnately compound leaves, caducous leaf-like stipules, showy and bright colored flowers, sepals color, different petals size and color etc. These characters were in agreement with those mentioned by Hooker (1875) and Backer (1963).

The histological characters of sepals and petals were similar with leaves. The lower surface of sepals was green and possesses stomata for photosynthesis. Wallis (1967) reported that the mesophyll of sepals is usually undifferentiated and resembles the spongy tissue of a foliage leaf. In this study, the mesophylls of sepals were undifferentiated into palisade and spongy layers. The histology of lobed petals was similar with lamina. The presence of papillae is agreed with Wallis (1967) who reported that this is the useful anatomical characters of petals. Clawed petals were very similar with petiole structure. Starch sheath was found around the vascular bundles. According to Borghi & Fernie (2017), these stored carbohydrates may be for the bright red color appearance of this flower.

In phytochemical study, alkaloids, saponins and reducing sugar were not found in petals. This study pointed out that the sepals possess more phytochemical constituents than the petals. Shabir, *et al*., (2011) have reported that IC_{50} value of this flower was 14.80 μg/ml in DPPH method. Vivek *et al*., (2013) also stated that IC_{50} value was 24.88 μg/ml. However, their reports were for all floral parts of this plant. In this study, the antioxidant activities of sepals and petals were separately reported. IC_{50} value for sepals is 6.44 μg/ml and 20.04 μg/ml for petals.

Gan *et al*., (2017) mentioned that polyphenols and alkaloids are major antioxidants. Phenolic compounds and flavonoids (including anthocyanin) are polyphenols (Miguel, 2011). In this study, these polyphenols were detected qualitatively in both parts but alkaloids were not present in petals. Therefore, it can be concluded that the more effective free radical scavenging activity of sepals may be due to the presence of more antioxidant compounds than petals. The quantitative determinations of antioxidant compounds from this flower will be studied in future. Further researches may also be carried out to isolate the pure antioxidant compounds from the floral parts of this plant, especially from sepals.

Acknowledgements

I would like to express my gratitude to Professor Dr Nu Nu Ye, Head of Botany Department, University of Mandalay for her supporting to do this research. I would also like to thank to Professor Dr Aye Pe, Head of Botany Department, University of Yangon for his permission to carry out this research.

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OPTIMIZATION OF SOME FERMENTATION PARAMETERS, IDENTIFICATION OF THE ENDOPHYTIC FUNGUS MHA-10 AND EXTRACTION OF CRUDE EXTRACT

Myo Htaik Aung[1], Kyaw Kyaw Lwin[2] and Mya Min Min Myo[3]

Abstract

Endophytic fungi is a rich source of novel organic compounds with interesting biological activities and a high level of structural diversity. In the present study, endophytic fungus MHA-10 was isolated from the leaves of *Dioscorea birmanica* Prain and Burkill. The pure fungal culture was inoculated in fermentation medium with different growth parameters. In the present investigation, the effect of pH was studied by varying pH 3, 4, 5, 6, 7, 8 and 9. The maximum antibacterial activity was recorded at pH 7 (21.86 mm, inhibitory zone) against *Bacillus subtilis*. Antibacterial activity was observed at different temperatures such as 20ºC, 25ºC, 30ºC, 35ºC and 40ºC. Maximum antibacterial activity by fungal isolate MHA-10 was recorded at 30ºC (19.84 mm, inhibitory zone) against *Bacillus subtilis*. In the comparison of static and shaking culture, the antibacterial activity of shaking culture (20.89 mm, inhibitory zone) was more than that of static culture (19.01 mm, inhibitory zone). Maximum antibacterial activity reached at 5 days fermentation period (22.38 mm, inhibitory zone) against *Bacillus subtilis.* In the paper chromatography study, ethyl acetate is suitable for the extraction of crude extract from the fermented broth. Based on the macroscopical and microscopical characters, fungus MHA-10 was identified as *Trichoderma* sp.

Keywords: endophytic fungi, growth parameters, *Trichoderma* sp.

Introduction

Endophytes are microorganisms that are present in living tissues of various plants, establishing mutual relationship without apparently any symptom of diseases (Strobel and Daisy, 2003). It has been known that endophytic fungi are important source of bioactive compounds (Pan *et al.,* 2008).

Several environmental factors, such as temperature, pH and incubation period, play a major role in the production of antimicrobial agents (Lin *et al.,* 2010).

pH is also a very important in parameter because inappropiate pH may change overall physiological and physical environment of microorganisms resulting in decrease in the production of desired product (Verma and Debnath, 2017).

Temperature is known to influence directly the overall growth and development of any organism. It affects the physiology and subsequently the synthesis of various metabolites (Pandey *et al.,* 2005).

Paper chromatography is one of the types of chromatography procedures which run on a piece of specialized paper. It is planar chromatograph system wherein a cellulose filter paper acts as a stationary phase on which the separation of compound occurs. Extraction is a separation process consisting in the separation of a substance from a matrix.

Thin layer chromatography is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identify of compound and the purity of a compound (Geiss, 1987).

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Many species in the genus *Trichoderma* can be characterized as opportunistic avirulent plant symbionts (Harman *et al.*, 2004). The fungal *Trichoderma* species inhabiting healthy tissues of host plants as endophytic fungi (Wu *et al.*, 2011).

The aim and objectives of this research were to find out the effect of pH, temperature, shaking and static and fermentation period on the antibacterial activity of fungal isolate MHA-10, to investigate extraction of crude extract from endophytic fungus by using solvent system and to identify the selected fungal isolate MHA-10.

Materials and Methods

Isolation of endophytic fungi

Endophytic fungi MHA-10 was isolated from the leaves of *Dioscorea birmanica* Prain and Burkill, which was collected from Mawlamyine University Campus.

The leaves were thoroughly washed in running tap water. Then the leaf segments were surface sterilized by immersion in 70% ethanol for 1 min and rinsed in sterile distilled water for 1 min. And then the materials were immersed in 70% ethanol for 30 seconds and finally rinsed in sterile distilled water for 1 min and blot-dry.

The surface sterilized leaf segments were evenly spaced in petridishes containing isolation medium (glucose 1.0 g, yeast extract 0.5 g, $MgSO_4$ 0.01 g, K_2HPO_4 0.01 g, agar 1.8 g, distilled water 100 mL) amended with 250 mg/L chloramphenicol. The petridishes were incubated at room temperature and monitored every day for the growth of endophytic fungal colonies from leaf segments. The hyphae, which grew out from leaf segments were isolated and brought into pure culture. The isolated endophytic fungi were identified down to genus level using standard manuals.

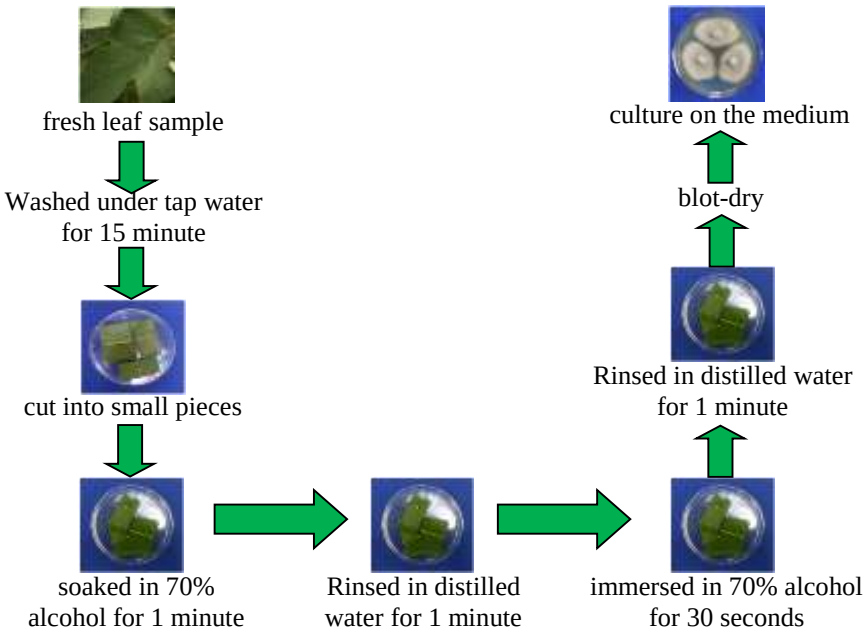


Figure 1 Procedure for isolation of endophytic fungi (BRBDC, 2015)

Fermentation of isolated fungus MHA-10

The isolated fungus MHA-10 was incubated into conical flask containing seed medium (glucose 1.0 g, yeast extract 0.5 g, $MgSO_4$ 0.001 g, KNO_3 0.1 g, distilled water 100 mL) for 3 days. After incubation, the seed culture (30%) was transferred into the conical flask containing

fermentation media (glucose 1.5 g, yeast extract 1.0 g, $MgSO_4$ 0.001 g, KNO_3 0.1 g, distilled water 100 mL). The fermentation period was 3-7 days.

Optimization of some parameters for maximum production of bioactive compounds

Effect of pH on the antibacterial activity of fermentation broth of fungus MHA-10 against *Bacillus subtilis*

The optimization of pH of the fermentation broth for antibacterial activity was done by carrying out the fermentation at seven different pH values viz, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0.

Effect of temperature on the antibacterial activity of fermentation broth of endophytic fungus MHA-10 against *Bacillus subtilis*

The optimization temperature for antibacterial activity was carried out at five different temperatures viz. 20ºC, 25ºC, 30ºC, 35ºC and 40ºC.

Effect of static and shaking condition

250 mL conical flask containing 100 mL of the fermentation medium was incubated on the shaker (150 rpm). Another flask was incubated under static condition without shaking (Figure 2).

Figure 2 Comparison of Static and Shaking culture of fermented broth

Paper chromatography (Tomita, 1988)

Paper chromatography was carried out to extract the crude extract from the fermented broth by the method of Tomita, 1988. The purpose of paper chromatography is to extract the crude extract using suitable solvent systems.

Solvents employed in Paper chromatography

1. 20% NH_4Cl
2. Water saturated n-BuOH
3. n-BuOH-acetic acid-water (3:1:1)
4. Water saturated ethyl acetate

Preparation of PPC

20% ammonium chloride, n-butanol, 3 n-butanol: 1 acetic acid; 1 water and ethyl acetate were used as solvents. The fermented broth samples (100 µL) were applied on the papers and allowed to dry. The paper was immersed 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.

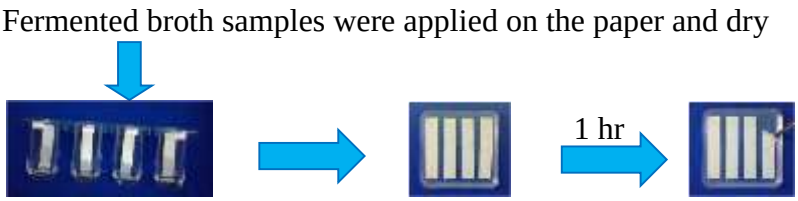


Figure 3 Preparation of paper chromatography

Extraction of the crude extract from fermented broth of the fungus MHA-10

Fermented broth of the fungus MHA-10 was filtered with filter paper to separate the mycelia and the filtrate. To the filtrate equal volume of ethyl acetate was added, shaked well for 30 min and the organic layer was separated and collected (Figure 4).

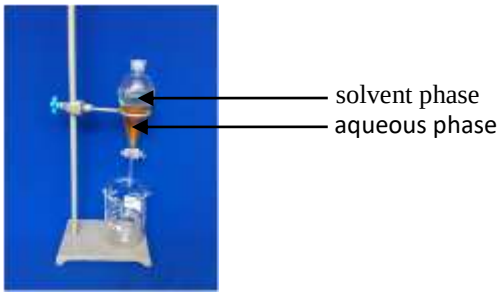


Figure 4 Extraction of the crude extract with ethyl acetate

Thin layer chromatography and Bioautographic Overlay Assay

(Touchstone, 1992 and Aszalos, 1980)

The obtained ethyl acetate extract samples (20 µL) were applied on the TLC plate and allow to dry. The TLC plates were developed in the solvents of chloroform and chloroform-methanol mixture (9:1, 8:2, 7:3) and Hexane and Hexane-ethyl acetate mixture (9:1, 8:2, 7:3). Then, bioautography was done to check the antibacterial activity of each. Each TLC plate was placed on assay agar plates, and then the plates were incubated for 24 hours. In this case, the inhibitory zone was measured yielding an R_f value for the corresponding antibacterial compound. The R_f value can be calculated as

$$R_f \text{ value} = \frac{\text{Distance of compound from origin}}{\text{Distance of Solvent front from origin}}$$

Results

Isolation of Endophytic Fungi

Fungus MHA-10 was isolated from the leaves of *Dioscorea birmanica* Prain and Burkill.

Habit

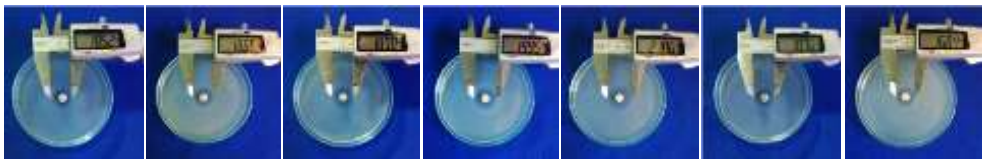
Figure 5 *Dioscorea birmanica* Prain and Burkill

Effect of pH on the antibacterial activity of fermentation broth of fungus MHA-10 against *Bacillus subtilis*

In the present study, pH 7 (21.86 mm) showed the highest antibacterial activity against *Bacillus subtilis* (Table 1, Figure 6).

Table 1 Effects of different pH on the antibacterial activity of selected fungus MHA-10 against *Bacillus subtilis*

pH	inhibitory zone, mm
3	18.52
4	18.66
5	18.98
6	19.95
7	**21.86**
8	18.76
9	16.84



pH-3 pH-4 pH-5 pH-6 pH-7 pH-8 pH-9

Figure 6 Effects of different pH on the antibacterial activity of selected fungus MHA-10 against *Bacillus subtilis*

Effect of temperature on the antibacterial activity of fermentation broth of endophytic fungus MHA-10 against *Bacillus subtilis*

Maximum antibacterial activity was recorded at incubation temperature 30ºC (19.84 mm) and it was followed by 25ºC (17.15 mm) (Table 2, Figure 7).

Table 2 Effect of temperature on the antibacterial activity of fungus MHA-10 against *Bacillus subtilis*

Temperature (ºC)	inhibitory zone, mm
20	14.24
25	17.15
30	**19.84**
35	16.23
40	13.98

20ºC 25ºC 30ºC 35ºC 40ºC

Figure 7 Effect of temperature on the antibacterial activity of selected fungus MHA-10 against *Bacillus subtilis*

Table 3 Differences between Static and Shaking activities of selected fungal isolate MHA-10 against *Bacillus subtilis*

Fermentation condition	Inhibitory zone, mm
Static	19.01
Shaking	**20.89**

The antibacterial activity of shaking culture (20.89 mm) was more than that of static culture (19.01 mm).


Static Culture

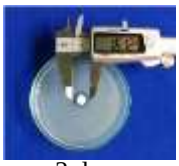

Shaking Culture

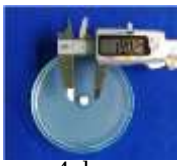
Figure 8 Differences between Static and Shaking activities of MHA-10 against *Bacillus subtilis*

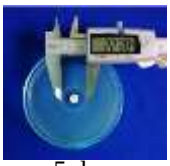
Table 4 Time course of fermentation for the antibacterial activity against *Bacillus subtilis*

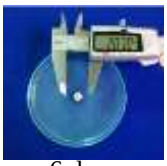
Fermentation period (days)	Inhibitory zone, mm
3 day	16.12
4 day	19.02
5 day	**22.38**
6 day	20.30
7 day	19.12
8 day	18.30
9 day	17.63
10 day	16.50

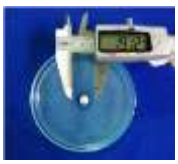
Fermentation was carried out with 84 hrs age and 30% size of inoculum, pH 7.0, 30ºC and FM-1 (glucose 2.0 g, yeast extract 1.0 g, $MgSO_4$ 0.001 g, KNO_3 0.1 g, distilled water 100 mL)


3 days


4 days


5 days


6 days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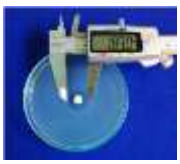
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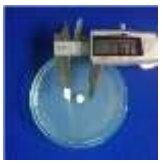

9 days

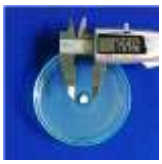
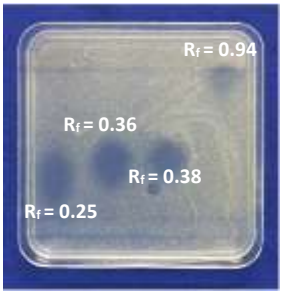

10 days

Figure 9 Antibacterial activity on fermentation period against *Bacillus subtilis*

Paper chromatography to extract crude extract

It was observed that R_f values are 0.25 in 20% NH_4Cl, 0.36 in n-butanol, 0.38 in n-butanol: acetic acid: water (3:1:1) and 0.94 in ethyl acetate. According to the R_f value (Figure 10) it was considered that active compound could be extracted with ethyl acetate.



1. 20% NH_4Cl
2. Water saturated n-BuOH
3. n-BuOH-acetic acid-water (3:1:1)
4. Water saturated ethyl acetate

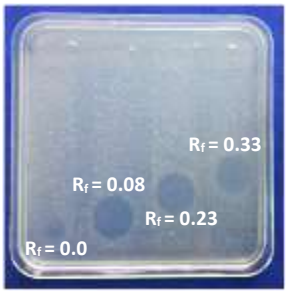
Figure 10 Paper Chromatography Bioautographic Assay

Thin layer chromatography

Thin layer chromatographies were developed by chloroform and chloroform methanol mixture (9:1, 8:2 and 7:3) and hexane and hexane ethyl acetate mixture (9:1, 8:2 and 7:3). The results were shown in Figure 11 and 12.

1. Chloroform only
2. Chloroform-methanol (9:1)
3. Chloroform-methanol (8:2)
4. Chloroform-methanol (7:3)

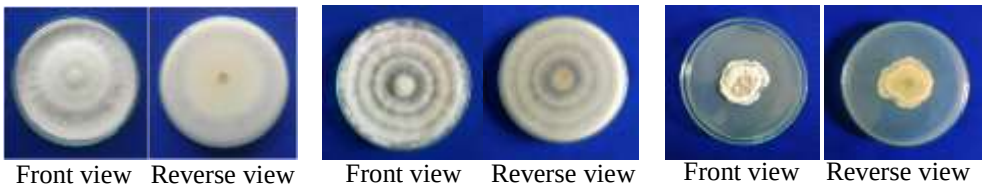
Figure 11 Thin Layer Chromatography with chloroform and chloroform-methanol mixture



1. Hexane only
2. Hexane-ethyl acetate (9:1)
3. Hexane-ethyl acetate (8:2)
4. Hexane-ethyl acetate (7:3)

Figure 12 Thin Layer Chromatography with Hexane and Hexane-ethyl acetate mixture

Identification of the fungus MHA-10



Front view Reverse view on PDA medium

Front view Reverse view on MEA medium

Front view Reverse view on YEA medium

Front view Reverse view on CA medium

Front view Reverse view on OA medium

Figure 13 Macroscopical character of fungus MHA-10

Table 5 Macroscopical characters of MHA-10 on different media

Medium	colour		size
	front	reverse	
MEA	white	pale yellow	9.0 × 9.0 cm
	1-2 concentric ring		
YEA	white	pale yellow	4.1 × 3.8 cm
	with concentric ring		
CA	white	white	9.0 × 9.0 cm
OA	white with dull green patch	white with dull green patch	9.0 × 9.0 cm
PDA	white	pale yellowish	9.0 × 9.0 cm

PDA - Potato Dextrose Agar, MEA - Malt Extract Agar
CA - Corn Agar, OA - Oatmeal Agar,
YEA - Yeast Extract Agar

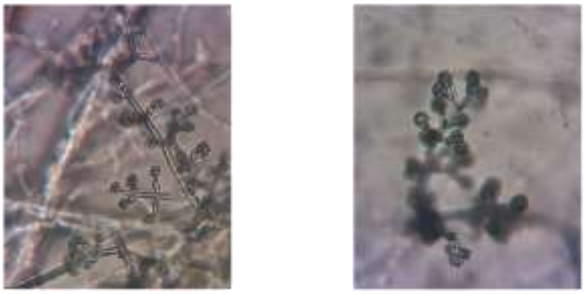


Figure 14 Photomicrograph of MHA-10 (X 400)

Figure 15 Chlamydospore X 400

Macroscopical characters of MHA-10

Colonies grow rapidly and mature in 7 days. On potato dextrose agar, the colonies are wooly. From the front, the color is white. As the conidia are formed, scattered yellow-green patches become visible. These patches may form concentric rings. Reverse is yellowish white.

Microscopical characters of MHA-10

Hyphae are hyaline, septate. Conidiophores are hyaline, branched and display a pyramidal arrangement. Phialides are hyaline, flask-shaped, and inflated at the base. They are attached to the conidiophores at right angles. The phialides are arranged in clusters. Conidia are one-celled and round in shape. They are smooth-walled and grouped in sticky heads at the tips of the phialides. The color of the conidia is green.

These above characters of strain MHA-10 are similar to those of *Trichoderma* (Barnett, 1969).

Key to the genus *Trichoderma* (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

B1 Prasitic on soil-inhabiting rhizopods or nematodes ----------(ZOOPAGALES)

B2 Not parasitic on small, soil-inhabiting animals ----------(MUCORALES)

C1 Conidia more or less coiled or spirally curved, hyaline or dark ----------(parts of Moniliaceae, Dematiaeae 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 ----------H2

H1 Conidiophores simple or sparingly branched; phialides, if present, not tightly clustered

* H2 Conidiophores mostly branched, sometimes simple, phialides, if present, in groups or clusters ----------I2

I1 Conidia catenulate

* I2 Conidia not catenulate ----------J2

J1 Large, conspicuous, rough-walled chlamydospores present

* J2 Large, rough-walled chlamydospores absent ----------K1

* K1 Conidia produced apically on phialides or branches of conidiophore ---------- L2

K2 Conidia attached both at apex and on sides of conidiophores or its branches

L1 Branches of conidiophore verticillate (at least the larger conidiophores)

* L2 Branches of conidiophore not verticillate, irregular ----------M2

M1 Aquatic on submerged leaves ----------Dimorphospora

* M2 Not aquatic ----------N1

* N1 Conidia held in heads by slime drops ----------O2

N2 Conidia not in slime drops, dry

O1 Conidiophore brush-like, similar to *Penicillium* ----------*Gliocladium*

* O2 Conidiophore branches spreading ----------*Trichoderma*

Classification of endophytic fungus MHA-10

Kingdom	Fungi
Phylum	Ascomycota
Class	Hyphomycetes
Order	Moniliales
Family	Moniliaceae
Genus	*Trichoderma*

Discussion and Conclusion

The antibacterial activity of endophytic fungi MHA-10 was observed on different pH (3, 4, 5, 6, 7, 8 and 9). The result indicated that pH 7.0 was suitable for maximum production of antibacterial activity. Similar reports were given by Verma *et al.,* (2017) and Hassan and Bakhiet (2017). Physical factors such as incubation temperature, can exert different effects on the growth and production phases of secondary metabolism (Rizk *et al.,* 2007). In the present study, maximum antibacterial activity was recorded at 30ºC. In the investigation of the static and shaking condition, shaking condition is the optimum condition for MHA-10 fermentation. Similar observations were made by Hassan and Bakhiet (2017).

Fermentation was undertaken with 84 hrs age and 30% size of inoculum, pH 7.0, temperature 30ºC and FM-1 for 8 days fermentation period. Maximum antibacterial activity was recorded at 5 days fermentation period (22.38 mm, inhibitory zone) against *Bacillus subtilis.*

In conclusion, process parameter like pH 7.0, temperature at 30ºC, 5 days of fermentation period under shaking condition were found to be optimum for maximal production of antibacterial metabolite.

In paper chromatography, four kinds of different solvents were used to observe the optimum extraction ability. According to R_f value, ethyl acetate showed the excellent extraction than other. Therefore, solvent No.4 ethyl acetate is suitable for the extraction of crude extract from the fermented broth.

In thin layer chromatography, chloroform and chloroform-methanol mixture (9:1, 8:2, 7:3) and hexane and hexane-ethyl acetate mixture (9:1, 8:2, 7:3) were used. The isolated MHA-10 was identified by studying the colony morphology on PDA medium and microscopic analysis of reproductive structure. The isolate grew rapidly on PDA medium forming a cottony white colony. The front color is white and the reverse color is pale yellowish. Conidiophores are hyaline and branched, conidia are one cell and globose. Conidia occurred in clusters. These characters were in agreement with those mentioned by Barnett (1969) and Kamala *et al.,* (2015). Based on the above observations, the isolate MHA-10 was identified and assigned to the genus *Trichoderma.*

Acknowledgements

I would like to express my gratitude to Dr Si Si Hla Bu, Rector, Pathein University, for her various guidance, suggestion and permissions to do the research. I am also grateful to thanks Dr Than Tun Pro-rector, Pathein University, for his suggestion and advices. I wish to express most sincere gratitude to Dr Wah Wah Lwin, Professor and Head, Department of Botany, Pathein University, for her guidance, invaluable suggestions and comments offered in writing this research. I also wish to express my thank to Dr Min Min Soe, Professor, Department of Botany, Pathein University, for her encouragement and suggestion for this paper. Many thanks are due to my supervisor, Dr Kyaw Kyaw Lwin, Lecturer, Department of Botany, Pathein University, for his advice, encouragement, understanding and cooperation of this research. My thanks are also extended to all of my friends for their understanding and kind help.

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HISTOLOGICAL CHARACTERS, NUTRITIONAL VALUES OF FRUITS FROM *ZANTHOXYLUM ARMATUM* DC. AND ITS ANTIMICROBIAL ACTIVITY

Yin Yin Khaing[1], Myat Myat Moe[2]

Abstract

Zanthoxylum armatum DC. is an aromatic medicinal plant belonging to the family Rutaceae has been undertaken. They were collected from Loikaw Township, Kayah State during from September to November 2019. The collected plants were identified, classified, and studied by the literature. The morphological characters of vegetative and reproductive parts and microscopical characters of the leaves and fruits were conducted to ascertain their correct identification. The plant was small tree with thorny branches. Leaves were distinctly trifoliate with wing leaf-stalk. Inflorescence were terminal, axillary and bracts presented. Petals were absent. For microscopical studies, lamina, midrib, petiole and fruits were examined by preparing free hand sections. The upper surface of the lamina was polygonal in shaped and lower surface was wavy. Stomata present only on lower surface were numerous and anomocytic type. In transverse section of lamina, calcium oxalate crystals were presented in palisade layer. The preliminary phytochemical tests were examined from the powdered fruits. Alkaloid, carbohydrate, flavonoid, glycoside and phenolic compound, saponin and tannin were present in this examination. The nutrient contents of powdered fruits were analyzed protein, fat, fiber and carbohydrate by using David Pearson and Kieldaha method. Antimicrobial activities of various crude extracts of fruits were carried out by using paper disc diffusion method with six test organisms. Acetone and ethyl acetate extracts indicated more effective against on Candida albicans than other tests organisms. Medicinal plants contained numerous biologically active compounds which are used for helpful in improving the human health.

Kewwords : *Zanthoxylum armatum*, thorny, anomocytic, *Candida albicans*

Introduction

Nature has been a source of medicinal agent for thousands of years. Herbal medicine represents one of the most important fields of traditional medicine all over the world. Different extracts from traditional medicinal plants have been tested to identify the source of therapeutic outcome. Developing countries still depend mainly on medicinal herbs due to their cheaper cost and their intervention in the treatment of various infectious diseases with lesser side effects (Butkhup *et al*, 2011).

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought about by the acknowledgment of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care (Taylor and Attaur, 1994).

Zanthoxylum armatum DC. is an aromatic tree or shrub with winged petiolate prickly imparipinnate compound leaves. Flowers are small and polygamous. Male flowers have 6 to 8 stamens with rudimentary ovary. Female flowers have 1-2 carpels. Fruit is drupe, splitting into two when ripe. Seeds are rounded and shining black. The plant grows in shady or semi shady habitat at altitude from about 800m up to 1500m. In many tropical countries, rural people traditionally harvest of leafy vegetables and fruits from wild because of its taste, cultural uses, as food supplements or to tide over food shortage (Kebu and Fassil, 2006).

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A family of about 140 genera and 1200 species, widely distributed in temperate and tropical regions of northern and southern hemispheres but most numerous in South Africa and Australia. The Rutaceae are distinguished by the presence of translucent pellucid dots in the foliage, the lobed ovary evaluated on a disc, and the outer stamens usually opposite the petals (Lawrance, 1964). The fruit of several species is used to make the spice Sichuan pepper.

Zanthoxylum armatum DC is small tree mostly with a strong pungent and aromatic smell. The seeds and barks are used in fever, dyspepsia and cholera. The fruits and thorn are used as remedy for toothache, also as stomachic and carminative. Various parts of *Zanthoxylum armatum* DC. are used in the preparation of tooth powder and medicinal purposes. It is an evergreen shrub or small tree with stem and branches, sharp prickles. It's also found throughout China, Japan, Pakistan, Nepal, and Malaysia. Its seeds and barks are used for the treatment of various diseases such as fever, cholera, heartburn or indigestion. Fruits and seeds of this plant are used in fever, dyspepsia and skin diseases. Leaves and barks were reported for hepatoprotective activity, anticancer and antidiabetic activity The nutritional values and mineral contents of these fruits were richer than that of the commercial fruits and could be used for nutritional purpose (http://florajournal.com).

A family consisting mainly of trees or shrubs and a very small proportion of herbs all of which flourishing in the tropics and sub-tropics. The ground tissues of both leaf and axis is nearly always characterized by the presence of secretory cavities appear to the naked eyes as transparent dots in the leaf. The usually abundant crystals of calcium oxalate may be either solitary or clustered or a mixture of both of these types (Melcheft & Chalk, 1950).

Bioactive constituents such as alkaloids, sterol, saponins, tannins, phenols and flavonoids were quantitatively evaluated in leaves, barks and fruits of *Zanthoxylum armatum* DC. The results bring out the presence of bioactive constituents in the fruits contained alkaloids, sterols, saponins, phenol and flavonoids (http://academicjournals.org).

Immature shoots are used as toothbrush and useful for curing gum diseases. Its fruits and seeds are edible and used as potherb species. Fruit is utilized for toothache, dyspepsia, as carminative and for stomachache. The seeds are applied as condiment and seasoning agent (Arshad and Ahmad, 2004).

Zanthoxylum armatum DC. (Rutaceace), also known as toothache tree residents an important the history of Indian system of medicines. It is used as carminative, stomachic and anthelmintic and in the intervention of toothache. It contains volatile oil with active constituents such as limonene and lagan. Its fruits branches and thorns are in general used as carminative, stomachic, and remedy for toothache. In India, different parts of the plants are used in Ayurvedic practices for the intervention of skin diseases, abdominal pain, anorexia, and ataxia. This review focuses on the detailed phytochemical composition and medicinal uses along with pharmacological activity of different parts (nopr.niscair.res.in/handle).

Aspergillus flavus is found through the world as saprophyte in soils and causes disease on many important agriculture crops. In mammals, pathogen can cause liver cancer through consumption of contaminated feed through invasive growth.

Bacillus species are bacteria used as a soil inoculate in agriculture and horticulture. *Candida albicans* is a genus of yeast that can cause fungal infection in human and other animals (Cruickshank *etal*.,1968).

Escherichia coli are a gram-negative bacteria and motile by peri trichous flagella or non-motile. The genus of *E. coli* occurs in the human intestinal tracts (Ronald, 1988).

Pseudomonas fluorescens (an antibiotic) can be produced, which has been found to be utile in treating of skin, ear, and eye disorders. *Pseudomonas fluorescens* is a common Gram-negative, rod-shaped bacterium.

Xanthomonas oryzae are slow-growing, mucoid and straw-colored to yellow in colour and those are fairly slow-growing, usually pale-yellow, round,

smooth, domed and mucoid. Both bacteria are Gram-negative rods, capsulated and motile with a polar flagellum. The bacterium gets in the leaf through stomata or wounds. Spread within a crop occurs by mechanical contact and in rain and irrigation water; under favorable warm wet conditions, rapid and terrible disease development can occur (Reddy,1984).

In this paper, morphological and microscopial characters of fresh specimens, preliminary phytochemcial tests, nutritional values and antimicrobial activity of the dried powdered fruits of *Zanthoxylum armatum* DC., were carried out. The aim of the present was studied to identify the plant of *Zanthoxylum armatum* DC., to examine the microscopical characters of leaves and fruits, nutritional values and to study the phytochemical analysis and antimicrobial activities of the fruits.

Materials and Methods

Botanical Studies

The specimens of *Zanthoxylum armatum* DC. were collected from Loikaw Township, Kayah State during from June to November 2019. After collection, the specimens were identified with the help of available literatures Hooker (1885), Kirtikar and Basu (1935), Dutta (1979), Dassanayake (1999). Both the vegetative and reproductive parts of the fresh specimens were used for the morphological and microscopical characters studies.

The samples were washed and dried at room temperature and then crushed into powder to study the powdered characteristic. For microscopical studies, leaves and fruits were examined by preparing free hand sections from the fresh specimens, according to the methods by Esau (1965), Metcalfe and Chalk (1950), Trease and Evans (1978), Pandey (2004) and Tandon (2011). Chloral hydrate solution was used as a clearing reagent. The presence of calcium oxalate crystals was tested by 80% sulphuric acid.

Chemical Studies

Preliminary Phytochemical Examination

Preliminary phytochemical study the fruits of *Zanthoxylum armatum* DC. has been conducted with test reagent in Department of Botany, Dagon University. The experiment was carried out to determine the presence or absence of alkaloids, amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch and tannin according to the methods described by using Vogel (1956), Marini-Bettalo (1981).

Nutritional values of Powdered fruits of *Zanthoxylum armatum* DC.

Nutritional values

The experimental process for the nutritional contents in powdered fruits of *Zanthoxylum armatum* DC. was carried out at the Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigiation, North Okkalapa Township, Yangon Region. According to the experiments carbohydrate, protein, fiber, fat and moisture were analyzed by using the method of David Pearson (1976) and Kjeldahl (1883).

Antimicrobial Activities

Microorganisms were divided into four main types, bacteria, fungi, virus and protozoa. The most microorganisms were free living and performed useful activity that benefits animals and plants life. Bacteria are pathogenic microorganisms with a relatively simple and primitive form of cellular organization and are smaller than those of protozoa and fungi (Cruickshank, 1968).

Various crude extracts of powdered fruits such as acetone, ethyl acetate, ethanol, methanol, pet-ether and distilled water extracts were used for antimicrobial activities. Screening of antimicrobial activity was done by paper disc diffusion assay according to Madigan and Martinko, 2005 at microbiology lab Department of Botany, University of Yangon. The six test organisms were utilized for antimicrobial activity. The assay medium (agar 2.0 g, sucrose 1.0g, pH 7.0) was utilized for these test organisms. Test organisms (0.3ml) was added to 1000 ml assay medium, then poured into plates. After solidification, about 0.2 ml of crude extract was impregnated on to the paper disc with the size of 6mm diameter on the test agar plate and these plates were incubated for 24-36 hours at 30C. After 24-36 hours, clear zones surrounding the test discs were measured.

Table 1 Type of microorganisms, their respective code numbers and diseases

No.	Test organisms	Source	Diseases
1.	*Aspergillus flavus*	-	Bronchitis
2.	*Bacillus subtilis*	JAP-0225215	Pathogenic group, anthrax in animals
3.	*Candida albicans*	IFO-1060	Skin infection, veginal candidiasis alimentart tract infection
4.	*Escherichia coli*	ACTT-25922	Cholera, diarrhea and vomiting urinary tract infection
5.	*Pseudomonas fluorescens*	-	Bacteria for leaf blight
6.	*Xanthomonas oryzae*	-	Bacteria for leaf blight

Results

Botanical Studies

Morphological characters of *Zanthoxylum armatum* DC.

Scientific name : *Zanthoxylum armatum DC.*
Myanmar name : Mak-kha
English name : Winged Prickly Ash
Family : Rutiaceae
Flowering and fruiting period : August- December
Distribution : Wildly grows in cold region Myanmar.
Part used : Fruits

Tree; stems woody with thorny branches. Leaves were distinctly trifoliate with the wing leaf-stalk. Leaflets were stalk less. Inflorescence racemose. Petals were absent

Microscopical Characters of leaves of *Zanthoxylum armatum* DC.

Lamina

In surface view, epidermal cells of both surfaces were parenchymatous cells, thin-walled and compactly arranged. Anticlinal walls of the lower surface were wavier than the upper ones. Stomata were absent on upper surface and abundant in lower surface. They were anomocytic type.

Stomata were elliptic in shape with very small pores; guard cells were reniform shape with chloroplast.

In transverse section, the lamina was dorsiventral and smooth cuticle present on both surfaces. The upper epidermal cell was one layer on both sides, rectangular in shape and the lower epidermal cells were barrel shaped. The mesophyll composed of palisade and spongy parenchyma. The palisade mesophyll was made up of two layers of vertically elongated cylindrical cells, which were closely packed with one another compactly arranged. The spongy mesophyll consisted of 3-4 layers of cells, irregular to isodiametric shape and loosely arranged. Calcium oxalated crystals were presented in palisade layer.

Midrib

In surface view, the epidermal cells were parenchymatous and compactly arranged and rectangular in shaped. In transverse section, convex at both sides covered with thin cuticle. Both epidermal cells were rounded shaped. The lower epidermal cells were similar to those the upper epidermal cells. Below the epidermis, the cortex was differentiated into collenchyma and thin-walled parenchyma cells. The angular types of collenchymatous cells were 1-2 layers in thickness towards the upper surface and 2-3 layers in thickness towards the lower surface. The parenchyma cells were 3 to 4 layers in thickness above the vascular bundle and 5 to 6 layers in thickness below the vascular bundle. They were thin- walled and irregularly rounded or oval in shape. Intercellular spaces were numerous druses crystals of calcium oxalate were present in both parenchymatous cells. The vascular bundle was rounded or oval in outline, collateral and closed type.

Petiole

In surface view, the epidermal cells were parenchymatous, thin-walled and mostly rectangular in shape and elongated along the length of the petiole. In transverse section, the petiole was oval in outline. The cuticle layer was thick. The epidermal cells were rounded- shaped. Hairs consist of thick or thin-walled multicellular of trichomes were present. The cortex was made up of collenchymatous and parenchymatous tissues. The collenchymatous type tissues consisted of 4 to 5 layers in thickness. The parenchymatous tissues composed of 8 to 9 layers in thickness. The parenchyma cells were oval to isodiametric in shape. Intercellular spaces were numerous among the tissue. Druses of calcium oxalate crystals were present in the cells. The vascular bundles were more or less rounded in outline and embedded in the parenchymatous tissues. Vascular bundles were arranged in shaped, collateral and surrounded by a bundle sheath. The xylem was present in the abaxial side and the phloem was present in the adaxial sides.

Microscopical characters of fruits of *Zanthoxylum armatum* DC.

Fruits

In surface view, epidermal cells of fruit surface were parenchymatous cell. In transverse section, covering with cuticle was presented. The fruit wall showed three well differentiated layers. The epicarp composed of one layer of selerenchymatous cells. Epidermal cells were compactly elongated. The mesocarp was made up of parenchyma tissue about 5 to 6 layers. Oil cavity were presented in mesocarp. The endocarp was found below the mesocarp and consists of 4 to 5 layers of parenchymatous cells.

Figure 1 Habit

Figure 2 Upper and lower surface view of leaves

Figure 3 Inflorescence

Figure 4 Flowers

Figure 5 L.S of Flower

Figure 6 T.S of Ovary

Figure 7 Fruits

Figure 8 Seeds

Morphological characters of *Zanthoxylum armatum* DC

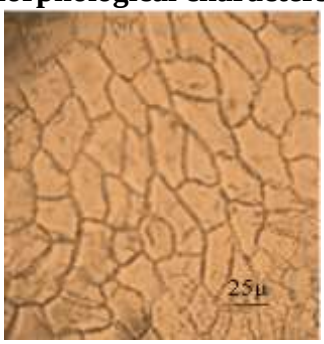


Figure 9 Upper surface view of lamina

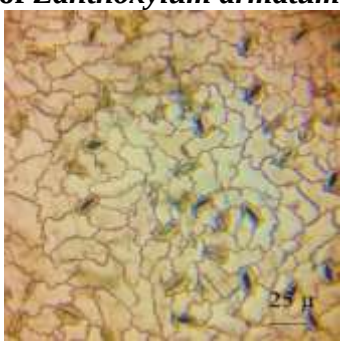


Figure 10 Lower surface view of lamina

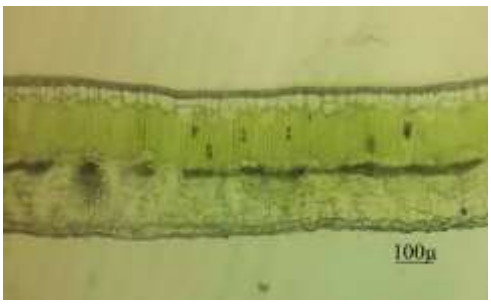


Figure 11 T.S of lamina

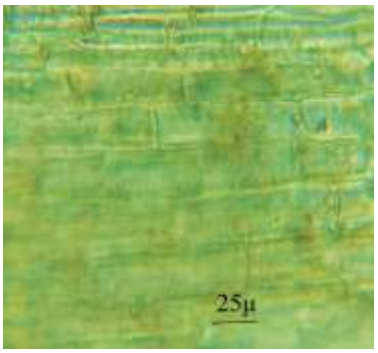


Figure 12 Surface view of midrib

Figure 13T.S of midrib

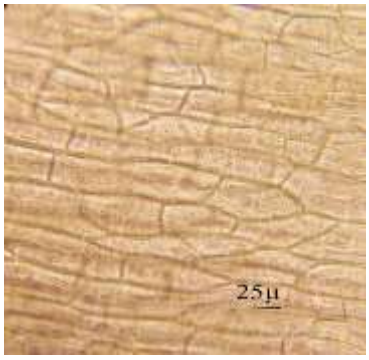


Figure 14 Surface view of petiole

Figure 15 T.S of petiole

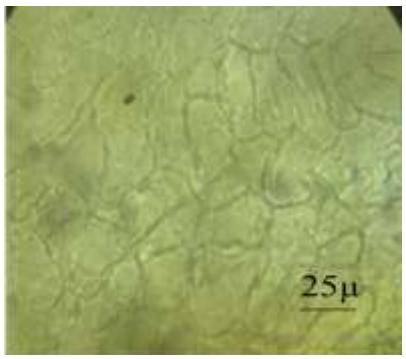


Figure 16 Surface view of fruit

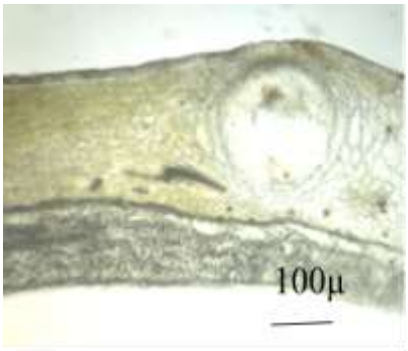
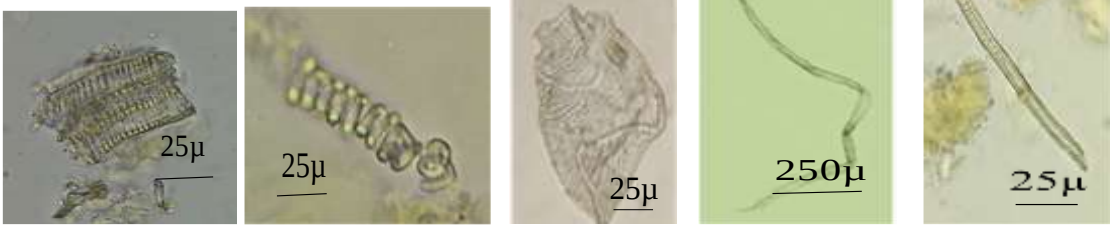


Figure 17 T.S of fruit

Microscopical characters of *Zanthoxylum armatum* DC.



Spiral Vessels Pitted Vessel Fiber Tracheid

Figure 18 Diagnostic Characters of Powdered fruits of *Zanthoxylum armatum* DC.

Chemical Studies

Preliminary Phytochemical Tests

The phytochemical investigation of the fruits *Zanthoxylum armatum* DC. indicated the presence of alkaloids, carbohydrate, glycosides, phenolic compound, saponin, tannins and flavonoid. α -amino acid, starch and reducing sugar were absent in fruits. The results of phytochemical examination were shown in Table (2).

Table 2 Preliminary phytochemical test of powdered fruits from *Zanthoxylum armatum* DC.

No	Test	Extract	Test Reagents	Observation	Result
1	Alkaloid	H_2O, 1%HCL	(1) Mayer's Reagent (2) Wagner's Regent (3) Dragendroff's reagent	White ppt Brown ppt Brown ppt	+ + +
2	α-amino acid	H_2O	Ninhydrin solution	Two layer	-
3	Carbohydrate	H_2O	10% α-naphthol+conc-H_2SO_4	Pink ring, ppt	+
4	Starch	H_2O	I_2solution	Brown color	-
5	Reducing sugar	H_2O	Benedict's solution	Reddish brown	-
6	Glycoside	H_2O	10%lead acetate solution	Pale brown ppt	+
7	Phenolic compound	H_2O	Ferric chloride	Black ppt	+
8	Saponin	H_2O	Distilled water	Frothing	+
9	Tannin	H_2O	Ferric chloride	Dark brown ppt	+
10	Flavonoid	H_2O	Mg turning, Conc HCL	Pale brown ppt	+

(+) = present (-) = absent

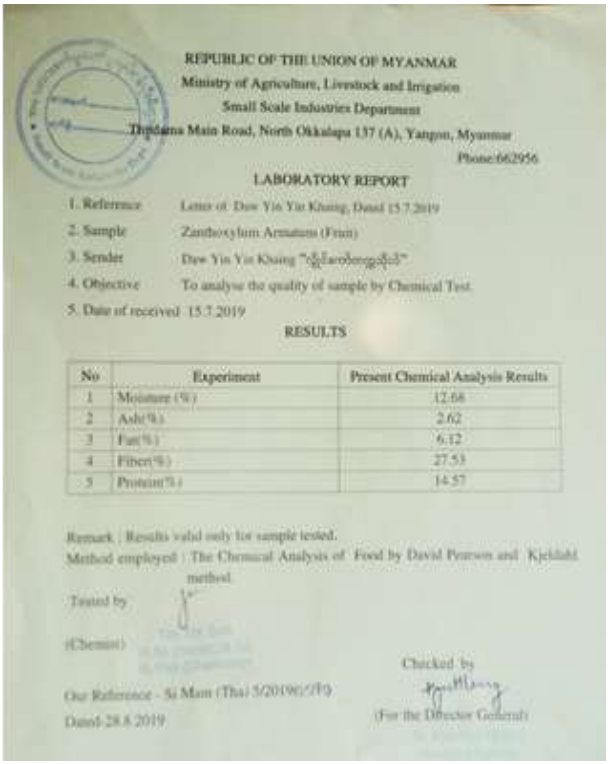
Nutritional values of Powdered fruits of *Zanthoxylum armatum* DC.

Nutritional values

The experimental work for the nutritional contents in powdered fruits of *Zanthoxylum armatum* DC. was carried out. One of the purposes of this research was to study the nutritive values of food content such as carbohydrate, fat, protein, fiber and protein of fruits have been analyzed. The results were shown in Figure (19) and Table (3).

Table 3 Determination of nutritional values from Powdered fruits of *Zanthoxylum armatum* DC.

No	Type of nutrients	Percentage %
1.	Protein	14.57
2.	Moisture	12.68
3.	Ash	2.62
4.	Fat	6.12
5.	Fiber	27.53
6.	Carbohydrate	36.48



REPUBLIC OF THE UNION OF MYANMAR
Ministry of Agriculture, Livestock and Irrigation
Small Scale Industries Department
Thiddana Main Road, North Okkalapa 137 (A), Yangon, Myanmar
Phone:662956

LABORATORY REPORT

1. Reference — Letter of Daw Yin Yin Khaing, Dated 15.7.2019
2. Sample — Zanthoxylum Armatum (Fruit)
3. Sender — Daw Yin Yin Khaing "ဒေါ်ရင်ရင်ခိုင်"
4. Objective — To analyse the quality of sample by Chemical Test
5. Date of received 15.7.2019

RESULTS

No	Experiment	Present Chemical Analysis Results
1	Moisture (%)	12.68
2	Ash(%)	2.62
3	Fat(%)	6.12
4	Fiber(%)	27.53
5	Protein(%)	14.57

Remark : Results valid only for sample tested.
Method employed : The Chemical Analysis of Food by David Pearson and Kjeldahl method.

Tested by
(Chemist)

Checked by
(For the Director General)

Our Reference - Si Mon (Tha) 5/20190(7/PQ
Dated 28.8.2019

Figure 19 Nutritional composition of powdered fruits of *Zanthoxylum armatum* DC.

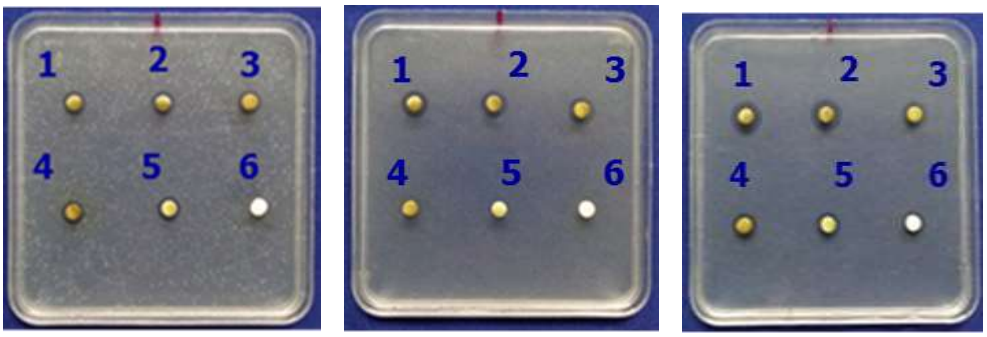
Antimicrobial activities of various fruits extracts

In the results, various crude extracts of powdered fruits *Zanthoxylum armatum* DC. showed the effective on five strains of microorganism. The acetone and ethanol extracts of the fruits were showed the highest activity, especially sensitive against on *Candida albicans*. The results were shown in Figures (20) and Table (4).

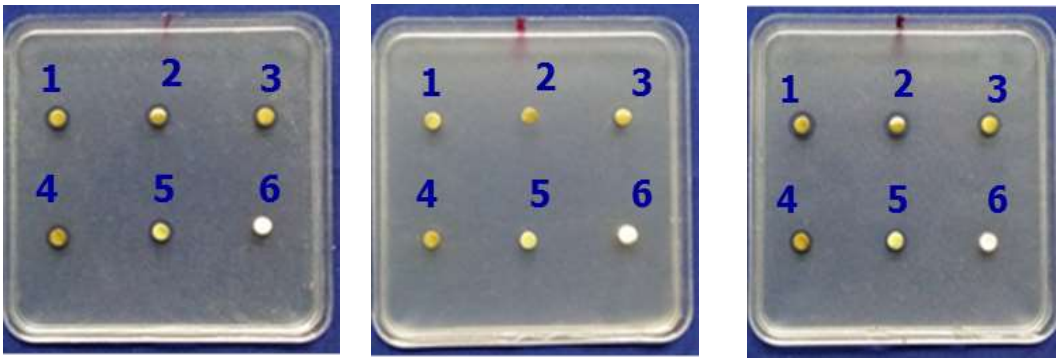
Table 4 Antimicrobial activity from various fruits extracts of *Zanthoxylum armatum* DC.

Extracts	Test Organisms					
	Aspergillus flavous	***Bacillus subtitis***	***Candida albicans***	***Escherichia coli***	***Pseudomonas fluorescens***	***Xanthomonas oryzae***
PE	8 mm	-	10mm	8 mm	-	8 mm
Acetone	8 mm	10 mm	12 mm	8 mm	-	10 mm
EtoAc	8 mm	10 mm	12 mm	8 mm	-	10 mm
EtOH	8 mm	10 mm	10 mm	8 mm	-	8 mm
MeOH	8 mm	-	10 mm	8 mm	-	8 mm
H_2O	-	-	8 mm	-	-	-

Paper disc size – 6 mm



Aspergillus flavous *Bacillus subtitis* *Candida albicans*



Escherichia coli *Pseudomonas fluorescens* *Xanthomonas oryzae*

1. Acetone, 2. EtoAc, 3. EtOH, 4. MeOH, 5. PE, 6. H_2O

Figure 20 Antimicrobial activity of various extracts from fruits of *Zanthoxylum armatum* DC.

Discussion and conclusion

In this paper, the morphological and microscopical studies of leaves, fruits, nutritional values and antimicrobial activity of *Zanthoxylum armatum* DC. have been undertaken.

In morphological study, the plant of *Zanthoxylum armatum* DC. is tree; stems woody with thorny branches. Leaves are alternate, palmate distinctly trifoliate with the wing leaf-stalk. Inflorescence racemose, bracteate, ebracteolate, petals were absent and female flowers have 1-2 ovary, 1- loculed, the ovule solitary and basal, stigma capitates. Fruits are small, ovoid. These characters are in agreement with those mentioned by Hooker (1885), Kirtikar and Basu (1935), Dutta (1979), Dassanayake (1999).

In microscopical study, stomata are abundant on the lower surface. They are anomocytic type. In transverse section of the lamina, palisade cells are present and cluster small of crystals are found in the palisade cells. In transverse section of petiole, the vascular bundles are arranged as a curved line. These characters are in agreement with those stated by Esau (1965), Metcalfe and Chalk (1950), Trease and Evans (1978), Pandey (2004) and Tandon (2011).

In phytochemical test on powdered fruits of *Zanthoxylum armatum* DC. has shown the presence of alkaloids, carbohydrate, glycosides, phenolic compound, saponin, tannins and flavonoid. These factors are described by (http://academicjournals.org).

The nutritional analysis of powdered fruits, protein (14.57%), fat (6.12%), fiber (27.53%) and carbohydrate (36.48%) are obtained from the powdered fruits. These data showed that the fruits are rich sources of nutritional values for human diet. Carbohydrates are body's source for plants and animals that depend on the plants for food. Fiber found in plant cells, cannot be digested but play an essential role in human health. Protein is a major constituent of almost every cell in the body. These factors are mentioned by Passmore (1974).

The antimicrobial activities of various fruits extracts of *Zanthoxylum armatum* DC. fruits such as acetone, ethyl acetate, ethanol, methanol, pet-ether and distilled water extracts were determined by paper disc diffusion method with six different microorganisms. Crude extracts of fruits were indicated that the antimicrobial activities showed against five microorganisms except *Pseudomonas fluorescens* The acetone and ethyl acetate crude extract of fruits showed the highest activity and especially sensitive against on *Candida albicans*.

In conclusion, the species of *Zanthoxylum* include many chemical constituents which are used for medicinal purposes. Hence, the family Rutaceae are not only the medicinal plants but also the economic ones. Therefore, it is sincerely hoped that this present study can be beneficial for the future research.

Acknowledgements

I would like to express my sincere gratitude to Professor Dr Myat Myat Moe, Head of the Botany Department, Dagon University, for providing all departmental facilities and valuable suggestions. I am grateful to Dr Sann Sann Oo, Professor & Head, Botany Department of Botany, Loikaw University for providing all kindly necessary instructions. I want to indebted their sincere thanks to Myanmar Academy of Arts and Science for their allowing submitting it this article.

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MORPHOLOGICAL CHARACTERS, ELEMENTAL ANALYSIS AND NUTRITIONAL VALUES OF *DIOSPYROS DISCOLOR* WILLD. (FRUIT)

Ohmmar Than*

Abstract

Diospyros discolor Willd is known as velvet apple belong to the family Ebenaceae, collected from Bogyoke Village, Thanlyin Township, Yangon Region from January to May 2020. The morphological characters of this plant were identified by using of the literatures. Elemental analysis and nutritional values were carried out by using the powder samples of these fruits. In morphological characters, it is dioecious, evergreen and young leaves are pinkish to pale green. Male flowers are 3-flowered axillary racemes and female flowers are solitary axillary cymes. The female flowers are slightly larger than the male flowers. Sensory characters of powdered fruits were pale-brown, pungent odour, sweet taste, texture with the granular. In elemental analysis, the concentration of potassium was found to be 75.988%. Moreover, in nutritional values of the fruits, carbohydrate is found to be the highest 80.69%.

Keywords: Morphological characters, elemental analysis, nutritional values.

Introduction

Ebenaceae family of only 3 genera and about 500 species distributed in the Indo-Malayan region. There are five genera according to Lawrence (1951), seven according to Hutchinson (1973), Willis (1973) and Kumanr and Subramanian (1987) recognize three genera and Takhtajan (1987) recognizes only two genera.

The Ebenaceae is a family of flowering plants belonging to order Ericales. It includes ebony and persimmon among about 768 species of trees and shrubs. The family is distributed across the tropical and warmer temperate regions of the world. *Diospyros discolor* (commonly known as velvet apple or velvet persimmon) is a tree of the genus Diospyros of ebony trees and persimmons. Its edible fruit has a skin covered in a fine, velvety fur which is usually reddish-brown and soft, creamy, pink flesh, with a taste and aroma comparable to a peach. (https://en.m.wikipedia.org)

Diospyros discolor taste is crisp when mature and will soften slightly as it ripens. The taste is sweet and the flavour has been likened to banana flavoured apples or strawberry - mango yogurt with hints of berries and bubblegum. Darker red fruits are sweeter than lighter colored varieties. (https://www.specialtyproduce.com)

Chemical elements are the basic building blocks of human lives for different functions. Although the elements mostly fall into the same category for plants and animals, there are a few exceptions. Some of the major and minor elements that human bodies use to function properly are potassium, calcium, iron, zinc, chlorine, copper, manganese, phosphorus, rubidium and sulphur. Minerals are essential for good health. In fact, it plays an important part of how human body obtains energy from our food. (Davis, 2003)

The most essential need of man is food. Food in the form of carbohydrate, fats and proteins are stored in the various parts such as roots, leaves, fruits and seeds. Fruits and nuts have a special place in the traditional culture and everyday life of the people. Fruits are life-enhancing medicines packed with vitamins, minerals, antioxidants and many phytonutrients. They have unique nutrition-profile that help human body free from diseases and keep it healthy. (May Thu, 2016)

Some of the health benefits of velvet apples include their ability to improve heart health, increase circulation, treat gastrointestinal disorders, clear skin irritation, build strong bones,

* Dr, Lecturer, Department of Botany, East Yangon University.

detoxify the body, boost the immune system, lower blood pressure and relieve respiratory distress. When topically applied or consumed the pulp of velvet apples has shown remarkable ability to reduce inflammation and irritation on the skin and is often turned to in alternative medicine as fastest way to heal skin conditions and burns. (https://www.health benefitstimes.com)

The purposes in the research paper are to obtain the knowledge of morphological characters, to analyze the concentration of elements and to reveal the nutritional values of the fruits of *Diospyros discolor* Willd by doing related tests.

Materials and Methods

The plant *Diospyros discolor* Willd was collected from Bogyoke Village, Thanlyin Township, Yangon Region, from January to May 2020 at the Department of Botany, East Yangon University. The identification of this plant was carried out by referring book of Flowering Plants (1998), Flora of Java (1965), Flora of Hong Kong (2007) and https:// www.biologydiscussion.com. The fresh sample of this fruits of edible parts (Fig.1a) thoroughly washed and cut into small pieces and dried at room temperature for two weeks. After that they were pulverized by grinding machine to get coarse powder (Fig.1b) and stored in air tight containers to prevent from moisture and air borne contamination for elemental analysis and nutritional value tests.

Figure 1(a) Pulp of fruits

Figure 1(b) Powder of fruits

Results

Morphological characters

Scientific Name - *Diospyros discolor* Willd

English Name - Velvet apple

Myanmar Name - Katte-par

Family - Ebenaceae

Diospyros discolor Willd is dioecious, evergreen, without any latex, deep tap root and much branched tree which grows up to 15 meters. Leaves are simple, alternate, oblong up to 30 cm long and 12 cm wide and coriaceous with entire margin, obtuse (rounded) base and acuminate apex. Leaf's upper surface is dark green, glabrous and glossy while lower surface is pale green silver hairy and petiole 1.5 cm long and densely pubescent. When young, leaves are pinkish to pale green colour. Inflorescence is 3 flowered axillary racemes in male flower and solitary and axillary cymes in female flower. Flower is creamy white, bractealate, short pedicel, bisexual, regular, actinomorphic, tetra-merous and hypogynous. Sepals are four, synsepalous, sepaloid, deeply lobed contorted, 1.0 cm long and 0.5 cm wide in male and 1.4 cm long and 0.8 cm wide in female and inferior. Petals are four reflexed lobe, synpetalous, petaloid (creamy white), 12 cm long

and 0.5 cm wide in male and 1.5 cm long and 0.6 cm wide in female, campanulate, coriaceous, contorted in bud and imbricate, inferior. Stamens are numerous in male and four in female flower. Filament 0.6 cm long and slender, epipetalous, anther dithecous, introrse, basifixed, longitudinal dehiscence and inferior. Carpels are none in male (pistillodes) two to eight in female, numerous carpellary, syncarpous, multilocular, one or two ovules in each locule, axile placentation, style four cornate at the base, stigma entire and ovary superior. Fruit is globular, 8 to 10 cm in diameter, orange to red with velvety fur. The fruits are often borne in pairs, very close together on opposite side of a branch. Seeds are oblong up to 3.5 cm long and 2 cm wide, two to eight seeds per fruit with brown colour. (Figure.2 and 3)

Male plant

(a) Habit (b) Leaves (c) Infloresence

(d) Flower (e) Sepals (f) Corolla

(g) Epipetalous stamens (h) Stamens

Figure 2 Morphological characters of *Diospyros discolor* Willd

Female plant

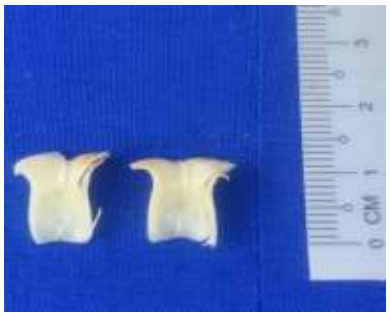
(a)Habit

(b) Inflorescence

(c) Flower

(d) Sepals

(e) Corolla



(f) Epipetalous stamens

(g) Carpel

(h) Fruits

(i) Seeds

Figure 3 Morphological characters of *Diospyros discolor* Willd

Sensory Characters

Colour	-	Pale-brown
Odour	-	Pungent
Taste	-	Sweet
Texture	-	Granular

Elemental Analysis

The determination of mineral elements such as potassium, iron, manganes and ruthenium were mentioned.

The results of EDXRF were shown in Table (1) and (Fig.4)

Table 1 Elemental analysis of the powdered fruits of *Diospyros discolor* Willd

No	Element	Concentration (%)
1	K	75.988
2	Fe	12.875
3	Mn	6.634
4	Ru	4.503

Nutritional values

Nutritional values of fruits of *Diospyros discolor* Willd was studied at the Food Industries Development Supporting Laboratory (FIDSL). From the result, it was found that moisture, ash, protein, fiber, fat, carbohydrate and energy.

The results were shown in Table (2) and (Figure.5)

Table 2 Nutritional values of the powdered fruits of *Diospyros discolor* Willd

No	Element	Result (%)
1	Moisture	6.72
2	Ash	2.80
3	Crude Protein	2.82
4	Crude Fiber	6.67
5	Crude Fat	0.30
6	Carbohydrate	80.69
7	Energy Value (Kcal/100ml)	339

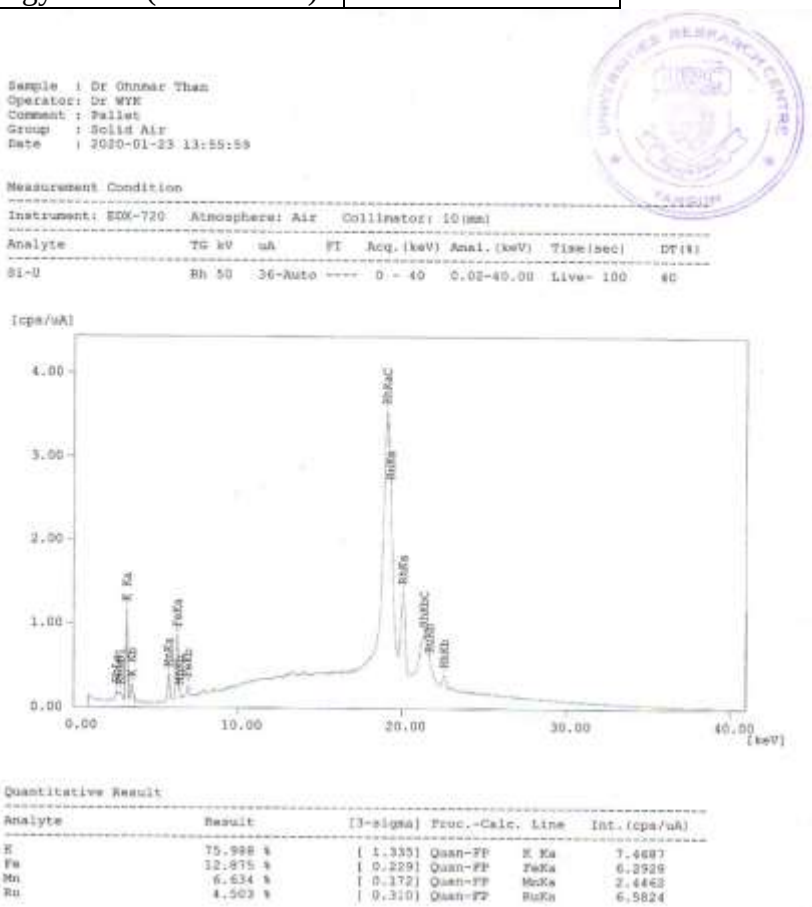
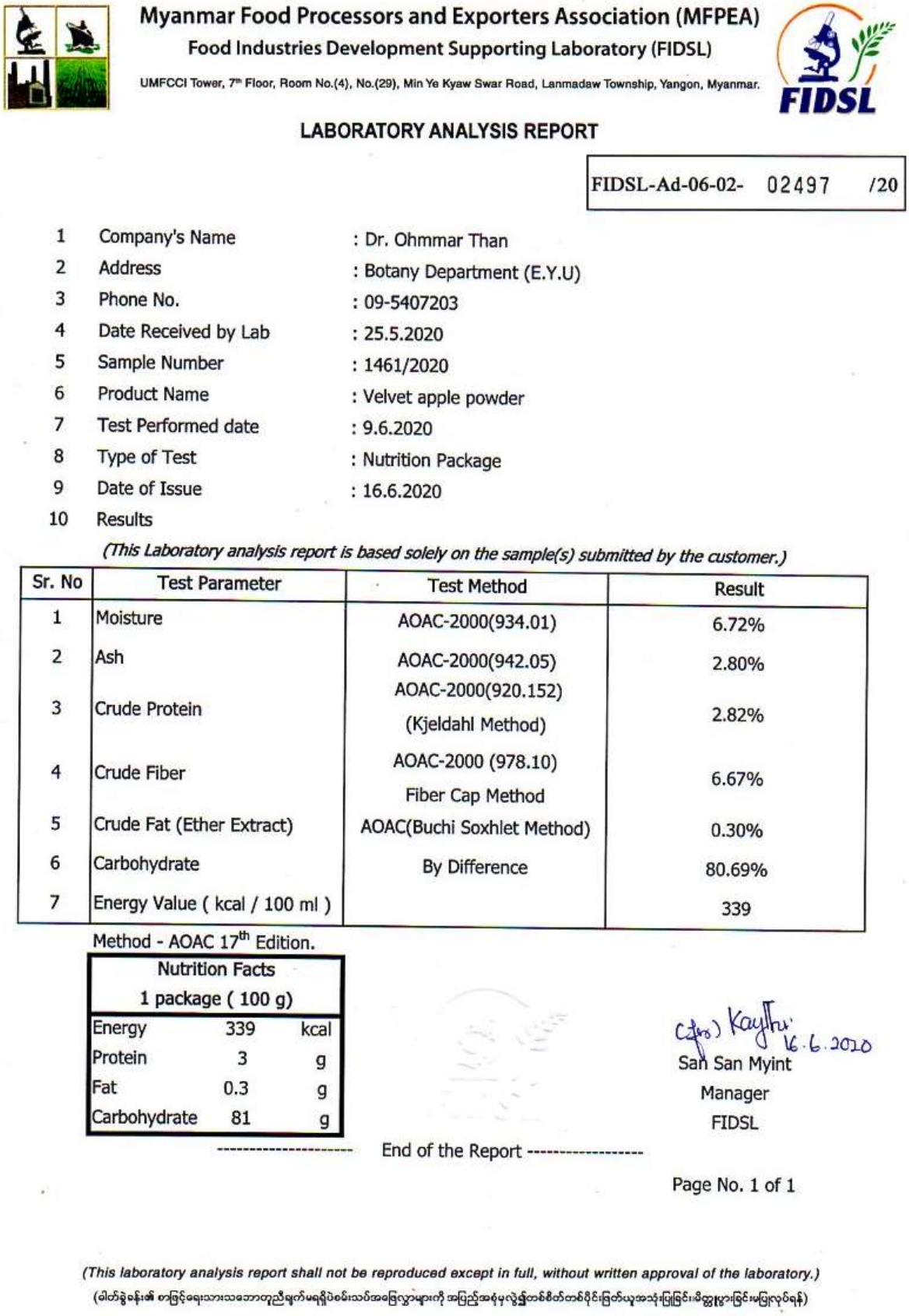


Figure 4 Elemental analysis of the powdered fruits of *Diospyros discolor* Willd



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-06-02- 02497 /20

1	Company's Name	: Dr. Ohmmar Than
2	Address	: Botany Department (E.Y.U)
3	Phone No.	: 09-5407203
4	Date Received by Lab	: 25.5.2020
5	Sample Number	: 1461/2020
6	Product Name	: Velvet apple powder
7	Test Performed date	: 9.6.2020
8	Type of Test	: Nutrition Package
9	Date of Issue	: 16.6.2020
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)	6.72%
2	Ash	AOAC-2000(942.05)	2.80%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	2.82%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	6.67%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	0.30%
6	Carbohydrate	By Difference	80.69%
7	Energy Value (kcal / 100 ml)		339

Method - AOAC 17th Edition.

Nutrition Facts 1 package (100 g)		
Energy	339	kcal
Protein	3	g
Fat	0.3	g
Carbohydrate	81	g

(Mrs) Kaythi 16.6.2020
San San Myint
Manager
FIDSL

---------- End of the Report ----------

Page No. 1 of 1

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(ဓါတ်ခွဲခန်းမှ စာဖြင့်ရေးသားသဘောတူညီချက်မရရှိပဲစမ်းသပ်အဖြေလွှာများကို အပြည့်အစုံမှလွဲ၍တစ်စိတ်တစ်ပိုင်းဖြတ်ယူအသုံးပြုခြင်း၊ဆိုင်းဘုတ်များခြင်းမပြုလုပ်ရန်)

Figure 5 Nutritional values of the powdered fruits of *Diospyros discolor* Willd

Discussion and Conclusion

Diospyros discolor Willd is a dioecious, evergreen tree over 30 m tall and crown conical. Leave's upper surface is dark-green, shiny, glabrous and lower surface is silver hairy and young leaves are pale-green to pinkish and silky-hair. (Fig.2b) Male and female flowers are produced on separate trees (Fig.2 and 3). Both flowers are necessary for pollination and fruit set. Fruiting generally occurs during summertime with fruits ripening from 2-4 months after flowering. Very beautiful dark-red coloured fruit with velvet-like skin. Fruit is about the size of an apple, with mildly sweet flavoured, somewhat mealy, flesh. Fruits are highly esteemed in some areas, but barely known in most parts of the world. (https://toptropical.com)

Some minerals are essential for a normal healthy body. In the fruits of *Diospyros discolor* Willd, the mineral element contents such as potassium (75.988%), iron (12.875%), manganese (6.634%) and ruthenium (4.503%) were observed in Table (1).

Potassium is a very important mineral for the proper function of all cells, tissues and organs in the human body. (Gregor, 2008). Potassium is needed for normal heart function. A high dietary intake of potassium has been shown to protect from a number of conditions, that effect the cardiovascular system, kidneys and bones (Sarit Anavi, 2013). The health benefits of potassium include relief from stroke, high blood pressure, heart and kidney disorders and anxiety and stress. (https://zerbos.com)

Iron is a mineral vital to the proper function of hemoglobin, a protein needed to transport oxygen in the blood. Iron also has a role in a variety of other important processes in the body. A shortage of iron in the blood can lead a range of serious health problems, including iron deficiency. (https://www.medicalnewstoday.com). It also increases the rate of healing of cells, boosts the speed of hair growth, makes circulation more effective and optimizes metabolism. (https://zerbos.com)

Manganese plays a vital role in the metabolism of nutrients by serving as a cofactor in a variety of chemical processes in human body. It is considered an essential nutrient because the body requires it to function properly. People use manganese as medicine (https://www. webmd.com). Manganese plays a variety of roles, such as aiding metabolism, helping regulate blood sugar, contributing to decrease inflammation, reducing premenstruate cramps and more. (https://www.healthline.com)

Ruthenium compounds are encountered relatively rarely by most people. All ruthenium should be regarded as high toxic and as carcinogenic. Compounds of ruthenium stain the skin very strongly. It seems that ingested ruthenium is retained strongly in bones. (https://www.lenntech.com)

Nutritional values are required in diet to maintain good health. The resultant data of nutrient contents in velvet apple (*Diospyros discolor* Willd) fruits are shown in Table-2. Velvet apples present the moisture content is 6.72%. Moisture assays can be one of the most important analyses performed on a food product. It helps regulate the body temperature. Velvet apples consist a large amount of ash is 2.80%. Ash refers to the inorganic residuce remaining after either ignition of organic matter in foodstuff. (May Thu, 2016). The contents of protein is 2.82% in velvet apples. *Proteins* are essential nutrients for human body. It is an important building block of bones, muscles, cartilage, skin and blood. As a fuel, proteins provide as much energy density as carbohydrates. Human body uses protein to build and repair tissues. (https://www. webmd.com)

Like many fruits, velvet apples present the amount of fiber and fat are 6.67% and 0.30%. Fiber has various health benefits. Fiber is important to digestion and regularity, weight management, blood sugar regulation, cholesterol maintenance and more. Fiber may reduce appetite and decreases the rise in blood sugar after big carbohydrate meal. Fat is essential for several bodily functions. It is an energy source and it protects the skeleton and nerves. Fat is necessary to create hormones and helps feel full after eating. (https://www. livescience.com)

The content of carbohydrate in velvet apples is the highest 80.69%. Carbohydrates are one of the main types of nutrients. They are the most important source of energy and needed to do work. Carbohydrate derivatives are involved in reproduction, the immune system, the development of disease and blood clotting. (https://www.medicalnewstoday.com)

Velvet apples fruits are most often eaten raw like normal apples, but is also included in various dessert dishes in certain beverage. The flesh fruits can be mixed with other tropical fruits for salads or desserts. Some people are used despite the unpleasant smell, because the velvet apples have high nutrient contents that can be very beneficial for a variety of health issues.

In conclusion the velvet apple fruits are packed with nutrients, minerals and other useful organic compounds (Table-1 and 2). The fruits are a major source of potassium (75.988%). So consuming the fruits lower cholesterol due to the fiber content (6.67%), velvet apples are a great diet choice for vascular health. The fruits can greatly decrease the risks of stroke, heart attack and blood clots. Thus the velvet apple fruits are more useful for Human diary diet or supplement food and health.

Acknowledgements

I grateful thank to Dr. Daw San Khaine, Professor and Head of Botany Department, East Yangon University for her valuable guidance and advice. I am grateful and deeply indebted to Dr. Khin Myo Thwet, Associate Professor, Taungyi University for her encourages this paper to do. I also thank to Dr. Khin Thu Zar, Associate Professor, Yangon University for her valuable suggestion.

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PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND NUTRITIONAL VALUE OF THE SMALLEST FLOWERING PLANT, *WOLFFIA ARRHIZA* (L.) HORKEL EX WIMMER. IN INNMA AREA, PYAY DISTRICT

Khin Myo Win[1], Khin Ohn Myint[2]

Abstract

Wolffia arrhiza (L.) Horkel ex Wimmer. is the smallest flowering plant. It is known as in local name Ye-au (water egg) and its belonging to the family of Lemnaceae. It is situated in the natural lake, Innma area of Pyay District. The *Wolffia* occurs in the form of colonies that form bright green mats over the surface of water. The specimens were collected on April, 2012 and September, 2019. In this paper, preliminary phytochemical examination and nutritional value of this plant has been undertaken. Preliminary phytochemical examination showed the presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate. Nutritional value of the plant contains energy (230 kcal), proteins (27.12%) and carbohydrates (26.55%). *Wolffia arrhiza* (L.) Horkel ex Wimmer. provides rich in proteinaceous contents.

Keywords: *phytochemical, nutritional values*

Introduction

In the plant Kingdom, the smallest flowering plant is also aquatic. It is *Wolffia*, belonging to the family Lemnaceae (duckweeds), known as water meal or water egg which is tiny globular plants without roots. Duckweeds, monocotyledonous aquatic plants, are represented by 37 species. The 37 species are categorized into five well-defined genera (Appenroth *et al.*, 2013). The flowering plants range in size from 1 mm (*Wolffia* sp.) to over 100 m tall (*Eucalyptus regans*) (Groombridge, 1992). *Wolffia* is a genus of eleven species which include the smallest flowering plants on Earth (Royal Botanic Gardens, Kew 2020). Wayne and Thorne, 1984 stated that the plant bodies of *Wolffia arrihza* are 0.4-1.3 mm long and 0.2-1.0 mm wide; upper surface intensely green; floating on or partially below water surface. Determination of *Wolffia* species on morphological basis alone is very difficult and sometimes not reliable (Landolt, 1994). *Wolffia* generally floats on the surface of the water. The plants are distributed throughout the world, particularly in warm temperate and tropical regions. It is native to Europe, Africa, and parts of Asia, and it is present in other parts of the world as a naturalized species (United States Department of Agriculture, 2018). It is greatly reduced flowering plant, without leaves or stems. Some botanists refer to the plant body as a "frond" or "thallus," The community plants of Ye-au includes giant duck weeds (*Lemna* sp.) and water fern (*Azolla* sp.) in the pond.

In Thailand, Laos and India, *Wolffia* has been harvested for food for many generations. The plant contains about 35% protein as the same protein content as soy beans. Water-egg plants (*Wolffia*) are used as proteinaceous foods as in Wolffia-muffins, Wolffia-tomato sandwichs, gourmet dishes and delicious Wolffia-apple pie la mode in India, Thai and Laos. The plant is widely used as the fodder for fishes, water birds, other invertebrates, buffalos, cows and pigs (Hillman and Culley, 1978). Chantiratikul *et al*., 2010 stated that the cultivated species of *Wolffia* contained 29.61% of protein.

Wolffia is eaten by herbivorous fish as well as a variety of waterfowl. It is also used both as fodder for cattle and pigs, and as a fertilizer because of its high phosphorus and nitrogen

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accumulation, in Africa, India, and Southeast Asia (National Academy of Sciences 1976). Carbohydrate is one of the most essential for factors for survival of human being and use for energy. Protein and fat are regulated the hormone in body activities. Protein plays an essential constituent of the diet of energy. Fiber helps digestion by stimulating muscular activity in the digestive tract (FAO, 1994). Flora of North America Editorial Committee (2000) notes that duckweeds are known to have a very high productivity and nutrient value. *Wolffia arrhiza* (L.) Horkel ex Wimmer. is found in the natural lake of Innma Inn near the Yetashe village in Innma Area of Pyay District.

In this paper, the microphotograph, morphological characters and vegetative propagation were also studied. In addition, the preliminary phytochemical test can be known for the constituents of bioactive compound. And then, the nutritional value of the plants was conducted. The main objectives are to know the outstanding character of *Wolffia arrhiza* (L.) Horkel ex Wimmer., to investigate the phytochemical constituents and to evaluate the nutritional values of this plant.

Materials and Methods

Study Area

The study area of the present work is located in latitude 18˚ 31' 50" N and longitude 95˚ 21' 30" E. It is located in Thegon Township, Bago Region (West).

Collection and Identification

The materials used in this study were collected from the natural lake of Innma Inn near the Yetashe village in Innma Area of Pyay District. The lake was full of water condition in raining season and it become drought in dry season. The specimens were collected on April 2012 for nutritional value and September, 2019 for phytochemical investigation. After collection, the specimens were used for identification in the Department of Botany, Pyay University. The morphological characters were studied with fresh specimens. The microscopical characters of the plants were examined under light microscope and then classification was also described. The morphological character and nomenclature were done according to Backer (1968), Datta (1969) and Lawrence (1951).

Preparation of powdered samples

The collected specimens were washed with water to remove impurities. After washing, the specimen was air dried for 30 days and ground to get powder and stored in air tight container to prevent moisture changes and contaminations.

Preliminary phytochemical test from powdered of plant

Phytochemical investigation of the dried powdered of *Wolffia arrhizal* (L.) Horkel ex Wimmer. was tested qualitatively for the presence or absence of chemical constituents namely alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate. Preliminary phytochemical examination was carried out in the Department of Botany, University of Yangon according to the method of Central Council for Research in Unani Medicine (1987) and Trease and Evans (2002). The results were shown in figure (4) and table (1).

Examining the Nutritional Value of powdered specimens

Nutritional value of powdered specimens such as protein, crude fat and carbohydrate were carried out in Food Industries Development Supporting Laboratory (FIDSL), Lanmadaw

Township, Yangon. The powdered samples have been determined according to the procedures given in the methods of Association of Official Analytical Chemists (AOAC) Horwitz (1980). The results were shown in Figure (5 and 6).

Results

Morphological Characters of water egg plant, *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Floating or submerged perennial herbs, without roots, the plant body reduced to a small or minute oval frond, oblong flat or globosely thallus, free floating, the largest frond 1.2 mm long and 0.5mm broad, the smallest frond 0.6 mm long and 0.275 mm broad; daughter segment or bud grow from retroverted pocket of the mother frond; colonial and primarily reproduces vegetative propagated; the flowers are not obtained in these collection Figure (1, 2 and 3).

Figure 1 Study area and collecting the water egg plants in Innma Inn

Figure 2 Micrograph of *Wolffia arrhiza* (L.) Horkel ex Wimmer. and selling the plants in the market

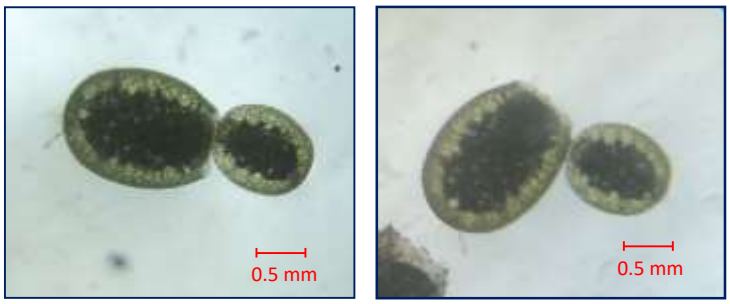


Figure 3 Vegetative Propagation showing budding stages of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Phytochemical Investigation of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

The investigation of these test observed that the presence or absence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate were shown in Figure (4) and Table (1).

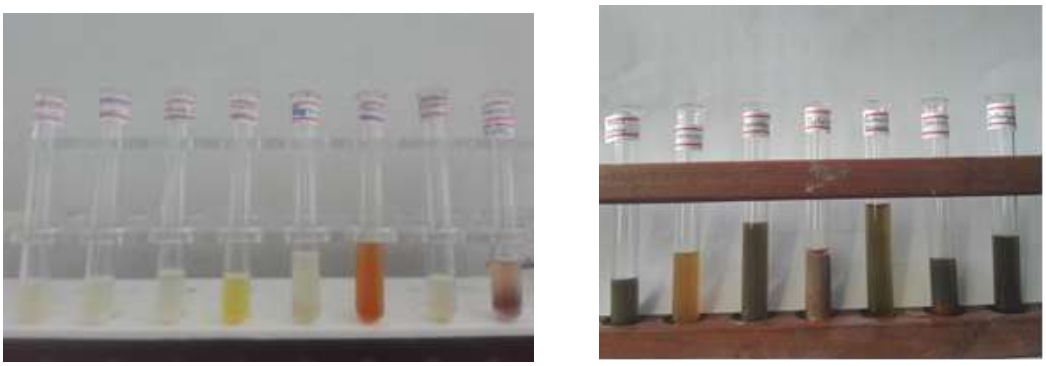


Figure 4 Phytochemical constituents of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Table 1 Preliminary Phytochemical investigation of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

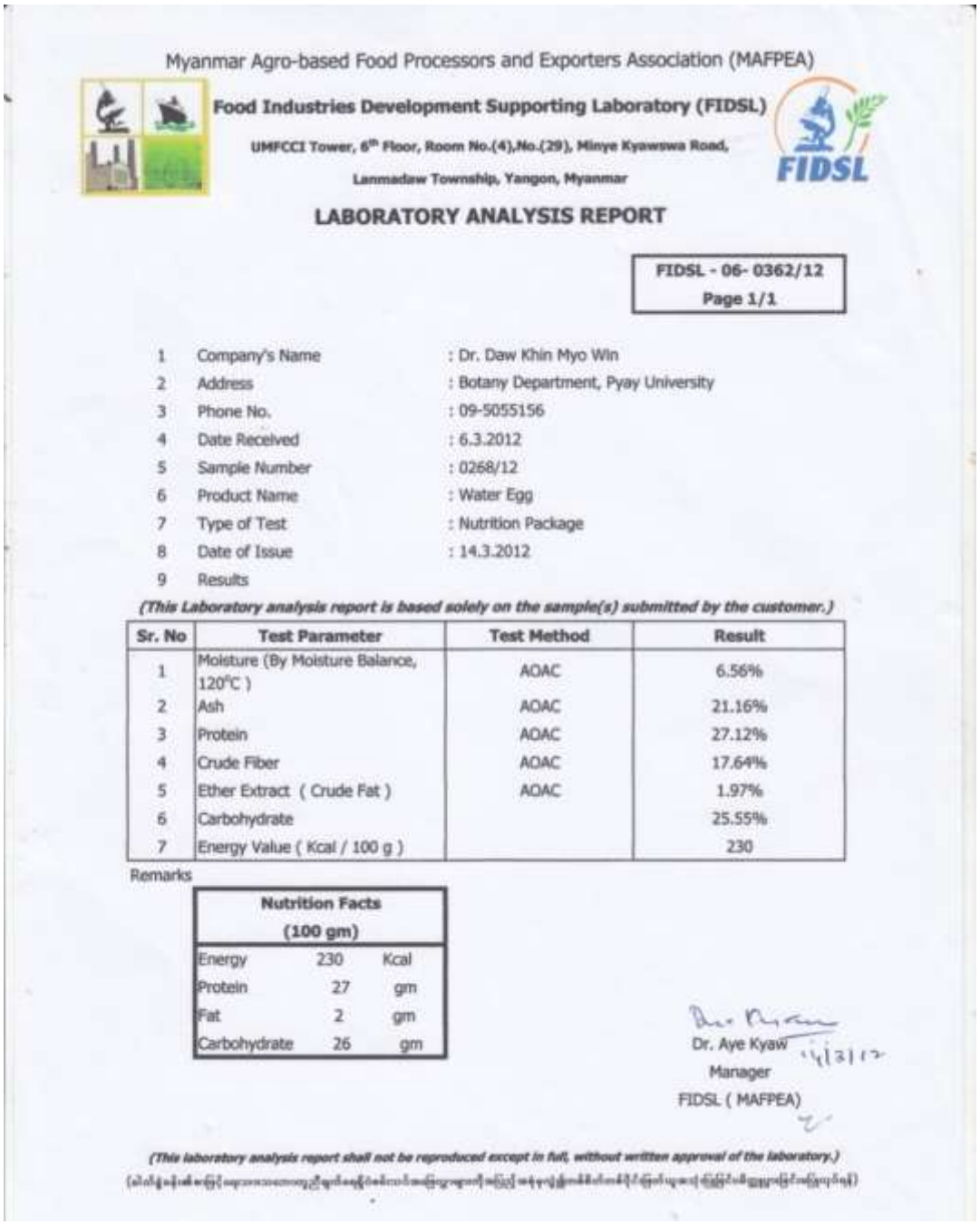
No.	Type of compounds	Extract	Test reagents	Observation	Results
1.	Alkaloid	Methanol	1%HCL+Mayer's reagent	White ppt.	+
		Methanol	1%HCL+Wagner's reagent	Reddish brown ppt.	+
		Methanol	1%HCL+Hager's reagent	Yellowish ppt.	+
2.	Glycoside	Methanol	1ml H_2O + NaOH	Yellow green color	+
3.	Phenolic compound	Methanol	2ml H_2O +10%FeC l_3	Green color	+
4.	Flavonoids	Methanol	Mg coil + HCl(dil.)	Reddish brown colour	+
5.	Steroid	Methanol	CH Cl_3 + H2So_4(conc.)	Green colour	+
6.	Tannin	Water	5%$FeCl_3$ + H_2So_4(dil.)	Yellowish brown ppt.	+
7.	Saponin	Water	Shaken with 2ml H_2O	Forthing	+
8.	α-amino acid	Water	Ninhydrin reagent	Pink spot	+
9.	Protein	Water	Millon's reagent(heated)	White ppt.	+
10.	Reducing sugar	Water	1ml H_2O and mixture equal part fehling's A and B	Brick red ppt.	+
11.	Starch	Water	Iodine	Brown ppt.	+
12.	Carbohydrate	Water	1 ml benedict's reagent and boil for few minute	Brick red ppt.	+

(+) = present ppts = precipitate

According to preliminary phytochemical test of *Wolffia arrhiza* (L.) Horkel ex Wimmer. presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate were observed.

Nutritional Value of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

The experimental work for the nutritional value was carried out at the Food Industries Development Supporting Laboratory (FIDSL). According to the experiment, moisture, ash, protein, crude fiber, crude fat, carbohydrate and energy value were found. The results were shown in Figure (5 and 6).



Myanmar Agro-based Food Processors and Exporters Association (MAFPEA)

Food Industries Development Supporting Laboratory (FIDSL)

UMFCCI Tower, 6th Floor, Room No.(4),No.(29), Minye Kyawswa Road,
Lanmadaw Township, Yangon, Myanmar

FIDSL

LABORATORY ANALYSIS REPORT

FIDSL - 06- 0362/12
Page 1/1

1	Company's Name	: Dr. Daw Khin Myo Win
2	Address	: Botany Department, Pyay University
3	Phone No.	: 09-5055156
4	Date Received	: 6.3.2012
5	Sample Number	: 0268/12
6	Product Name	: Water Egg
7	Type of Test	: Nutrition Package
8	Date of Issue	: 14.3.2012
9	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 (By Moisture Balance, 120°C)	AOAC	6.56%
2	Ash	AOAC	21.16%
3	Protein	AOAC	27.12%
4	Crude Fiber	AOAC	17.64%
5	Ether Extract (Crude Fat)	AOAC	1.97%
6	Carbohydrate		25.55%
7	Energy Value (Kcal / 100 g)		230

Remarks

Nutrition Facts (100 gm)		
Energy	230	Kcal
Protein	27	gm
Fat	2	gm
Carbohydrate	26	gm

Dr. Aye Kyaw
Manager
FIDSL (MAFPEA)

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)

Figure 5 Laboratory analysis report of nutritional package of water egg plants tested at Food Industries Development Supporting Laboratory (FIDSL) Yangon.

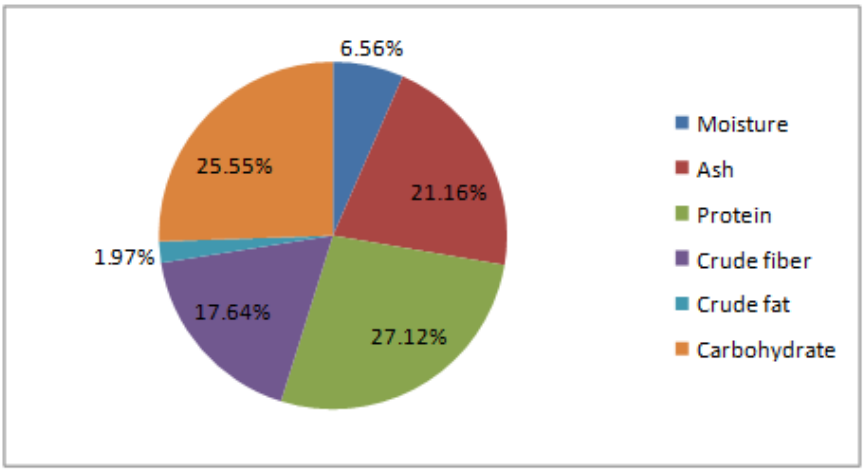


Figure 6 Nutritional contents of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

According to this result, the content of protein, and carbohydrate were higher than the other; ash and crude fiber moderately presence; moisture and crude fat were found as small amount in *Wolffia arrhiza* (L.) Horkel ex Wimmer.

Discussion and Conclusion

In this paper, *Wolffia arrhiza* (L.) Horkel ex Wimmer. belonging to the family Lemnaceae. The plant were very small, free floating, perennial herbs, without roots, the plant body reduce to a small or minute, oval or oblong thallus which act as leaves. These characters were in agreement with those described by Backer (1968), Datta (1969) and Lawrence (1951). *Wolffia* is associated with giant duck weeds (*Lemna* sp.) and water fern (*Azolla* sp.) in the natural lake of Innma Inn. In this investigation, the plant bodies of *Wolffia arrihza* are 0.6 - 1.2 mm long and 0.275 - 0.5mm wide; upper surface green; floating on water surface. These parameters were similar to those given by Wayne and Thorne (1984). In this present paper, the water egg plants is failed to get the flowers of this tiny aquatic plants because absent of Scanning Electron Micrograph (SEM) during collecting period. The vegetative propagation by budding was thoroughly studied and photomicrographs of the budding stages were taken and presented.

In this study, the preliminary phytochemical investigation of the methanol and aqueous extracts of the whole plant of *Wolffia arrhiza* (L.) Horkel ex Wimmer. showed the presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate.

One of the purposes of this research was to study the nutritional contents of water egg plant such as the content of protein, and carbohydrate were higher than the other; ash and crude fiber moderately presence; moisture and crude fat were found as small amount in the *Wolffia arrhiza* (L.) Horkel ex Wimmer. Chantiratikul *et al*., 2010 stated that the cultivated species of *Wolffia* contained 29.61% of protein. In this research, *Wolffia arrhiza* (L.) Horkel ex Wimmer. contains 27.12%. The constituent of proteins were in similar to those mentioned by Chantiratikul *et al*., 2010.

Water-egg plants (*Wolffia*) are used as proteinaceous foods as in Wolffia-muffins, Wolffia-tomato sandwichs, gourmet dishes and delicious Wolffia-apple pie la mode in India, Thai and Laos. The plant is widely used as the fodder for fishes, water birds, other invertebrates, buffalos, cows and pigs (Hillman and Culley, 1978).

According to phytochemical investigations and nutritional contents, the presence of protein and carbohydrate in this plant which can strongly used for food in our country. In Myanmar, especially Innma, Shwedaung and Pyay Township people collect these plants and eat as frying and delight foods. The taste somewhat likes a sweet cabbage.

In conclusion, they are well grown in neutral pH. Water egg plants are widely grown in the lakes of Innma area, Shwedaung area and Pyay Township in Myanmar. It would be substituted of high priced meat and some vegetables for the people. Therefore water egg plants should be consumed because of its protein content value. And the information of the water egg plants should be published in journals and let them know it can be eaten and it can be partially fulfilled the meats of protein in daily diet cheaply. It is important to educate the local people to conserve and maintain the natural products of *Wolffia arrhiza* (L.) Horkel ex Wimmer.

However, it is expected that the present paper will give the knowledge of phytochemical constituents and nutritional value for some information of the smallest flowering plants of the world which is located in Innma, Shwedaung and Pyay Township of Myanmar.

Acknowledgements

We would like to express our profound gratitude to Dr. Kyaw Kyaw Khaung, Rector of East Yangon University for advice and encouragement to fulfill this work. We wish to express my deep gratitude to Professor Dr. Daw San Khine, Head of the Botany Department and Dr. Thida Htoo, Professor, East Yangon University, who taught our not only the subject matter of Botany but also the essence of research and for their generous help and permission in this paper. We are grateful to Dr. Aye Pe, Head of the Botany Department, University of Yangon for his encouragement to conduct this paper. The special thanks to Professor U Mg Mg Myint (Retired) Head of the Department of Botany, Mawlamyine University, for his various suggestion to complete this research. Our special thanks go to Dr. Mee Mee Myint Shein (Retired), Professor and Head, Department of Botany, Pyay University, for her guidance and advice in this research.

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THE STUDY ON WILD ORCHIDS AT YEE-AYE RESERVED FOREST OF KALAW TOWNSHIP IN SOUTHERN SHAN STATE (Part – 2)

Moe Sandar Shein[1], Tin Moe Aye[2], Khin Swe Swe Htun[3]

Abstract

The present work is concerned with the study on wild orchids of natural habitat in Yee Aye Reserved Forest in Kalaw Township. The Yee Aye Reserved Forest is situated in Kalaw Township of Taunggyi District and also the southern west part and 5.5 miles distance from Kalaw city. In this recent study 6 genera and 11 species were recorded from study area. The Yee Aye Hill wetland located in the centre of Yee Aye Reserved Forest. Most of the wild orchids were collected around the area of this Hill wetland. Epiphytic genera namely ***Bulbophyllum, Coelogene, Dendrobium, panisea, Luisia*** and ***Vanda*** were collected. Photographs have been taken to record habits of orchids in nature. The collected specimens were classified, identified and described with colour photographs of their natural habitats and inflorescence. The morphological characters have been emphasized and artificial keys from the tribe to the species have been constructed and GPS location system.

Keywords: Wild Orchids, Yee Aye Reserved Forest, Hill wetland, Epiphyte, Lithophyte, artificial keys.

Introduction

The family Orchidaceae are largest family among Angiospermae, Manocotyledonae. Some botanist estimated about 35000 orchids among flowering plants. Orchidaceae grow well throughout the world. They can thrive in tropical, subtropical and temperate regions except in ice capped regions and deserts. The most wild orchids have distributed various regions of Myanmar that is tropical, subtropical and temperate regions, especially they have grown in temperate regions. Now The study area is Kalaw Township in Taunggyi district of Southern Shan State. Kalaw Township is located on the east by Shwe Nyaung Township, on the west by Thazi Township, on the south by Pin Laung Township, on the north by Pindaya Township, and it lies between North latitude 20°25′-21˚0′ and East latitude 96°20-97˚10′. There are various types of forest in this township that is hill evergreen forest, lower deciduous forest, Indine forest and mixed deciduous forest. Six reserved forest are controlled by Forest Department. Among them the invest gold area is Yee Aye reserved forest in the recent study. Which forest is Hill evergreen forest type (The hill evergreen forest is found in the north at altitudes of over 1,000 meters above sea level. In other regions they are found area of high altitudes. This type of forest is less dense than the tropical evergreen forest because it has less large trees. This type of forest too is quite cool because it is found at high altitudes. The hill evergreen forest is very important to the preservation of water sources. Trees are mainly shrubs mixed with some pines. Smaller plants in the forest include ground orchids and other tropical plants such as wild roses, violets and lilaes. In addition there are small plants that grown on the larger trees such as moss and orchids. Hill evergreen forest, where the wild orchids have grown on the various plants which are (Thit-ya) ***Shoreaob longifolia*** Klall., (Thit-el) ***Castanea sativa*** **Mliler** (Pyin-ma) ***Lagerstromia speciera*** Pers (Pyin-Ka-doe), ***Pyliadolabri formis*** Benth and ***Dipterocarpus obtusifolius*** Teysm. ex. Miq (In-pin). Yee Aye reserved forest situated in Northern East of Pin Laung Township and North by Shwe Nyaung Township and Southern West part between Kalaw and Tharzi Township 5.5 miles far from Kalaw city. The area of these forest is about 1952 acres and altitude of 1465 meters and lies between

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North latitude 20°36′ and East longitude 96°31′. Genus ***Bulbophyllum, Coelogene, panisea, Dendrobium, Luisia*** and ***Vanda*** have been found in this area.

In this recent study, (2) Subfamilies belong to (3) Tribes (4) Subtribes (6) genera and (11) species have been collected from this study area including epiphyte and lithophytes .The classification and taxonomic description of collected specimens are provided with coloured photographic and artificial keys of Tribe, Subtribe, genera and species are also constructed.

Methodology

The specimens were collected from Kalaw Township of Taunggyi District. All these specimens were colourful photographed to record their actual habitat and the nature of inflorescence in 2018-2019. The collected specimens were classified according to Dresseler's classification R. Dresseler's (1927) and identified by Seidenfaden (1992) Grant: B (1966), Nantiya Vaddhanaputi (2006), Hooker, J.D. (1954), Seidenfaden and Smitch (1965), Dassanayake, N.D. (1981), Flora of China Vol. 25 (2013) and Flora of Thailand Vol. XI & XII. Part I & II (2014) methods. Herbarium specimen well prepared and submitted to Botany Department, University of Yangon.

Results

In this paper (2) subfamily, (3) tribes, (4) subtribes (6) genera and (11) species have been collected from study area. According to Seidenfaden and Wood (1992).

Key to the Subfamily

1. Pollinia soft, waxy, without stalk or with caudiculae only rarely with stipe. Anther erect and earlier ontogeny ---------- **(I) Epidendroideae**

1. Pollinia cartilaginous or bony, usually with stipe. Anther incumbent already from earliest stages in ontogeny often strongly deflexed at maturity. ---------- **(II) Vandoideae**

Key to Tribe of Subfamily Epidandroideae

1. Pseudobulb with single internode. Inflorescence terminal. Column long with wings with caudicle, pollinia 2. ---------- **Coelogyneae**

1. Pseudobulb slender with many internode. Inflorescence lateral, column stout without wings and caudicle, pollinia 8 ---------- **Epidendreae**

Key to the Subtribe of Tribe Epidendreae

1. Pseudobulb several shapes. Inflorescence terminal or lateral ---------- **Dendrobiinae**

1. Pseudobulb usually ovoid. Inflorescence arising from the base of the pseudobulb ---------- **Bulbophyllinae**

I. Subfamily Epidendoideae

Subfamily	Tribe	Subtribe	Genus	Species	Myanmar name
Epidendroideae	Coelogyneae	Coelogyninae	*Coelogyne*	*lentiginosa*	aiEi;jzLrS;u
	Epidendreae	Dendrobiinae	Dendrobium	*thysiflorum*	wpciv;a&
				primulium	oi;uLuL
				sutepense	None
				trigonopus	aZmi;vm;o;opc
		Bulbophyllinae	*Bulbophyllum*	*lobbii*	oZiu,
				refractum	oZiyeum
				kanbuerance	oZiyeum ye;a&mi
			panisea	*uniflora*	None

In this recent study only one genus C*oelogyne* was collected under Subtribe Coelogyninae.

1. *Coelogyne* Lindl.

Sympodial epiphyte with creeping rhizome. Pseudobulb oblong ovate. Leaves two alternate. Inflorescence from the base of the pseudobulb. Sometime terminal, with persistent peduncular bracts. Flower medium. Sepals and petals free. Lip trilobe. Column long and slender with large wings around the stigmatic surface. Anther two cells. Pollinia 4.In this recent study only species collected from study area.

1.1 *Coelogyne lentiginosa* (Lindl.) Kuntze

Habit

Inflorescence

Flower

1.1 *Coelogyne lentiginosa* (Lindl.) Kuntze

Pleione lentiginosa (Lindl.) Kuntze (1891)

Epiphyte. Rhizome with dense scale sheaths. Pseudobulbs linear oblong, 4 angled, about 6.00-8.00 cm long and 2.00 cm width, glabrous. Leaves elliptic lanceolate acute, 20-30.00 cm long and 3.5-4.00 cm width, shortly grooved at the base, petaloid, glabrous. Inflorescences with 6-8 flowers, erect, arising from the new pseudobulbs with persistent lanceolate floral bracts. Flower yellow with white lip, about 4-5.00 cm across, pedicel pale green, lanceolate floral bracts. Dorsal sepal and lateral sepals subsimilar, oblong acute with veins. Petals linear lanceolate about 2.00 cm long and 0.7 cm width lip trilobed, thin membranous margin, side lobes oblong obtuse, reddish brown margin, midlobe shortly clawed nearly semicircular, white with orange blotches, three keels

on lip starting away from the base ending in middle of mesochile column green, about 0.6 cm long and 0.2 cm wide with wings, rosetellum orange. Pollinia 4, suborbicular.

Myanmar Name - Ngwe Hinn Phyu Myo Kwe (aiEi;jzLr$;u)

Occurrence - Myanmar, Yee-Aye reserved forest, Wet-Phyu-Yee reserved forest N 20˚34' 22", E 96˚30' 36"

Distribution - Myanmar, Thailand (Flora of Thailand Vol.12, 5) (**Seidenfaden,** 1992) Tenasserium, Moulemein (Grant, B., 1964)

Ecology - Epiphyte, Lithophyte, Hill evergreen forest, deciduous forest, Lowland forest, alt 1280 m. Flowering period December- January

Key to the species of Genus *Dendrobium*

1. Pseudobulb fusiform, flower golden yellow. Sepals with distinct keels on the back. Sidelobes of lip green with red stripe. ---------- **3. *D. trigonopus***

1. Pseudobulb slender,flower white.Sepals not with keel ---------- 2

   2. Inflorescence pendulous with many flowers. Sepals and petals white, rounded. Lip golden yellow. ---------- **1. *D. thrysiflorum***

   2. Inflorescence short with 1-2 flowers on white sheath covered. Sepals and petals pale purple with veins, oblong acute. Lip pale sulphur yellow. ---------- **2. *D. primulinum***

2.1. *Dendrobium thrysiflorum* Rchb.f.

Habit

Inflorescence

Flower

2.1. *Dendrobium thrysiflorum* Rchb.f. In III. Hort. Xxii. t. 207: Garterfl. t. 1022: FL.Mag. N. S. t. 449: Veitch. Mahar.

Epiphyte, evergreen. Stem erect, clavate with four ridges narrowly at the base, about 20-30.00 cm long, yellow. Leaves oblong ovate acute, subterminal, glabrous about 6-8.00 cm long 25-3.00 cm wide. Inflorescence lateral, pendulous with many flowers in upper portion of pseudobulbs, peduncle white. Flower large, white, about 5.00 cm across with white pedicle, bract revolute with strong recurved, fragrant. Dorsal sepal ovate obtuse, about 1.5 cm long and 1.2 cm wide, lateral sepals obliquely oblong ovate acute, white, thin texture. Lateral sepals broader than the sepals, ovate, slightly undulate in margin with ciliate on upper surface, white and thin texture. Lip large, golden yellow the whole of lip, funnel shaped, retuse and at epichile, pubescent on abaxil, no ciliated at the base of both sides, about 2.00 cm long and 1.5 cm wide. Colum curved, yellow, about 1.2 cm long 0.3 cm wide. Operculum pale yellow. Pollinia 4.

Myanmar Name - Ta-Khing-Lone-Shwe,Tagun Lone Ngwe (wpciv;a&)
Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest.
N 20˚34' 12.6", E 96˚31' 8.12"
Distribution - NE India, Laos, Myanmar, Thailand, Vietnam (**Flora of China**, 2014) (**Holttum,** 1964)
Ecology - Epiphyte, Hill evergreen forest. 1412 meter. Flowering period March-April

2.2. *Dendrobium primulinum* Lindl.


Habit


Inflorescence


Flower parts


Pollinia

2.2 *Dendrobium primulinum* Lindl.

***D nobile var. pallidiylora* Hook.k**

Epiphyte. Stem stout pendulous and not too long abaxil 15-30.00 cm long 1.00 cm width, covered with white sheath. Leave broadly oblong acute, obliquely emarginated, glabrous. Flower solitary on two flowers on the leafless stem and pale purple with pale sulphur yellow lip about 3-5.00 cm across. Pedicel white or pink. Sepals and petals subequal oblong obtuse, 1.5-1.8 cm long and 0.6 cm width, pale purple. Pollinia 4 in masses, waxy with vescidium, without stripe.

Myanmar Name - Thin-Kyu-Kyu
Occurrence - Myanmar, Yee- Aye reserved forest, N 20˚36' 40", E 96˚31' 87"
Distribution - Himalaya, Burma, Thailand and China (**Seidenfaden,** 1992), Tropical Himalaya, Nepal (Grant. B., 1966) Assam, eastern Himalaya, Nepal, Andaman island, Myanmar, Thailand, Laos, China, Vietnam (Flora of Thailand, 2011)
Ecology - Epiphyte, Hill evergreen forest, mixed deciduous forest, alt 960 m. Flowering period April – May.

2.3. *Dendrobium trigonopus* Rchb.f. in Gard. Chron. 1887


Habit


Inflorescence


Flower parts


Pollini

2.4. ***Dendrobium trigonopus*** **Rchb.f. in Gard. Chron. 1887**

D velutinum **Rolye.**

Stem short, cluster, fusiform, yellow when mature about 10-12.00 cm long, 1.00 cm width. Sheath hispidulous in young. Leaves 1-3 subterminal, oblong, ligulate, acute, thickly leathery, black hair on the midvein. Inflorescence 1or 2 and with 2 to 3 flowers on leafly pseudobulb, peduncle short, about 1-2.00 cm with ovate triangular floral bracts. Flower golden yellow, about 3.00 cm across, thick and waxy with trigonous ovary. Dorsal sepal and lateral subequal, ligulate, lanceolate with strongly keeled, about 2.00 cm long and 0.6 cm width, both margin slightly incurved ascending on upper portion, lateral sepals adnate to the column foot, mentum obtuse. Petals ovate oblong acute with veins, broader than the sepals. Lip trilobed, erect, yellow with pale green patch in the centre, lateral lobed quadrate or semiorbicular with reddish brown stripes, midlobe orbicular, disk with papillose. Column short, anther cap shape. Pollinia 4 in masses, oblong obtuse.

Myanmar Name - None

Occurrence - Myanmar, Yee- Aye reserved forest, Mee-Nel taung mountain forest (Hopone Township) N 20˚36' 40", E 96˚31' 87",

Distribution - Myanmar, Thailand, China (**Seidenfaden,** 1992), China, Loas, Myanmar, N-Thailand, Vietnum (Flora of China-Vol. 25) and China, Thailand, Vietnum, Myanmar (Dassanayake, 1981)

Ecology - Epiphyte, Hill evergreen forest, mountain forest alt 1416 m. Flowering period March- April.

Subtribe Bulbophylllinae Schle.

Epiphyte. Pseudobulb single or internode, widely separated or rhizome, sometimes reduced in size. Leaves duplicate, articulate, sometimes reduced to scale. Inflorescence lateral, simple or spiral distinchous, one to many flowers. Flower small to large, resupinate. Lip often hinged at base .Column with a prominent foot. Anther terminal, incumbent, 2 celled. Pollinia 2 to 4, naked, with visidium or visidia or stripe. Stigma entire.

Only two genus ***Bulbophyllum*** and ***Sunipia*** of Subtribe Bulbophylllinae was found in study area.

Key to the genus of Subtribe Bulbophyllinae

1. Lip hinged to the column foot, in most cases very mobile ---------- ***Bulbophyllum***

1. Lip not hinged to the column foot, not completely mobile ---------- ***panisea***

3. ***Bulbophyllum*** **Thou.**

Rhizome usually long and creeping, only attached with roots to the substratum, bearing pseudobulbs, each of a join, at its top carrying a single leaf, only rarely two. Pseudobulb close or distinct, vary in size. Inflorescence one to many flower arising at the base of the pseudobulb. Flowers single or closed head flower much varying in size from small to quite large. Sepals equal or lateral sepals much larger than the dorsal, joined to the column foot to form mentum, free, spreading or their edges more or less joined or connate; petals always smaller than the sepals. Lip almost nearly mobile, usually fleshy, tongue-shaped, straight or curved, papillose or warty. Column short with conspicuous wings, column foot curved forward. Pollinia 4.

Key to the species of Genus *Bulbophyllum*

1. Flower solitary and expended, sepals and petals pale yellow with brown stripe of sepals and petals ---------- **1. *B. lobbii***

1. Flower greenish yellow with brown stripe. Two lateral sepals oblong acute, join straightly, greenish yellow sepals with brown stripe, not papillose and twisted. Lip pale yellow with purple papillose on epichile. ---------- **2. *B. refractum***

3.1. *Bulbophyllum lobbii* Rchb.f.

Habit

Inflorescence

Flower

3.1. *Bulbophyllum lobbii* Lindl.in Bot.Reg.1847, sub.t.29.

***B.henshallii*, Lindl.in Gard.Chron1852,422.**

***Bulbophyllum siamense* Rchb.f.I.c.1867,572.**

Epiphyte, pseudobulb ovoid in young oblong with depress in mature, well spaced equal apart on stout rhizome. Leaf simple, oblong ovate notch at the tip, leaf texture fleshy with petiole, about 15-20.00 cm long and 5-7.00 cm wide. Flower solitary, broadly expended, pale yellow, about 5-6.00 cm across with yellow petiole about 3.00 cm long and 0.2 cm wide. Dorsal sepal erect oblong acute, yellow with 10 reddish brown stripe, about 2.00 cm long and 0.8 cm wide, lateral sepals obliquely ovate acute, about 1.5 cm long and 1.00 cm wide. Lateral petals oblong acute, smaller than the sepals, colour like as petals. Lip tongue-shaped, projection toward and curved down ward an epichile, brightly yellow patch on the mesochile and pale reddish brown stripe on epichile, join to the column foot. Column stout with two distinct yellow horns. Operculum white. Pollinia 4 in pairs.

Note : This species was revealed as synonym in A Checklist of the trees, shrub, herbs, and climbers of Myanmar. (Kress *et,al.,* 2003)

Myanmar Name - Thazin Kywe (oZiu ,)

Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest.
N 20˚35' 125", E 96˚32' 20.5"

Distribution - Assam, Myanmar, Thailand, Malaya, Indonisia, Phillipines (**Seidenfaden,** 1992) Myanmar (U Nyan Tun, 2014), Thailand (**Nantiya Vaddhanaphutt**, 2005)

Ecology - Epiphyte, Hill evergreen forest 1420 m. Flowering period March

3.2. *Bulbophyllum refractum* (Lindley) HG. Reichenbach

Habit

Inflorescence

Flower

Petals

3.2. *Bulbophyllum refractum*(Lindley) HG. Reichenbach, Ann. Bot.Syst6:259, 1861

Bulbophyllum wallichii **Lindl. Gen. Sp. Orchid Pl. 59.1830**

Epiphyte. Pseudobulb crowded on the rhizome, globose ovoid about 2.00 cm long and 2.3 cm wide, wrinkled. Leaves sessile, deciduous, oblong acute, 6-8.00 cm long and 1-2.00 cm wide. Inflorescence erect and nodding in flower portion, rising from the base of the leafless pseudobulb with many flowers floral bract and yellowish green. Flower greenish yellow about 3.00 cm long and 1.00 cm wide, black yellow, lanceolate acute. Sepals 3, dorsal sepal oblong acute, hooked, greenish yellow with purple line and ciliate at the edges. Lateral Sepal narrowly oblong acute, base adnate to the column foot, their lower edges connate to each other, greenish yellow with fine purple lime. Petals 3, lateral petals obliquely ovate triangular pale yellow with irregular fimbriate purple, smaller than the sepals. Lip fleshy, subligulate, obtuse at open, pale yellow with purple spot, margin and epichile with brownish purple, papillose, attached to the end of column foot, versatile. Column cylindrical, white. Anther cap purple.

Note : ***Bulbophyllum refractum*** was revealed as synonym in Floral of China, 2013. and A Checklist of the trees, shrub, herbs, and climbers of Myanmar. (Kress *et al*, 2003)

Myanmar Name - Thazin Panka (oZiyeum)

Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest.
N 20˚35", E 96˚33',

Distribution - NE and NW India, Myanmar, Nepal, Thailand, Vietnam, (Floral of China 2016), Thailand (**Nantiya**, 2006)

Ecology - Epiphyte, Hill evergreen forest, 1425 m alt. Flowering period March -April.

4. *Panisea*

Epiphyte, rhizome creeping. Pseudobulbs well spaced on creeping rhizome. Pseudobulb with two leaves. Scape arising laterally from the base of pseudobulb with usually few to many flowers. Flowers small to medium, floral bracts small to large. Sepals similar. Petals smaller than the sepals. Lip unlobed. Column short, rostellum 2 lobed, reflexed, anther terminal. Pollinia 4 in pairs, waxy, subglobose with divided stripe.

4.1. *Panisea uniflora* (Lindl.) Lindl.

Habit

Inflorescence

Flowers

Pollinia

4.1. *Panisea uniflora* (Lindl.) Lindl.

Epiphyte or Lithophyte. Rhizome creeping. Pseudobulbs apart on rhizome, cylindric to obpyriform, ((2.00-3.5 cm long and 1.00-1.5 cm wide) with terminal pair leaf. Leaves subsessile, blade narrowly oblong acute, about 8-10.00 cm long and 0.8 cm wide. Scape from the base of pseudobulb with 1-2 flowers, 15.00 cm long and 0.2 cm wide and pale brown scale like sheath. Flowers green yellow with orange lip, 1.5 cm across, pedicle, 1.00 cm long and 0.1 cm wide, floral bract ovate acute. Dorsal sepal oblong acute 1.2 cm long and 0.5 cm wide, delicate texture, like transparency, recurved, lateral sepals similar petals oblong ovate, obtuse with mid veins, a little shorter than the sepals. Lip orange broadly obovate 1.5 cm long and 1.00 cm wide, thickened in hypochile with three distinct keels and margin undulate, broadly expended in epichile, slightly notch on the top. Column greenish yellow, stout with wings, about 0.8 cm long and 0.2 cm wide. Rostellum capitate, yellow, anther cap protruding forward. Pollinia 4 in pairs with stipe.

Myanmar Name - None

Occurrence - Myanmar, Kalaw and Pin-daya township, Yee-Aye reserve forest N 20˚36" 12', E 96˚32'

Distribution - Myanmar

Ecology - Epiphyte or Lithophyte. On the tree trunk, Alt. 1211 alt. deciduous forest and Hill evergreen forest. Flowering period – April - May

II. Subfamily Vandoideae

Subfamily	Tribe	Sutribe	Genus	Species	Myanmar Name
Vandoideae	Vandeae	Sarcanthinae	*Vanda*	*liacina*	r;v;ri;ao;
			Luisia	*teres*	none

Tribe Vandeae Lindelay

Habit monopodial, stem short or elongated. Leaves dithecious, rarely spiral, duplicate, laterally flattened. Inflorescence lateral, simple or branched one to many flowers. Flower small to large with spurred. Colum with prominent foot. Anther terminal. Pollinia two or four with definite stipe and visidium. Stigma entire.

Vandeae is the only major group of orchids in which all members are monopodial. There is no pseudobulbs. In this recent study only Subtribe Sarcanthinae under Tribe Vandeae has occurred in this study area.

Subtribe Sarcanthinae

Habit monopodial, stem short or elongated. Leaves disthecious, rarely spiral, laterally flattened. Inflorescence lateral, simple or branched, one to many flowers. Flower small to rather large, may be jointed, saccate, or deeply spurred. Column with prominent foot, Anther terminal. Pollinia two or four with definite stipe and viscidium. Stigma entire. In this study only one genus *Lucia* was collected under the subtribe Sarcanthinae.

Key to the genera of Subtribe Sarcanthinae

1. Leaves strap-shape. Scape long. Sepal and petals narrowly at the base and sometime tessellate. Column foot present. Rostelllum projection.---------- ***Vanda***

1. Leaves long slender terete. Scape very shop. Sepals and Petals always not tessellate. Rostellum not projection.---------- ***Luisia***

5. *Vanda* R. Broum

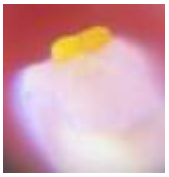
All epiphytes, leaves distichous, thick and lathery, more or less strap shaped. Inflorescence lateral erect or pendulous. Sepals and petals wide-spreading and resemble each other. The lip is saccate or spur and fleshy, entire, or 3-lobed continuous at the base of the column. Column short, thick, pollinia masses, bilobed.

5.1. *Vanda lilacina* Teijsm. & Binnend.

Habit

Flowers

Flowers parts



Pollinia

5.1. Vanda lilacina Teijsm. & Binnend.

***V. laotica* Guill. Bull. Soc. bot. Fr. 77:335, 1930**

***Sarcochilus caligaris* auct. Non Ridl,: Guillaumin 1959.**

Stem 2.3 cm long and 1.5 cm in diameter. Leaf blade fleshy apex unequal lobe, 8-10.00 cm long. Inflorescence erect with 8-10 flowers, peduncle 8-10.00 cm long and 0.2 cm in diameter, floral bract 2-3, ovate triangular, acute, pale brown. Flower pale purple widely open, about 25.00 cm across with pale purple pedicle about 1.5 cm long. Lateral sepals obovate acute, white about 1.20 cm long and 0.8-1.0 cm wide. Lateral petals ovate acute, spathulate excurved at apex; white. Lip trilobes, midlobe of lip squarish, frontage slightly expended with white tinged, and notch at apex, convex in middle with 4 callus, central callus long from the base to the apex, side-lobes triangular acute with purple sport-spur short, laterally compressed, straight, light green. Colum short pale purple spot each side, about 0.5 cm long and 0.2 cm in diameter. Pollnia 2, subglobose, anther cap pale purple.

Myanmar Name - None

Occurrence - Myanmar, Kalaw and Pin-daya township, Yee-Aye reserve forest N 20˚34' 56.9", E 96˚3.5' 45"

Distribution - Myanmar, Thailand, China (Seidenfaden, 1992)

Ecology - Epiphyte. On the tree trunk, Alt. 1211 m alt. deciduous forest and Hill evergreen forest. Flowering period – April

6. *Luisia* Gaud

Epiphyte. Stem long. Leaves terete. Pseudobulb absent. The root vermiform. Flower spicate on a short, dense scape very short and thickened. Sepals and petal free, equal or petals larger than the sepals. Lip fleshy, fixed immovably to the base of column, distinctly divided by grove into a basal hypochile and epichile, basal part more or less hollow, sometime in the distinct side lobes, apical part usually longer, often wrinkled grooved longitudinally. Colum short, foot absent. Pollinia 2, with short broad stipe.

6.1. *Luisia teres* (Thunberg) Blume, Rumphia 4:50, 1849

Habit

Inflorescence

Flowers

6.1 *Luisia teres* var. *botanensis* (Fukuyama) T.P. Lin

***Luisia teres* (Thunberg) Blume, Rumphia 4:50, 1849**

***Epidendrum teres* Thunberg in Murray**

Epiphyte. Stem pendulous or erect with internodes 3-3.5 cm leaves terete, obtuse. Inflorescence 2-3 flowers on upper portion. Floral bract ovate, acute. Flower medium size, fleshy, greenish yellow, brownish yellow pale stripe on the back. Dorsal sepal ovate oblong acute, lateral sepals slightly longer than the dorsal sepal, carinate on the back, about 0.8-1.00 cm long. Petals falcate elliptic, obtuse, fleshy greenish yellow, tinged, pale purple on the back. Lip broadly spreading not distinct boundary epichile and hypochile, more broadly undulate on epichile, hypochile slightly concave, subquadrate lobes at the base, longitudinal ridges at the apex on the back of epichile, furcately bilobed. Column short and stout, rostellum distinct. Pollinia 2, waxy, globose.

Note : *Luisia teres* regard as new record in Myanmar. (2019)

Myanmar Name - None

Ecology - Epiphyte. Alt. 1411 m alt. Hill evergreen forest. Flowering period –May-June

Occurrence - Myanmar, Kalaw and Pin-daya township, Yee-Aye reserve forest N 20˚34' 56.3", E 96˚3.5' 57"

Distribution - Myanmar 2019, China and Hongkong (Flora of China vol. 25, 2013)

Discussions and Conclusions

This paper based on some collected wild orchids specimens. The present list is (2) subfamily, (3) tribe, (4) subtribe, (6) genera and (11) species. The subfamily Epidendroideae includes (2) tribe, (3), subtribe, (4) genera and (9) species. Genus ***Coelogyne, Dendrobium, Bulbophyllum*** and ***Panisea*** have been collected from study area. In recent studyone species of genus ***Coelogyne*** was collected in this study area that is ***Coelogyne lentiginosa***, which midlobe shortly clawed nearly semicircular, white with orange blotches, three keels on lip.Three species of genus ***Dendrobium*** are ***D. thrysiflorum* Rchb.f.**, ***D. Primulium* Lindl.**, ***D. trigonopus* Rchb.f.** and ***Dendrobium thrysiflorum* Rchb.f.** contains pendulous inflorescence with many flowers and golden yellow lip with pubescent. ***D. sutepense* Rolfe ex.** and ***D***. ***Primulium* Lindl.** have orbicular papillose lip and fragrant. ***D.trigonopus* Rchb.f.** has trilobe yellow lip with pale green patch in the centre and trigonous ovary. Three species of genus ***Bulbophyllum*** are ***B. lobbii* Rchb.f.**, ***B. refractum* (Lindley) HG. Reichenbach,** ***B. kanburiense* Seidenf.** and ***B. lobbii* Rchb.f.** which distinct character is oblong ovate large leaves, large solitary pale flower with brown stripes. ***B. refractum* (Lindley) HG. Reichenbach.** has greenish yellow flower with faint brown stripe, two lateral sepals join straightly and lip with purple papillose on epichile. ***B. kanburiense* Seidenf.** contains lanceolate acute two lateral pinkish purple sepals join twisted and dense papilllase at the base. ***Panisea uniflora*** Lindl. has cylindric to obpyriform pseudobulb and lip orange broadly, thickened in hypochile with three distinct keels and margin undulate, broadly expended in epichile, slightly notch on the top. In subfamily Vandoideae, (1) tribe, (1) subtribe and only two genus was collected from study area. Two genus of subtribe Sarcarthinae under tripe Vandeae is genus *Vanda* and *Luisia*. Genus ***Luisia*** is ***Luisia teres*** L. has lip broadly spreading not distinct boundary on epichile and hypochile slightly concave, subquadrate lobes at the base, longitudinal ridges at the apex on the back of epichile, fulcately bilobed. In this paper, all collected species are epiphyte and lithophytes.

In recent study some species ***Dendrobium thysiflorum* R.chb.f , *Bulbophllum siamense* R.chb.f**, ***Bulbophllum lobbii*** and ***B. refractum*** were only found in Chin, Mon, Thaninthayi, Kachin and Rakhing by (Kress *et al*, 2003) and also found in this study area. Among them ***D. thyrsiflorum*** regard as a native in Myanmar (**Holttum, 1964).** ***Luisia teres*** L. collected as a new record in Myanmar. Botanical collection are still needed to cover the whole floristic diversity of Myanmar, because botanical exploration have sharply decreased in Myanmar 1950. (Kress *et al*, 2003) Compared with neighboring countries with intensive orchids studies, Myanmar orchids flora have lagged behind being well documented and studied. So the orchidologist will have to find out continuously to get update current wild orchids information and report to government for protection of our living jewels.

Acknowledgements

An author wish like to thank Dr. Aye Aye Tun, Rector of Bago University, for allowing me to undertake this research paper.I also thankful to Dr. Yin Yin than, Prorector, Bago University. I am also grateful to U Kyaw Myo Naing (Forest Department, Kalaw Township) for his kind help, helping with forest type literature and collecting of specimens during field trip.

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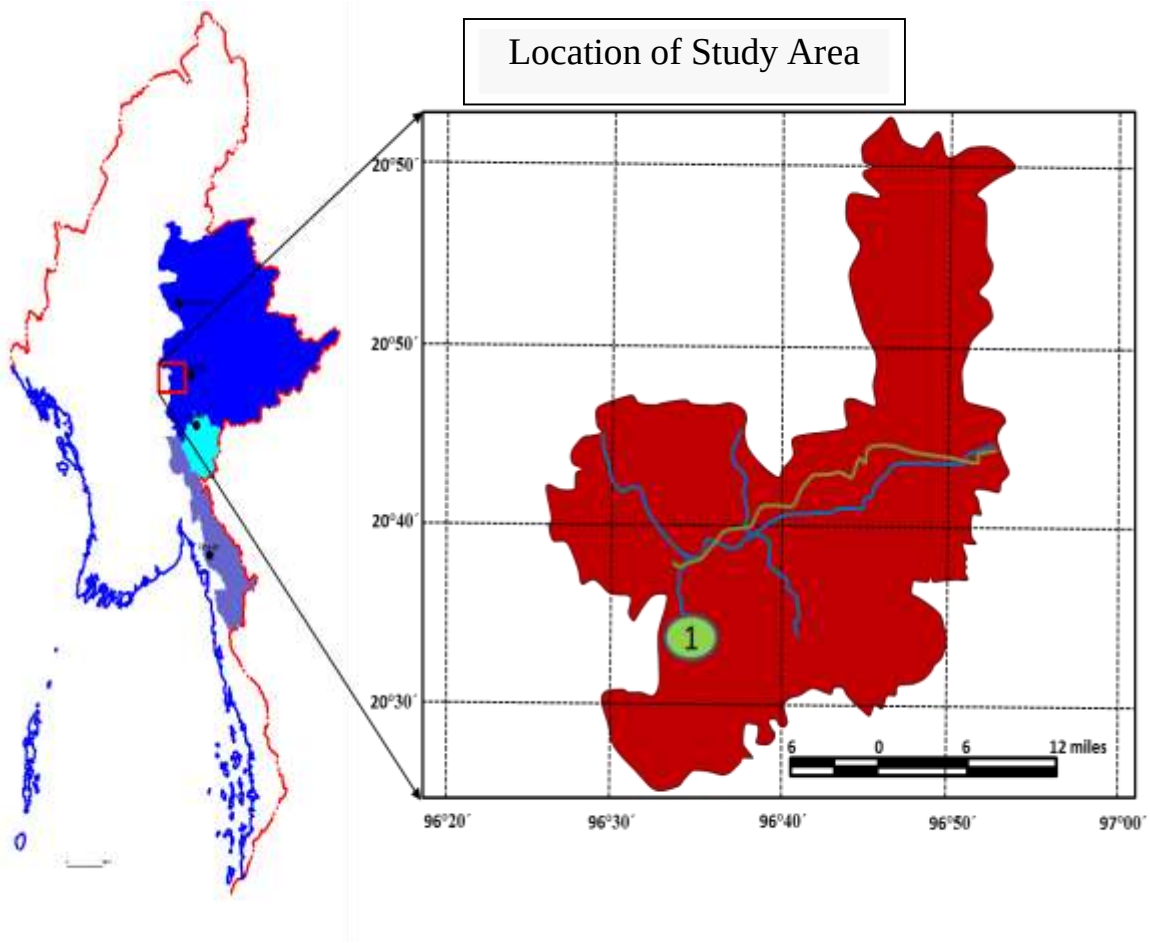
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IDENTIFICATION OF ISOLATED INDIGENOUS BACTERIA FROM NODULES OF *PISUM SATIVUM* L.

Win Win Khaing[1], Mya Win[2], Maw Maw Than[3]

Abstract

The present study is to focus describe and identify the specific bacteria from the nodules of *Pisum sativum* L. The plant samples were selected from the cultivated field of Kachin State and it carried out to the laboratory of Agricultural Microbiology Section, Agricultural Research Center, Yezin, Nay-pyi-daw. This research was conducted from November 2018 to December 2019. The media YEMA, CRYEMA and BTBYEMA were used as selective media for isolate bacteria. After culturing, colony characters and cell morphological characters of isolate bacteria were studied. Colonies of isolates were sticky appearance, circular, convex (raised), smooth, entire margin and white (translucent). Cell morphological characters were medium size, rod shaped, motility and gram-negative. Moreover, the characterization was done by biochemical tests. As results, the isolated indigenous bacteria were faster grower. The positive chemical reaction were showed Indole, Methyl red, Catalase, Urease hydrolysis, starch hydrolysis, Glucose peptone agar, Glucose, Sucrose and Mannitol tests. The negative chemical reactions were showed in lactose fermentation, Citrate utilization, Gelatin hydrolysis, Methyl blue and Mannitol salt agar experiments. According to the character results, isolated indigenous bacteria from nodules of *Pisum sativum* L. was confirmed as genus *Rhizobium.*

Keywords: Root nodules, *Pisum sativum*, Biochemical, *Rhizobium*

Introduction

Pulses, fresh or dry, had a high nutritional values due to their high contents of carbohydrates, proteins, vitamins and minerals (Smart, 1990). The garden pea (*Pisum sativum* L.) is a cool season legume. A major resource of food in Myanmar and vital component of our daily dishes. Food legumes serve as a feed crop in farming systems and fetch higher prices compared to cereals. These crops are being grown more to supplement farmers' incomes. Pulses play an important and different role in food systems and in the diet of the poor, they are the best crop to reduce poverty and hunger, improving human health, nutrition and enhancing ecosystem resilience Akibode and Maredia(2011).

The crops from legumes are an important crop for consumption in developing countries. These are considered a vital crop for achieving food and nutritional security for both poor producers and consumers in many parts of the tropics countries, particularly where meat is scarce. Because they are higher in protein than any other food plant and are close to animal meat in quality. In fact, they are often called poor man's meat being an inexpensive source of high quality protein and also play an important nutritional role in supplying those essential amino acids.

Legume-*Rhizobium* symbiosis is the most promising plant bacterium association for immediate increase in grain yield through biological nitrogen fixation Gresshoff *et al.*, (2014) Desta *et al*., (2015) stated that by the inoculation of adaptable effective legume-*Rhizobium* effect on pulses production can be increase from lower point.

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Rhizobium plays a very important role in agriculture by inducing nitrogen fixing nodules on the roots of legumes such as peas, beans and clover. In 19th century, the scientific demonstration of this symbiosis was started and established that bacteria are present in legume root nodules and which are responsible for fixing atmospheric nitrogen Zsbrau, (1999). The *Rhizobium* species live inside the root nodules of host legumes so they are beneficial for the growth of the plants Oblisami, (1995). They easily colonize in the plant root and promote solubilizing activity, nitrogen fixation and biocontrol activity Deshwal *et al*., (2011).

Rhizobium inoculation increased the root nodulation through better root development and more nutrient availability, resulting in vigorous plant growth and dry matter production which resulted in better flowering, fruiting and pod formation and ultimately there was beneficial effect on seed yield Sardana *et al*., (2006) Rhizobia have been classified and characterized on the basis of biochemical tests Gachande and Khansole, (2011). The interest in biological nitrogen fixation and rhizobia-legumes symbioses, particularly those involving economically important legume crops in terms of food and forage is essentially for sustainable agricultural practices Laranjo *et al.*, (2014). The excessive use of nitrogen fertilizers increased the total costs of crop production, created pollution and increased deterioration of soil fertility Marschner, (1995). Use of these fertilizers has led to worldwide ecological problems as well as affects the human health Vitousek, (1997). Biological nitrogen fixation (BNF) micro-organisms is the cheapest and most environment friendly organisms that can be fixed aerobic nitrogen in the soil and interacting with leguminous plants. This symbiotic relationship reduces the requirements for nitrogenous fertilizers during the growth of leguminous crops Gauri *et al*., (2011). The rhizobia are a group of Gram-negative bacteria that form species-specific symbioses with legume plant Bhatt *et al*., (2013).

The present study was investigated identification of isolated indigenous bacteria from nodules of *Pium sativum* L. (Garden pea). The aim and objectives of the present research is to study nature isolated indigenous bacteria and to evaluate their characteristics performed by various biochemical tests.

Materials and Methods

The fresh and healthy root nodules of *Pisum sativum* L. (garden pea) plants were collected from different places of Mogoung, Nanmati and Sahmaw in Kachin state. Plants possessing healthy nodule with pink colour were selected and the nodule samples were collected in plastic capped tube containing desiccant material, such as silica gel cover with a cotton plug. And then, carried out to the laboratory of Microbiology Section, Department of Agricultural Research Center, Yezin, Nay-Pyi-Taw during November 2018 to December 2019.

Table 1 Location of the samples collected sites

Collected Sites	Area	Location	Collected date
MOGAUNG	Kachin	25˚18′ 16.3″N 96˚56′11.2″ E	29. 11.2018
NANMATI	Kachin	25˚ 22′ 49 .2″N 97˚ 00′ 38 .3″ E	29. 11.2018
SAHMAW	Kachin	25˚ 13′ 37.2″N 96˚47′ 33 .4″ E	30. 11.2018

Figure 1 Root with nodules

Figure 2 Nodules in tube filled with dehydrated silica gel for transportation

Surface sterilization of the nodules

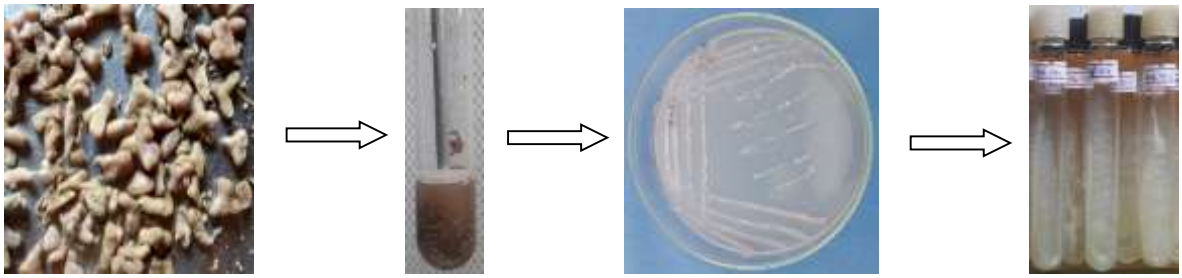
The samples of root nodules from *Pisum sativum* L. collected from different localities of Kachin state. Pea plants were uprooted carefully and as to get intact are obtained. Healthy pea nodules were detached from the root and further isolation of root nodulating rhizobia was carried out. The detached root nodules were washed in tap water to remove the adhering soil particles from nodule surface and surface treatment of nodules done with 95% alcohol for 30 sec. Nodules were dipped in 0.1% mercuric chloride ($HgCl_2$) solution for 30 sec and later washed successively ten times with sterilized distilled water to remove the traces of toxic $HgCl_2$. Surface sterilized nodules were transferred in test tube containing 5 mL of sterilized distilled water.

Extraction of indigenous root nodules bacteria

In this experiment, the selected plants have a healthy root nodules with pink color and are used as a material for isolation and further study the morphological characters of indigenous bacteria strain. These nodules were crushed with the help of sterilized glass rod to achieve a pink color suspension contain bacteriods. These were direct streaked on the media YEMA, CRYEMA and BTBYEMA. The plates were sealed by parafilm to avoid contamination and incubated at 28˚C. Typical isolated colonies were re-streaked on fleshy prepared YEMA slants, and use in order to obtain pure cultures for experiment. Finally, the pure culture slants were stored at 4˚C in refrigerator for further experiment works.

Colony morphological characterization of isolated indigenous bacteria

Colony morphology was studied by observing of various features such as size, shape, color, margin, elevation, surface and consistency. Microscopic examination was done by using Gram staining as described by (Arora,2003)



(A)Healthy fleshy root (B)Crushed root nodule (C)Culture on YEMA (D) Pure culture nodule maintain in slant

Figure 3 Extraction of indigenous bacteria from root nodules of *Pisum sativum* L.

Biochemical tests of isolated indigenous bacteria

All the collected samples were used in process of different biochemical tests such as Indole production test, Methyl red test Elsheikh E.A and wood, M (1989)., Methyl blue Wei *al.*, (2003), Citrate utilization Lupwayi and Hague (1994), Urea hydrolysis Lindstrom and Lehtomaki (1988), starch hydrolysis De O Liveria *et al*., (2007),Gelatin hydrolysis(Hunter *et al.*, 2007)., Glucose peptone agar(Kucuk *et al.*, 2006), Mannitol salt agar, Catalase test MacFaddin (2000), Glucose fermentation,Sucrose fermentation,Lactose fermentation Somasegaran & Hoben (1985) and Mannitol fermentation.

Results

Morphological characters of *Pisum sativum* L.

Scientific name - *Pisum sativum* L.
Family - Fabaceae
Sub-family - Papilionoidae
Common name - Sa-daw-pe
English name - Garden pea, Pea

(A)Cultivated field (B)Garden pea plant (C) Pod with seeds

Figure 4 Habit of ***Pisum sativum*** L**.**

Morphological characters of *Pisum sativum* L.

Pisum sativum L. is an herbaceous annual, with a climbing hollow stem. Leaves are alternate, pinnately compound, and consist of large leaf-like stipules. Flowers have five green fused sepals and five white to reddish-purple petals of different sizes. Fruit grows into a pod. that often has a rough inner membrane. The pod is a seed container which composed by two sealed valves and spilled along the seam which connects the two valves. Seeds are round, smooth, and green color.

Characteristics of nodules sample

Nodules are found on lateral roots of *Pisum sativum* L. as well as found a few Mostly on taproots. The nodules are about 2-5 mm in diameter. Active nitrogen fixing nodules contain a protein which is called leghemoglobin. When the nodules crushed the turned pink color because of the presence of leghemoglobin.

(A)Nodule

(B) L.s section

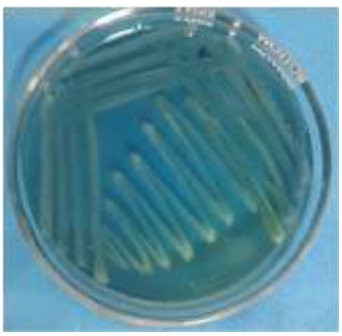
(C)Nodules

(D) L.s section

Figure 5 Transverse section of nodules of garden pea

(A)YEMA

(B)CRYEMA



(C)BTBYEMA

Figure 6 Isolation of bacteria culture on different medium

(A)Single colony(400X)

(B)Motility test

(C)Gram staining

Figure 7 Morphological characters of isolated indigenous bacteria

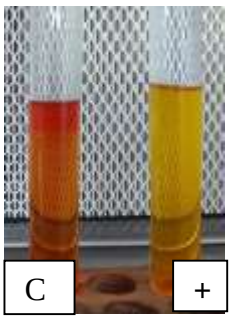
Table 2 Morphological colony characterization of isolated indigenous bacteria

Sr. No.	Colony character	MG	NM	SH
1.	Size (mm)	2-3	2-4	2-4
2.	Shape	Circular	Circular	Circular
3.	Color	white and translucent	white and translucent	white and translucent
4.	Margin	entire	entire	entire
5.	Elevation	convex (raised)	convex (raised)	convex (raised)
6.	Surface	Smooth	Smooth	Smooth
7.	Opacity	Opaque	Opaque	Opaque
8.	Consistency	Sticky	Sticky	Sticky
9.	Motility	Motile	Motile	Motile
10.	Gram nature	(- ve)	(- ve)	(- ve)

Table 3 Biochemical characterization of isolated indigenous bacteria

Sr. No	Biochemical Tests	MG	NM	SH
1.	Indole production test	+	+	+
2.	Methyl red test	+	+	+
3.	Methyl blue test	-	-	-
4.	Citrate utilization test	-	-	-
5.	Urease Hydrolysis test	+	+	+
6.	Starch Hydrolysis test	+	+	+
7.	Gelatin Hydrolysis test	-	-	-
8.	Glucose Peptone Agar test (GPA)	+	+	+
9.	Mannitol Salt Agar test	-	-	-
10.	Catalase test	+	+	+
11.	Glucose fermentation	+	+	+
12.	Sucrose fermentation	+	+	+
13.	Lactose fermentation	-	-	-
14.	Mannitol fermentation	+	+	+

(+) =Positive reaction (-) = Negative reaction



(A) Glucose fermentation

(B) Lactose fermentation

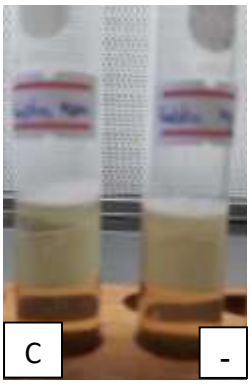
(C) Glucose Peptone Agar

(D) Citrate utilization

(E) Indole test

(F) Methyl red

(G) Methyl blue

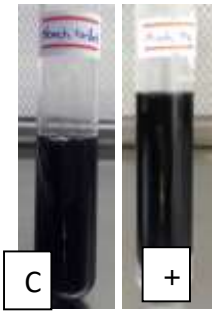


(H) Gelatin hydrolysis

(I) Urea hydrolysis

(J) Catalase test

(K) Mannitol salt agar



(L) Starch hydrolysis

Figure 8 Biochemical characterization of isolated bacteria

Discussion and Conclusion

The present study deals with identification of isolated indigenous bacteria from nodules of *Pisum sativum* L. The colonies of isolated *Rhizobium* bacteria were obtained by culture on the media YEMA, CRYEMA and BTBYEMA incubated after 3 days at 28˚C. The colonies were large (2-4 mm in diameter) mucilaginous, circular, convex with smooth edges **and** glistening translucent or white. This findings are in close agreement with Vincent, (1970) and Holt *et al.,* (1994). The isolated colonies were white and translucent, mucilaginous, circular, convex with smooth edges and sticky characters. However, the isolated bacteria were failed to absorb congo-red color in the CRYEMA and blue color in the BTBYEMA that is similar to the statements by researchers Shetta *et al.* (2011).

According to the results, colonies of isolated bacteria were appeared after 3 days and which are found like the fast grower species. Therefore, these findings are consistent with the findings of researchers Bala *et al* (2011). Microscopic examinations of the isolates were observed rod-shaped, pink colour gram- negative in nature and non-spore forming. These findings are similar to the nature of the findings of researchers Singh *et al*. (2008).According to biochemical tests results, the starch hydrolysis test was showed a positive response and the results are in line with the statement of researchers De Oliveria *et al.,* (2007).According to the results of Kucuk et al. (2006) Rhizobial cells have grown in GPA media. It has been suggested that Rhizobium uses glucose as a carbon source. The current result is consistent with the results of Kucuk *et al.*

However, pure *Rhizobium* isolates are unable to grow on lactose that can grow best on glucose, mannitol and sucrose. Therefore, the current findings are similar to the findings of the Somasegaran & Hoben (1985). In this experiment, It was observed that the rhizobial cells did not produce gelatinase enzymes as a medium containing gelatin.So, the result is agreement by findings of Hunter *et al.,* (2007) that negative gelatinase activity is a sign of Rhizobium. Besides, Current research has shown negative reactions to the growth of microorganisms in the methylene blue test and the gentian test.

These results consistent with the findings of Wei *et al.* (2003). In the catalase test, the formation of bubbles was clearly demonstrated. This finding is supported by the statement of MacFaddin (2000). Also in urea hydrolysis test, the isolated bacteria had a positive reaction and similar to the finding of Lindstrom and Lehtomaki (1988). In citrate utilization test the isolated bacteria reacted negatively reaction and in the line with the statement of Lupwayi and Hague. (1994). In Indole and Methyl red tests, the isolated bacteria were showed positive reaction and agreement with the findings of Elsheikh and Wood (1989). Mannitol salt agar test showed a negative reaction.

According to the results of the research, the isolated indigenous bacteria can be identified as *Rhizobium* species based on morphological and biochemical characteristics. These findings allow us a new scope for extensive research in Agricultural Biotechnology. The present study provides the valuable knowledge for young scientists to apply microbiology that can be applied and make available information for preparation of biofertilizer.

Acknowledgement

I am highly indebted to my advisor Dr. Mya Win, Associate Professor, Department of Botany, West Yangon University for his guidance and constant supervision as well as for providing necessary information regarding the study. I express heartfelt thank to Dr. Maw Maw Than, Deputy Director and Head, Agricultural Microbiology Section, Department of Agricultural Research center, Yezin, Nay- Pyi -Taw for financial support and providing space in micro-lab to achieve this work.

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PALYNOLOGICAL STUDY ON SOME SPECIES OF CONVOLVULACEAE FROM SHWEDAUNG TOWNSHIP

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Abstract

The pollen morphology of twelve species belonging to six genera of Convolvulaceae familiy were studied in the present paper. The pollen grains of all specimens were collected from Shwedaung Township, Bago Region (west). The pollen morphological characteristics of all species were studied. The aperture type and sculpture pattern of each grain were examined by electric microscope. Three types of aperture (tricolpate, pentacolpate and porate) and four types of exine sculpture echinate, granulate, reticulate and striato-reticulate are found. The outline of pollen images for each species were presented by polar and equatorial view and then were recorded by photomicrographs of clear cut pollen images and types of habit and flower.

Keywords: pollen grains, exine sculpture, flower.

Introduction

Palynology is the science of the study of plant pollens, spores, microscopic planktonic organisms, in both living and fossil form. Pollen morphology is one of the most important and fundamental branches of palynology. The study of pollen morphology helps in the confirmation of relationship and affinities between the related taxa. Pollens of related families and genera are usually of more or less the same type. The number of apertures on the wall, size and shape of the pollen grains etc, play an important role in identification and relationships of plants at various taxonomic levels (Nair, 1964).

Palynology is the study of pollen and spores of plants. Spores and pollen grains have a number of morphological and ultrastructural features. These palynological features have provided a wealth of characters that have been important in inferring phylogenetic relationship of plants (Simpson, 2006).

The morphological studies of pollens are very important. It is also used in the field agriculture, forestry, archaeology, and plant geography. The examination of pollen grains, both recent and ancient can be of value in a range of scientific studied (Moore *et al*., 1991).

The family Convolvulaceae comprise a large number of species (about 50 genera and over 1200 species; Lawrance, 1968). In Myanmar, about 133 species from 26 genera had been recorded by the previous studies of Kress *et al*. (2003).

The aims of this research are to study the pollen morphology of the collected species, to support some information into the features use for pollen identification and to provide the valuable pollen characters that can be used in plant classification and identification.

Materials and Methods

The specimens were collected from Shwedaung Township, Bago region (west). Shwedaung Township is situated on the eastern bank of Ayeyarwady River in western of Bago Region. It lies between 18°20' 10" and 18°45' 0" north latitudes and 95°2' 20" and 95°23' 0"east longitudes.

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All the specimens were recorded while flowering. Describing and classifying of the species were used fresh specimens. Identification of specimens were accomplished in accordance with the taxonomic procedures. By using floristic literatures of Lawrence (1968), Backer (1963-1968), Ali and Nasir (1979-1990), Gilbert (1994), Dassanayake (1983-2000) and HU.Qi-ming & WU Delin (SCBG), (2007-2009). Myanmar names were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003).

For the pollen study, pollen samples of the specimens were freshly collected from the anthers in blooming flowers. Pollens of each species were stored in glass vials with 1cc of glacial acetic acid/glass bottle with 99.9% alcohol and the specimen was labeled with its specific name. The pollen sample in glacial acetic acid were acetolysed by the standard acetolysis method of Erdtman (1952). The anther specimen in a glass vial were crushed with a glass rod and 1cc of glacial acetic acid were added then transferred into a test tube and 5-9 drops of concentrated sulphuric acid were added depending on the amount of pollen materials. The test tubes were put in a water-bath for 15-30 minutes at 70-80˚c.

The fluid in the test tubes were stirred frequently and after boiling, it were centrifuged with distilled water and decanting the clear parts. These were carried out repeatedly for 3 or more times. Then glycerine jelly with safranin were added to the polliniferous materials according to the method of Kisser formula (Erdtman, 1952).

For the pollen study, the storage bottles were warmed in water bath and a drop of polliniferous jelly were taken out and placed on the glass slide and then covered with a glass coverslip. A glass slide mounted with pollen sample was examined under electric light microscope with (x400) and photomicrograph. The samples of pollen grains for each species were measured and studied. The shape, size, and exine sculpture of the pollens were studied, recorded by photographs.

Results

In pollen morphology, the 12 species belonging to the 5 genera of Convolvulaceae family were identified and studied the morphological characteristics of pollen grains.

Pollen Morphology of Study Species

1. *Argyrera barbigera* Choisy. Mem. Soc.Phys. Geneve 6: 424 (1833 publ. 1834)

Myanmar Name : Min-go-kha

English Name : Notknown

Flowering period : December to March

Polyporate (about 50), pantoporate, spheroidal, large, 70-80 µm in diameter; amb circular; pori circular, about 5 µm in diameter, inter poral spaces 7.5-10.0 µm ; exine about 5 µm in thick, sexine thicker than nexine; sculpturing echinate, spine 7.5-10.0 µm in length, straight, pointed.

2. *Camonea vitifolia (*Burm.f.) A.R.Simoes & Staples. Bot. J.Linn.Soc. 183:583 (2017)

Myanmar Name : Not-known

English Name : Grape glory

Flowering period : February to April

Pentacolpate, zonocolpate, suboblate, medium, 37.5-42.5 x 45.0-47.5µm in length and breadth; amb rounded; colpi longicolpate, 25-30 x 5-10 µm in length and breadth; exine 1.7-2.5 µm thick, sexine thicker than nexine; sculpturing reticulate.

3. ***Evolvulus alsinoides*** **(L.) L. Sp. Pl. ed. 2: 392 (1762)**

Myanmar Name : Kyauk-hkwe-pan

English Name : Not-known

Flowering period : October – December

Tricolpate, zonocolpate, suboblate, large, 42.5-47.5 x 19.0-22. µm in length and breadth; amb rounded triangular; colpi longicolpate (38.5-43.5 x 17.5-18.5 µm in length and breadth; exine 3.7-3.8 µm thick, sexine thicker than nexine; sculpturing striato-reticulate.

4. ***Ipomoea alba*** L. p. Pl.: 161 (1753)

Myanmar Name : Nwe-kazon-phyu

English Name : Moon flower

Flowering period : September to December

Polyporate, (about 136), pantoporate, spheroidal, very large, 105-140 µm in diameter; amb circular; pori circular, 5.0-10 µm in diameter, inter poral spaces 7.5-10 µm in width,; exine 6.2-7.5 µm thick, sexine thinner than nexine; sculpturing echinate, spines 5.0-7.5 µm in length, rounded, globoid.

5. ***Ipomoea carnea*** Jacq. Enum. Syst. Pl.: 13 (1760)

Myanmar Name : Lathar-pan

English Name : Pink morning glory

Flowering period : October to December

Polyporate, (about 30), pantoporate, spheroidal, large-very large, 95-110 µm in diameter; amb circular; pori circular, 2.5-5.0 µm in diameter, inter poral spaces 10 - 12.5 µm in width; exine 3.7-5.0 µm thick, sexine thicker than nexine; sculpturing echinate, spines 10.0-12.5 µm in length, rounded, slightly curved.

6. ***Ipomoea hederifolia*** L., Syst. Nat. ed.10, 925 (1759)

Myanmar Name : Myat-lay-ni-yaing

English Name : Star Ipomoea or Scarlet creeper

Flowering period : October to December

Polyporate, (about 50), pantoporate, spheroidal, large-very large, 100 - 112.5 µm in diameter; amb circular; pori circular, 3.7 - 5.0 µm in diameter, inter poral spaces 10 - 11.2µm in width; exine 5.0 - 7.5 µm thick, sexine thicker than nexine; sulpturing echinate; spines about 5.0 µm in length, subacute, straight.

7. ***Ipomoea nil*** **(L.) Roth.** Catal. Bot. 1: 36 (1797)

Myanmar Name : Pan-kha-mauk

English Name : Blue morning glory

Flowering period : October to December

Polyporate, (about 60), pantoporate, spheroidal, 87.5-107.5 µm in diameter; amb circular; pori circular; exine 6.2-7.5 µm thick, sexine thicker than nexine; sculpturing echinate, spines 5.0 - 10 µm in length, subacute, straight.

8. ***Ipomoea obscura***(L.) Ker Gawl. Bot. Reg. 3: t. 239 (1817)

Myanmar Name : Not known

English Name : Obscura morning glory

Flowering period : August to December

Polyporate, (about 30), pantoporate, spheroidal, large, 55-70 µm in diameter; amb circular; pori circular, 5.0-7.5 µm in diameter; exine 3.7-5.0 µm thick, sexine as thick as nexine; sulpturing echinate; spines 5.0-7.5 µm in length, pointed, straight.

9. ***Ipomoea triloba*** L. Sp. Pl.: 161 (1753)

Myanmar Name :Yoikha-ma-shokthwe

English Name : Aiea morning glory

Flowering period : September to December

Polyporate, (about 46), pantoporate, spheroidal, large, 55.0 - 67.5 µm in diameter; amb circular; pori circular, 2.5-5.0 µm in diameter; exine 2.5 -5.0 µm thick, sexine thinner than nexine; sculpturing echinate; spines 5.0-7.5 µm in length, pointed, straight.

10. ***Merremia aegyptia*** (L.) Urb. Symb. Antill. 4: 505 (1910)

Myanmar Name : Not-known

English Name : Hairy woodrose

Flowering period : October to December

Tricolpate, zonocolpate, suboblate, medium, 40-45 x 45-50 µm in length and breadth; amb rounded triangular; colpi longi colpate, 30-32.5 x 2.5-5 µm in length and breadth; exine 2.5-3.7 µm thick, sexine thicker than nexine; sculpturing striato-reticulate.

11. ***Merremia gemella*** (Burm.f.) Hallier f. Bot. Jahrb. Syst. 16: 552 (1893)

English Name : Not-known

Myanmar Name : Not-known

Flowering period : October – December

Tricolpate, zonocolpate, oblate spheroidal, medium, 35-37.5 x 40 µm in length and breadth; amb rounded triangular, colpi longicolpate, 10 x 2.0 -3.0 µm in length and breadth; exine 2.5 - 5.0 µm thick, sexine as thick as nexine; sculpturing granulate.

12. ***Operculina turpethum*** (L.) Silva Manso. Enum. Subst. Braz: 16. 1836.

Myanmar Name : Nyan-nwe; Kyar-hin

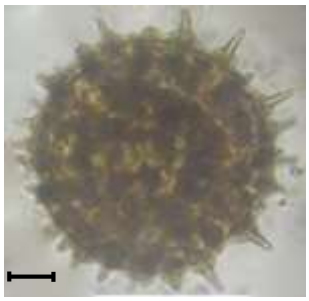
English Name : Transpaent woodrose

Flowering period : December to March

Tricolpate, zonocolpate, oblate spheroidal, large, 53.0-58.0 x 55.0-60.0 µm in length and breadth; amb rounded triangular; colpi longi colpate, 32.0-40.0 x 3.0 µm in length and breadth; exine 2.0-3.0 µm thick, sexine thicker than nexine; sculpturing striato-reticulate.

1. ***Argyrera barbigera*** **Choisy.**

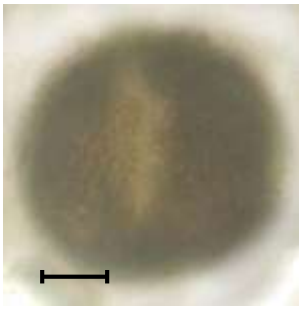


Habit Flower Surface view Surface view

2. ***Camonea vitifolia (***Burm.f.) A.R.Simoes & Staples.



Habit Flower Polar view Equatorial view

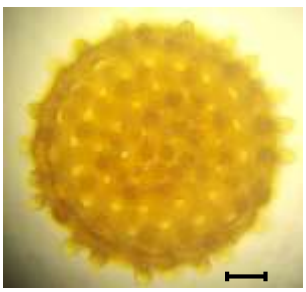
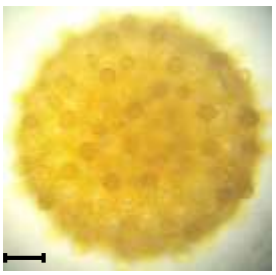
3. ***Evovulus alisnoides*** L.




Habit Flower Polar view Equatorial view

4. ***Ipomoea alba*** L.



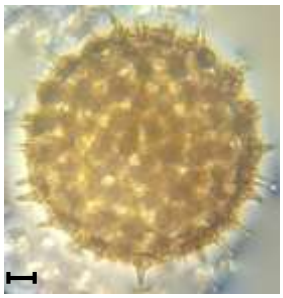
Habit Flower Surface view Surface view

5. ***Ipomoea carnea*** Jacq.


Habit


Flower

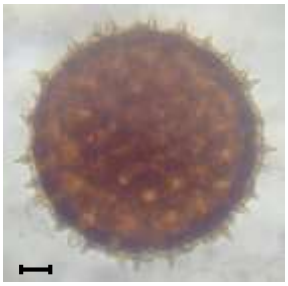

Surface view

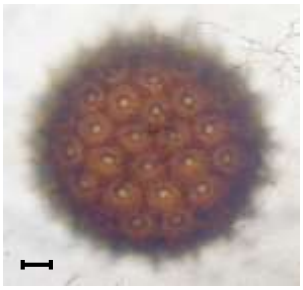

Surface view

6. ***Ipomoea hederifolia*** L.


Habit


Flower

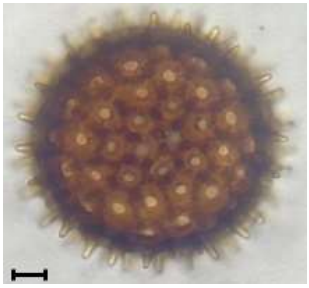

Surface view

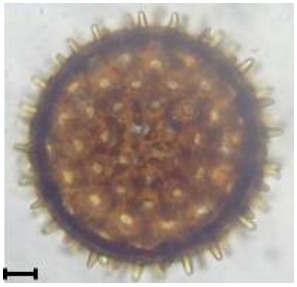

Surface view

7. ***Ipomoea nil*** (L.) Roth.


Habit


Flower

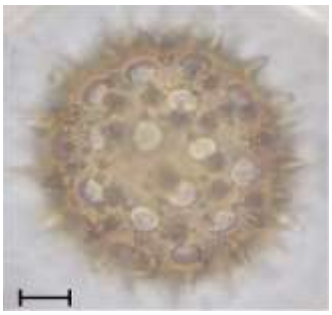

Surface view

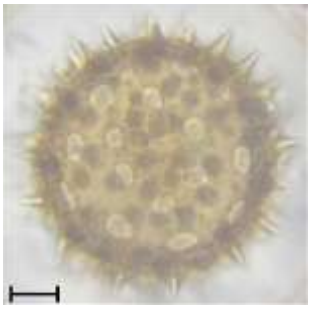

Surface view

8. ***Ipomoea obscura***(L.) Ker Gawl.


Habit


Flower


Surface view

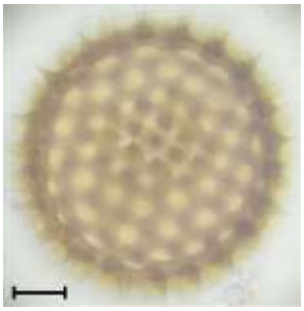

Surface view

9. ***Ipomoea triloba*** L.


Habit


Flower


Surface view


Surface view

10. ***Merremia aegyptia*** (L.) Urb.


Habit


Flower


Polar view


Equatorial view

11. ***Merremia gemella*** (Burmf.) Hallier.


Habit


Flower


Polar view

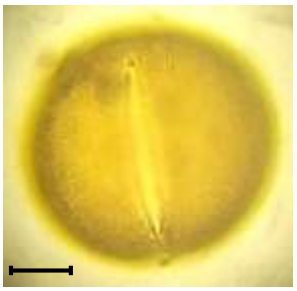

Equatorial view

12. ***Operculina turpethum*** (L.) Silva Manso.


Habit


Flower


Polar view


Equatorial view

Discussion and Conclusion

Family Convolvulaceae belonging to Order Solanales is one of the largest family of flowering plants. This family is widely distributed throughout the world but especially in the tropic and subtropic on both hemispheres and a few in temperate region. It is composed of about 1500 taxa (Cronquist; 1981). In Myanmar, about 133 species from 26 genera had been recorded by the previous studies of Kress *et al*. (2003). In the present paper, pollen morphology of 12 species belonging to 6 genera of family Convolvulaceae has been studied.

In the classification of taxa, taxonomic characters of pollen morphology are apertures type, number, position, sculpture, shapes and grain size. The types of all pollen grains occur in Convolvulaceae are colpate (sometimes colporate), rupate, rugate or forate (porate). The *Ipomoea* type- grains polyporate, very large, crassinexinous, spiniferous. The sexine consist of more or less rod like elements. These rods often coalesce with the spines to form basal rootlets (Erdtman *et al*., 1961).

In the present paper, the type of pollen grains are colpate and porate. Among the colpate grains, tricolpate grains are found in *Evovulus alisnoides* L., *Merremia aegyptia* (L.) Urb., *M gemella* (Burmf.) Hallier. and *Operculina turpethum* (L.) Silva Manso. Pentacolpate grains are found in *Camonea vitifolia* **(**Burm.f.) A.R.Simoes & Staples.. The rest of all *Ipomoea* species and *Argyrera barbigera* Choisy. are polyporate grains.

Hesse *et al*., (2009) stated that pollen size varies less than 10µm to more than 100 µm. In this research, smallest pollen (35-37.5 x 40 µm in length and breadth) is found in *Merremia gemella* (Burmf.) Hallier f. and the largest pollen (105-140 µm in diameter) is found in *Ipomoea alba* L.

In this study, the sculpture pattern of the pollens were observed as granulate, echinate, reticulate and striao-reticulate. The granulate sculpture is found in *Merremia gemella* (Burmf.) Hallier f. The reticulate sculpture is found in *Camonea vitifolia* **(**Burm.f.) A.R.Simoes & Staples.. The striao-reticulate sculpture found in *Evolvulus alsinoides* L., *Merremia aegyptia* (L.) Urb., and *Operculina turpethum* (L.) Silva Manso.

The echinate sculpture is the distinct character of pollen grains in the family of Convolvulaceae representing the genus *Argyrera* and *Ipomoea*. The length and shape of spines in echinate sculpture of *Ipomea* varies according to the species. In the present study, the spines varies within range of 5.0- 12.5 µm in length. Among them, the largest spine of *Ipomoea carnea* Jacq. is 10.0-12.5 µm in length and the smallest spine about 5.0 µm in *Ipomoea hederifolia* L.

In the present study, the thickness of the exine in Convolvulaceae range was found between 1.7 µm and 7.5 µm in thick. The thinnest exine found in *Merremia* species and the thickness exine found in *Ipomoea* species.

Therefore, aperture and exine sculpture forms useful taxonomical characters in systematic study of Convolvulaceae. Hence, the pollen characters may support additional identification and classification of flowering plants. The present result will provide valuable information for the further studies of palynology.

Acknowledgements

We are deeply indebted to Dr. Aung Aung Min, Pro-Rector and Dr. Thwe Lin Ko, Pro-Rector, Pyay University, for their permission and encouragement for this research paper. We wish to express our deep gratitude to Dr. Nyo Nyo Thaung, Professor and Head, Department of Botany, Pyay University, for their encouragement and suggestions.

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TAXONOMIC STUDY ON TWELVE SPECIES OF ANGIOSPERMAE IN THARTHANAR-2500 HILLOCK, LASHIO AREA

Tin Tin Maw*

Abstract

The present study deals with the members of Angiospermae growing in Tharthanar-2500 Hillock, Lashio area. Some species are collected during December 2019 to June 2020. Some flowering plants from Tharthanar-2500 Hillock have been collected, identified and then morphological characteristic were studied. In this study, twelve species belonging to twelve genera of nine families were identified and systematically arranged according to APG IV system, 2016 (Angiosperm Phylogeny Group). Artificial key to the species, detail taxonomic descriptions of the individual species has also been described with relevant photographs. In addition, their flowering period, Myanmar names and English names were also described.

Keywords: Taxonomy, Angiospermae, Tharthanar-2500 Hillock, Lashio area

Introduction

Lashio area is situated in Northern Shan State of Myanmar. Tharthanar 2500 Hillock is located in Lashio area. Lashio area is bounded by Humon village in the east, Ei nine village in the west, Mehan and Man ai villages in the south and Swan kwai village in the north. It lies between 22°53'-23°02' North Latitude and 97°42'-97°48' East Longitude. Lashio area lies 855 meter above sea level. The area is about 4832 kilometer square.

During the period from December 2019 to June 2020, an average monthly rainfall is 76. 86 mm and 6 rainy days. This area almost gets no rain fall in February and March. The average maximum temperature is 29.64° C and average minimum temperature is 13.77° C. The coldest month of this area is December (8° C). The warmest month is May (33° C). The maximum percentage of humidity in December and January is 86 and the minimum percentage of humidity in May is 35 (Meteorology and Hydrology Department, Lashio). The climate condition is warm and wet, good rainfall and moderate temperature than the middle part of Myanmar. The soil type is mostly red loamy.

Tharthanar 2500 Hillock is in the mountain deciduous forest region. The natural vegetation of Tharthanar 2500 Hillock consists of herbs, shrubs, climbers, twiners, vines and woody trees. In the present study twelve species belonging to twelve genera of nine families under subclass Magnoliidae had been identified and fully described. The families Proteaceae, Fabaceae, Rosaceae, Moraceae, Cucurbitaceae, Elaeocarpaceae, Cornaceae, Rubiaceae and Apocynaceae are found in this area.

The aim and objectives of the present research are mainly to record the knowledge on the natural resources in study area, to get valuable information of Angiospermae to be used for other researchers and to provide for learning in botany.

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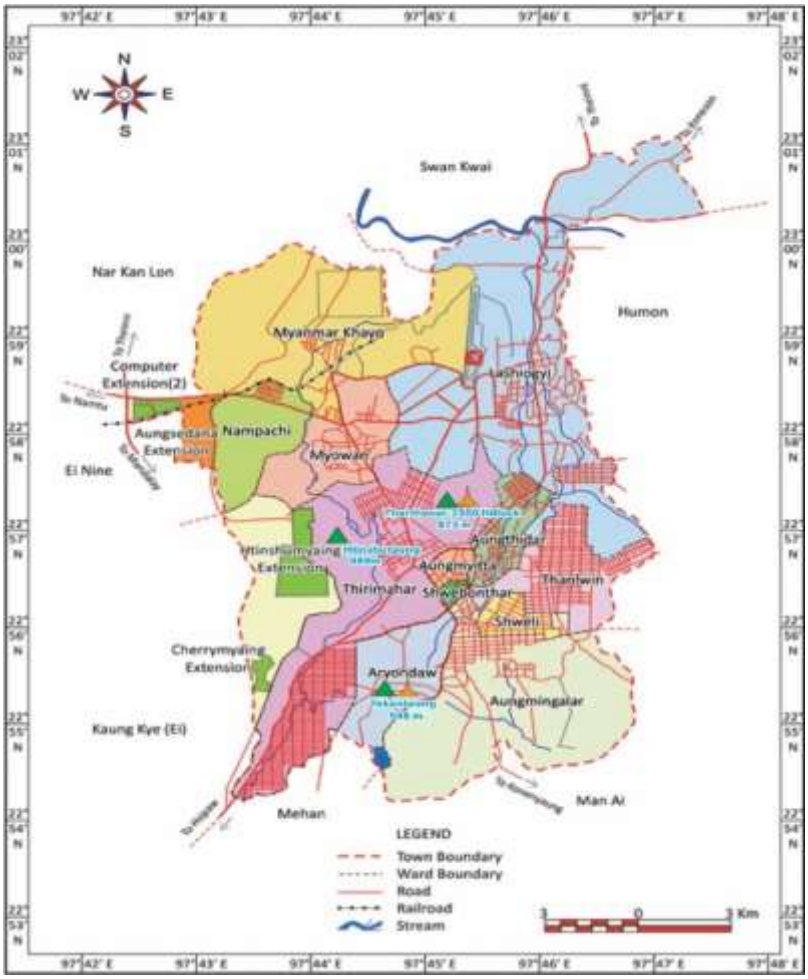

Source: Department of Geography, Lashio University

Figure 1 Location map of Lashio area

Materials and Methods

Some members of Angiospermae were collected from Tharthanar-2500 Hillock, Lashio area. The specimens were collected from December 2019 to June 2020. The specimens were kept immediately into the plastic bags to identify and classify systematically. The collected specimens had been observed and noted in detail. And then the collected specimens were identified with the references of Flora of British India (Hooker, 1875, 1878 & 1885), Flora of Java (Backer, 1965), Flora of Ceylon, (Dassanayake, 1980, 1981, 1983, 1991, 1995, 1997, 1998 & 1999), Flora of Hong Kong, (Qi-ming, 2008), Flora of China (Wu. *et al*., 2010). In addition to construction of artificial key to the species, all the resulting species are systematically arranged into families according to APG IV system, 2016 (Angiosperm Phylogeny Group) and genera and species according to alphabetically. The specimens were recorded by photographs.

Results

Table 1 List of the collected species (Subclass: Magnoliidae)

Super order	Order	Family	Scientific name	Myanmar name
Eudicots	Ranunculales	Proteaceae	1. *Grevillea robusta* A. Curn.	Khardaw-hmi
Rosids	Fabales	Fabaceae	2. *Acrocarpus fraxinifolius* Whight & Arn.	Ye-tama
			3. *Adenanthera pavonina* L.	Ywe-gye
			4. *Biancaea sappan* (L.) Tod.	Thein-nyet
			5. *Caesalpinia minax* Hance	Unknown
	Rosales	Rosaceae	6. *Rubus alceifolius* Poiret	Unknown
		Moraceae	7. *Broussonetia papyrifera* (L.) L' Herit.	Hmaing-kan-setku
	Cucurbitatales	Cucurbitaceae	8. *Momordia dioica* Roxb.	Kyet-hin-kha-cho
	Oxalidales	Elaeocarpaceae	9. *Elaeocarpus robustus* Roxb.	Kaya-hmwe
Asterids	Cornales	Cornaceae	10. *Alangium chinese* (Lour.) Harms	Taw-posa
	Gentianales	Rubiaceae	11. *Pavetta indica* L.,	Myet-hna-pan
		Apocynaceae	12. *Telosma pallida* (Roxb.) Craib	Gwedauk-thein

Taxonomic descriptions

1. Proteaceae (Juss, 1789)

1. 1. *Grevillea robusta* A. Curn. ex R.Br., Port. Nov. 24.1830.

Myanmar name : Khardaw hmi
English name : Silver oak, Silk oak
Flowering period : March to May

Perennial, tall robust trees, 10-30 m high; twigs brownish with more or less appressed whitish hair. Leaves simple, alternate, exstipulate; petioles 2-6 cm long, brownish-tomentose; blades deeply pinnatipartite, deltoid-ovate to oblong-ovate, 15-33 cm by 9-15 cm, pinnae 5-11 pairs, 2-12 cm long, the pinnae deeply pinnatifid with entire or incision; lobes lanceolate or linear-oblong, 2.5-8 cm by 0.5-1 cm, acute at the base, recurved along the margin, acuminate at the apex, olive green above, silver grey silky hairy beneath. Inflorescences terminal raceme, 5-15 cm long, many flowered; peduncles solitary or a few together on lateral branchlets, 2-3 cm long, densely tomentose. Flowers orange to golden-yellow, 0.5-1 cm in diameter, zygomorphic; pedicels slender, 1-1.5 cm long, glabrous. Perianth tubular, hooded, 4-lobed, 1.5-2 cm long, orange or golden-yellow to lemon-yellow with dark red inner base; lobes narrowly spathulate, 5-7 mm by 1-2 mm. Stamens 4, free, epitepalous, sessile; anthers dithecous, about 1 mm long; floral disk semi-annular; gynophore about 2-3 mm long. Ovary superior, ovoid, 1.5 mm by 1.5 mm, unilocular with 1-to 4 ovules on pendulous placentae, glabrous; style slender, 1-2.5 cm long, dilated at the apex, lemon-yellow, glabrous; stigma rhomboid-ovoid, 1 mm long, thick, greenish-yellow. Fruits follicular, compressed-ovoid, 1.5-2 cm by about 1 cm, 2-seeded, dehiscent, silver grey to olive green, glabrous. Seeds ovate, 1-1.5 cm by 0.5-1 cm, broadly winged, thin. (Figure. 2. A)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 57' 58.4", E 97° 44' 54.3", 869 meter, Dr. Tin Tin Maw, March 16, 2020, collected no. 3.

2. Fabaceae (Lindl. 1836)

2.1. ***Acrocarpus fraxinifolius*** Whight & Arn., Mag. Zool. Bot. 2: 547. 1838.

Myanmar name : Ye-tama

English name : Pink ceder, Australian ash, Indian ash

Flowering period : January to February

Deciduous trees, upto 40 m high; bole buttressed; bark thin light-grey or yellowish-grey; blaze dull red. Leaves bipinnate compound, alternate; stipules triangular, free lateral, caducous; rachis 13-15 cm long, stout, glabrous, pulvinate; 3-5 pairs, paripinnate, 20-30 cm long, slender, glabrous; leaflets 10-16 per pinnae, opposite; petiolules upto 4 mm long, slender, glabrous; blades elliptic, elliptic-lanceolate or elliptic-oblong, 3.5-17 cm by 1.5-7.5 cm, oblique and acute or obtuse at the base, entire along the margin, acuminate at the apex, glabrous, coriaceous; lateral nerves 7-15 pairs, pinnae slender, prominent, intercostae reticulate, faint. Inflorescences dense, axillary racemes, erect, many-flowered; peduncles 15 cm long. Flowers red, 1-1.5 cm in diameter, actinomorphic, deflexed; bracts small, caducous; pedicels 4-10 mm long. Calyx campanulate, 5-lobed; tube 3-4 mm by 3-4 mm, lobes ovate or triangular, 2.5-5 mm long, equal, short-hairy outside. Petal 5, free, oblong, narrow subequal, 5-10 mm by 3-4 mm, short-hairy. Stamens 5, free, exserted, crimson coloured; filaments 1.5-1.8 cm long, anthers dithecous. Ovary superior, oblong to linear, 1.2-1.5 cm long, stipitate, unilocular, with many ovules on the marginal placentae, hairy; style short, incurved; stigma minute. Pods flat, ligulate, 8-17 cm by 1.5-2.5 cm, 5 to 15-seeded, dehiscent, narrowly winged. Seeds obovate, oblique, 6.5 mm by 5 mm, smooth, compressed, brownish. (Figure. 2. B)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 56' 54.7", E 97° 44' 46.7", 829 meter, Dr. Tin Tin Maw, February 28, 2020, collected no. 2.

2.2. ***Adenanthera pavonina*** L., Sp. Pl. 384. 1753. Hance, J. Bot. 22: 365. 1884.

Myanmar name : Ywe-gye, Ywe-ni

English name : Bead tree, Chek-long, Coral wood, Red sandalwood

Flowering period : May to July

Small to medium-sized trees, up to 40 m high; stems and branches terete, glabrous. Leaves bipinnate compound, paripinnate, alternate, to 38 cm long; stipules filiform, about 0.5 mm long, caducous; petioles 1.5-2.5 cm long; racheae 20-38 cm long; glabrous, pinnae 3-5 pairs; leaflets 5-9 on each side of pinnae, alternate, elliptic to ovate or obovate-elliptic, 1.5-4 cm by 1.4-2.4 cm, broadly cuneate at the base, entire along the margin, rounded to truncate at the apex, thinly chartaceous, glabrous or slightly puberulous on both surfaces. Inflorescences axillary racemes, 14-22 cm long, many-flowered, glabrous or slightly puberulous, often with a few scattered glandular hairs; peduncles 3-4 cm long. Flowers white to yellow, 7-10 mm in diameter, actinomorphic; bracts lanceolate, about 0.5 mm long, puberulous; pedicels 5-7 mm long. Calyx subcordate to broadly cup-shaped, 5-teethed, glabrous or sparsely appressed puberulous; tube about 1 mm long; teeth rounded. Petals 5, free, oblong, 3-5 mm by 1-1.2 mm, acute at the apex, glabrous or sparsely appressed puberulous. Stamens 10, free, exserted; filaments filiform 3-4 mm long; anthers dithecous, with a stipitate gland. Ovary superior, linear, 2.5-3 mm by 1 mm, unilocular, with many ovules on the marginal placentae, glabrous or few scattered hairy; style filiform, 2.5-3 mm long; stigma simple. Pods linear-falcate, 22-25 cm by 1-1.5 cm, 15 to 25-seeded,

brown, contorted to spirally twisted after dehiscence. Seeds ellipsoid, uniformly bright scarlet, 8-9 mm by 7-9 mm. (Figure. 2. C)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 57' 11.6", E 97° 44' 34.4", 830 meter, Dr. Tin Tin Maw, June 5, 2020, collected no. 5.

2.3. ***Biancaea sappan*** (L.) Tod., Hort. Bot. Panorm. 1: 3. 1875.

Caesalpinia sappan L., Sp. Pl. 381.1753.

Myanmar name : Tein-nyet; Sun-the

English name : Sappan Wood, Indian redwood

Flowering period : December to February

Small trees or shrubs, up to 10 m high; stems terete, minutely tomentose, glabrescent, sparsely armed with short, straight or recurved prickles. Leaves bipinnate compound, paripinnate, alternate; stipules spiniform, about 3-5 mm long, usually recurved; petioles 1.5-2.5 cm long; racheae 25-40 cm long; with prickles, pinnae 9-14 pairs; leaflets 10-20 paired, opposite, asymmetrically oblong with excentric costa, 1-2.5 cm by 3-8 mm, obliquely truncate or obtuse at the base, entire along the margin, obtuse at the apex, glabrous on both surfaces. Inflorescences supra-axillary or terminal racemes, many-flowered; peduncles 12-40 cm long. Flowers yellow, 2-2.5 cm in diameter, zygomorphic; bracts lanceolate, about 5-12 mm long, puberulous, caducous; pedicels 1-2 cm long. Sepals 5, free, cucullate, the lowest one about 10 mm long, the others 7 mm long, leathery, ciliate or glabrous. Petals 5, free, suborbicular, 9-11 mm by 7-10 mm, clawed, yellow, tinnged with pinkish at base. Stamens 10, free, exserted; filaments 1.5 cm long, densely pubescent in lower part; anthers dithecous, glabrous. Ovary superior, obliquely oblong, 7-10 mm by 3-4 mm, stipitate, unilocular, with 3-6 ovules on the marginal placentae, greyish tomentose; style filiform, 1.5 cm long, hairy; stigma truncate. Pods obliquely oblong, 7-10 cm by 3- 4 cm, 2 to 4-seeded, beaked, black at maturity, puberulent when young but essentially glabrous. Seeds oblong to elliptic, 15-18 mm by 8-11 mm, slightly compressed, brown to black. (Figure. 2. D)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 56' 51.6", E 97° 45' 53.8", 869 meter, Dr. Tin Tin Maw, December 25, 2019, collected no.1.

2.4. ***Caesalpinia minax*** Hance, J. Bot. 22: 365. 1884.

Myanmar name : Unknown

English name : Unknown

Flowering period : April to July

Climbers, prickly, puberulent, throughout up to 8 m high; stems terete, puberubent, spiny. Leaves bipinnate compound, paripinnate, alternate, to 65 cm long; stipules subulate, hard; about 5-7 mm long; petioles 1-1.2 cm long; racheae 25-48 cm long; with prickles, pinnae 5-10 pairs; leaflets 6-12 paired, opposite, elliptic or oblong, 2-4.5 cm by 1.2-2 cm, puberulent on midvein, rounded and slightly oblique at the base, entire along the margin, obtuse-rounded or acute at the apex, glabrous on both surfaces. Inflorescences terminal racemes or panicles, many-flowered; peduncles 9-30 cm long. Flowers White, 2-3 cm in diameter, zygomorphic; bracts ovate-lanceolate, about 1.5-2 cm by 0.8-1 cm, puberulous, shortly acuminate at the apex; pedicels 1.5-2 cm long. Sepals 5, free, oblong, about 1.5-2 cm long, densely yellowish velutinous. Petals 5, free, obovate, about 1.8-11 cm by 1.2 cm, contiguous at base, obtuse-rounded at apex, white, tinged with purple spots, hairy beneath and marginally. Stamens 10, free, inserted; densely pubescent in lower part, filaments linear, 1.7-2 cm long; anthers dithecous, 2 mm long, glabrous. Ovary superior, oblong, 3-4 mm by 1-1.5 mm, unilocular, with 5-8 ovules on the marginal placentae, dense small spines;

style filiform, 2-2.5 mm long, slightly longer than stamens, glabrous; stigma truncate. Pods oblong, 7.5-13 cm by 4-4.5 cm, 3 to 8-seeded, obtuse-rounded at apex, with 0.5-2.5 cm beak, densely needle-like spiny. Seeds elliptic, slightly concave on one side, about 1.8 cm by 1 cm, with cyclic veins. (Figure. 2. E)

Specimen examined : Northen Shan State; Lashio area, Tharthanar-2500 Hillock, N 22° 56' 55.1", E 97° 44' 53.4", 872 meter, Dr. Tin Tin Maw, June 12, 2020, collected no. 7.

3. Rosaceae (Juss. 1789)

3.1. *Rubus alceifolius* Poiret in Lamarck, Encycl. Meth. 6: 247. 1804. 1897.

Myanmar name : Unknown
English name : Giant bramble, Wild raspberry
Flowering period : June to August

Shrubs scandent, to 5 m high, with arching or climbing branches; branchlets brown or reddish brown, densely spreading straight tomentose-villous, with sparsely recurved prickles to 5 mm. Leaves simple, alternate; stipules oblong to orbicular, 1-1.7 cm long, deeply digitately or pinnately divided margin, with linear or linear-lanceolate lobes, hairy; petioles 2.5-4.5 cm long, yellowish gray to rustly tomontose-villous, with sparsely minute prickles; blades suborbicular or broadly ovate, 6-15 cm by 6.5-18 cm, palmately 5 veined, thinly leathery, cordate with incision to 3 or 4 cm deep at the base, shallowly 5-7- lobed and unevenly coarsely serrate along the margin, obtuse or acute at the apex, sparsely villous and distinctly bullate between veins above, yellowish-gray to rustly tomentose and villous along vein beneath. Inflorescences terminal, narrow cymose panicles or subracemes, 3-7-flowered, 6-11 cm long, sometimes flowers few in axillary clusters; peduncles 1-2 cm long, tomentose-villous, with minute recurved prickles. Flowers white, about 2-3 cm in diameter, actinomorphic; bracts 1-2 cm long, pannatifid to pinnatipartite, with linear or lanceolate lobes; bracteoles 2, oblong, 1.5-2 cm long, pinnately divided margin; pedicels slender, 0.5-2 cm long, tomentose-villous, with minute recurved prickles. Calyx campanulate, 5-lobed, tomentose-villous; tube cupular, 0.5 cm by 1 cm; lobes broadly ovate to triangular-ovate, 1.2-1.5 cm by 5-8 mm, apex and margin of outer sepals palmately or pinnately laciniate, inner sepals entire and shortly pointed. Petals 5, free, suborbicular to broadly obovate, 5-9 mm by 5-9 mm, clawed. Stamens many, free, shorter than petals; filaments flattened, about 5 mm long, hairy; anthers dithecous, linear, about 1 mm long, with few long hairs. Pistils numerous, longer than stamens. Ovary inferior, ellipsoid, 1-1.5 mm by 1 mm, unilocular, with two ovules on the basal placentae, glabrous; style filiform, 7-9 mm long, glabrous; stigma capitate. Fruits aggregate berry, subglobose, 1.5 cm by 1.8 cm, 1-seeded drupelets, red when ripe; drupelets small, subglobular, black, rugose. (Figure. 2. F)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 57' 03.8", E 97° 45' 0.8", 863 meter, Dr. Tin Tin Maw, June 30, 2020, collected no. 12.

4. Moraceae (Link. 1831)

4.1. *Broussonetia papyrifera* (L.) L' Herit. ex Vent., Tabl. Reg. Veg. 3: 547. 1799.

Morus papyrifera L., Sp. Pl. 2: 986. 1753.
Myanmar name : Hmaing-kan-setku, The-le
English name : Paper mulberry, Tapa cloth tree
Flowering period : February to April

Deciduous, medium sized trees, 10-20 m tall, dioecious; bole small buttresses; bark smooth, dark grey; branchlets 1.5-3 mm thick, short, soft hairy. Leaves simple, alternate or

opposite or whorled; stipules ovate, 5-15 mm by 4-10 mm, acuminate, membranous, slightly ribbed, densely hairy, caducous; petioles 2-12 cm long, hairy; blades obliquely broad ovate to ovate-elliptic or oblong, 4-18 cm by 3-12 cm, rounded or cordate and asymmetric at the base, entire or serrulate to finely dentate, unlobed or palmately 3-lobed along the margin, acuminate at the apex, scabrous and sparsely pubescent above, densely soft hairy beneath especially along the veins. Male catkins axillary or crowded at terminal of young shoots, cylindrical, 3-9 cm long, many-flowered, pale yellow; peduncles 1-2.5 cm long, hairy; bracts lanceolate, pubescent. Male flowers: minute, actinomorphic, sessile; calyx triangular-ovate, 1.5-2 mm by about 1 mm, 4-lobed, whitish hairy outside; stamens 4, 3-3.5 mm long, anthers dithecous, globose. Female heads globose, 1-1.5 cm in diameter, solitary, many-flowered, greenish; peduncles 0.5-2 cm long, hairy, bracts clavate, apically pubescent. Female flowers: minute, actinomorphic; calyx tubular, 4-lobed, apically connate with style, about 2 mm long; ovary superior, ovoid, about 0.5 mm long, unilocular, with one ovule on the pendulous placentae, stipitate; styles filiform, about 5-6 mm long, pink; stigma linear, pubescent. Etaerio of drupes, globose, 2-3 cm in diameter, orange-red, drupelets oblongoid, 2- 2.5 mm long, 1-seeded. (Figure. 2. G & H)

Specimen examined: Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 57' 02.3", E 97° 44' 54.5", 881 meter, Dr. Tin Tin Maw, March 27, 2020, collected no. 4.

5. Cucurbitaceae (Juss. 1789)

5.1. ***Momordia dioica*** Roxb. ex. Willd. Sp. Pl. 4: 605.1805.

Myanmar name : Kyet-hin-kha-cho, Hinga-baung
English name : Spiny gourd, bristly balsam pear, prickly carolaho
Flowering period : Jun to October

Perennial, climbing herbs, dioecious, with tuberous roots; stems slender, much branched, furrowed, glabrous and shining; tendrils simple, elongate, striate, glabrous. Leaves simple, alternate, petioles slender, 2.5-5 cm long, puberulous, channelled above; blades ovate-cordate, 5-15 cm by 5-12 cm, deeply cordate at the base, shortly denticulate along the margin, acuminate at the apex, minutely punctate, especially beneath. Male flowers axillary, solitary, pale yellow or white, 3-6 cm in diameter, actinomorphic; bracts cupuliform, 1.5-2.5 cm by 2.5-3.5 cm, foliaceous, enclosing the flowers bud, strongly nerved, corrugate, oblique, emarginate at the apex, pubescent; peduncles 5-10 cm long, finely patent-pubescent. Calyx 5-lobed, distinct, linear-lanceolate, 1-1.3 cm by 7-9 mm, pubescent. Petals 5, basally connate, oblong lanceolate, 4-6 cm by 2.5-3 cm, pale yellow with black spots at the base, finely pubescent. Stamens 5, inserted; filaments short, slender, black; anthers dithecous, one monothecous, yellow above and black beneath. Female flowers axillary, solitary, pale yellow or white, 3-6 cm in diameter, actinomorphic; peduncles 5-23 cm long, finely patent-pubescent. Calyx and petals similar to male flowers. Ovary inferior, oblong-ovoid, unilocular, with many ovules on the parietal placentae, densely long soft-papillose; style very short; stigma bifid. Fruits baccate, ellipsoid or oblong-ovoid, 2.5-7 cm by 3.5-4.5 cm, shortly beaked, densely soft spines, orange or yellow, splitting into 3 valves when ripe. Seeds many, broadly ellipsoid, 1 cm long, slightly compressed, enclosed in a red pulp, irregularly corrugated, black. (Figure. 2. I & J)

Specimen examined: Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 56' 58.8", E 97° 44' 54.7", 879 meter, Dr. Tin Tin Maw, Jun 8, 2020, collected no. 6.

6. Elaeocarpaceae (Juss. ex DC. 1824)

6.1. ***Elaeocarpus robustus*** Roxb., Fl. Ind. ed. 2, 2: 597. 1832.

Myanmar name : Kaya-hmwe, Taw-magyi
English name : Cylon olive, India olive
Flowering period : Jun to July

Trees, 15-25 m high; barks lenticellate, warty on mature one, greenish grey; stem and branches terete, glabrescent. Leaves simple, alternate; stipules triangular, 5 mm by 3 mm, pubescent; petioles 1-3 cm long, thickened at both ends, often with 2 glands near apex, grey puberulous; blades elliptic-oblong to ovate-oblong, 5-24 cm by 3-10 cm, broadly cuneate to rounded at the base, repand-serrate along the margin, acute to acuminate at the apex, rusty pubescent when young, glabrous, coriaceous. Inflorescences axillary racemes, many-flowered, 4-14 cm long; peduncles 0.5-2 cm long, pubescent; flower buds oblong-ovoid, acute at apex. Flowers white, 5-7 mm in diameter, actinomorphic, fragrant; pedicels about 5-7 mm long, grey puberulous. Sepals 5, free, lanceolate or ovate, 5-6 mm long, acute at the apex, greenish yellow, densely villous along the margin. Petals 5, free, cuneate-oblong or obtriangular, laciniate at the apex, ciliate along the margin, 4-8 mm by 5 mm. Stamens 25-40, free; filaments filiform, about 1-3.5 mm long, glabrous or minutely puberulous; anthers dithecous, oblong, about 1 mm long; disc thick, 5-lobed, yellow, tomentose. Ovary superior, oblong to ovoid, 1-1.5 mm by 1.5 mm, trilocular, with one ovule in each locule on the axile placentae; style slender, about 2 mm long, hairy; stigma simple. Fruits drupaceous, ovoid-ellipsoid or oblong-ovoid, 3.5 cm by 3 cm, 1-seeded, greenish-yellow; pyrenes 2-3 locular, with 2 longitudinal grooves prominently rugose. (Figure. 2. K)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 56' 54.7", E 97° 44' 46.7", 829 meter, Dr. Tin Tin Maw, June 28, 2020, collected no. 11.

7. Cornaceae (Dumort. 1829)

7.1. ***Alangium chinese*** (Lour.) Harms, Ber. Deutsch. Bot. Ges. 15: 24. 1897.

Stylidium chinese Lour., Fl. Cochinch. 221.1790.

Myanmar name : Taw-posa, Saga-thein, Letkadon, Tabuya
English name : Alangi, Chinese Alangium
Flowering period : May to July

Shrubs or small trees, 3-5 m high; branchlets pubescent when young, glabrescent. Leaves simple, alternate, exstipulate; petioles 1.5-3.5 cm long, reddish, puberulous; blades ovate or orbicular to cordate, 10-22 cm by 5-14 cm, papery, usually oblique and often rounded or subrounded or triangular at the base, entire or with few shallow lobes along the margin, acuminate at the apex, glabrous above, tufted pubescent at axil of vein beneath. Inflorescences axillary cymes, 3-15-flowered, 5-6 cm long; peduncles 2-3 cm long, pubescent. Flowers creamy-white to pale yellow, 2 cm in diameter, actinomorphic, sweetly scented, golden hairy; bracts linear, 3 mm long; pedicels slender, 3-4 mm long, pubescent. Calyx cup-shaped, 6-8-lobed; tube 2 mm by 2 mm; lobes shortly dentate. Petals 6-8, free, lanceolate, 1-1.5 cm by 1 mm, roll backwards. Stamens 6-8, about as many as petals, free; filaments flattened, short, 2-3 mm long, pubescent inside; anthers dithecous, linear-oblong with a long connective, 8-9 mm long; disk cushion-like. Ovary inferior, ovoid, 1 mm by 1.5 mm, uni-bilocular, with one ovule in each locule on the pendulous placentae; style slender, 8-9 mm long, pale-yellow, glabrous; stigma capitate, 2-4-lobed, 1-1.5 mm long, pale-yellow. Fruits drupaceous, ovoid ellipsoid, 5-13 mm by 5-7 mm, 1-seeded, longitudinally grooved, crowned by a persistent disk and cup-shaped calyx, dark violet. Seeds compressed. (Figure. 2. L)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 27' 05.5", E 97° 44' 58.8", 872 meter, Dr. Tin Tin Maw, June 23, 2020, collected no. 10.

8. Rubiaceae (Juss. 1789)

8.1. ***Pavetta indica*** L., Sp. Pl. 110. 1753.

Myanmar name : Myet-hna-pan, Za-gwe-pan

English name : White pavetta, Hill pavetta, Indian pavetta

Flowering period : April to Jun

Perennial erect shrubs to small trees, up to 3 m high; stems and branches terete, glabrous, usually with pallid peeling bark. Leaves simple, opposite and decussate; stipules cupular to broadly triangular, 3-6 mm long, glabrous; petioles 1-2 cm long; blades elliptic to broadly elliptic, 11-15 cm by 5-7.5 cm, acute at the base, entire along the margin, obtuse or subacute at the apex, glabrous above, soft hairy beneath. Inflorescences terminal or axillary corymbose cymes; peduncles 3-5 cm long, glabrous. Flowers white, 1-1.5 cm in diameter, actinomorphic, fragrant; bracts often fused in pair; pedicels slender, 3-10 mm long. Calyx short, tubular, 4-lobed; tube tubular, 1-1.5 mm long; lobes triangular, 5 mm long. Corolla salverform; 4-lobed, tube1.5-1.7 cm long, glabrous outside, pilose inside; lobes oblong, 5-9 mm by 2-3 mm, acute to mucronate at apex. Stamens 4, free, exserted, at the throat of corolla-tube; filaments filiform, about 1 mm long,; anther dithecous, linear-oblong, 4-5 mm long, sagittate at base, often twisted, cream turning to black. Ovary inferior, ovoid, about 1 mm long, bilocular, with solitary ovule in each locule on the axile placentae, glabrous; style filiform, 2-3 cm long, glabrous; stigma oblong, greenish. Fruits baccate, globoid, 7-10 mm in diameter, 2-seeded, black. Seeds subgloboid, plano-convex. (Figure. 2. M)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 57' 04.8", E 97° 44' 59.8", 874 meter, Dr. Tin Tin Maw, June 15, 2020, collected no. 8.

9. Apocynaceae (Juss. 1789)

9.1. ***Telosma pallida*** (Roxb.) Craib, Bull. Misc. Inform. Kew. 1911: 418. 1911.

Asclepias pallida Roxb., Fl. Ind. 2: 49. 1824.

Myanmar name : Gwedauk-thein, Taw-daung-da-late, Swe-daw-nwe

English name : Telosma vine

Flowering period : June to September

Perennial, herbaceous, high-climbing vines; latex watery; stems slender, slightly tumid at the nodes, pale green with velvety hairs. Leaves simple, opposite and decussate, exstipulate; petioles 1.5-2.5 cm long, with velvety hairs; blades ovate, 4-9 cm by 2.5-6.5 cm; cordate at the base, entire along the margin, acuminate at the apex, membranous, sparsely hairy above, glabrous beneath except on the veins. Inflorescences axillary, erect or pendulous subumbelliform cyme, single or in pair, 10-16 flowered; peduncles 8-10 mm long. Flowers pale green, 2-2.5 cm in diameter, actinomorphic, slightly fragrant; bracts lanceolate, 5 mm by 2 mm, sparsely velutinous on both surfaces; pedicels filiform, 8-10 mm long, with velvety hairs. Calyx campanulate, 5-lobed, hirsute; lobes ovate-lanceolate or oblong, 4-5 mm by 1.5 mm, membranous with distinct veins, imbricate, obtuse at the apex. Corolla salver-shaped, 5-lobed, greenish, sparsely hairy both inside and outside; tubes very short, swollen at the base; lobes oblong, 9 mm by 3 mm, membranous, with outwardly rolled margin, obtuse at the apex, overlapping to the right in bud, rotate and twisted near the tip in bloom. Corona staminal, 4.5 mm by 2.5 mm, exserted above corolla tube; coronal scales 5, flattened, adnate to the back of anthers, not proceeding above anthers tip, erect, transversely bifid, swollen at the base with constriction just above it. Stamens 5, free, inserted, adnate to the

base of corolla tube; filaments connate into a tube; anthers dithecous, basifixed, the tips fleshy, scale-like, applied against the stigma; pollinia solitary in each cell, subcylindric-clavate, with a narrow hyaline margin, much larger than corpusculum, yellow, erect, waxy; corpusculum oblong-ovate, reddish brown; caudicles simple, membranous, shorter than the corpusculum. Ovary superior, composed of 2 distinct carpel, bilocular, with many ovules on the axile placentae; styles 2; stigma capitate-globose, protruding above the anthers tip. Follicles solitary, lanceolate, 10-16 cm by 1.5-2 cm, echinate with long soft bristles. Seeds 8 mm by 4 mm, comose, with thin membranous wings. (Figure. 2. N)

Specimen examined : Northen Shan State, Lashio area, Tharthanar-2500 Hillock, N 22° 57' 06", E 97° 44' 55.5", 875 meter, Dr. Tin Tin Maw, June 23, 2020, collected no. 9.

An artificial key to the studied species:

1. Ovary superior ---------- 2
1. Ovary inferior ---------- 9

2. Leaves simple ----------3

2. Leaves compound ---------- 6

3. Placentation pendulous ----------4

3. Placentation axile ---------- 5

4. Plants monoecious; flowers zygomorphic; fruits follicular ----- 1. *Grevillea robusta*

4. Plants dioecious; flowers actinomorphic; fruits etaerio of drupe ---------- ---------- 7. *Broussonetia papyrifera*

5. Trees; leaves alternate; inflorescences racemes; flowers white; petals laciniate; stamens many; fruits drupaceous ---------- 9. *Elaeocarpus robustus*

5. Climbing vines; leaves opposite and decussate; inflorescences subumbelliform cymes; flowers pale green; petals not laciniate; stamens 5; fruits follicular ---------- 12. *Telosma pallida*

6. Plants without prickles; flowers actinomorphic ---------- 7

6. Plants with prickles; flowers zygomorphic ---------- 8

7. Flowers red; stamens 5; pods ligulate ---------- 2. *Acrocarpus fraxinifolius*

7. Flowers white to yellow; stamens 10; pods linear-falcate ---------- 3. *Adenanthera pavonina*

8. Climbers; petal white, tinged with purple spots; pods densely needle-like spiny ---------- ---------- 4. *Caesalpinia minax*

8. Small trees or shrubs; petals yellow, tinged with pinkish base; pods glabrous ---------- ---------- 5. *Biancaea sappan*

9. Leaves opposite and decussate; stamens 4, turning to black ---------- 11. *Pavetta indica*

9. Leaves alternate; stamens 5 or 6-8 or many, not turning to black ---------- 10

10. Plants dioecious ; placentation parietal; fruits densely soft spines ---------- ---------- 8. *Momordia dioca*

10. Plants monoecious; placentation basal or pendulous; fruits glabrous ----------11

11. Plants with prickles; petals not roll backwards; fruits aggreate berry ---------- ---------- 6. *Rubus alceifolius*

11. Plants without prickles; petals roll backwards; fruits drupaceous ------- 10. *Alangium Chinese*

Discussion and Conclusion

The present study deals with the plants growing in Tharthanar 2500 Hillock, Lashio area. Totally, twelve species belonging to twelve genera of nine families under subclass Magnoliidae had been studied in the present paper. All the species presented in this study are dicotyledonous plants.

The families in this research paper are Proteaceae, Fabaceae, Rosaceae, Moraceae, Cucurbitaceae, Elaeocarpaceae, Cornaceae, Rubiaceae and Apocynaceae under the subclass Magnoliidae. They are arranged according to the classification of APG IV system, 2016. Among the species in the present study, the species of *Broussonetia papyrifera* (L.) L' Herit and *Momordia dioica* Roxb. are commonly found in this area. The species of *Acrocarpus fraxinifolius* Whight & Arn. and *Alangium chinese* (Lour.) Harms are rarely found.

Among the twelve species, *Rubus alceifolius* Poiret is shrub; *Caesalpinia minax* Hance, *Momordia dioica* Roxb. and *Telosma pallida* (Roxb.) Craib are climbers and the rest species are trees. Except *Acrocarpus fraxinifolius* Whight & Arn., *Adenanthera pavonina* L., *Biancaea sappan* (L.) Tod., and *Caesalpinia minax* Hance are compound leaves and others are simple. Flowers of *Grevillea robusta* A. Curn., *Biancaea sappan* (L.) Tod., and *Caesalpinia minax* Hance are zygomorphic, but the rest species are actinomorphic. Except *Rubus alceifolius* Poiret, *Momordia dioica* Roxb., *Alangium chinese* (Lour.) Harms and *Pavetta indica* L. are inferior ovaries, others are superior ovaries. *Momordia dioica* Roxb. is parielal and *Rubus alceifolius* Poiret is basal placentation, *Grevillea robusta* A. Curn, *Broussonetia papyrifera* (L.) L' Herit and *Alangium chinese* (Lour.) Harms are pendulous placentation, *Elaeocarpus robustus* Roxb., *Pavetta indica* L. and *Telosma pallida* (Roxb.) Craib are axile placentation while the others are marginal placentation. Fruits of *Grevillea robusta* A. Curn and *Telosma pallida* (Roxb.) Craib are follicular, *Rubus alceifolius* Poiret, *Momordia dioica* Roxb. and *Pavetta indica* L. are baccate, *Broussonetia papyrifera* (L.) L' Herit, *Elaeocarpus robustus* Roxb. and *Alangium chinese* (Lour.) Harms are drupaceous, but those of others species are pods.

Momordia dioica Roxb. is found in the study area used for edible. *Grevillea robusta* A. Curn, *Acrocarpus fraxinifolius* Whight & Arn., and *Elaeocarpus robustus* Roxb. are used for timber production plants. *Broussonetia papyrifera* (L.) L' Herit is used for quality paper making plants. All twelve species are also medicinally important plants. *Biancaea sappan* (L.) Tod. included the IUCN (International Union for the Conservation of Nature) Red list of threatened species (2011).

According to the data collected, it can be noted that twelve species from twelve genera are distributing. The collected species are identified and described with comments on their scientific names, Myanmar names and coloured plates. It is hoped that this research of present investigation have contributed towards a better understanding of twelve species distributed in Tharthanar 2500 Hillock for its paper utilization in the other researchers in various field of study. Finally, it is also hoped that this research paper will provide invaluable taxonomic data and information to be used for learning in botany.

Figure 2

A. *Grevillea robusta* A. Curn.
B. *Acrocarpus fraxinifolius* Whight & Arn.
C. *Adenanthera pavonina* L.
D. *Biancaea sappan* (L.) Tod.
E. *Caesalpinia minax* Hance
F. *Rubus alceifolius* Poirt
G. & H. *Broussonetia papyrifera* (L.) L' Herit.
I. & J. *Momordia dioica* Roxb.
K. *Elaeocarpus robustus* Roxb.
L. *Alangium chinese* (Lour.) Harms
M. *Pavetta indica* L.
N. *Telosma pallida* (Roxb.) Craib.

Acknowledgements

I would like express my sincere to Dr. Kyaw Tun, Rector and Dr. Hla Hla Tin, Pro-rector, Lashio University, for their permission to conduct this research paper. I wish to express deepest thanks to Dr. Khin Thet Kyaw, Professor and Head, Dr. Nwe Nwe Hnin, Professor, Department of Botany, Lashio University, for their invaluable suggestion and permission to this paper.

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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF SOIL FUNGI

Wunna Tun[1], Zar Zar Yin[2] and Yee Yee Thu[3]

Abstract

In this study, three different soil samples were collected from three Townships (North Oakkala, Mayangone and Insein) in Yangon Region. The soil type from North Oakkala and Insein was clay loam whereas that from Mayangone was sandy clay. Twenty-four soil fungi were isolated from three different soil samples by the serial dilution method. The forms of fungal colonies were circular, irregular, filamentous and rhizoid. The elevation of colonies was raised, convex, flat, umbonate while the margins of colonies of isolated fungi were entire, undulate, filiform, curled and lobate. Antimicrobial activity of isolated fungal strains was evaluated by the agar well diffusion method with eight test organisms: *Agrobacterium tumefaciens, Escherichia coli, Staphylococcus aureus, Pseudomonas fluorescens, Bacillus subtilis, Bacillus pumilus, Malassezia furfur* and *Candida albicans.* Among 24 isolates, seventeen strains showed different levels of antimicrobial activities. Among seventeen active strains, isolated fungal strains WT-14, 17 and 24 were moderately antimicrobial activity while seven strains WT-1, 2, 4, 7, 9, 13 and 15 showed highly antimicrobial activity against all test organisms. According to the morphological and microscopical characters of bioactive seven strains, six of them (strains WT-1, 2, 4, 9, 13 and 15) were identified as *Aspergillus* sp. while strain WT-7 was *Rhizoctonia* sp.

Keywords: Antimicrobial activity, Identification, Morphological characters, Soil fungi

Introduction

Soil provides ecosystem services critical for life: soil acts as a water filter and a growing medium; provides habitat for billion of organisms, and supplies most of the antibiotics used to fight diseases (Soil Science Society of America). Biodiversity loss also occurs in soil in which there is great diversity of living organisms that depends on the vegetation, as well as on the quantity of organic material produced (Fierer and Jackson, 2006).

The microorganisms plays significant role in soil ecosystem. Estimate point to 1.5 million fungal species worldwide of which only about 99.000 has been described using classical taxonomic approaches (Hawksworth, 1991; 2001). Fungi are very vital for the soil ecosystem since they play a key role in different essential processes including organic matter decomposition (Christensen, 1998). The novel discoveries of microorganisms can produce as a potential source of new antibiotics in the recent decades (Ullah *et al*., 2017). Fungi produce many antibiotics, having antibiotics and antifungal activity, which are widely used as drugs over the world especially the penicillin, cephalosporin and fusidic acid (Dobashi *et al*., 1998).

The objectives of this paper were to isolate the soil fungi on four different media Blakeslee's Malt Extract Agar, Czapek Dox Agar, Potato Dextrose Agar and Low Carbon Agar, to study the colony morphology and the cultural characteristics of isolated soil strains, to observe the antimicrobial activity on eight test organisms and to identify possible genus of active soil fungi.

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Materials and Methods

Collection of soil samples

The soil samples (15 cm depth) were collected from three different Townships in Yangon Region, during July 2019, and put sterilized polythene bags after removing the surface soil for the isolation of fungi. Soil colour, soil type and their location were shown in Table 1.

Table 1 Collected soil samples from three different townships of Yangon Region

Sample	Place	Soil Color	Soil Type	pH	Location
1	North Oakkala	Red	Clay Loam	7.02	N16º53.665" E 96º08.257"
2	Mayangone	Brown	Sandy Clay	6.27	N16º52.307" E 96º08.412"
3	Insein	Red	Clay Loam	6.40	N16º53.080" E 96º06.608"

Isolation of soil fungi by serial dilution method (Dubey, 2002)

Each soil sample (1.0 g) was introduced into a conical flask containing 99 mL of distilled water. The flask was then shaken 30 minutes in order to make the soil particles free from each other. This solution was serial diluted from 10^{-3} to 10^{-7} dilution in separate test tubes and 0.5 mL each of the above dilution was separately transferred into sterile petri dishes under aseptic condition. The sterilized medium in conical flask was cooled down to about 45ºC and separately poured into each of the petri-dish containing the respective solid dilutions.

The inoculated plates were shaken clock-wise and anticlock-wise direction for about 5 minutes so as to make uniform distribution of the fungi inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at 27º-30º C for 3-6 days at the Laboratory of Biological Development Center of Pathein University.

Four different culture media for the isolation of fungi (Ando, 2004)

1. Blankeslee's Malt Extract Agar (BMEA medium)

Malt extract 20.0 g, Peptone 1.0 g, Glucose 20.0 g, Agar 18.0 g, DW 1 L, pH 6.5

2. Czapek-Dox Agar (CzA medium)

Sucrose 30.0 g, $NaNO_3$ 2.0 g, K_2HPO_4 1.0 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 .7H_2O$ 0.01g, Agar 18.0 g, Distilled Water 1L, pH 7 .0

3. Potato Dextrose Agar (PDA Medium)

Sucrose 30.0 g, $NaNO_3$ 2.0 g, K_2HPO_4 1.0 g, $MgSO_4 .7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 .7H_2O$ 0.01g, Agar 18.0 g, Distilled Water 1 L, pH 7

4. Low Carbon Agar (LCA) medium for first culture

Glucose 2.0 g, Sucrose 2.0 g, K_2HPO_4 1.0 g, $MgSO_4 .7H_2O$ 0.5 g, KNO_3 1.0 g, KCl 0.5 g

Agar 18.0 g, DW 1 L, pH6.

After autoclaving (121ºC for 30mins), Chloranphenicol was added into the medium.

Morphological characters of isolated fungi (Ando, 2004)

After incubating fungi on the plate cultures for 5 days, colony morphological characters such as the surface color and the reverse color, the elevations of colonies and the margins of colonies of all isolated fungi were photographed and measured.

Antimicrobial activities by agar well method (Collin, 1965)

Isolated stains were tested by agar well method for their preliminary antimicrobial activities. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3-6 days old culture fermented broth ($20\mu L$) were incubated at room temperature for 24-28 hours. After 24-28 hours of incubations, the clear zones (inhibitory zones) were measured. Therefore, the diameter of clear 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 2).

Table 2 Eight kinds of test organisms used for antimicrobial activity

Test Organisms	Code Numbers	Disease
Agrobacterium tumefaciens	NITE 09678	Crown gall disease
Escherichia coli	AHU 5436	Diarrhoea, abdominal pain
Staphylococcus aureus	AHU 8456	Boil and food poisoning
Pseudomonas fluorescens	IFO 94307	Septicemia
Bacillus subtilis	IFO 90571	Fever
Bacillus pumilus	IFO 90571	Fever
Malassezia furfur	AUS 01020	Dandruff, Dermatitis
Candida albicans	NITE 09542	Candidasis, Skin disease

NITE = National Institute of Technology Evaluation

Results

Isolation of fungi from soil samples

In present research work, the soil samples were collected from three different places: North Oakkala, Mayangone and Insein Townships in Yangon Region. The soil type of North Oakkala was clay loam and pH value of 7.02. Mayangone Townships was sandy clay and pH value of 6.27, and Insein Townships was clay loam and pH value of 6.40 respectively.

All isolated fungal strains were temporarily named as WT-1 to WT-24. These total of 24 fungal isolates were obtained; five strains from soil sample 1, seven strains from soil sample 2 and twelve strains from soil sample 3. Soil fungal strains were isolated on four different media: 11 strains were isolated on BMEA Medium, 8 strains on PDA Medium, 4 strains on LCA Medium and 1 strain on CzA.

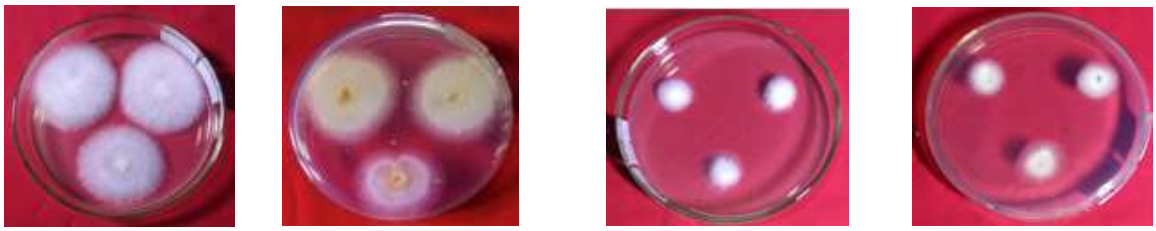
Morphological characters of isolated fungi

The surface color of strains WT-1, 11, 17, 18, 19, 22 and 23 was white. The surface colors of other strains were green, greenish white, dark green, pale green, black, white black and yellow. The reverse color of WT-1 was white and the other strains were white black, green, greenish white, pale green, black and cream. The forms of colonies were circular, irregular, filamentous and rhizoid. The elevations of fungal colonies were raised, convex, flat, and umbonate. The margins of

fungal colonies were entire, undulate, filform, curled and lobate. These results were shown in Table 3 and Figures 1 to 12.

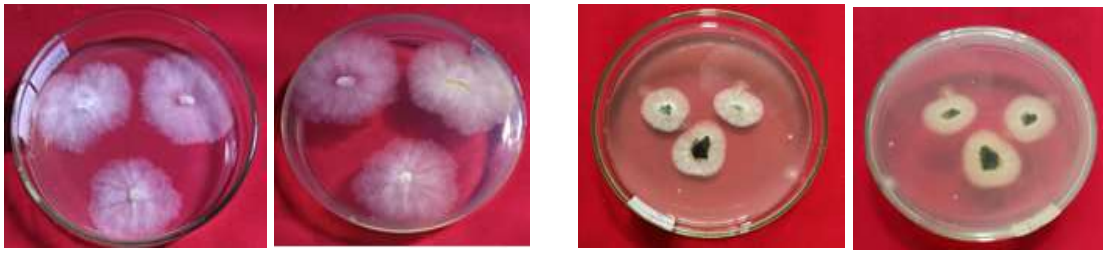
Table 3 Morphological character of isolated fungi

Strain No.	Surface color	Reverse color	Form	Elevation	Margin	Size
WT-1	White	Yellowish White	Circular	Raise	Undulate	Large
WT-2	White	White	Circular	Flat	Entire	Small
WT-3	White	Cream	Circular	Raised	Entire	Small
WT-4	Black White	Black White	Irregular	Convex	Entire	Medium
WT-5	Black White	Greenish White	Filamentous	Flat	Undulate	Medium
WT-6	Greenish White	Greensih White	Circular	Flat	Entire	Medium
WT-7	Black White	Greenish White	Circular	Raised	Entire	Medium
WT-8	White	Yellowish White	Circular	Flat	Undulate	Small
WT-9	White	Yellowish White	Circular	Raised	Undulate	Large
WT-10	Black White	Yellowish White	Circular	Flat	Entire	Large
WT-11	Black White	White	Circular	Raised	Entire	Small
WT-12	Pale Green	Pale Green	Irregular	Convex	Entire	Large
WT-13	White	Cream	Filamentous	Filamentous	Undulate	Medium
WT-14	Dark Green	Dark Green	Circular	Flat	Entire	Medium
WT-15	White	White	Circular	Raised	Entire	Large
WT-16	Greenish White	White	Circular	Flat	Undulate	Small
WT-17	Green	White	Circular	Raise	Undulate	Large
WT-18	Black	White	Circular	Flat	Entire	Small
WT-19	Black	White Black	Circular	Raised	Entire	Small
WT-20	Pale Green	White	Irregular	Convex	Entire	Medium
WT-21	Greenish White	White	Filamentous	Flat	Undulate	Medium
WT-22	Dark Green	Cream	Circular	Flat	Entire	Medium
WT-23	Black White	Cream	Circular	Raised	Entire	Medium
WT-24	Cream	Pale Yellow	Circular	Flat	Undulate	Small



Front view (WT-1) Reverse view Front view (WT-2) Reverse view

Figure 1 Colony morphology of isolated soil fungi WT-1 and WT-2

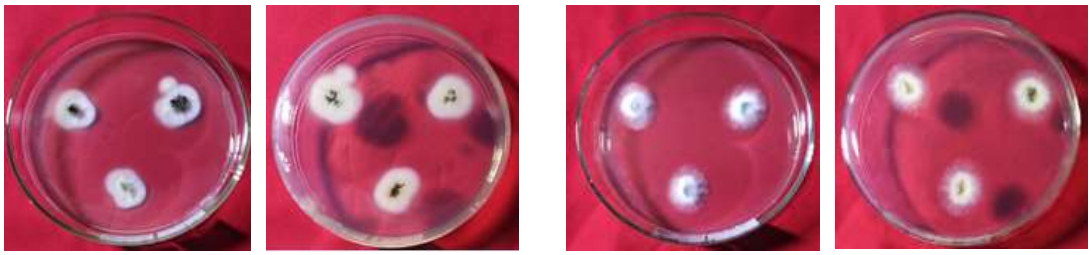


Front view (WT-3) Reverse view Front view (WT-4) Reverse view

Figure 2 Colony morphology of isolated soil fungi WT-3 and WT-4

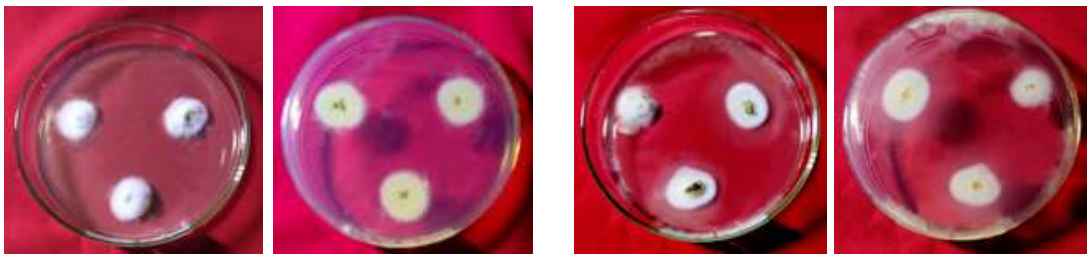
Front view (WT-5) Reverse view Front view (WT-6) Reverse view

Figure 3 Colony morphology of isolated soil fungi WT-5 and WT-6



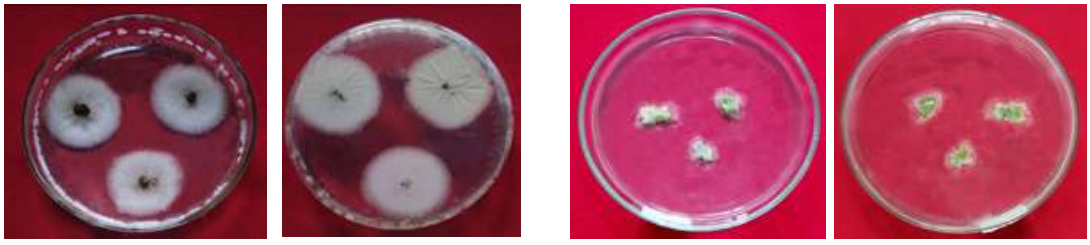
Front view (WT-7) Reverse view Front view (WT-8) Reverse view

Figure 4 Colony morphology of isolated soil fungi WT-7 and WT-8



Front view (WT-9) Reverse view Front view (WT-10) Reverse view

Figure 5 Colony morphology of isolated soil fungi WT-9 and WT-10

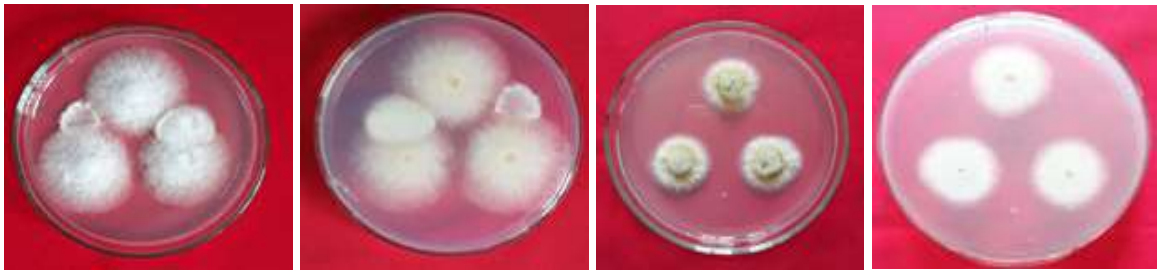


Front view (WT-11) Reverse view Front view (WT-12) Reverse view

Figure 6 Colony morphology of isolated soil fungi WT-11 and WT-12

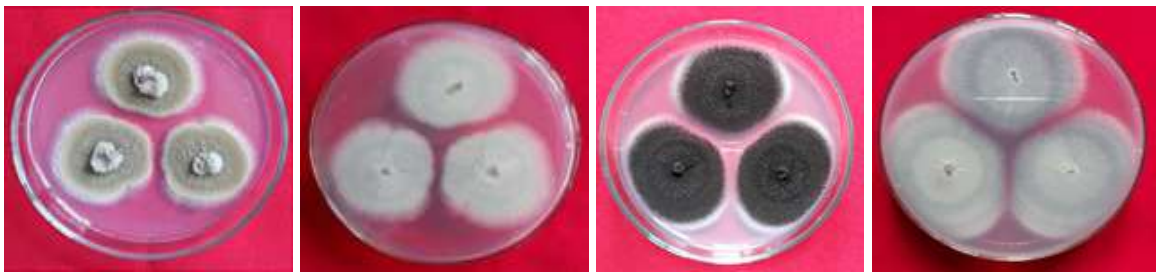
Front view (WT-13) Reverse view Front view (WT-14) Reverse view

Figure 7 Colony morphology of isolated soil fungi WT-13 and WT-14



Front view (WT-15) Reverse view Front view (WT-16) Reverse view

Figure 8 Colony morphology of isolated soil fungi WT-15 and WT-16



Front view (WT-17) Reverse view Front view (WT-18) Reverse view

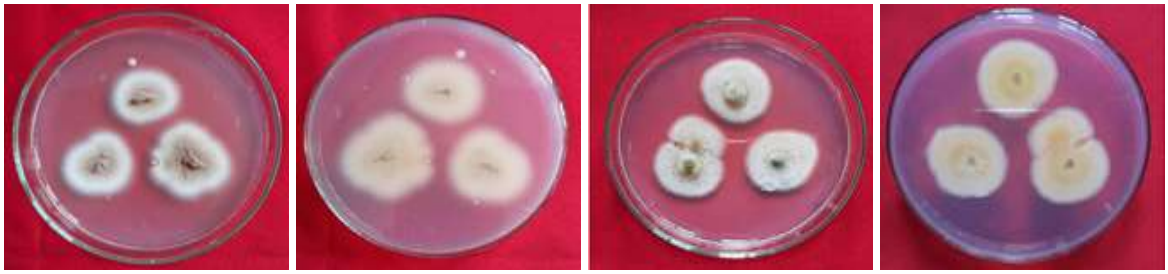
Figure 9 Colony morphology of isolated soil fungi WT-17 and WT-18

Front view (WT-19) Reverse view Front view (WT-20) Reverse view

Figure 10 Colony morphology of isolated soil fungi WT-19 and WT-20

Front view (WT-21) Reverse view Front view (WT-22) Reverse view

Figure 11 Colony morphology of isolated soil fungi WT-21 and WT-22



Front view (WT-23) Reverse view Front view (WT-24) Reverse view

Figure 12 Colony morphology of isolated soil fungi WT-23 and WT-24

Antimicrobial activity of all isolated fungal strains

All fungal strains were tested their antimicrobial activity by using eight test orgainsms. Among 24 isolates, seventeen strains showed different levels of antimicrobial activity. Among seventeen strains, seven isolated fungi (WT-1, 2, 4, 7, 9, 13 and 15) showed highly antimicrobial activity against all test organisms. Fungal strain WT-4 showed the highest antimicrobial activity (24.8 mm and 26.4 mm) on *Bacillus pumilus* and *Bacillus subtilis* respectively. Strains WT-9 and WT-13 also exhibited highly antimicrobial activity (25.1 mm) and WT-7 showed (23.6 mm) on *Bacillus subtilis.* Strain WT-15 also exhibited high antimicrobial activity (22.3 mm) against *Agrobacterium tumefaciens* respectively. Moreover, strains WT-1 and WT-2 showed highly antifungal activity (19.2 mm) and (21.9 mm) against *Candida albicans.*

Identification of Active Fungal Strains

Microscopic characters of strains WT-1, 2, 4, 9, 13 and 15

Conidiophores were upright, simple, terminating in a globose or clavate swelling, bearing phialides at the apex. Conidia 1 celled, globose, often variously colored in mass. Therefore, these strains WT-1, 2, 4, 9, 13 and 15 were identified as *Aspergillus* sp. (Figures 13−18).

Figure 13 Microscopic characters of WT-1

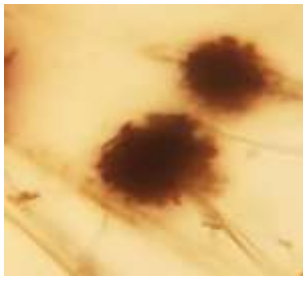


Figure 14 Microscopic characters of WT-2

Figure 15 Microscopic characters of WT-4

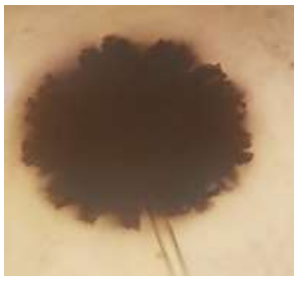


Figure 16 Microscopic characters of WT-9

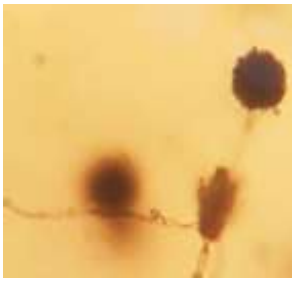


Figure 17 Microscopic characters of WT-13

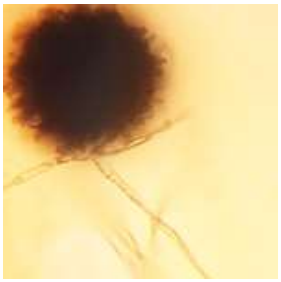


Figure 18 Microscopic characters of WT-15

Microscopical characters of strain WT-7

Mycelium hyaline was white. Cells of mycelium usually long, septa of branches and set off from the main hypae. Asexual fruit bodies, conidia absent, chlamydospore-like cells in chains. This fungus WT-7 was identified as *Rhizoctonia* sp. (Figure 19).

Figure 19 Microscopic characters strain WT-7

Antimicrobial activity of seven fungal strains

Strain WT-1 showed moderate activity at 5th, 6th and 7th day fermentation. Strain WT-2 showed high activity at 3rd, moderate activity at 4th and 5th day fermentation. Strain WT-4 showed high activity at 5th, 6th and 7th day fermentation. Strain WT-7 showed moderate activity at 3rd, 4th and 5th day fermentation. Strain WT-9 showed moderate activity at 3rd, 4th and 5th day, and high activity at 6th and 7th day fermentation. Strain WT-13 showed moderate activity at 5th, 6th and 7th day and high activity at 3rd day and 4th day. Strain WT-15 showed moderate activity at 3rd day to 7th day on *Agrobacterium tumefaciens* (Table 4 & Figure 20). All seven active strains showed antimicrobial activities on other test organisms: *Escherichia coli, Staphylococcus aureus, Pseudomonas fluorescens, Bacillus subtilis, Bacillus pumilus, Malassezia furfur* and *Candida albicans* (Tables 5-11 and Figures 21-27).

Table 4 Antimicrobial Activity of isolated fungal strains against *Agrobac. tumefaciens*

No	Isolated Fungi	Fermentation period (Day) and Inhibitory Zone(mm)				
		3rd-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	10.0	13.4	16.6	16.0	15.1
2	WT-2	20.7	17.7	17.6	13.0	12.0
3	WT-4	17.2	19.6	21.6	28.2	22.4
4	WT-7	16.9	16.4	16.1	14.0	12.5
5	WT-9	18.0	18.2	18.2	21.4	23.0
6	WT-13	19.4	19.2	17.2	16.5	16.2
7	WT-15	14.5	17.0	16.9	16.9	15.5

12-14 mm = weak activity, 15-19 mm = moderate activity, > 20 mm = high activity

A B C D

A: WT-4 against *A. tumefaciens* at 6th day of fermentation period
B: WT-4 against *A. tumefaciens* at 7th day of fermentation period
C: WT-9 against *A. tumefaciens* at 6th day of fermentation period
D: WT-9 against *A. tumefaciens* at 7th day of fermentation period

Figure 20 Antimicrobial activities of selected fungi against *Agrobacterium tumefaciens*

Table 5 Antimicrobial activity of isolated fungal strains against *Escherichia coli*

No.	Isolated Fungi	Fermentation period (Day) and Inhibitory zone (mm)				
		3rd-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	-	21.6	18.0	16.1	14.4
2	WT-2	-	16.0	16.0	17.2	14.6
3	WT-4	12.9	16.6	18.3	15.7	13.7
4	WT-7	9.6	15.1	15.0	14.1	13.7
5	WT-9	14.0	16.7	14.2	13.1	13.0
6	WT-13	11.1	14.2	14.0	13.2	13.6
7	WT-15	12.7	15.7	16.0	16.2	15.8

A B C D

A: WT-9 against *Escherichia coli* at 4th day of fermentation period

B: WT-9 against *Escherichia coli* at 5th day of fermentation period

C:WT-15 against *Escherichia coli* at 6th day of fermentation period

D:WT-15 against *Escherichia coli* at 7th day of fermentation period

Figure 21 Antimicrobial activities of selected fungi against *Escherichia coli*

Table 6 Antimicrobial Activity of isolated fungal strains against *Staphylococcus aureus*

No.	Isolated Fungi	Fermentation period (Day) and Inhibitory Zone(mm)				
		3rd-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	-	16.7	12.4	-	-
2	WT-2	-	14.1	22.2	17.8	-
3	WT-4	10.8	15.2	22.5	14.3	13.3
4	WT-7	-	13.7	18.6	-	-
5	WT-9	13.3	15.2	20.0	19.0	13.7
6	WT-13	-	16.6	22.0	-	-
7	WT-15	-	12.2	19.1	16.4	13.7

A B C D

A: WT-2 against *Staphylococcus aureus* at 5th day of fermentation period
B: WT-2 against *Staphylococcus aureus* at 6th day of fermentation period
C:WT-13against *Staphylococcus aureus* at 4th day of fermentation period
D:WT-13 against *Staphylococcus aureus* at 5th day of fermentation period

Figure 22 Antimicrobial activities of selected fungi against *Staphylococcus aureus*

Table 7 Antimicrobial Activity of isolated fungal strains against *Pseudomonas fluorescens*

No.	Isolated Fungi	Fermentation period (Day) and Inhibitory Zone(mm)				
		3rd-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	14.9	22.1	18.0	16.2	14.0
2	WT-2	21.3	22.1	16.1	14.5	12.3
3	WT-4	27.1	26.4	18.2	20.6	14.7
4	WT-7	23.6	16.2	16.0	15.1	14.6
5	WT-9	25.1	23.0	18.3	18.8	16.4
6	WT-13	22.5	25.1	20.0	17.2	15.7
7	WT-15	21.4	18.7	18.1	19.4	15.2

A B C

A: WT-4 against *Pseudomonas fluorescens* at 6th day of fermentation period
B: WT-4 against *Pseudomonas fluorescens* at 7th day of fermentation period
C:WT-15against *Pseudomonas fluorescens* at 6th day of fermentation period

Figure 23 Antimicrobial activities of selected fungi against *Pseu. fluorescens*

Table 8 Antimicrobial Activity of isolated fungal strains against *Bacillus subtilis*

No.	Isolated Fungi	Fermentation period (Day) and Inhibitory Zone(mm)				
		3rd-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	-	-	-	-	-
2	WT-2	13.8	14.5	15.1	16.9	12.3
3	WT-4	-	-	-	20.6	16.3
4	WT-7	-	-	-	-	-
5	WT-9	15.7	17.1	18.5	25.1	16.4
6	WT-13	-	-	-	-	-
7	WT-15	-	-	17.0	19.4	14.7

A B C D

A: WT-2 against *Bacillus subtilis* at 3rd day of fermentation period
B: WT-2 against *Bacillus subtilis* at 7th day of fermentation period
C:WT-9against *Bacillus subtilis* at 6th day of fermentation period
D:WT-9against *Bacillus subtilis* at 7th day of fermentation period

Figure 24 Antimicrobial activities of selected fungi against *Bacillus subtilis*

Table 9 Antimicrobial activity of isolated fungal strains against *Bacillus pumilus*

No	Isolated Fungi	Fermentation period (Day) and Inhibitory Zone(mm)				
		3rd-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	15.3	13.7	17.2	12.3	12.7
2	WT-2	18.4	18.5	22.0	19.2	15.0
3	WT-4	22.0	18.5	28.4	23.2	24.7
4	WT-7	17.5	16.8	15.0	14.1	11.8
5	WT-9	19.9	16.7	22.8	23.4	30.3
6	WT-13	19.9	18.3	22.8	15.5	18.1
7	WT-15	18.0	16.7	21.4	22.2	20.8

A B C D

A: WT-2 against *Malassezia furfur* at 4th day of fermentation period
B: WT-2 against *Malassezia furfur* at 5th day of fermentation period
C:WT-9 against *Malassezia furfur* at 3rd day of fermentation period
D:WT-9 against *Malassezia furfur* at 4th day of fermentation period

Figure 25 Antimicrobial activities of selected fungi against *Bacillus pumilus*

Table 10 Antimicrobial Activity of isolated fungal strains against *Malassezia furfur*

No	Isolated Fungi	Fermentation period (Day) and Inhibitory Zone(mm)				
		3th-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	11.9	12.6	16.7	15.0	13.9
2	WT-2	18.7	14.4	22.7	19.8	19.0
3	WT-4	18.7	19.9	19.4	19.0	18.0
4	WT-7	16.8	17.4	14.7	12.5	10.5
5	WT-9	16.8	21.4	19.7	19.0	18.1
6	WT-13	19.2	16.0	20.9	18.2	17.0
7	WT-15	19.2	16.0	20.9	18.6	18.1

A B C D

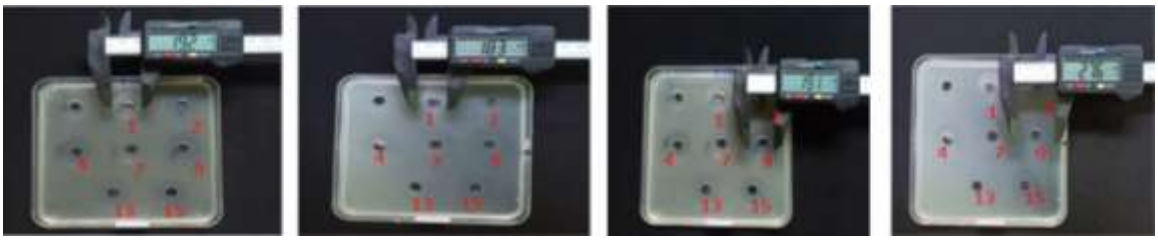
A: WT-1 against *Malassezia furfur* at 4th day of fermentation period
B: WT-1 against *Malassezia furfur* at 5th day of fermentation period
C:WT-7 against *Malassezia furfur* at 3rd day of fermentation period
D:WT-7 against *Malassezia furfur* at 4th day of fermentation period

Figure 26 Antimicrobial activities of selected fungi against *Malassezia furfur*

Table 11 Antimicrobial Activity of isolated fungal strain against *Candida albicans*

No	Isolated Fungi	Fermentation period (Day) and Inhibitory Zone(mm)				
		3rd-day	4th-day	5th-day	6th-day	7th-day
1	WT-1	9.6	18.6	19.2	18.3	13.3
2	WT-2	19.4	21.9	21.8	13.6	13.0
3	WT-4	23.3	23.3	20.6	18.7	14.2
4	WT-7	15.8	15.8	15.9	15.0	14.0
5	WT-9	17.3	18.0	19.1	27.6	16.6
6	WT-13	16.3	23.4	21.5	18.0	16.3
7	WT-15	20.7	19.6	19.6	22.2	14.7

12-14 mm = weak activity,15-19 mm = moderate activity, > 20 mm = high activity



A B C D

A: WT-1 against *Candida albicans* at 5th day of fermentation period
B: WT-1 against *Candida albicans* at 6th day of fermentation period
C:WT-9 against *Candida albicans* at 5th day of fermentation period
D:WT-9 against *Candida albicans* at 6th day of fermentation period

Figure 27 Antimicrobial activities of selected fungi against *Candida albicans*

Discussion and Conclusion

In the present study, 24 fungal strains were isolated from three different Townships in Yangon Region. Fungal strains WT-1 to WT-5 were isolated from soil in South Oakkala, WT-6 to WT-12 from soil sample in Mayangone and WT-13 to WT-24 from soil sample in Insein. A total of 24 isolated fungi, 11 strains were isolated from BMEA Medium, 8 strains from PDA Medium, 4 strains from LCA Medium and 1 strain were isolated from CzA. Moreover, physicochemical properties of soil from different locations of Yangon Area were analyzed. The soil samples were pH slightly base (6.27−7.02). The temperature of the soil was high (30- 45ºC), with great variation

in moisture content (2.28 – 2.64), organic carbon (0.58- 4.07) and organic nitrogen (0.18 – 0.27), and texture.

Among 24 isolates, 7 strains showed highly antimicrobial activity. These six active strains were identified as *Aspergillus* sp. and the other one was *Rhizoctonia* sp. Morphological and microscope characters of isolated strains were investigated according to Barnett (1998) and other literatures. Seth *et al*., (2016) isolated and identified soil fungi such as *Aspergillus* spp., *Penicillum* spp., *Fusarium* spp., and *Rhizoctonia* spp. from cultivated area in India. Moira (1961) isolated soil fungi and identified these strains to study their growth in pure culture. Gaddeya, *et al.*, (2010) isolated soil mycoflora in different crop fields for producing bioactive compounds. Farid and Nareen (2013) isolated and identified *Aspergillus* sp., and other fungi from soil and some medicinal plants. Ana et al., (2010) isolated several soil borne microbes such as mycorrhizal fungi. Azaz (2003) also isolated *Aspergillus* sp., *Penicillum* sp., and *Rhizoctonia* sp., from the fields.

All isolated strains showed different antimicrobial activity on eight test organisms. According to the results of antimicrobial activity, bioactive strains WT-1, 2, 4, 7, 9, 13 and 15 showed highly antimicrobial activity on eight test organisms. Prabavathy and Nachiyar (2012) stated that the fungus *Aspergillus* sp., had antimicrobial activity. Many others researchers isolated soil fungi for producing bioactive agents. Devi & Joshi (2012) stated that most of soil fungi (e.g., *Aspergillus* sp. and *Rhizoctonia* sp.) had antimicrobial activity on some test organisms (*Escherichia coli*, *Pseudomonaous aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida krusei*). Srividya, *et al.* (2010) stated that the soil fungus showed antimicrobial activity against *Escherichia coli*, *Pseudomonaous aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida krusei*. Yee Yee Thu (2006), Kyawt Kyawt Aung (2014), Hnin Wit Mhone (2018) and Soe Soe Yu Hnin (2018) have also isolated many fungi including *Aspergillus* and *Rhizoctonia*, etc. from soil fungi and endophytic fungi.

It was concluded that the 24 soil fungi were isolated on four different media. Seven strains of them possessed highly antimicrobial activity on eight test organisms caused serious diseases and infections on man and plants. Therefore, these seven active strains will be chosen to continue fermentation studies for extraction and isolation of the bioactive compounds.

Acknowledgements

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

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EFFECTS OF CARBON AND NITROGEN SOURCES FOR THE GROWTH OF SOIL FUNGUS YY-13 AND ITS FERMENTATION OPTIMIZATION

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Abstract

In this study, fungal strain (YY-13) isolated from soil sample in Minhla Area, Magway Region were intended to produce antibacterial metabolites. In addition, it was focused on the growth and fermentation conditions of isolated soil fungus (YY-13) against *Escherichia coli* by using agar well diffusion method. Moderate growth and the best antibacterial activity of YY-13 were observed both in carbon and nitrogen sources. The addition of potato powder as a carbon sources resulted better growth and the inhibition zone reached (32.78 mm) in glucose. In the nitrogen sources, the maximum growth of YY-13 was found in peptone and the highest antibacterial activity was obtained by using the sodium nitrate (28.39 mm). The fermentation conditions of YY-13 were studied by the effect of age, size of inoculum, temperature, pH, static and shaking culture. In this investigation, YY-13 was found that 25% of size of inoculums and 84 hrs of age of old culture were suitable conditions. And then, the effects of different temperature and pH range were also determined. According to these studies, temperature 30 ˚C and pH-6 were found to be the best conditions of antibacterial activity against *E.coli*. In the comparison of static culture and shaking culture of YY-13, the static culture exhibited the higher antibacterial activity (26.91 mm) than that of shaking culture (20.38 mm).

Keywords: YY-13, Antibacterial activity, *Escherichia coli*, carbon and nitrogen sources, fermentation conditions

Introduction

Natural products are important sources in the drug discovery process. Accordingly, new ecological niches should be explored for natural bioactive agents in pharmaceutical, agricultural, and industrial fields. These products should be renewable, ecofriendly and easily obtainable. The most importance of natural products are plants, animals, marine microorganisms (sponge, corals and algae), and microorganisms (bacteria, actinomycetes, and fungi) (Liu, *et al*., 2004).

The presence of more than 200,000 natural metabolites presenting various bioactive properties (Berdy, 1974) demonstrates the important of natural product in new drugs discovery. The use of microorganisms has created a huge revolution in many aspects of human's life as witnessed by numerous studies conducted by scientists in different fields. Microorganisms are used in production of pharmaceutical products, especially antibiotics (Philippe, *et al*., 2009).

Antibiotics have an important role in human health. Their necessity emerged from the spread of various diseases. As a result, scientists are trying to produce and discover more antibiotics (Rolain, *et al.*, 2000). Soil fungi have been the most studied of fungi, and typical soil genera such as *Acremonium*, *Aspergillus*, *Fusarium* and *Penicillium* have shown ability to synthesize a diverse range of bioactive compounds. About one third of metabolites derived from fungi belong to *Aspergillus* and *Penicillium* (Berdy, 1974).

Modifying fermentation parameters such as time, temperature, pH, and nutrients can help expanding the range of those secondary metabolites (Pfefferle and Gurtler, 2000). The presence of antimicrobial properties may indicate a larger activity spectrum, including antitumor and antiparasitic characteristics (Demain, 1999).

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The purpose of the present study was to screen the ability of YY-13 to produce antibacterial compounds using different growth parameters namely, pH, temperature, static and shaking culture.

Materials and Methods

Source of Soil Fungi

The fungal strain (YY-13) isolated from soil sample in Minhla Area, Magway Region, were intended to produce antibacterial metabolites.

Study on Antimicrobial Activity

The isolated fungus YY-13 was cultured on BMEA medium for 5 days. The isolated fungus were inoculated into 25 mL seed medium and incubated at room temperature for 3 days (Ando, 2004). After 3 days, 25 mL seed culture was transferred into the 75 mL of fermentation medium and incubated at room temperature. Fermentation was carried out for 3-10 days.

Screening of Antimicrobial Activity by Agar Well Diffusion Method (Collins, 1965)

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, cork borer was to make the wells (wells-8 mm). The fermentation broth (20 μL) was carefully added into the wells and incubated at room temperature for 24 to 48 hours. The diameter of the zones of inhibition around each well was measured and recorded after 24 to 48 hours incubation.

Table 1 Test Organisms Utilized in the Antimicrobial Activity of Isolated Soil Fungi

No.	Test Organisms	Diseases
1	*Escherichia coli* AHU5436	Diarrhoea
2	*Bacillus subtilis* IFO 90571	DNA topoisomerase I
3	*Bacillus pumilus*IFO 12092	Wound and burn infection, fever
4	*Candida albicans* NITE 09542	Candidasis
5	*Staphylococcus aureus* AHU8465	Food poisoning, Methicillin Resistance
6	*Malassezia furfur* AUV 0255	Dandruff, Seborrhoeic dermatitis

The Effects of Carbon and Nitrogen Sources for the growth of Fungus YY-13

In this study, carbon sources such as glucose, xylose, sucrose, mannitol, lactose, fructose, maltose, glycerol, tapioca powder, molasses, soluble starch, potato powder, wheat powder and arabinose were used in each of 1.0 g. Nitrogen sources such as asparagine, malt extract, peptone, gelatin, casein, yeast extract, sodium nitrate, urea, ammonium nitrate, potassium nitrate, ammonium chloride and ammonium sulphate were utilized (each 1.0 g).

The Effect of Age and Size of Inoculum for the Fermentation (Omura, 1985)

In this study, the selected fungus YY-13 was cultured on BMEA medium at room temperature for 5 days and then was transferred into seed medium. Inoculation period 3 to 10 days were used for the production of antimicrobial metabolite and the procedure of seed culture medium was also used as the previous method. And then, seed culture was transferred to 100 mL conical flask containing of fermentation medium and incubated at room temperature. The inoculum age of

fermentation were studied by 48 hrs, 60 hrs, 72 hrs, 84 hrs, 96 hrs and 108 hrs. In the study of sizes of inoculum, (5%, 10%, 15%, 20%, 25%, and 30%) were utilized with 84 hr age of culture. Fermentation was done and antimicrobial activity was tested by agar well diffusion method.

Study on Different Carbon Sources Utilization for the Fermentation

Carbon sources (each 1.0 g or 1.0 mL) such as glucose, xylose, sucrose, mannitol, lactose, fructose, maltose, glycerol, tapioca powder, molasses, soluble starch, potato powder and arabinose were used. Fermentation were incubated at 30˚C for 6 days.

Basal fermentation medium used in carbon source

Yeast extract 0.3 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.1 g, DW 100 mL and pH 6.5

Study on Different Nitrogen Sources Utilization for the Fermentation

Nitrogen sources (each 1.0 g or 1.0 mL) such as malt extract, peptone, asparagine, yeast extract, gelatin, casein, sodium nitrate, potassium nitrate, urea, ammonium nitrate, ammonium chloride and ammonium sulphate were used. Fermentation were incubated at 30˚C for 6 days.

Basal fermentation medium used in nitrogen source

Glucose 0.3 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.1 g, DW 100 mL and pH 6.5

The Effect of Shaking and Static Culture for the Fermentation

100 mL conical flask containing 25 mL of the best fermentation medium was incubated on the shaker for 6 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.

The Effect of pH and Temperature on Culture of Fungus YY-13

In this study, optimum pH was studied by varying the medium pH as 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. The different pH of fermentation medium was adjusted by using 0.1 M HCl and 1 M NaOH. In the effect of temperature, the selected fungus YY-13 was inoculated and incubated at five different temperatures by using 20 ˚C, 25 ˚C, 30 ˚C, 35 ˚C and 40 ˚C. The fermentation medium was assayed for antimicrobial activity.

Results and Discussion

The Effects of Carbon and Nitrogen Sources Utilization for the growth of Fungus YY-13

In the growth morphology on various carbon sources, good growth of YY-13 was found on potato powder, moderate growth soluble starch, xylose, glucose and lactose.

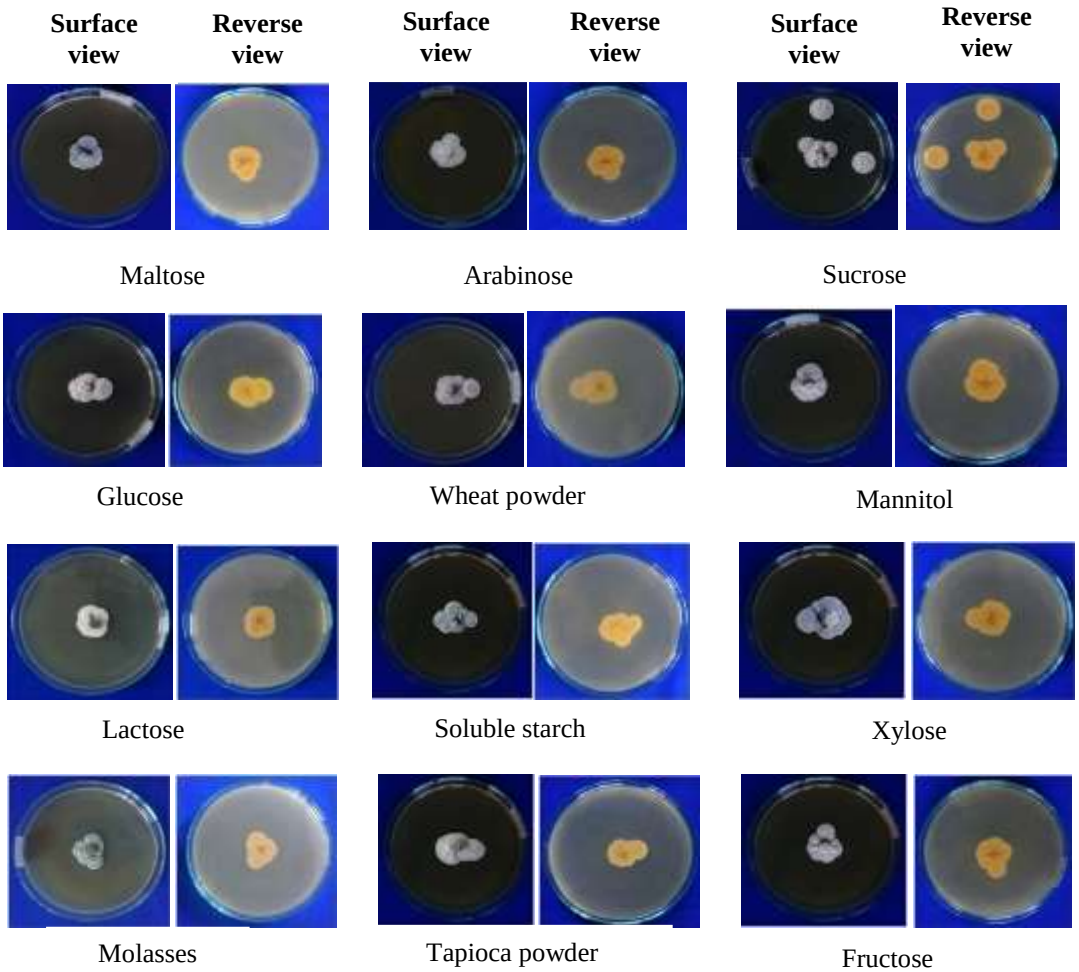
In the nitrogen sources, YY-13 were good growth on peptone, potassium nitrate, sodium nitrate, yeast extract and casein, moderate growth of ammonium chloride, asparagine, malt extract, gelatin, ammonium sulphate, ammonium nitrate, and while urea were poor growth.

As many issued (Buchanan *et al.,* 1984) and (Calvo *et al.,* 2002) support, simple sugar such as glucose, fructose, sucrose enhanced growth as well as secondary metabolite production by microorganisms slightly than complex carbon sources like starch, galactose, xylose, mannitol, etc.

Table 2 Morphological Characters of Fungus YY-13 on various Carbon Sources

No.	Carbon sources	Color		Growth size (mm)
		Surface colour	Reverse colour	
1	Glucose	White	Pale yellow	29.20-30.21
2	Tapioca powder	Pale green	Yellow	25.46-26.32
3	Potato powder	Pale green	Pale yellow	**34.03-35.06**
4	Glycerol	White	Pale yellow	24.99-26.22
5	Arabinose	Pale green	Pale yellow	24.39-25.13
6	Soluble starch	Pale green	Pale yellow	28.39-29.32
7	Maltose	Pale green	Pale yellow	23.70-24.76
8	Mannitol	White	Pale yellow	26.54-27.04
9	Molasses	Deep green	Yellow	24.80-25.68
10	Sucrose	Pale green	Pale yellow	20.50-22.37
11	Xylose	Pale green	Yellow	28.35-29.44
12	Wheat powder	Pale green	Pale yellow	23.33-24.62
13	Fructose	White	Pale yellow	26.64-27.92
14	Lactose	White	Pale yellow	27.21-28.68

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



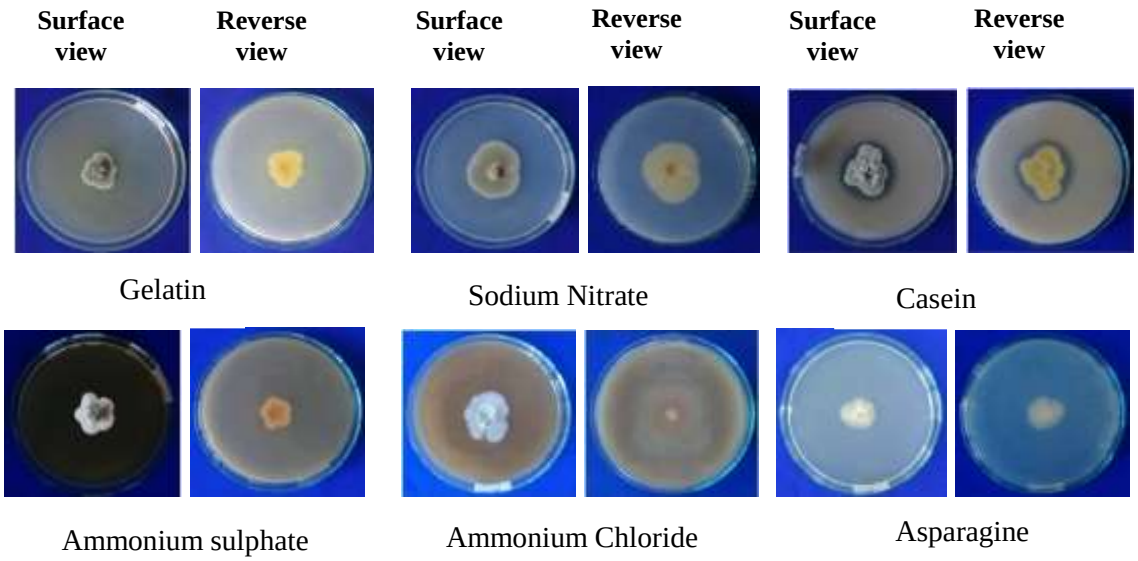
Potato powder Glycerol

Figure 1 Morphological characters of fungus YY-13 on various carbon sources

Table 3 Morphological Characters of Fungus YY-13 on various Nitrogen Sources

No.	Nitrogen sources	Color		Growth size (mm)
		Surface colour	Reverse colour	
1	Ammonium Nitrate	Grey	Pale orange	18.40-18.65
2	Potassium Nitrate	Grey	Pale orange	37.84-39.94
3	Sodium Nitrate	Grey	Pale orange	36.88-38.41
4	Malt Extract	White	Pale gray	29.20-30.06
5	Gelatin	White	Pale yellow	23.32-24.22
6	Yeast Extract	White	Gray white	24.39-25.08
7	Peptone	Brown	Pale yellow	38.65-40.79
8	Casein	White	Pale yellow	32.94-33.16
9	Ammonium Chloride	White	Pale gray	26.54-27.47
10	Asparagine	Pale yellow	White	22.91-20.64
11	Ammonium Sulphate	White	Pale grey	19.71-20.63
12	Urea	Whitish grey	Pale yellow	11.34-12.12

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



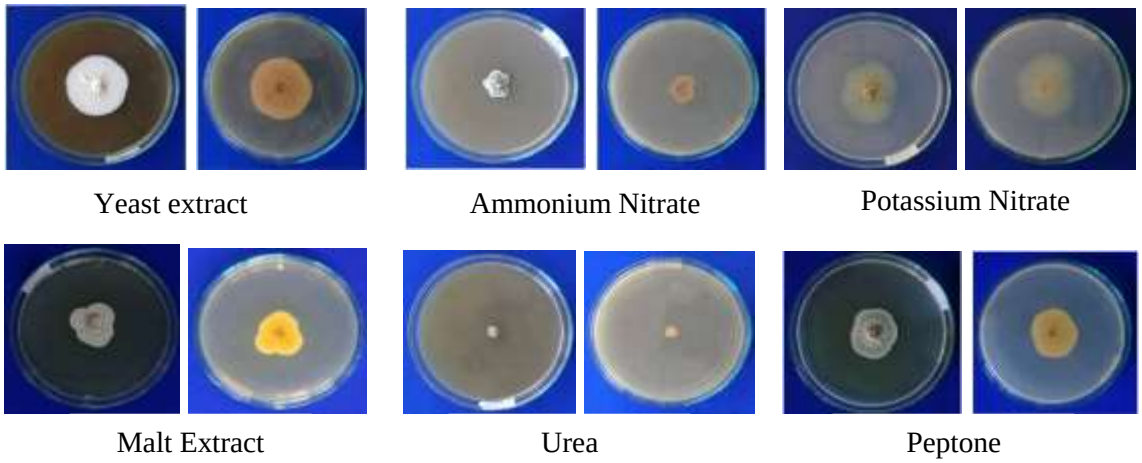


Figure 2 Morphological characters of fungus YY-13 on various nitrogen sources

Effects of Age of Inoculum on Fungus YY-13 against *E.coli*

In the effect of age of inoculum, the antimicrobial activity of fungus YY-13 was determined in different age of culture (48 hrs, 60 hrs, 72 hrs, 84 hrs, 96hrs and 108 hrs). In 84 hrs age, YY-13 showed the highest antibacterial activity (33.08 mm) followed by (32.28 mm) in 72 hrs on *E. coli*. Jain, 2010 reported that the variations in the fermentation environment often result in alteration in antibiotic production. (Table-4 and Figure-3)

Table 4 Effect of Age of Inoculum on Fungus YY-13

Ages of inoculum (hrs)	Inhibition Diameter Zone (mm)
48	22.33
60	23.81
72	32.28
84	**33.08**
96	31.37
108	30.85

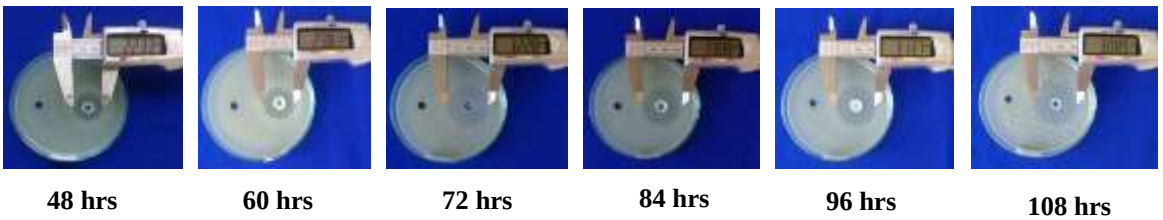


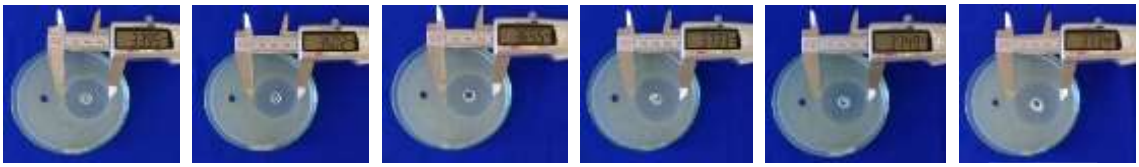
Figure 3 Inhibition zone of fungus YY-13 at different age of inoculum

Effect of Size of Inoculum on Fungus YY-13 against *E.coli*

In the study of size of inoculum, different size of inoculum (5%, 10%, 15%, 20%, 25% and 30%) 84 hrs seed culture were used. The best antibacterial activity of YY-13 was obtained by using 25% size of inoculum followed by 20% and 30% on *E. coli*, respectively. (Table-5 and Figure-4)

Table 5 Effect of the Size of Inoculum on Fungus YY-13

Size of inoculum (%)	Inhibition Diameter Zone (mm)
5	33.95
10	36.02
15	36.55
20	37.33
25	**37.49**
30	37.34



5% 10% 15% 20% 25% 30%

Figure 4 Inhibition zone of fungus YY-13 at different size of inoculum

Effect of Antibacterial Activity on Carbon Sources Utilization of YY-13 against *E.coli*

There are variation in the level of antibacterial activity, when the different carbon sources were tested in fermentation. The effects of carbon sources were different for the maximum antimicrobial metabolites productions. The highest antibacterial activity were observed by the addition of glucose (32.78 mm) and tapioca (31.23 mm) followed by potato powder (31.15 mm) and glycerol (30.78 mm) against *E. coli*. These results were shown in Table-6 and Figure-5. Bhavana, *et al.*, 2014 who observed glucose as favourable carbon source for maximum antimicrobial compound and mycelium growth of *Streptomyces carpaticus* MTCC 11062. So, it could be assigned that carbon sources was the best in the fermentation process used in this study.

Table 6 Effect of Different Carbon Sources Utilization of YY-13 against *E. coli*

No.	Carbon sources	Inhibition Diameter Zone (mm)
1.	Lactose	22.69
2.	Glycerol	30.78
3.	Glucose	**32.78**
4.	Mannitol	29.72
5.	Fructose	27.23
6.	Sucrose	29.04
7.	Maltose	30.04
8.	Arabinose	30.62
9.	Soluble starch	30.37
10.	Molasses	29.41
11.	Xylose	28.28
12.	Tapioca powder	31.23
13.	Potato powder	31.15
14	Wheat powder	27.98

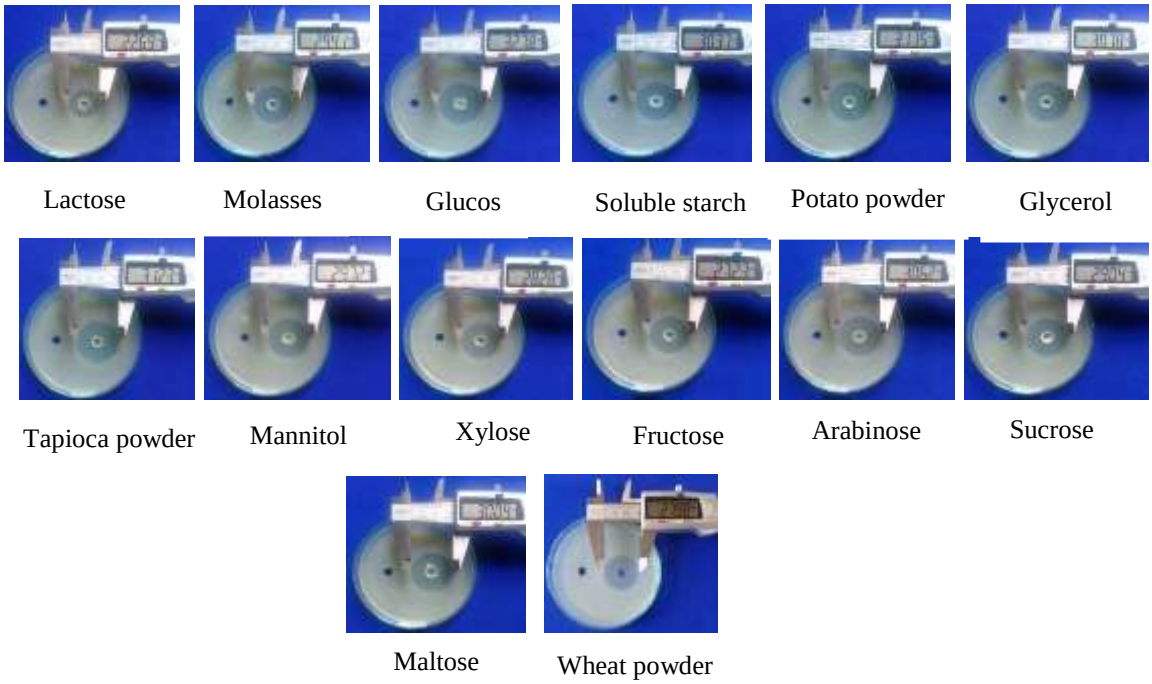


Figure 5 Inhibition zone of fungus YY-13 on various carbon sources

Effect of Antibacterial Activity on Nitrogen Sources Utilization of YY-13 against *E.coli*

In this study, the addition of malt extract, potassium nitrate, sodium nitrate, gelatin, casein displayed the greatest activity. These results were shown in Table-7 and Figure-6. Ismaiel, *et al.*, 2010 reported that sodium nitrate as best nitrogen sources for the production of metabolite by fungus *Fusarium roseum*. According to the observation, it could be denoted that nitrogen sources was the best in the fermentation medium.

Table 7 Effect of Different Nitrogen Sources Utilization of YY-13 against *E. coli*

No.	Carbon sources	Inhibition Diameter Zone (mm)
1.	Ammonium Nitrate	16.38
2.	Potassium Nitrate	26.02
3.	Sodium Nitrate	**28.39**
4.	Malt Extract	25.61
5.	Gelatin	21.42
6.	Yeast Extract	17.05
7.	Peptone	17.93
8.	Casein	20.00
9.	Ammonium Chloride	14.04
10.	Asparagine	16.38
11.	Ammonium Sulphate	12.10
12.	Urea	+

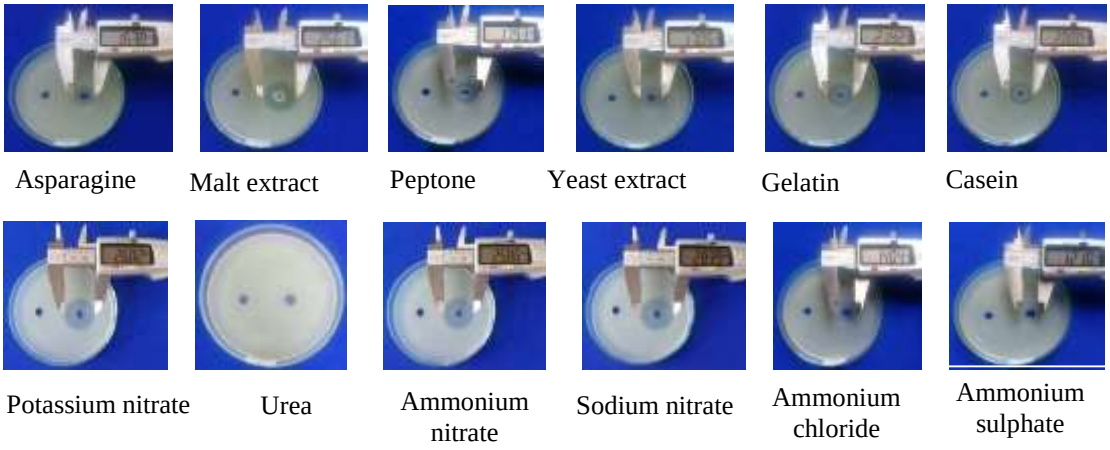


Figure 6 Inhibition zone of fungus YY-13 on various nitrogen sources

Comparison between Shaking Culture and Static Culture of Fermentation Optimization

In this investigation, the comparison between shaking culture and static culture were carried out. The maximum production of antimicrobial metabolite of shaking culture showed the inhibitory zone 20.38 mm against *E. coli.* The static culture of fermentation broth showed activity 26.91 mm and 25.76 mm against *E. coli* (Figure-7). Stinson *et al.*, 2003 observed that maximum growth and production of antimicrobial agent was recorded after the fungus reached its stationary phase. From the observation, it could be remarked that the finding results were agreeable with literature.

(A) Static (B) Shaking

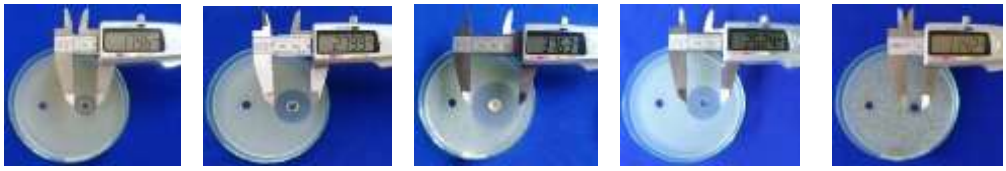
Figure 7 Inhibition zone of YY-13 in shaking and static culture

Effects of Incubation Temperature on Culture of YY-13

In this study, the effect of incubation temperature on antibacterial activity of YY-13 was determined by changing the temperature at 20 ˚C, 25 ˚C, 30 ˚C, 35 ˚C and 40 ˚C. The optimum temperature was found at 30 ˚C (37.63 mm) against *E. coli.* Incubation temperature is known to influence directly the overall growth and development of any organism. It affects the physiology and subsequently the synthesis of various metabolites (Pandey, *et al.*, 2008). The finding of present study (Table-8 and Figure-8) could be assigned that it is agreeable with the literature preview on Gunasekaran and Poorniammal, 2008 have reported highest secondary metabolite production at a temperature of 30 ˚C in their study.

Table 8 Effects of Incubation Temperature on Culture of YY-13

Temperature (˚C)	Inhibition Diameter Zone (mm)
20	17.96
25	27.99
30	**37.63**
35	26.24
40	11.42



20 ˚C **25 ˚C** **30 ˚C** **35 ˚C** **40 ˚C**

Figure 8 Inhibition zone of YY-13 at different temperature

Effect of pH Utilization for YY-13

In the present study, the antimicrobial activity of YY-13 was investigated by varying the pH, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. The maximum antimicrobial activity of YY-13 (38.10 mm) against *E. coli* was found at pH 6.0. These results are shown in Table-9 and Figure 9. Similarly, the effect of culture medium on mycelial growth, metabolite profile and antimicrobial compound yield by a marine derived fungus *Arthrinium* c.f. *saccharia* was investigated by Maio *et al*., 2006.

Table 9 Effect of Different pH on culture of YY-13

pH	Inhibition Diameter Zone (mm)
4.0	35.10
4.5	36.46
5.0	37.68
5.5	37.78
6.0	**38.10**
6.5	37.89
7.0	37.26
7.5	36.81
8.0	35.92

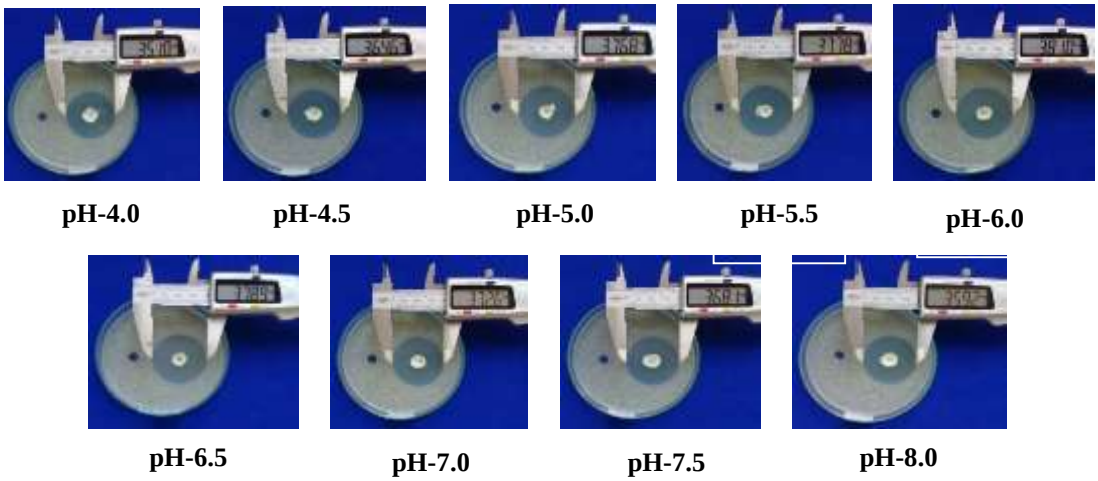


Figure 9 Inhibition zone of YY-13 at different pH

Conclusion

In this study, colony morphology and maximum metabolite production of YY-13 were investigated. The supplement of carbon and nitrogen sources effect the growth of colony morphology. In the fermentation studies, it was found that 84 hrs age of inoculum and 25% of size of inoculum were suitable for fermentation. The antibacterial substance production of YY-13 was influenced by addition of glucose and sodium nitrate.

In this study, the optimum temperature of incubation was found at 30 ˚C against *E. coli* (37.63 mm). In the present study, the highest antibacterial activity of YY-13 were found at pH 6.0 against *E. coli* (38.10 mm). The fermentation broth was studied at two conditions such as shaking culture (20.38 mm) and static culture (26.91 mm) on *E. coli*.

It can be concluded that this research may be supported for maximum production of antibacterial metabolite. In fact, this paper can contribute to the prevention and treatment of the bacterial diseases related to the *E. coli*.

Acknowledgements

We would like to thank Dr Nilar Myint, Acting Rector and Dr Mar Lar, Pro-Rector, Hinthada University, for giving the permission to write this paper. We also wish to express our deepest gratitude to Dr Thi Thi Aye, Professor, Department of Chemistry, Hinthada University for her enthusiastic support and valuable suggestions and also for providing the facilities needed in writing this paper.

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EXTRACTION OF ANTIBACTERIAL METABOLITES PRODUCED BY SLECTED SOIL FUNGUS (KM-16)

Kyae Mhon Win[1], Ei Ei Mon[2], Mar Mar Aye[2], Yin Yin Mya[3], and Thandar Aung[3]

Abstract

In the study, soil fungi were isolated from soil samples which were collected from the Pathein University Dhamma Yone campus and then air-dried at room temperature. After three days, ten different soil samples were isolated by feeding method and physical treatment dilution method. Thirty-one soil fungi were isolated from ten different soil samples. Among them isolated soil fungus KM-16 showed the highest antimicrobial activity against *Escherichia coli*. In this investigation, selected soil fungus (KM-16) was utilized by using paper chromatography for extracting of antibacterial metabolites. The extraction of antibacterial metabolites was carried out by Thin Layer Chromatography and antibacterial metabolites could be isolated from 20 Liters of fermented broth of KM-16.

Keyword: Soil Fungi, Feeding Method and Physical Treatment Dilution Method

Introduction

Soil is considered as one of the most suitable environments for microbial growth (Cavalcanti *et al.,* 2006). Fungi are fundamental for soil ecosystem functioning. Especially forest and agriculture soils play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization (Haqeeqat and Nasreen, 2016). Fungi are one of the most common microbes living around us in the environment which could be pathogenic and sometimes life threatening. Lots of antifungal agents have been discovered so far and many are still the procedure to be defined as an effective fungicide.

The need for new, safe and more effective antifungals is a major challenge to the pharmaceutical industry today, especially with the increase in opportunistic infections in the immunocompromised host (Thakur *et al*., 2007).

Materials and Methods

Sample collection, storage and transportation

Ten different soil samples were collected from ten different places around the University Campus (Pathein) during June 2019. The samples were taken near the tree by digging 6 cm depth under the soil. The soil sample were collected in sterile glass container, sealed and carefully placed in plasticbags and brought to the laboratory. The soil textures were measurement at Department of Agriculture (Land Use) Soil Interpretationin Yangon Township.

Isolation of soil fungi from different soil samples

The soil microorganisms were isolated by two methods, namely feeding method (Phay and Yamamura, 2005) and physical treatment dilution method (Hayakawa and Kobayashi, 2005) on different media such as low carbon agar (LCA)(Ando 2004), soil extract agar(SEA)(Ando 2004),potato glucose agar(PGA)(Ando 2004) and Czapek- dox agar medium.

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Isolation of Soil Fungi by Feeding Method

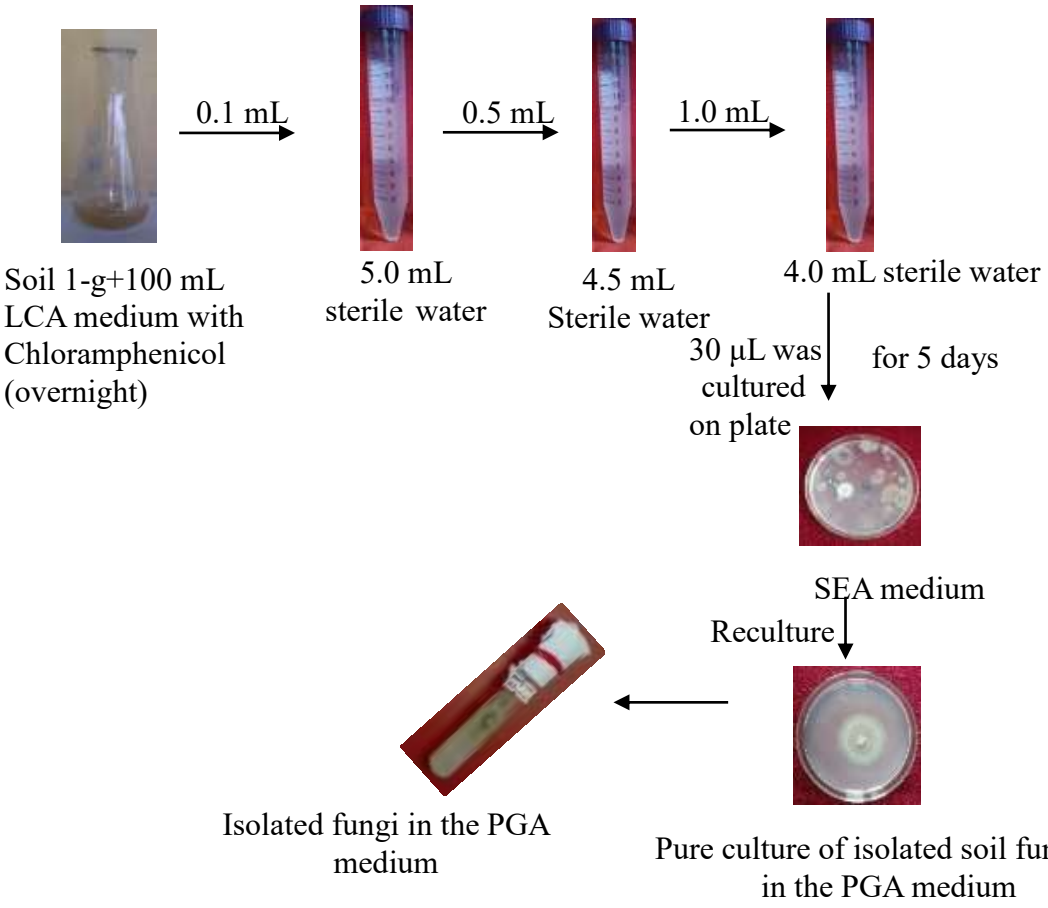


Figure 1 Procedure for isolation the fungi by using feeding method

The collected soil sample was air - dried at room temperature for 3 days. The soil sample was grounded and sieved in 2mm screen. One gram of soil sample was added LCA medium with chloramphenicol (overnight), and then prepared with 0.5 ml, 4.5 mL and 4 mL sterile water. The dilution series were cultured on SEA medium with chloramphenicol medium and incubated for 1 to 5 days at room temperature. After 6 days incubation, small piece colonies were appeared on the medium and transferred to a fresh PGA medium. Pure colonies were preserved into slant cultured containing in PGA medium (Figure.1).

Isolation of Soil Fungi by Physical Treatment Dilution Method

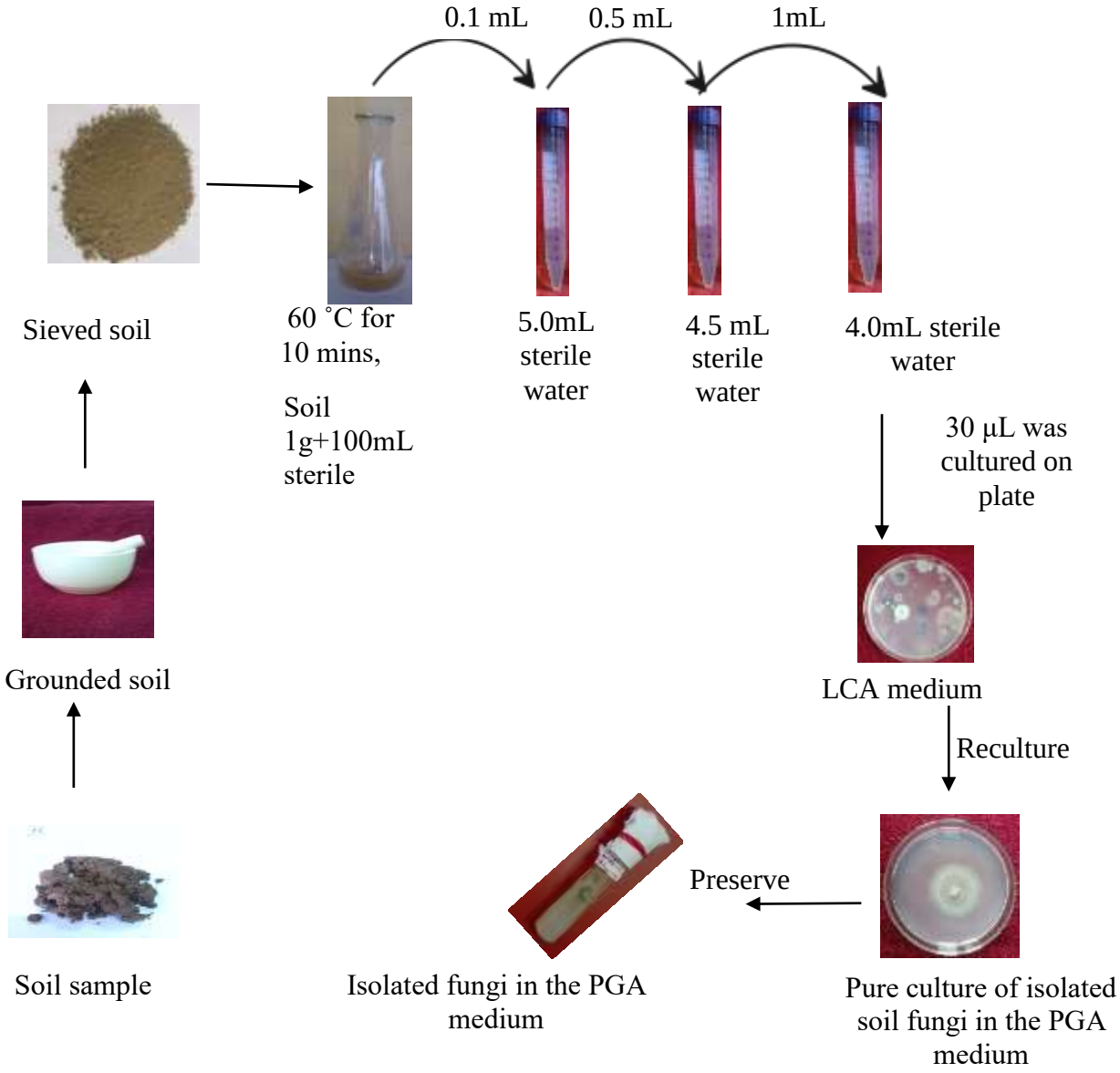


Figure 2 Procedure for isolation the fungi by using physical treatment dilution method

The collected soil sample was air - dried at room temperature for 3 days .The soil sample was grounded and sieved in 2mm screen. The soil sample was placed in the hot air oven at 60°C for 10 min. Soil sample was suspended series diluted with sterile water. The dilution were cultured on LCA medium and inoculated for 1 to 5 days at room temperature. Pure colonies were incubated into slant culture containing in PGA medium (Figure. 2)

Preliminary study of the activities of isolated microorganisms

The isolated soil microorganisms were inoculated into seed medium and incubated for 3 days at room temperature. Seed culture were transferred into the fermentation medium. It took 4 to 10 days for carrying out and it was tested with test organisms. After the end of fermentation, the fermented broth was used to check the antimicrobial activity against test organisms by paper disc diffusion assay. Paper disc having eight millimeter diameter were utilized for antimicrobial assays. The fermented broth (10-30 µL per disc) were dissolved and allowed to dry.

Assay Method

One percent of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates were incubated at room temperature for 24-36 hours.

Clear zones (inhibitory zones) surrounding the paper discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms. The test organisms used in paper disc diffusion assay were supported by Department of Biotechnology of Pathein University for the cooperation research.

Paper Chromatography

Paper chromatography was carried out to extract the antibacterial compound from the fermented broth by the method of Tomita, (1988). The purpose of paper chromatography is how to extract the bioactive compound with which the suitable solvent systems.

The filter paper (Toyo Advantech Japan) 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 metabolites.

The obtained fermented broth samples were applied on the paper and allowed to dry. The paper were 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 the paper was taken out, and then the plates were incubated for 24- 36 hours. In this case, the inhibitory zone was measured yielding an R_f value for the corresponding metabolites.

Thin Layer Chromatography

The EtOAc extracted residue is necessary to be separated and purified more. Thus, TLC was developed by the methods of Touchstone, 1992 and Aszalos, 1987.

R_f values reported were acquired on Merck Kiesel gel GF_{254} silica gel precoated alumuinium plates (Merck), which were utilized for analytical preparative purpose.

The obtained EtOAc extracted samples (20µL) were applied on the TLC plates and allowed to dry. The TLC plates were developed in the solvent Hexane: EtOAc and Chloroform: EtOAc (100/1 v/v), (80:1 v/v), (60:1 v/v), (40:1 v/v), (20:1 v/v), (10:1 v/v), (5:1 v/v), (2:1 v/v) and (1:1 v/v).

The R_f values of isolated compounds were measured. Location of spot was made by viewed directly under UV 254 nm and 365 nm light or by treating with visualizing agents

Results and Discussion

In the course of the investigation of isolation, thirty one different fungi, KM-01 by feeding method and KM-02 to KM-31 by physical treatment dilution method) were isolated from the ten different soil samples of Pathein University (DhammaYone) campus. Among them, selected soil fungus (KM-16) showed antibacterial activities against *Escherichia coli* (Table 2 and Figure 3).

Table 2 Antibacterial activity of selected soil fungus(KM-16)

Sr.No	Isolated fungus	Antibacterial activity *Escherichia coli*
1	KM-16	20.59 mm clear zone

Figure 3 Antimicrobial activity of selected soil fungus (KM-16) against test organism (*E.coli*)

Morphology and Photomicrograph of selected soil fungus

Surface view of colony morphology

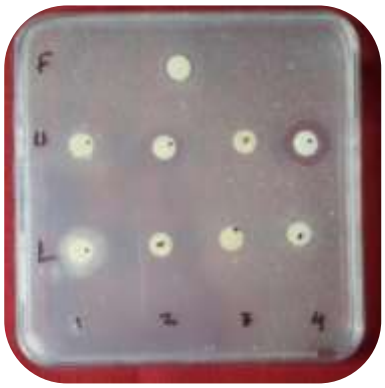
Reverse view of colony morphology

Photomicrograph (x 400)

Figure 4 Morphology and Photomicrograph characters of selected soil fungus KM-16

Paper Chromatography

According to the R_f value of paper chromatography bioassay (Figure. 5) and comparism of butanol extract and ethyl acetate extract, it was considered that the antibacterial compound of strain KM-16 could be extract from the fermented broth by ethyl acetate.



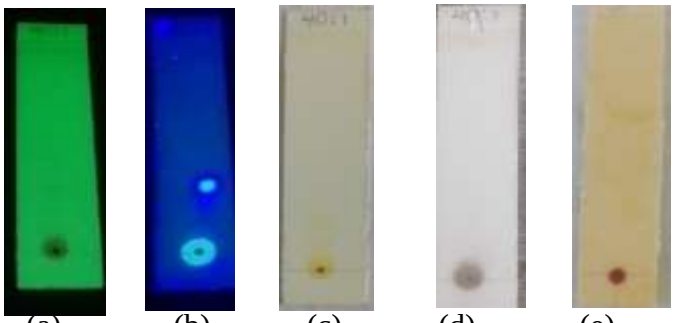
1 20% NH_4Cl 3 n-BuOH-aceticacid-water 3:1:1

2 n-BuOH satutated with water 4 EtOAc saturated with water

Figure 5 Paper chromatography bioautographic assay

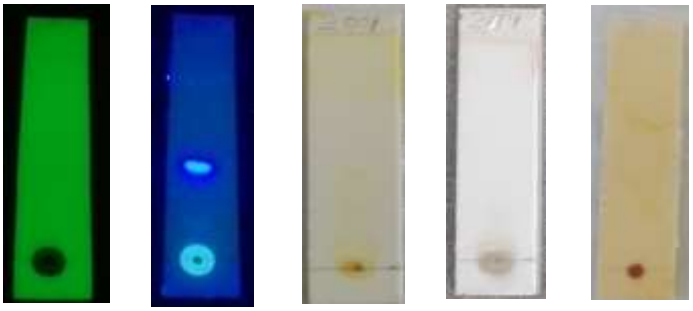
Study on Thin Layer Chromatography of Ethyl Acetate Extract of *Aspergillus nidulans*

According to the results of TLC (Figure. 6-11), it may be considered that *Aspergillus nidulans* product was isolated to purify by silica gel column chromatography with n- hexane and ethyl acetate mixture as eluting solvents.



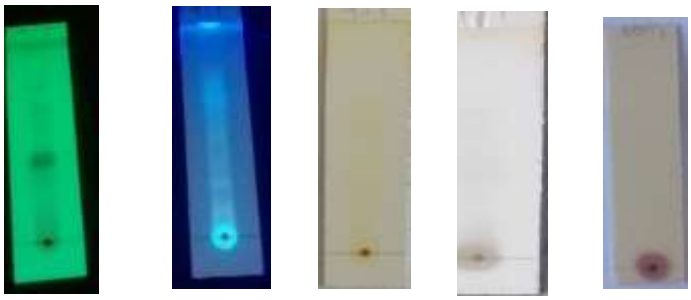
(a) (b) (c) (d) (e)

Figure 6 TLC chromatogram (a) under UV light 254 nm, (b) under 365 nm, (c) I_2 vapour, (d) 5% H_2SO_4 and (e) 1% $FeCl_3$ of n-hexane :ethyl acetate (40:1 v/v)



(a) (b) (c) (d) (e)

Figure 7 TLC chromatogram (a) under UV light 254 nm, (b) under 365 nm, (c) I_2 vapour, (d) 5% H_2SO_4 and (e) 1% $FeCl_3$ of n-hexane :ethyl acetate (20:1 v/v)

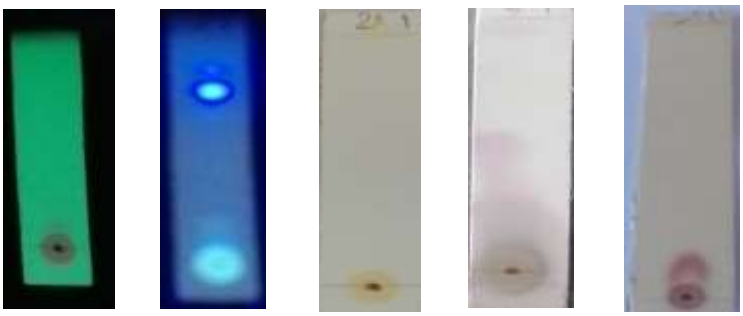


(a) (b) (c) (d) (e)

Figure 8 TLC chromatogram (a) under UV light 254 nm, (b) under 365 nm, (c) I_2 vapour, (d) 5% H_2SO_4 and (e) 1% $FeCl_3$ of n-hexane:ethyl acetate (10:1 v/v)

(a) (b) (c) (d) (e)

Figure 9 TLC chromatogram (a) under UV light 254 nm, (b) under 365 nm, (c) I_2 vapour, (d) 5% H_2SO_4 and (e) 1% $FeCl_3$ of n-hexane :ethyl acetate (5:1 v/v)



(a) (b) (c) (d) (e)

Figure 10 TLC chromatogram (a) under UV light 254 nm, (b) under 365 nm, (c) I_2 vapour, (d) 5% H_2SO_4 and (e) 1% $FeCl_3$ of n-hexane :ethyl acetate (2:1 v/v)

(a) (b) (c) (d) (e)

Figure 11 TLC chromatogram (a) under UV light 254 nm, (b) under 365 nm, (c) I_2 vapour, (d) 5% H_2SO_4 and (e) 1% $FeCl_3$ of n-hexane :ethyl acetate (1:1 v/v)

Conclusion

A number of antibiotics drugs have been discovered from soil inhabiting microorganisms. Antibiotics produced by fungi are widely used in current chemotherapy (Dobashi, 1998). Therefore, the isolation and screening of effective soils were investigated.

Thirty-one fungi were isolated from these 10 different soil samples. Most of soil are sandy loam. In these fungi, most of them are members of the *Aspergillus spp*. Optimal fermentation conditions are very important for maximal productivity of antibiotics (Cruegar, 1989). Moreover, the further investigation for extraction of antibacterial metabolite will be taken. According to the R_f value (Figure. 5), it was considered that antibacterial metabolite is suitable ethyl acetate solvent.

Therefore, solvent No. 4, ethyl acetate is suitable for the extraction of antibacterial metabolite from the fermented broth. The extraction of antibacterial compound was carried out by column chromatographic to get 9.5 g/ 20 liters.

Various chromatographic techniques have been used for successful fractionation and purification of biologically active compounds from variety of sample. Column-chromatography is one of the most popular and widely used separation techniques to characterize both organic and inorganic materials suggesting is potential usefulness in chemical analysis of complex extract material (Vivek *et al*., 2016).

In the screening of extracted substance, according to the TLC results (R_f value), gradient elution was performed successively with increasing polarity n-Hexane:EtOAc, (40:1 v/v), (20:1 v/v), (10:1 v/v), (5:1 v/v), (2:1 v/v) and (1:1 v/v) (Figure.6-11). Keeping in view the above justifications, the purification of antibacterial metabolites was carried out by using column chromatographic method. Identification of isolated compounds by modern spectroscopic techniques such as UV, FT IR and GC-MS would be carried out in further research.

Acknowledgements

We would like to thank to the Department of Higher Education, Ministry of Education in Myanmar, for giving us the opportunity to do this research. Our deepest gratitude is expressed to Dr Si Si Hla Bu, Ractor, Pathein University, for her encouragement, kind guidance, and kind help to do this research. We wish to thank the Myanmar Academy of Arts and Science for allowing the present of this paper.

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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.

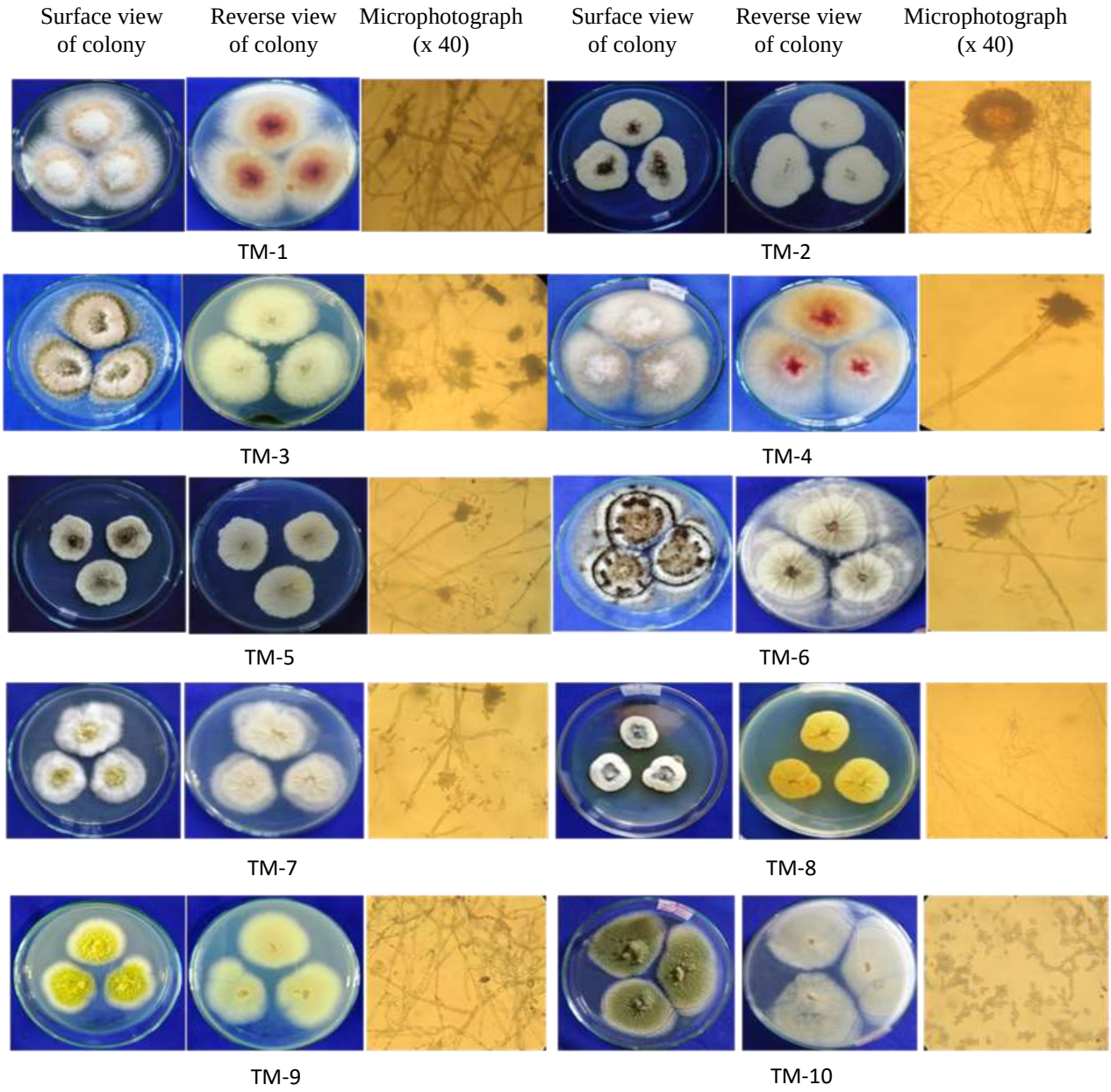


Figure 1 Morphology and their microscopical characters of isolated fungi TM-1 to TM-10

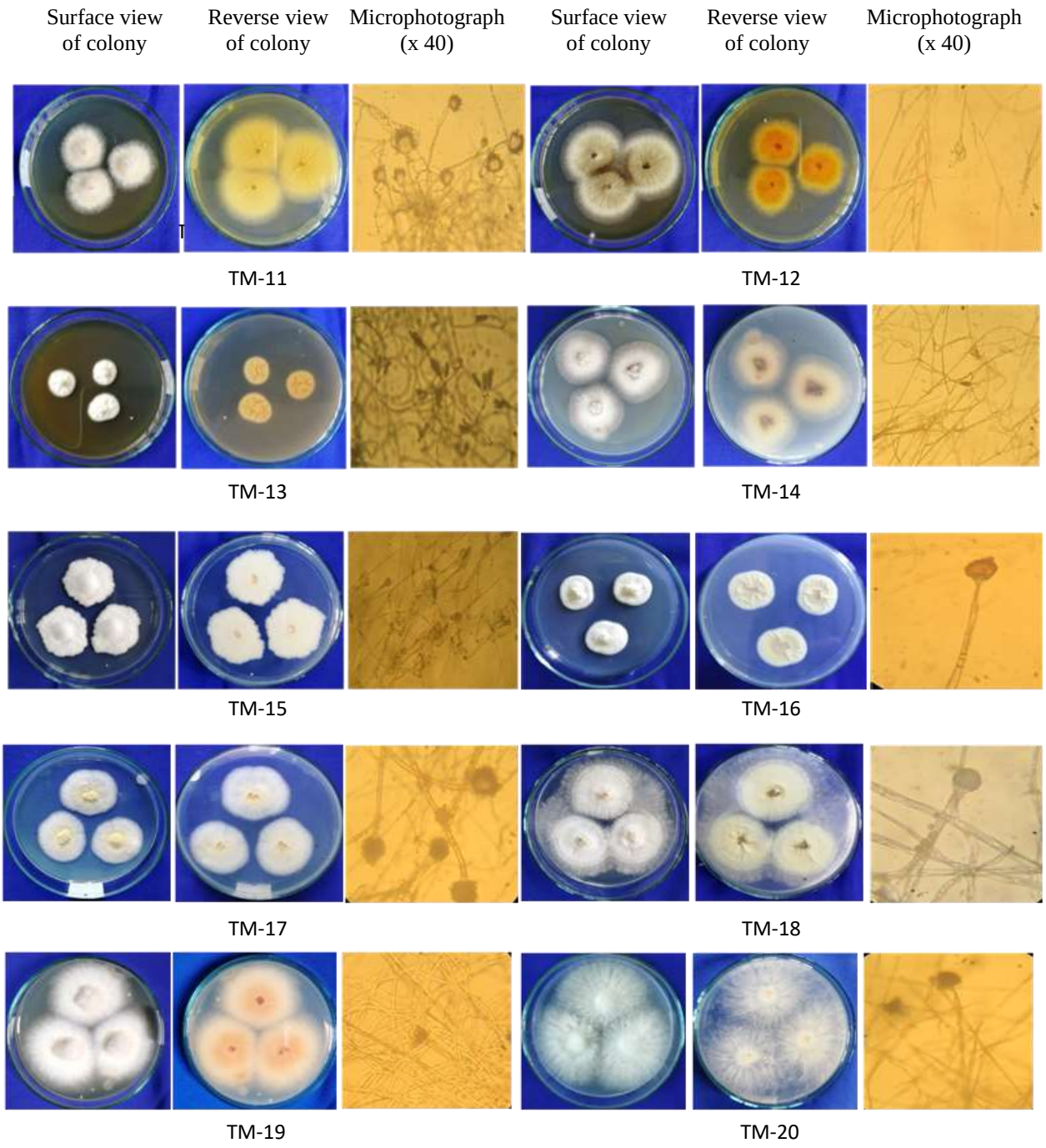


Figure 2 Morphology and their microscopical characters of isolated fungi TM-11 to TM-20

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

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

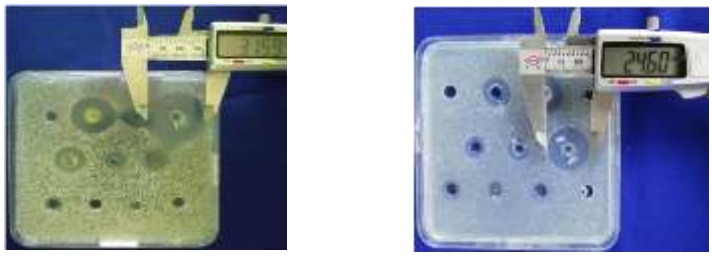
Table 11 Antibacterial Activity of Isolated Fungal strains against *Agrobacterium tumefaciens*

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.

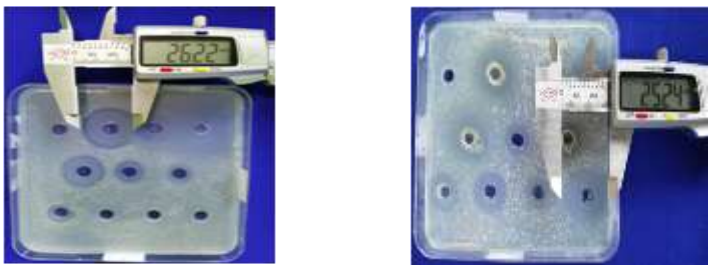


TM-14 TM-16

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*

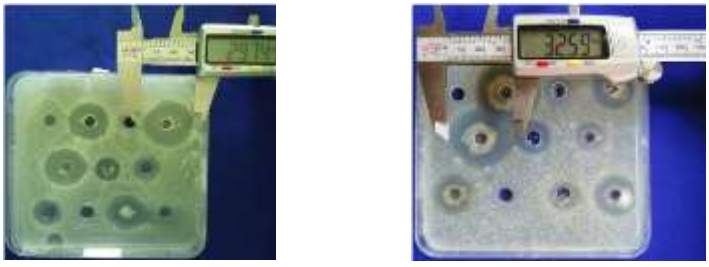


TM-4 TM-20

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*

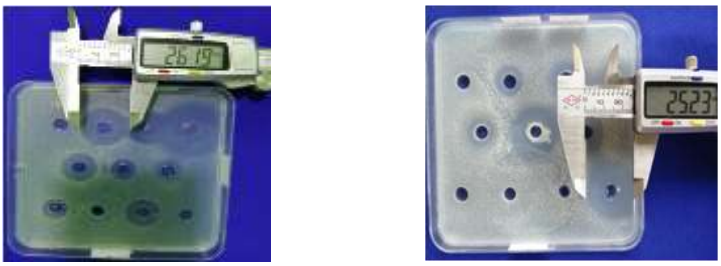


TM-14 TM-16

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 5th day, (22.94 mm) on *Bacillus subtilis* at 5th day, (24.30 mm) on *Bacillus pumilus* at 5th day and (23.66 mm) on *Candida albicans* at 6th day. TM-16 showed the highest antimicrobial activities (33.68 mm) on *Candidas albicans* at 4th day, (27.67 mm) *on Malassezia furfur* at 5th day and then (32.59 mm) on *Pseudomonas fluorescens* at 5th day and (22.70 mm) on *Staphylococcus aureus* at 4th 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.

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. Seconrdly, I'm very grateful to my supervisiors, Dr Than Than Oo, Professor, Department of Chemistry, Pathein University and Dr. Zar Zar Yin, Professor, Department of Botany, University of Yenangyaung for their valuable instructions, constructive suggestions and insightful supervisions for the successful completion of this research paper. And then, I would like to record my deep thank to Professor Dr. War War Lwin, Department of Botany, Pathein University.

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EXTRACTION, ISOLATION AND CHARACTERIZATION OF ANTIFUNGAL METABOLITES FROM THE FERMENTED BROTH OF *PENICILLIUM PURPUROGENUM* (MF-12)

Moe Moe Aye[1], Yin Yin Mya[2], Nant Si Si Htay[3]

Abstract

In this study, twenty six fungi were isolated from nine different soil samples for the production of antibiotics. The antimicrobial activities of isolated fungi were tested by ten test organisms. The bioactive metabolites of *Penicillium purpurogenum* were extracted by using *n*-BuOH solvent. The antifungal activity of *n*-BuOH extracts from the fermented broth of *P. purpurogenum* against *C. albicans* was examined. Separation of bioactive compounds by fractionations of the crude extract in silica gel column chromatography eluted with co-solvent in gradient was able to separate almost all impurities. From the analysis, four compounds (I, II, III and IV) were obtained. Characterization and classification of four compounds were performed by some chemical reagent tests and some modern spectroscopic techniques such as UV and FTIR. The isolated antifungal compounds could be applied as medicine to treat infectious diseases such as candidiasis, dermatophytosis, meningitis and arthritis which are related to infections of *C. albicans.*

Keywords: microbes, antibiotics, antifungal compounds, *C. albican,* candidiasis

Introduction

Most of the naturally occurring antibiotics have been isolated from soil microorganisms. Secondary metabolites are small molecules that are not directly involved in metabolism and growth of the organism. Both plants and fungi are known for producing a large number of chemically diverse secondary metabolites. It is well known that fungi remain one of the most important resources for the discovery of new bioactive compounds against bacteria, fungi, insects and nematodes as well as antitumor compounds (Pelaez, 2005).

Microorganisms that are able to producing secondary metabolites have a diverse chemical structure and biological activities (Stachelhaus *et al*., 1995). Some soil filamentous fungi such as *Penicilium* produce many bioactive small molecules, or secondary metabolites, that range from beneficial bioactive compounds (antibiotics, anticancer, anti-infective, antimicrobial, and antioxidant) to harmful toxins (Tajick *et al*., 2014). Fungi have provided several bioactive compounds and chemical models currently used as pharmaceuticals, and the soils are traditionally the main source of fungal genetic resources for bio-prospection programs (Adrio and Demain, 2003).

The fungi species of genus *Penicillium* are very attractive organisms for production of useful protein and biologically active secondary metabolites. These organisms have the ability to secrete various compounds with different biological activities, such as antibiotics, antitumor, antifungal, anti-tuberculosis, as well as pesticides. Pharmaceutical industry is now facing a scarcity of new antibiotics; therefore, to ensure the availability of effective drugs in the future, it is important to increase efforts for identification of new antibiotics (Marasabessy *et al.,* 2017). In the last 25 years, a steady increase of microbial resistance to antibiotics has become a serious threat to global public health (Marasabessy *et al.,* 2017).

Several species of the yeast genus *Candida* are capable of causing candidiasis. They are members of the normal flora of the skin, mucous membranes and gastrointestinal tract. Superficial

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candidiasis is established by an increase in the local census of *Candida* and damage to the skin or epithelium that permits local invasion by the yeast and *pseudophae*. Systemic candidiasis occurs when *Candida* enters the blood stream and phagocytic host defenses are inadequate to contain the growth and dissemination of the yeast. From the circulation, *Candida* can infect the kidneys, attach to prosthetic heart valves, or produce *Candida* infections almost anywhere (e.g., arthritis, meaningitis, endophthalmitis).

Fermentation procedures have to be developed for the cultivation of microorganisms under optimal condition and for the production of desired metabolites or enzymes by the microorganisms. The proper cultivation and transfer of inoculum are essential for the production of both primary and secondary metabolites. The pre-culture (seed culture) media and culture condition often have to be designed for optimal yields. However, the kinetics of product formation is not necessary correlated with the length of the lag phase. The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production (Stanbury *et al*., 1997). Biomass was separation of fermentation broth. Fermentation processes are a central process step in industrial biotechnology. The chemical, pharmaceutical, and food industries rely on fermentation to transform bacteria, yeasts or molds into valuable products and materials.

Bioautography is a technique that combines thin layer chromatography (TLC) with bioassay in situ (Shitu *et al*., 2006). It can be used for the screening of separated components of natural product extracts. The purification of bioactive metabolites from fermented broth of microorganisms largely depends upon the physicochemical properties of metabolites.

For the past 50 years, fungal secondary metabolites have revolutionized natural product research, affording drugs and drug leads of enormous medicinal and agricultural potential. For instance, penicillins (e.g. penicillin G, 1.1) and cephalosporins (e.g. cephalotin, 1.2), β-lactam antibiotics firstly isolated from *Penicillium* and *Acremonium* species, are still among the world's blockbuster drugs, representing about 50% of the total antibiotic market in 2009 (Aly *et al.,* 2011).

The antifungal agent griseofulvin (1.3, Fulvicin), that was isolated from the mold *Penicillium griseofulvum* is approved for the treatment of dermatophyte infections of the skin, nails and hair of humans (Aly *et al*., 2011). Certain fungi are of great importance in our daily life. For instance, the metabolic physiology of yeast has been used since ancient times for preparing cheese, bread, and alcoholic beverages. Antibiotic is a drug used to treat infections caused by bacteria that can cause illness to humans and animals. Antibiotic functions to inhibit or destroy the bacterial cells that cause certain disease. Soil microorganisms have continually been screened for their useful biological active metabolites, such as antibiotics since long ago. Therefore, this study was an attempt to discover novel antibiotics from microbes in soil samples.

The aim of the present study is to isolate the soil microorganisms from different soil samples, to study the antimicrobial activities of isolated soil fungi, to extract, isolate and characterize antifungal metabolites from the fermented broth of selected fungus *P. purpurogenum* and to screen some of their biological activities.

Materials and Methods

Collection of soil samples and isolation of soil microorganisms

Nine different soil samples were collected from Mawlamyinegyun Township, Ayeyarwady Region during June, 2015. The soil samples were analyzed by laboratory method and seven bacteria and twenty six fungi were isolated from soil samples by using physical and chemical treatment methods (Phay & Yamamure, 2005) and soil dilution method (Ando, 2014).

Antimicrobial activities of isolated soil microorganisms

The antimicrobial activities of isolated soil microorganisms were tested by paper disc diffusion assay method (NITE, 2005). The isolated soil microorganisms were inoculated into seed medium and incubated for 3 days at room temperature. Five percent of seed medium were inoculated into the fermentation medium. The fermented broth was used by paper disc diffusion assay to check the antimicrobial activity against ten test organisms, namely *Agrobacterium tumefaciens* NITE 09678, *Aspergillus parasciticus* IFO5123, *Bacillus subtilis* IFO90571*, Candida albicans* NITE09542, *Micrococcus luteus* NITE83297, *Salmonella typhi* HU9743*,Escherichia coli* AHU5436*, Saccharomyces cerevisae* NITE52847*, Pseudomonas fluorescens* IFO94307 *and Staphylococcus aureus* AHU8465 which are provided by NITE & Kyowa Hakko Co. Ltd. Paper disc having eight millimeter diameter were utilized for antimicrobial assays.

Study on the solvent systems for the extraction of antifungal metabolites by bioautograph method

The purified fermented broth were extracted with four different organic solvents watery saturated ethyl acetate, watery saturated *n*-Butanol, 20 % of ammonium chloride, *n*-Butanol : acetic acid : water (3:1:1 v/v) were used for extraction of antimicrobial metabolites from fermented broth of *P. purpurogenum*. Antifungal activity of each extract extracted with different solvents was determined out by using bioassay.

Extraction of antifungal metabolites by using separation method

Selected soil fungi *P. purpurogenum* were inoculated in a fermentation medium prepared in distilled water containing Glucose (0.7g),Yeast extract (0.6g), Polypeptone (0.3g), K_2HPO_4 (0.01g), KNO_3 (0.1g), $CaCO_3$ (0.1g), pH (6.0).The fermented broth (1L) was incubated agitation for 6 days at 25 °C. Once the incubation period was complete, mycelia were filtered by using sand column. The pure fermented broth was submitted to a liquid-liquid extraction (1:1) with *n*-BuOH by using (250 mL) separation funnel and shaken vigorously for 15 minutes and kept without any disturbance for another 10 minutes to separate the solvent from aqueous phase. The upper (organic phase) and lower layer (aqueous phase) were separated and tested bioassay. The organic phase was evaporated with a vacuum rotary evaporator at a temperature of 50-55 ºC until the organic solvent completely evaporated, leaving a dried crude extract in a rotary evaporator flask.

Separation and purification of the active antifungal metabolites from *n*-BuOH crude extract

A glass chromatographic column (50×3 cm) with a tap attached was clamped so that it was perfectly vertical. The column was packed by the wet method, using PE (only). The column was plugged by pushing a small piece of cotton wool through the solvent with a glass rod. Care was taken so that no air bubbles were trapped in the cotton wool. Silica gel (ca.50 g) was measured and placed in a beaker and made into slurry by mixing with pet ether and the suspension was thoroughly stirred. A portion of the slurry was poured into the column and at the same time the tap was opened so that the solvents flowed at a slow but constant rate. As the column material slowly settled to the bottom, the column was lightly tapped with a rubber tubing around the outside wall so as to achieve an air bubble free, uniform packing. Column materials sticking to the upper walls of the column were washed down with the solvent. When the level of solvent had fallen to a few millimettres above the top of the silica gel column, the tap was closed.

A 4.0 g of *n*-BuOH crude extract was dissolved in *n*-BuOH and mixed with a little amount of silica gel. The mixture was allowed to evaporate with continuous agitation so that a free flowing dry silica gel on which the sample was uniformly adsorbed. By careful pouring of the adsorbed gel down the small funnel and adjusting the position of the lower end of the tube, a uniformed layer of

adsorbed gel placed onn the top of the column. The top of the layer was wet with solvent. A piece of cotton wool was placed between the solvent and the column gel. The column was then completely filled with the solvent system and fraction was started; flow rate was adjusted to about one drop per five seconds. Gradient elution was performed successively with increasing polarity (PE only, PE:EtOAc, 10:1; 10:2; 10:3; 10:4; 2:1; 1:1 and followed by PE: EtOAc: MeOH in ratio 2:3:1 v/v). Successive fractions obtained were combined on the basic of their behaviour on TLC. Finally 29 main fractions (F_1 to F_{29}) were collected. Fraction F-21 was occurred as compound mixture, fraction F-23 and F-26 were occured as solid materials. Other fractions occurred as mixtures.

Antifungal activity of each fractions were examined in bioassays to determine the fractions containing the active compound. Fractions F-21, F-23 and F-26 were found to have antifungal activity against *C. albicans*. Other fractions were found to be inactive against the tested *C. albicans* and so were disposed.

Successive fraction F-21 containing compound mixtures was further chromatographed (column size 30×1.5 cm) on small scale as the above procedure using eluted solvent PE:EtOAc in ratio (5:1 and 3:1) and PE:EtOAc:MeOH (2:3:1) finally provided two compounds I and II. Fraction F-23 was evaporated, washed with PE only and then PE:EtOAc (2:1 v/v) and then crystallized from MeOH, yielded 0.1625 %, (6.5mg) of compound III as colourless needle shape crystals. Fraction F-26 was evaporated, washed with PE (only) and then PE:EtOAc (2:1 v/v) and then crystallized from MeOH, yielded 0.1125 %, (4.5 mg) of compound IV as colourless solids.

Bioassay for antifungal activity

Fractions of 1-29, each fraction was re-dissolved with 2 ml methanol and put on paper disc. A 20 ml GPA assay medium (Goucose, peptone and agar) was suspended with 0.1 ml *C. albicans* spores suspension in water and shaken gently, then poured into a sterile petri dish and allowed to solidify. Bioassay for measuring the antifungal activity was carried out by the so-called paper disc diffusion method, done according to the following procedures.

After solidification, paper discs impregnated on the agar plates. The test plates were incubated at 27 ºC for 24-48 hours. Clear zones (inhibitory zones) were observed surrounding the paper disc indicated the presence of bioactive metabolites which inhibit the growth of *C. albicans*. The inhibition zone diameter was measured with (digital caliper) three times in three different directions, and then averaged. No inhibitory diameter zone of other fractions were deposited.

Physicochemical characterization of isolated compounds

The isolated compounds were subjected to TLC analysis and their R_f values were determined. GF_{254} silica gel precoated aluminium plate (Merck) was empolyed and the chromatogram was developed in the appropriate solvent system. After the TLC plate was dried, the R_f values of isolated compounds were measured. Localization of spot was made by viewing directy under UV 254 nm and 365 nm light or by treating with visualizing agents.

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, EtOAc, Hexane, $CHCl_3$, MeOH, EtOH, Acetone and PE in order to know their solubilities.

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 Vanaline sulphuric acid, Anisaldehyde sulphuric acid, Lieberman Burchard,, 5% H_2SO_4, 5% $FeCl_3$, and I_2 vapour to study their behaviour on TLC. Isolated

compounds were also treated with Hydroxyl ammonium chloride to examine their type of compounds.

Classification and identification of isolated compounds

The isolated compounds were structurally identified by modren spectroscopic techniques such as UV-visible and FTIR spectrometry.

Results and Discussion

In this study, the soil samples were taken near the tree by digging under 4 cm depth from surface of soil. Tangjang, *et al*., 2009, where they found out that there was greater amounts of bacterial and fungal populations in the top soil (0-10 cm) if compared to that of other depths. Alexander, 1997, said that physicochemical analysis of soil showed that pH range of soil conditions ranging from 5.1 to 7.5 and soil textures were determined the fungal population. In this study, the most soil samples were indicated that acid range 5.15 to 6.41. The soil textures were clay, silt clay and silt clay loam. During the investigation period seven bacteria and twenty six fungi (MF-1 to MF-26) were isolated from the nine soil samples. Seven bacteria were not used in this study.

In this study, antimicrobial activity of fungal isolates was screened for research and selection of new antimicrobial metabolites with using paper disc diffusion assay method. Twenty-six soil fungi were tested by using ten test organisms, namely *A. tumefaciens*, *A. paraciticus*, *B. subtilis, C. albicans*, *M. luteus*, *S. typhi, E. coli, S. cerevisae, P. fluorescens and S. aureus*. Mostly soil fungi were against *A. tumefaciens*, *B. subtilis, C. albicans*, *M. luteus*, *S. typhi*, *E. coil, S. cerevisae,and S. aureus*. In this study, soil fungi MF-12 was selected for further investigation depending on the experimental data of antifungal activities.

The selected fungus MF-12 was identified to the genus level and to the species when possible on the basis of macro-morphology and micromorphology characteristics on LCA medium, according to references keys of (Ando, 2016). Colonies on PGA medium (7 days, room temperature) was 47.45 mm in diameter. Mycelium was white to pale at the margins and greyish in the center. Sporulation was heavy with greyish green colors, reverse was orange with cream to white margins and deep orange in the center. The vegetative characters of genus *Penicillium* sp is a mycelium, which consists of freely branched, hyaline coloured and septate hyphae.

The mycelium may grow superficially forming a weft upon the substrate. The hyphae cells contain usually a single nucleus. The conidiophores are unbranched. At the ends of the unbranched conidiophores, clusters of finger-shaped metulae are developed two stage branched (Biverticillate-asymmetrical). Each metullae finally terminated in a tuft of uninucleated flask-shaped branches, which are called sterigmata or phialides. The conidiophore along with its branches (metullae), sterigmata or phialides and conidia in basipetal chains, looks like a brush-like structure, known as the *penicillus* (brush).

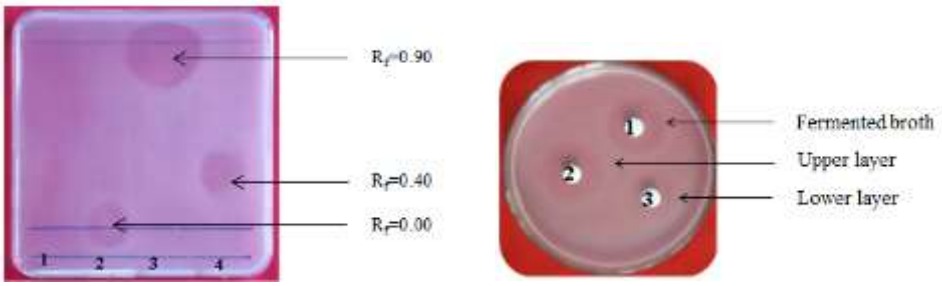
Based on the morphologically and microscopically characters and according to Barnett, 1956; Domsh 1980, Watanabe, 2002 and Ando, 2016, the selected fungus MF-12 was identified as *Penicillium purpurogenum* Stoll 1904 (Figure.1).

Front view of colony Reverse view of colony Micromorphology (10 × X 40)

Figure 1 Colony characters (7 days) and micromorphology of *P. purpurogenum*

Andriy, *et al*., 2016 describe that the antimicrobial activity of the fungal extracts derived from their mycelia. Geweely, *et al*., 2011 said that *P. purpurogenum* inhibits the growth of all bacterial species tested. Amna *et al*., 2009 reported that the species of *Penicillium* sp. was the most potent fungal producers of antibacterial compounds. Moreover, fungus *Penicillium* is one of the antifungal against all fungi tested. In this research, several clinically important yeasts *C. albicans* was selected for the antifungal activity of *P. purpurogenum*.

In the paper chromatography, by using (1) NH_4Cl, (2) EtOAc, (3) *n*-BuOH and (4) *n*-BuOH: CH_3COOH: H_2O (3:1:1). The antifungal secondary metabolites were detected from the fermented broth of *P. purpurogenum* by using bio-autography method. An overall view of bio-autography has been depicted in Figure 2(A). According to the R_f values of bio-autography, it was suitable for the extraction of antifungal compound of *P. purpurogenum* metabolites by using *n*-BuOH solvent. Antifungal metabolites from the fermented broth of *P. purpurogenum* were extracted by using separation method. In the preparation, the filtrate of fermented culture, upper and lower layer were tested by using assay medium containing test organisms.



(A) (B)

Figure 2 (A) Extraction of antifungal metabolites by bio-autography, (B) bioassay

In this study, the filtrate of fermented broth showed that (ID=26.70 mm) and upper layer (ID=31.85mm) and lower layer (ID=16.34mm) were optimized and described in Figure 1 (B). Therefore, the upper of metabolites were collected for the separation and purification of antifungal compounds. 0.8 g/L of dry reddish brown crude extract was obtained. The reddish brown crude extract obtained after *n*-BuOH extraction was subjected to thin layer chromatography to detect the various components present in the crude extract of *P. purpurogenum*.

The various metabolites present in the crude extract were detected by thin layer chromatography. By using the solvent system, PE (only), PE: EtOAc (10:1; 10:2; 10:3; 10:4; 2:1; 1:1) and PE: EtOAc: MeOH (1:1.5:0.5) for silica gel column chromatography, 29 fractions (F_1-F_{29}) were obtained. Bioassay for measuring the antifungal activities of fractions 1 to 29 were carried out by bioassay method and the results are shown in Table 1 and Figure 3. Isolation of some bioactive compounds from fraction $F_{21.1}$ to $F_{21.11}$ was done out by paper disc diffusion method. Among the fractions, F_1-F_{20}, F_{22}, $F_{24\text{-}25}$, $F_{27\text{-}29}$ were discarded because these fractions did not show the antifungal activity. Fractions F_{23} and F_{26}, compounds III and IV and fraction $F_{21.11}$, compounds I and II were obtained. Compound I (colourless needle shape crystals, 0.1517 %,), compound II (pale yellow solids, 0.12%), compound III (colourless needle shape crystals, 0.1625 %) and compound IV (colourless solids, 0.1125 %,) were isolated from *n*-BuOH extract of *P. purpurogenum* metabolites.

Table 1 The average diameter of the inhibition zone of fractions F_1-F_{29}

Fraction	Inhibition diameter zone (mm)	Fraction	Inhibition diameter zone (mm)
F_1 to F_2	-	F_{21}	18.43
F_3	15.47	F_{22}	-
F_4	-	F_{23}	22.21
F_5	14.56	F_{24}	-
F_6 to F_{15}	-	F_{25}	-
F_{16}	+	F_{26}	18.21
F_{17} to F_{20}	-	F_{27} to F_{29}	-

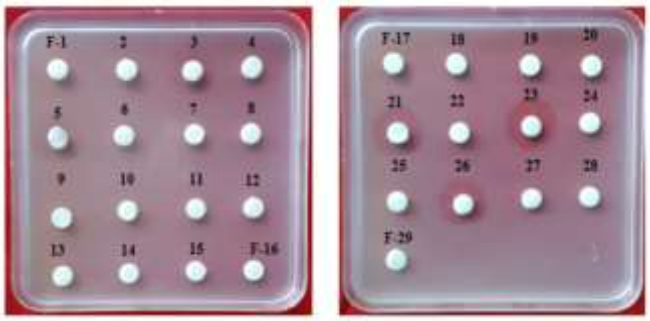


Figure 3 The inhibition diameter zone of fraction F_1 to F_{29}

The isolated compound I was classified by chemical reagent tests and UV, FT IR spectroscopy. Its R_f values was found to be 0.33 in PE:EtOAc (3:1 v/v) solvent system. It gave a yellow with iodine vapour, violet spot with anisaldehyde-sulphuric acid followed by heating and greenish blue colouration with Liebermann Burchard reagent on TLC and gave deep red colouration on testing with ethanolic hydroxylamine hydrochloride reagent which is the test for ester and so it could be classified as a steroid which contained an ester group. No absorption was observed in readily accessible UV region.

The FT IR spectrum at compound I showed the absorption bands at 3323 cm^{-1} for –OH stretching vibration of alcohol. Absorption band at 2921 and 2853 cm^{-1} were due to asymmetric and symmetric C-H stretching vibration of $–CH_2$ and $–CH_3$ groups. The corresponding C=O stretching vibration of cyclic ketone was observed at 1717 cm^{-1}. The C=C stretching vibration of olefinic group was found at 1642 cm^{-1}. The corresponding –CH bending vibrations of $–CH_2$ and $–CH_3$ were shown at 1450 and 1375 cm^{-1}. The corresponding C–O–C stretching vibration of ester was observed at 1106 cm^{-1}. The corresponding –C–O stretching vibration of alcohol was observed in 1044 cm^{-1}. The corresponding =C–H bending vibration was observed at 877 cm^{-1}. All the above information inferred the isolated compound I as a steroid that contained an ester group.

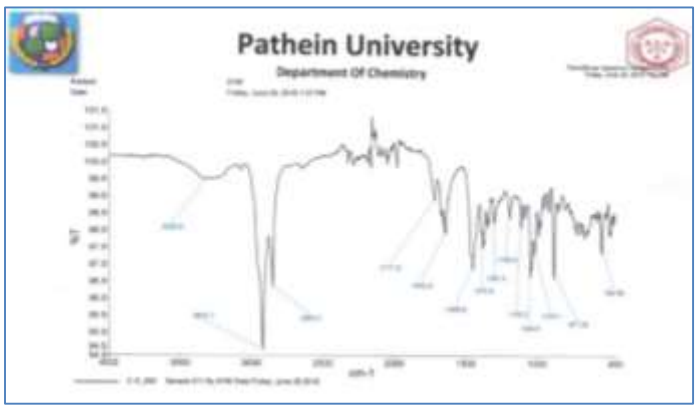


Figure 4 FT IR spectrum of isolated compound-I from $F_{21.11}$

The R_f value of compound II was found to be 0.35 in the solvent system of PE: EtOAC (2:1 v/v). According to the result obtained from chemical test compound II developed into cherry red on TLC while spraying with 5%H_2SO_4 followed by heating, yellow with iodine vapour, violet with anisaldehyde-sulphuric acid followed by heating and green colouration with Liebermann Burchard reagent and so it could be classified as a steroid. It was UV active indicating the presence of conjugated double bond.

The FT IR spectrum at compound II showed the bands at 3373 cm^{-1} due to stretching vibration of O–H. Absorption band at 2960 cm^{-1} and 2874 cm^{-1} were due to asymmetric and symmetric C–H stretching vibration of -CH_2 and -CH_3 groups. The corresponding C=C stretching vibration was observed at 1660 cm^{-1}. The banding vibration of methyl parts and methylene parts were noticed by the medium intense bands at 1450 and 1306 cm^{-1}. The corresponding C–O stretching vibration of cyclic alcohol was shown as weak intense band at 1037 cm^{-1}. From the physicochemical properties, melting point, R_f values and FT IR spectral data, isolated compound II was classified as a steroid.

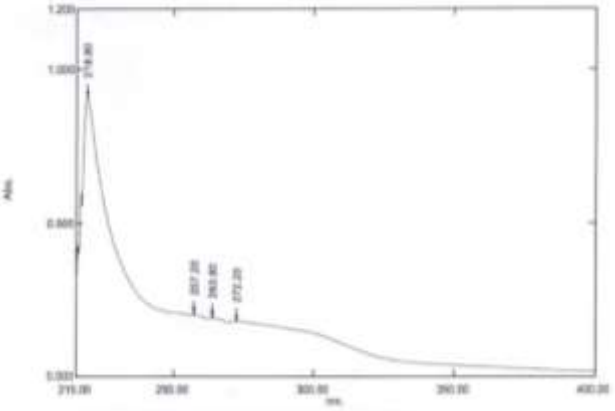


Figure 5 UV-Vis spectrum of isolated compound-II from $F_{21.11}$

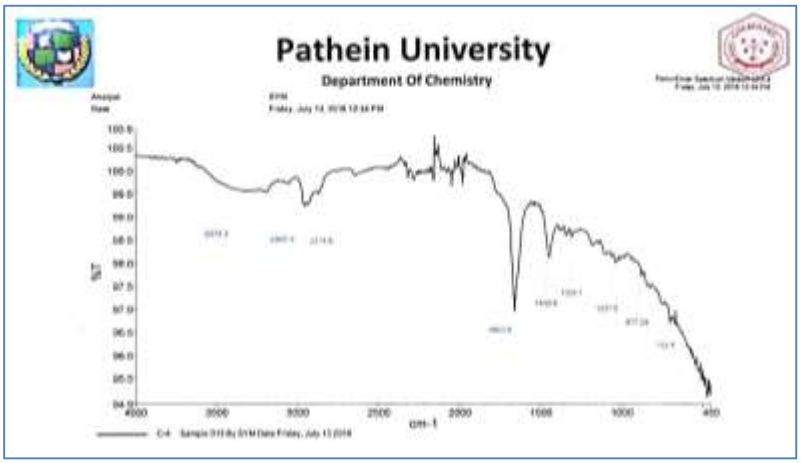


Figure 6 FT IR spectrum of isolated compound-II from $F_{21.11}$

The R_f values of compound III was found to be 0.86 in PE:EtOAC (1:2 v/v) solvent system. According to the result obtained from chemical test, compound III developed into yellow on TLC with iodine vapour and it decolourized 1% $KMnO_4$. It was UV active indicating the presence of conjugated double bond.

The FT IR spectrum of compound III showed the bands at 3334 cm^{-1} due to stretching vibration of O-H. Absorption band showed at 2914 and 2846 cm^{-1} due to asymmetric and symmetric C-H stretching vibrations of -CH_2 and -CH_3 groups. The aromatic C=O stretching was

observed at 1671 cm^{-1}. The corresponding C=C stretching in aromatic ring was observed at 1575 and 1543 cm^{-1} respectively. The banding vibration of methyl parts and methylene parts were noticed by the medium intense bonds at 1464 cm^{-1} and 1429 cm^{-1} respectively. The C-O stretching vibration of alcohol was observed by 1041 cm^{-1}. The =CH bending vibration was found that 877 cm^{-1}. The corresponding C-H out of plane bending vibration of benzene ring at 720 cm^{-1}. From the physicochemical properties, R_f values and FT IR spectral data, isolated compound III from fraction F_{23} was classified as an aromatic derivative containing carbonyl and alcohol functional group.

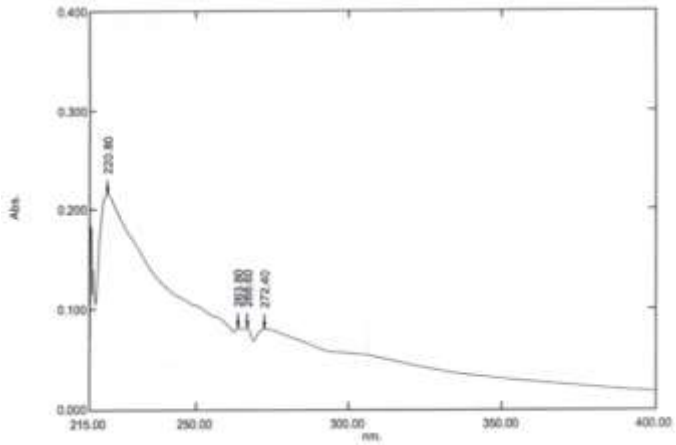


Figure 7 UV-Vis spectrum of isolated compound-III from F_{23}

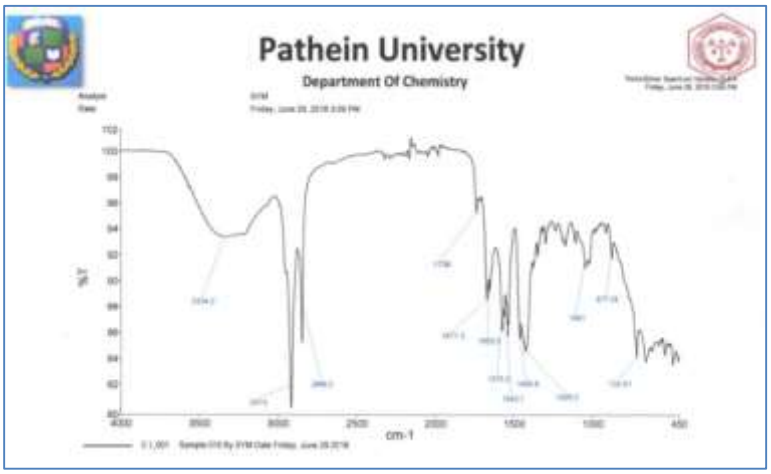


Fig. 8 FT IR spectrum of isolated compound-III from F_{23}

The R_f value of compound IV was found to be 0.6 in the solvent system of PE:EtOAC (1:2 v/v). According to the result obtained from chemical test, compound IV was developed into yellow on TLC chromatogram with shown iodine vapour and did not develop any colour with anisaldehyde-sulphuric acid and libermannburchard test followed by heating. It was UV active indicating the presence of conjugated double bond.

The FT IR spectrum of compound IV showed the bands at 3366 cm^{-1} due to O–H stretching vibration of alcohol. Absorption band at 3089 cm^{-1} was found to be C–H stretching vibration of aromatic compound. The C–H stretching vibration of $–CH_2$ and $–CH_3$ was observed at 2921 and 2849 cm^{-1}. The corresponding C=C stretching in aromatic ring was shown at 1575 and 1511 and 1404 cm^{-1}. The corresponding C–H bending vibration of $–CH_3$ was shown at 1379 cm^{-1}. The C–O stretching vibration of alcohol was observed at 1037 cm^{-1}. From the physicochemical properties, R_f values and FT IR spectral data, isolated compound IV was classified as an aromatic derivative.

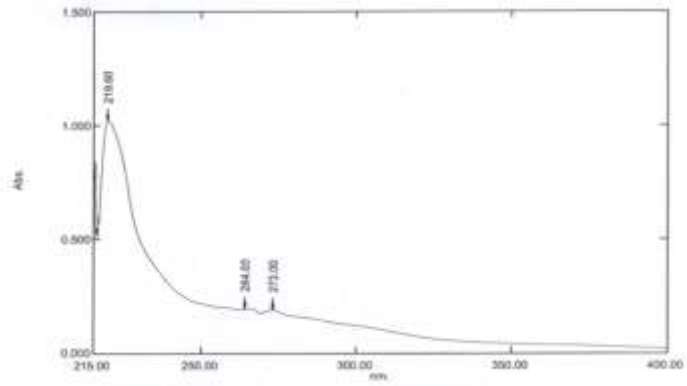


Figure 9 UV-Vis spectrum of isolated compound-IV from F_{26}

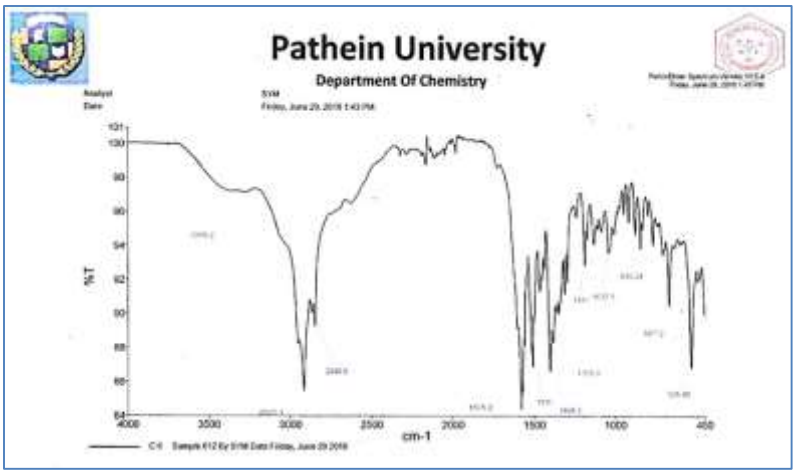


Figure 10 FT IR spectrum of isolated compound-IV from F_{26}

Aromatic steroids are lipids that contain at least one or more aromatic ring (s) in a steroid skeleton. The aromatic steroids can be produced from microorganisms, fungi, and marine invertebrates, and also they were found in plants, animals, marine sediments, and karst deposits. Biologically active aromatic steroids likely have an anti-tumor, anti-inflammatory, and neuron protection activity. This review emphasizes the role of aromatic steroids as an important source and potential leads for drug discovery and they are of great interest to chemists, physicians, biologists, pharmacologists, and the pharmaceutical industry (Valery, *et al.*, 2018). Biological activity of mono, di and tri aromatic steroids isolated from plants, fungi, invertebrates, marine sediments, and oils.

Penicillium is a genus of ascomycetous fungi of major importance in the environment, food and drug production **(**Tiwari, 2011). Fungi produce a wide range of secondary metabolites such as antibiotics, toxins, alkaloids, fatty acids, ketones and alcohols during active cell growth. Nose, *et al.,* (2000) purified two novel antifungal antibiotics, PF 1163 A (1) and B (2) from the fermentation broth of *Penicillium* sp. which showed potent growth inhibitory effect on *C. albicans*. The IR spectra of 1 and 2 showed the absorption peaks characteristic for hydroxyl group (3400 cm^{-1}), lactone or ester carbonyl (1740 cm^{-1}) and amide carbonyl (1635cm^{-1}). Therefore, *Pencillium* sp. may be used to treat bacterial and fungal infections including respiratory infection, pneumonia and bacterial throat infections, treatment of typhoid fever and other intestinal infections like dysentery, tuberculosis, sexually transmitted diseases like gonorrhea and syphilis (Tiwari, *et al*., 2011).New bioactive metabolites continue to be discovered from these fungi nowadays indicating their current importance as sources of high amounts of novel bioactive molecules to be used by pharmaceutical industry (Petit*, et al.,* 2009).

Conclusion

Based on the results from this study, metabolites of *P. purpurogenum* showed antifungal activity against on the diseases of *C. albicans.* The isolated antifungal compounds could be applied as medicine to treat infectious diseases such as candidiasis, dematophytisis, meningitis and arthritis which are related to infections of *C. albicans.* The results have been proved that metabolites of *P. purpurogenum* contain the potential antifungal component that may be used for the medicine and pharmaceutical industry. Further research is needed for the determination of chemical structures and identification of the antifungal compounds.

Acknowledgements

The authors would like to thank the Department of Higher Education, Ministry of Education, Myanmar, for the permission of doing this research and also to the Myanmar Academy of Arts and Science for allowing to present this paper. My deepest gratitude is expressed to Dr Nyunt Phay, Director General, Department of Monitoring and Evaluational (Education), Ministry of Education, for his invaluable instruction, suggestion, adivice, guidance and encouragement to do this research work. I would like to tender my thank to Dr Mar Lar, Pro-rector, Hinthada University for her permission to do this research. I am so thank to Dr Ohn Mar Tin , Professor and Head and Dr Thi Thi Aye, Professor, Department of Chemistry, Hinthada University for their encouragements and permission to do this research.

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ANTIBACTERIAL ACTIVITIES OF LEAF EXTRACTS FROM *CODARIOCALYX MOTORIUS* (HOUTT.) H.OHASHI

Aye Aye Myat[1], Thein Kywe[2]

Abstract

Codariocalyx motorius (Houtt.) H.Ohashi belonging to family Fabaceae was collected from Pyin Sar village, Pyin Oo Lwin Township during the flowering and fruiting periods from August to November, 2017. The botanical identification, preliminary phytochemical screening, physicochemical evaluation, elemental analysis and antibacterial activities of leaf extracts were carried out. According to preliminary photochemical examinations, the flavonoids, glycosides, phenolic compounds, polyphenols, saponins, amino acids, carbohydrates, reducing sugars and tannins were present while alkaloids, phytosterols and cyanogenic substances were absent. Physicochemical characterization showed that water soluble ash was found more than acid insoluble ash. The studied species showed more amounts of water extractable matter than ethanol, ethyl acetate and pet ether extractable matter. The elemental analysis showed that potassium, calcium, sulphur, iron, manganese, zinc and copper were present. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were detected according to microdilution method. The antibacterial activities were determined with four extracts, namely pet ether, ethyl acetate, ethanol and aqueous against on gram-positive bacteria; *Enterococcus faecalis*, *Staphylococcus aureus* and gram-negative bacteria; *Eschericha coli*, *Pseudomonas aeruginosa*. The MIC values were between 3.9 mg ml^{-1} to 125 mg ml^{-1} and MBC were 31.25 mg ml^{-1} to >250 mg ml^{-1}. This plant showed more sensitive effects on gram-positive bacteria than gram-negative bacteria. These results showed that *C. motorius* (Houtt.) H.Ohashi may be useful to treat diseases caused by *Enterococcus faecalis* and *Staphylococcus aureus* infections.

Keywords: *Codariocalyx motorius* (Houtt.) H.Ohashi, Morphology, Phytochemical, Physicochemical, Elemental, Antibacterial.

Introduction

Medicinal plants are nature's gift to human beings for disease free healthy life. The use of herbs as medicine is the oldest form of health care known to humanity and has been used in cultures throughout history (Barnes *et al*. 2007).

Herbal medicines usually contain in a range of pharmacologically active compounds. Bioactive compounds are normally accumulated as secondary metabolites in all plant cells. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Selvamohan *et al*. 2012).

Increasing development of drug resistance in human pathogens as well as the appearance of side effect of synthetic drugs needs to develop new antimicrobial drugs from natural sources (Mondel *et al.* 2004). This situation has forced to search for new antimicrobial sources like medicinal plants (Doshi *et al*. 2011). Prevention of bacterial infections, using plant extracts, is highly desirable due to low cost, environmental friendliness, and effectiveness against certain bacteria, compared to antibiotics which might be harmful to the environment (Cheng *et al.* 2014).

Codariocalyx motorius (Houtt.) H.Ohashi, commonly known as Dancing plant or Se ka myin or Shik kho pin and are widely distributed in Myanmar (Kress *et al*. 2003), is famous for its rapid movement of lateral leaflets. This plant is popularly used in Indian traditional and folk medicine since its leaves have diuretic, febrifugal and tonic properties and roots are used as a remedy for asthma, coughs, as antidysenteric and as emollient. It possess a remarkable wound

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healing effect (Vipin *et al.* 2015). It has traditionally been used in Chinese Medicine to treat various ailments such as rheumatism, cough, malaria, pyrexia, dysentery, hepatitis, haemoptysis (Ma *et al.* 2011). *C. motorius* (Houtt.) H.Ohashi has been demonstrated to possess wound healing activity (Gopalakrishnan *et al*. 2015, Gopalakrishnan & Rajameena. 2012), antimicrobial properties (Kalirajan *et al*. 2012), antithrombotic and anticoagulant activities (Vipin *et al*. 2015) and anti-oxidant activity (Chidambaram *et al*. 2013, Gopalakrishnan & Rajameena 2014). This plant is used in Myanmar traditional medicine, such as, vitamin B deficiency diseases, abscesses and wound healing activities.

The description of morphological characters and phytochemical constituents, physicochemical properties, elemental analysis and antibacterial activity of leaf extracts of *C. motorius* (Houtt.) H.Ohashi were carried out in present study.

The aim of the present research was to study the antibacterial activities of various leaf extracts of *C. motorius* (Houtt.) H.Ohashi. The objectives were to study the morphological characters, to investigate the qualitative and quantitative analysis and to detect the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of leaf extracts of *C. motorius* (Houtt.) H.Ohashi.

Materials and Methods

Sample collection and identification

The plant specimens were collected from Pyin Sar village, Pyin Oo Lwin township, Mandalay region during their flowering and fruiting period from August to November 2017. The collected specimens were identified with the help of literatures: Hooker (1879), Nian-he (2009) and Xu Langran (2010).

Phytochemical investigation

The preliminary phytochemical tests of the powdered leaf were carried out at Research Division, University of Traditional Medicine, Mandalay according to Harbone (1998) and Raaman (2006) methods.

Physicochemical properties

Physicochemical properties were determined for the quality control parameter of medicinal purposes (WHO, 2011) at Research Division, University of Traditional Medicine, Mandalay.

Elemental analysis

Elemental concentration were analyzed by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF) at Chemistry Department, Western Yangon University and Atomic Absorption Spectrophotometer (AAS) at Amtt Laboratory Department, Yangon.

Antibacterial activities

Antibacterial activity of pet ether, ethyl acetate, ethanol and aqueous extracts of *C. motorius* (Houtt.) H.Ohashi were tested by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using microdilution method with resazurin (Sarker *et al.* 2007). Twelve concentrations (0.12 to 250 mg ml^{-1}) of various extracts were tested in *vitro* antibacterial activity against four pathogenic bacterial strains. The ciprofloxacin was used as positive control. Test organisms used in this study were supplied from Upper Myanmar Public Health Laboratory, Mandalay and Biotechnology Research Department, Kyaukse. The test organisms were *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. The bacteria

concentration was (5×10^5 $CFUml^{-1}$). The antibacterial activity test was done at Medical Laboratory Technology Department, University of Medical Technology, Mandalay.

Results

***Codariocalyx motorius* (Houtt.) H.Ohashi** J. Jap. Bot. 40: 367. 1965

Desmodium motorium (Houtt.) Merr., J. Arnold Arbar. 19(4): 345–346.1938.

Hedysarum motorium Houtt., Nat. Hist. 2(10):246.1779.

Desmodium gyrans (L.f.) DC. Prodr. 2: 326. 1825.

Family : Fabaceae

Myanmar name : Say ka myin or Ship kho pin

English name : Nra lap or Dancing plant

Flowering time : August to January

Fruiting time : October to January

Annual, erect herbs, 0.5 – 1.0 m high; stems and branches slender, glabrous. Leaves pinnately trifoliolate compound, alternate; stipules lanceolate, 3.0 - 4.0 mm long; petioles terete, 0.8 – 1.8 cm long; stipels 2.0 - 3.0 mm long; leaflets oblong-lanceolate to lanceolate, the terminal one larger than the two lateral leaflets, 1.5 - 7.5 cm by 0.2 - 2.0 cm, rounded or obtuse at the base, entire along the margin, acute at the apex, glabrous above and densely pubescent beneath. Inflorescences terminal racemes, 2- to 5-flowered, peduncles 4.5 - 10 cm long, sparsely pubescent. Flowers bisexual, zygomorphic, pentamerous, hypogynous, purple, 1.0 - 1.3 cm in diameter; pedicels 2.0 - 3.0 mm long, pubescent; bracts ovate, 5.0 - 7.0 mm long; bracteoles ovate-linear, about 2.0 mm long. Calyx campanulate, 2-lipped; tube about 2.0 mm long; upper lip 2-lobed, orbicular, about 5.0 mm long; lower lip 3-lobed, lobes lanceolate, 2.0 - 5.0 mm long, densely pubescent. Corolla papilionaceous; standard obovate, 5.0 - 7.0 mm by 4.0 - 6.0 mm; wings linear-oblong, 4.0 – 5.0 mm; keels obtuse, 5.0 – 6.0 mm long. Stamens 10, diadelphous, (9)+1; staminal tubes about 7.0 mm long, glabrous; anthers dithecous, dorsifixed, oblong, dehiscing longitudinally. Carpel 1; ovary superior, oblongoid, 6.0 - 7.0 mm long, unilocular with few ovules in the locule on the marginal placentae, pubescent; styles filiform, 3.0 - 4.0 mm long, glabrous; stigma penicillate. Pods oblongoid, 4 - 10 jointed, compressed, 2.0 - 3.5 cm long, densely pubescent. Seeds oblongoid, green, glabrous.

Specimen examined: Mandalay Region, Pyin Oo Lwin Township, Pyin sar Village, 22°02'N, 96°28' E, elevation 1000 m; 17 September 2017; Aye Aye Myat, collection no. 1, 2, 3

Habit

Inflorescenc

Flower as

L.S of flower

Stamens Carpel Fruits Seeds

Figure 1 Morphological characters of *Codariocalyx motorius* (Houtt.) H.Ohashi

Phytochemical constituents

Phytochemical constituents showed that flavonoids, glycosides, phenolic compounds, polyphenols, saponins, amino acids, carbohydrates, reducing sugars and tannins were present while alkaloids, phytosterols and cyanogenetic substances were absent in studied species.

Physicochemical properties

Quantitative evaluation revealed that physicochemical parameter are pH 6.3%, ash content 6.45%, acid insoluble ash 3.38%, water soluble ash 93.55%, water extractable matter 22.64%, ethanol extractable matter 14.81%, ethyl acetate extractable matter 3.05% and petroleum ether extractable 1.61%.

Elemental analysis

According to the EDXRF, the macro elements consists of potassium 1.145%, calcium 0.805% and sulphur 0.208%. The microelements of iron 0.023%, manganese 0.009%, zinc 0.002% and copper 0.002% were found. According to AAS, the contents of toxic elements, lead and cadmium were not detected.

Antibacterial activity

The results of antibacterial activity of pet ether, ethyl acetate ethanolic and aqueous extracts of *C. motorius* (Houtt.) H.Ohashi was presented in Table 1. The positive control was used in ciprofloxacin.

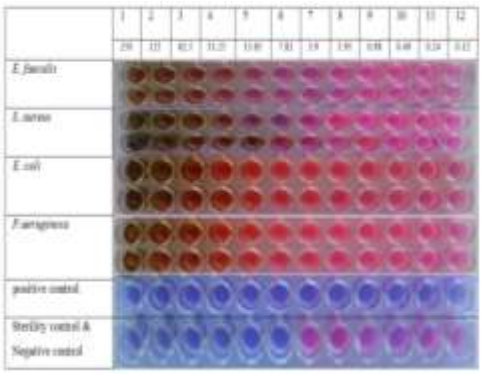


Figure 2MIC of pet ether extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria

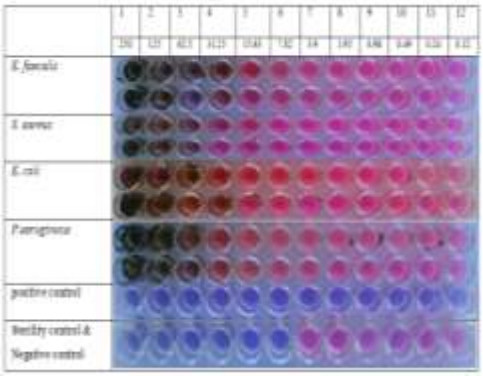


Figure 3 MIC of ethyl acetate extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against

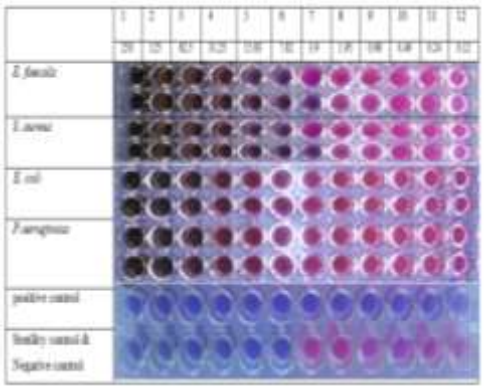


Figure 4 MIC of ethanolic extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria

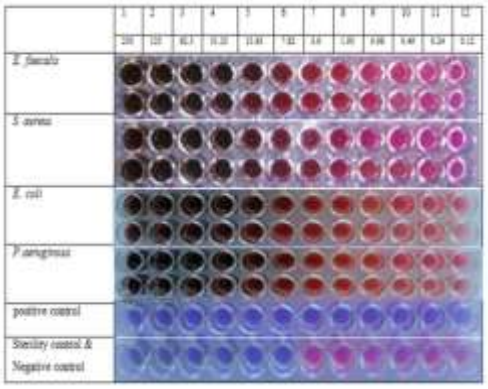
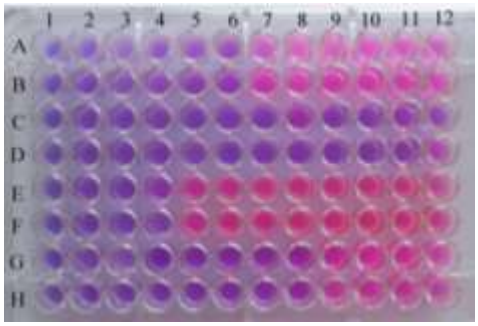


Figure 5 MIC of aqueous extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria



A, B = *Enterococcus faecalis*

C, D = *Eschericha coli*

E, F = *Pseudomonas aeruginosa*

G, H = *Staphylococcus aureus*

* pink colour indicates growth and blue means inhibition of growth

Figure 6 MIC of Antibiotic (Ciprofloxacin) against on bacteria

E .faecalis *S. aureus* *E-coli* *P. aeruginosa*

Figure 7 MBC of pet ether extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against bacteria

E .faecalis *S. aureus* *E-coli* *P. aeruginosa*

Figure 8. MBC of ethyl acetate extracts from leaf of *C. motorius* (Houtt.)H. Ohashi against bacteria

E .faecalis *S. aureus* *E-coli* *P. aeruginosa*

Figure 9 MBC of ethanolic extracts from leaf of *C. motorius* (Houtt.) H. Ohashi bacteria

Figure 10 MBC of aqueous extracts from leaf of *C. motorius* (Houtt.) H. Ohashi against

Figure 11 MBC of Antibiotic (Ciprofloxacin) against on bacteria

Table 1 Antibacterial activity of MIC and MBC values for various leaf extracts from *Codariocalyx motorius* (Houtt.) H.Ohashi

Tested Microorganisms	pet ether extract (mg ml^{-1})		ethyl acetate extract (mg ml^{-1})		ethanolic extract (mg ml^{-1})		aqueous extract (mg ml^{-1})		Ciprofloxacin (mg ml^{-1})	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis ATCC 29212	3.9	62.5	31.25	62.5	15.62	125	31.25	31.25	$1x10^{-3}$	$1x10^{-3}$
S. aureus ATCC 25923	7.81	125	62.5	125	7.81	125	15.62	62.5	$2.5x10^{-4}$	$5x10^{-4}$
E. coli ATCC 25922	125	125	62.5	125	62.5	250	15.62	>250	$5x10^{-4}$	$1x10^{-3}$
P. aeruginosa ATCC 27853	125	125	62.5	62.5	62.5	125	31.25	>250	$4x10^{-3}$	$8x10^{-3}$

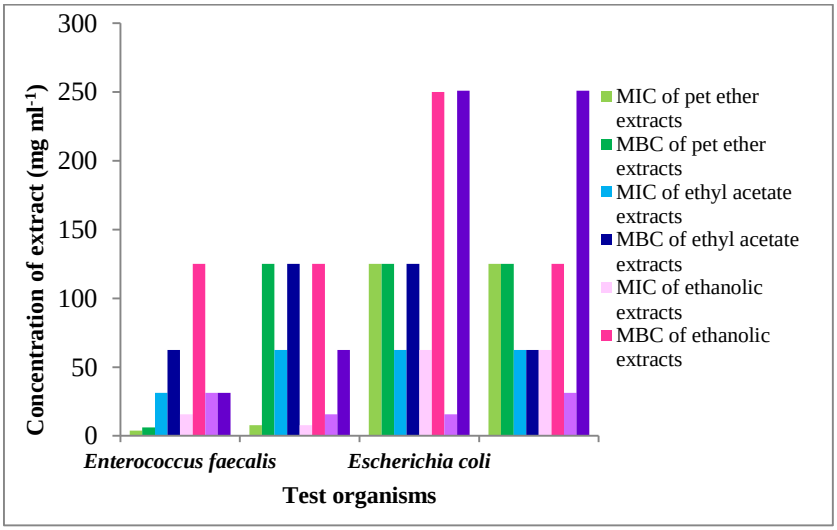


Figure 12 Antibacterial activity of MIC and MBC values for various leaf extracts from *Codariocalyx motorius* (Houtt.) H.Ohashi

Discussion and Conclusion

The antibacterial activities of pet ether, ethyl acetate ethanolic and aqueous extracts of leaf from *Codariocalyx motorius* (Houtt.) H.Ohashi was determined by microdilution method with resazurin. In addition, phytochemical constituents, physicochemical properties, elemental and heavy metal analysis of this plant was studied.

In the present study, the habit of *C. motorius* (Houtt.) H.Ohashi was annual herbs. Pinnately trifoliate compound leaves and the blades were oblong lanceolate. Inflorescences were terminal racemes with zygomorphic flowers. Papilionaceous corolla, stamens uniform and diadelphous (1+9), unilocular, few ovules with marginal placentation and sessile ovary were observed in studied species. The pods were curved and compressed which are agreed with those stated by Hooker (1885), Nian-he (2009) and Xu Langran (2010).

Phytochemical analysis is the best extraction method to indicate the presence of active constituents in herbal medicine. This species showed that the presence of flavonoids, glycosides, phenolic compounds, polyphenols, saponins, amino acids, carbohydrates, tannins, reducing sugar while alkaloids, phytosterol and harmful cyanogenic substance were absent. Due to the presence of effective bioactive compounds, this species possessed antibacterial and numerous pharmacological activities. Therefore, this plant plays a significant role in the treatment of various diseases.

In this study, the amount of water soluble ash was higher than acid insoluble ash. The low content of acid insoluble ash showed these plant extracts less amount of impurities. The extractable values were maximum in water extract followed by ethanol, ethyl acetate extract and least in pet ether extract which showed larger quantities polar phytoconstituents. These physicochemical data are pharmaceutically importance in herbal medicine.

Elemental analysis was done by Energy Dispersive X-Ray Fluorescence (EDXRF) Spectrophotometer. The macroelements; the content of potassium was mostly found. The second largest was calcium and the third was sulphur. Microelements; iron, manganese, zinc, copper were present. Moreover, the leaves powder of these plants were analysed by Atomic Absorption Spectroscopy (AAS) to know the present or absent of heavy metal. According to AAS, toxic elements; lead and cadmium were not present. Therefore this plant may be used in deficiency symptom of these elements and safely for long time.

In this study, antibacterial activities were determined by microdilution method using resazurin, as an indicator. This resazurin indicated the detection of bacterial growth. The results of present study showed that twelve different concentrations of leaf extracts were tested for their antibacterial activities. These bacteria were gram-positive bacteria; *Enterococcus faecalis*, *Staphylococcus aureus* and gram-negative bacteria; *Eschericha coli*, *Pseudomonas aeruginosa*.

The MIC results showed that all plant extracts (pet ether, ethyl acetate ethanolic and aqueous extracts) prevented the growth of all tested bacteria. The concentration for inhibition of growth of the test bacteria ranged from 3.9 mg ml^{-1} to 31.25mg ml^{-1} with the lowest MIC value against *Enterococcus faecalis* followed by *Staphylococcus aureus* (7.81 mg ml^{-1} to 62.5mg ml^{-1}) and *Eschericha coli* (15.62 mg ml^{-1} to 125mg ml^{-1}) while the highest *Pseudomonas aeruginosa* (31.25 mg ml^{-1} to 125 mg ml^{-1}). The MBC results showed that growth of the gram-positive bacteria was killed with a concentration ranged from 62.5 mg ml^{-1} to 125 mg ml^{-1} and gram-negative bacteria was from 62.5 mg ml^{-1} to >250 mg ml^{-1}. From these results, gram-negative bacteria are more resistant compared with gram-positive bacteria. Therefore, the plant extracts were more effective gram-positive bacteria than gram-negative bacteria. However, all of plant extracts possess antibacterial effects on both gram positive and gram negative bacteria. Kalirajan *et al*. (2012) reported that the methanol and aqueous extracts of *C. motorius* (Houtt.) H.Ohashi were tested for the antibacterial

activities by plate hole diffusion assay. They mentioned that methanol extract of this plant showed efficient antibacterial activity against *Eschericha coli* and *Staphylococcus aureus.* The aqueous extract was found to be effective against *Staphylococcus aureus*. Therefore, all of plant extracts have a deadly or killing effects on all test organism.

This result can be scientific evidence for the antibacterial activity of the leaf extracts of *C. motorius* (Houtt.) H.Ohash. Therefore, this plant may be used to treat many diseases caused by *Enterococcus faecalis* and *Staphylococcus aureus* infection.

Acknowledgements

We would like to express our deepest gratitude to Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay, for her permission to do this research work. We are thankful to Dr Soe Soe Aung, Professors, Department of Botany, University of Mandalay, for their suggestion and proper guidance in this research. We are thankful to Dr Zarni Htun Lwin (Associate Professor, University of Medicine Mandalay) and U Win Min Than (Associate Professor, University of Medical Technology) for their kind help in the antibacterial study.

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THE EFFECTS OF PH AND FERMENTATION MEDIA ON THE ANTIBACTERIAL ACTIVITY OF SECONDARY METABOLITE PRODUCING FROM MARINE DERIVED FUNGI*

S Aung Myo Htay[1], Khin Thandar Linn[2]

Abstract

The seagrass, *Enhalus acoroides*, was collected from Pho Htaung Gyaing, Shwe-Thaung-Yan Sub Township on June, 2019 for the isolation of marine derived fungi. The isolation was undertaken by Surface Sterilization Method. Six endophytic fungi were isolated and their antibacterial activities were tested against three test organisms. In the present study, three isolates showed the antibacterial activity against *Escherichia coli* and among them, fungus FE- 05 showed the highest activity on *Escherichia coli*. Therefore, fungus FE - 05 was selected for the investigation of the effects of pH and fermentation media on the antibacterial activity against *Escherichia coli*. In this study, the maximum antibacterial activity of secondary metabolite produced from fungus FE- 05 was observed in Potato Yeast Extract Medium under pH 6.5. According to identification result, the selected fungus FE - 05 was identified as *Cladosporium* sp.

Keywords: antibacterial activity, *Cladosporium* sp., endophytic fungi, *Escherichia coli*, seagrass

Introduction

Various researchers stated that endophytic fungi were a good source of bioactive natural products. Most of the investigations on endophytic fungi have been isolated from terrestrial plants (Naik et *al.*, 2008, Andrade- Linare et *al.*, 2011). However, bioactive natural compounds produced from endophytic fungi of marine plants including seagrass species have been rarely studied (Sakayaroj *et al.*, 2010).

Seagrasses are a relatively small group of flowering plants and distributed all along the three Coastal Regions of Myanmar, namely the Rakhine Coastal Region, the Ayeyarwady Delta and the Gulf of Mottama Coastal Region and the Tanintharyi Coastal Region. In Myanmar, twelve species of seagrasses were recorded (Soe-Htun et *al*., 2009).

Seagrasses played important roles in marine ecosystem. They served in stabilizing soil particles, reducing wave energy and providing a large shelter for a variety of marine animals (Hori et *al*., 2009). Some species of seagrasses were used as traditional medicine such as malaria and skin disaeases in India (Kumar et *al*., 2008).

Escherichia coli are the common facultative anaerobes inhabit the gastrointestinal tract of humans and animals (Ketia et *al.*, 2012). Most of *E. coli* strains are harmless but other strains can cause diseases such as watery diarrhea, bloody diarrhea and urinary tract infections (Nataro and Kaper, 1998).

Since microorganisms grow in unique and extreme habitats, they may have the capability to produce unique and unusual metabolites (Supaphon et *al*., 2014). For this reason, the objectives of the present research were to isolate and screen the endophytic fungi for antibacterial activities and to observe the effects of pH and fermentation media on the antibacterial activities of secondary metabolites produced from endophytic marine derived fungi.

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Materials and Methods

Sampling Site and Samples Collection

Figure 1 Map showing the location of sample collected area

The whole plant samples of *Enhalus acoroides* (Linnaeus f.) Royle were collected at Pho Htaung Gyaing, Shwe-Thaung-Yan Sub Township (Lat. 17 ˚10' 46" N, Long. 94˚ 28' 13" E) about thirteen miles up away from Chaungtha Beach of Ayeyarwady Region. Specimen collection was carried out on June, 2019. All specimens were collected in the intertidal zone and gloves were worn during collection. Specimens were transferred directly to sterile plastic bags containing seawater to prevent contact of plant tissue from air. The specimens were transported to the laboratory of Marine Science Department and processed immediately for the isolation and cultivation of fungi. Alternatively, some specimens were stored in refrigerator at - 80˚C for identification and future studies.

Isolation of endophytic fungi

The isolation was carried out by Surface Sterilization Method (NITE, 2004). Seagrass samples including leaves and rhizomes were washed thoroughly by running tap water. The plant parts (leaves and rhizomes) were surface- sterilized in 70 % ethanol for 3 minutes and rinsed into sterile water. The samples were dried on sterilized paper. The dried samples were then cut at the edge and then, placed onto the Glucose Yeast Extract Agar (GYA) medium (NITE, 2004) containing o.3g /ml of Penicillin G. Plates were incubated at room temperature for 3-7 days until the outgrowths of endophytic fungi were observed. The fungi were subcultured to produce pure culture on Low Carbon Agar (LCA) medium (NITE, 2004) and then stored in the slant for further investigation such as the screening of antibacterial activity.

The media used for the isolation of endophytic marine fungi are as follow.

GYA Medium (Glucose Yeast Extract Agar Medium)	
Glucose	2.0 g
Yeast extract	0.5 g
Agar	1.8 g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5
(after autoclaving Penicillin G was added to the medium.)	

Low Carbon Agar (LCA) Medium (NITE, 2004)	
Glucose	1.0 g
Sucrose	0.2 g
K_2HPO_4	0.1 g
$MgSO_4.7H2O$	0.05 g
KNO_3	0.1 g
KCL	0.05 g
Agar	1.8 g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5
(after autoclaving Penicillin G was added to the medium.)	

Study on the Antibacterial Activities

Preliminary studies for antibacterial activities against three tests were carried out by the paper disc diffusion assay method (NITE, 2004). The three test organisms, *Staphylococcus aureus*, *Pseudomonas fluorescence* and *Escherichia coli* were obtained from the laboratory of BRBDC of Pathien University.

Procedure for antibacterial activity test

A cut of mycelium from seven days old culture of each plate was cultured in a conical flask containing 50 ml of seed medium and incubated at the temperature of 25˚C. After three days, 5% of seed medium was taken by sterile pipette and poured into another conical flask containing 150 ml of fermentation medium and also incubated at the temperature of 25˚C. After 7 days, a sterile paper disc (8 mm in diameter) was impregnated in the fermentation medium and dried at least for 10 hours. About 20 ml of sterilized assay medium was poured into each sterile Petri plates and added o.5 ml of liquid culture of corresponding test organisms and allowed to solidify. And then, each dried paper disc was placed in order onto the assay plate. All the plates were incubated at 25˚C for 24 hours. After 24 hours incubation, the plates were observed for the formation of clear inhibition zone around the paper disc. The clear zone was examined by measuring the diameter of the clear zone with the aid of a digital clipper. All assays were carried out in triplicate.

Media used for antimicrobial activity test

Seed Medium	
Glucose	2.0 g
Yeast extract	1.0 g
NaCl	0.1 g
K_2HPO_4	0.001g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5

Fermentation Medium (Potato Glucose Yeast Extract Medium)	
Potato	20g
Glucose	2.0 g
Yeast extract	0.5g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5

Assay Medium	
Glucose	1.0g
Peptone	0.3g
Agar	1.8 g
Distilled Water	100ml
pH	6.5

Study on the effect of fermentation medium for the antibacterial activity of secondary metabolite

To investigate the effect of fermentation medium, the antibacterial activity of secondary metabolite produced from the selected isolated fungus was studied in four fermentation broths (Endo and Inaba, 2004) namely, Glucose Yeast Extract Medium, Potato Glucose Yeast Extract Medium, Glucose Malt Extract Medium and Glucose Malt Extract Peptone Medium. Four kinds of fermentation medium are as follows.

I. Glucose Yeast Extract Medium	
Glucose	2.0 g
Yeast extract	0.5g
$CaCO_3$	0.1 g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5

II. Potato Glucose Yeast Extract Medium	
Potato	20g
Glucose	2.0 g
Yeast extract	0.5g
$CaCO_3$	0.1 g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5

III. Glucose Malt Extract Medium	
Glucose	2.0 g
Malt extract	0.5g
$CaCO_3$	0.1 g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5

III. Glucose Malt Extract Peptone Medium	
Glucose	2.0 g
Malt extract	0.5g
Peptone	0.5g
$CaCO_3$	0.1 g
Distilled Water	80 ml
Seawater	20 ml (30 ‰)
pH	6.5

In this study, 100 ml of each broth was taken in 250 ml conical flasks. These flasks were autoclaved at 121°C, for 1 hour. After autoclaving, each flask was inoculated with five mm disk of the fungus inoculum grown on PDA medium. The inoculated flasks were incubated at 25°C for 7 days under stationary condition. The broth was filtered through sterilized Whatman filter paper No.1 and the culture filtrates were then tested for antibacterial activity against test pathogens by using paper disc diffusion assay.

Study on the effect of pH on the antibacterial activity of secondary metabolite

The optimization of pH of the fermentation media on the antibacterial activity of secondary metabolite was done by carrying out the fermentation study at five different pH values 5.0, 5.5, 6.0, 6.5 and 7.0 (Furtado *et al*., 2005). For each pH value, 100 ml of Potato Glucose Yeast Extract Medium (adjusted to desired pH by using either 1N NaOH or 0.1 N HCI) was taken in 250 ml conical flasks. These flasks were autoclaved at 121°C for 1hour. Three replicates were used for each pH values. A cut of mycelium (five mm diameter) from seven days old colony of selected fungus was added as an inoculum in each flask. The inoculated flasks were incubated at 25°C for 7 days under stationary condition. The filtration was done through sterilized Whatman filter paper

No. 1 and various filtrates were tested for antibacterial activity against the test pathogens by using paper disc diffusion assay.

Identification of endophytic fungi

Identification was achieved by means of observation on macroscopic features and detail microscopic characteristics of colonies. The microscopic examinations of selected fungus was done on MEA medium under microscope (Olympus, CX 41) in Marine Science Department and identified according to Ando and Inaba (2004).

Results

Classification of seagrass species collected from study area

The seagrass species was collected from Pho Htaung Gyaing, Shwe- Thaung- Yan Sub-township. The classification of the recorded seagrass species was referenced according to Soe-Htun et *al.* (2009).

Phylum- Tracheophyta
Class- Magnoliopsida
Order- Alismatales
Family- Hydrocharitaceae
Genus - Enhalus
Species- *E. acoroides*
(Linnaeus f.) Royle, 1839.

Description – Plant erect; the rhizome thick, about 1-2cm in diameter with tough black fibers; shoots pronounced at the node, with 3-6 leaves; leaf blades flat and linear, 70-180 cm long, 0.8-2.0 cm wide, with 35-55 nerves and ribs at the margin, apex obtuse, base narrow without lingual, margin slightly serrulate in young leaves.

Figure 2 Habit of *Enhalus acoroides* (Linnaeus f.) Royle.

Isolation of Marine Derived Endophytic Fungi from *Enhalus acoroides*

In the prescent study, six endophytic fungi were isolated from the seagrass, *Enhalus acoroides* by Surface Sterilization Method (Table 1). Two fungi were isolated from rhizome of the collected seagrass plant and four fungi from leaf (Table 1, Figure 3).

Table 1 Isolated fungi from *Enhalus acoroides* by surface sterilization method

Sample	Part use	Isolated fungi	Fungi No.
Enhalus acoroides	Rhizome	2	FE-01,02
	Leaf	4	FE- 03,04,05,06
Total isolated fungi		6	6


FE-01 (Front side)


FE-01 (Reverse side)


FE-02 (Front side)


FE-02 (Reverse side)


FE -03 (Front side)


FE -03(Reverse side)


FE -04 (Front side)


FE -04 (Reverse side)


FE -05 (Front side)


FE -05 (Reverse side)


FE -06 (Front side)


FE -06 (Reverse side)

Figure 3 Morphologies of endophytic fungi isolated from *Enhalus acroides*

Antibacterial Activities of Isolated Fungi against Three Test Organisms by Paper Disc Diffusion Assay

Antibacterial activities of six isolated fungi were carried out by the paper disc diffusion assay method against three test organisms, namely *Staphylococcus aureus*, *Pseudomonas fluorescence* and *Escherichia coli*. In the present study, the three fungi (one fungus isolated from rhizome and two fungi from leaf) showed distinct clear zone against only *Escherichia coli*. All of isolated endophytic fungi did not show any activities on the other test organisms, *Staphylococcus aureus* and *Pseudomonas fluorescence* (Table 2).

Table 2 Antibacterial Activity of Six Isolated Fungi against Three Test Organisms by Paper Disc Diffusion Assay (7 days fermentation)

Fungi No.	Test Organisms		
	Staphylococcus aureus	***Pseudomonas fluorescence***	***Escherichia coli***
FE-01	no activity	no activity	23.52 ±0.33 mm
FE -02	no activity	no activity	no activity
FE -03	no activity	no activity	no activity
FE -04	no activity	no activity	24.25 ±0.35 mm
FE -05	no activity	no activity	27.75 ±0.43 mm
FE -06	no activity	no activity	no activity

± standard deviation (SD)

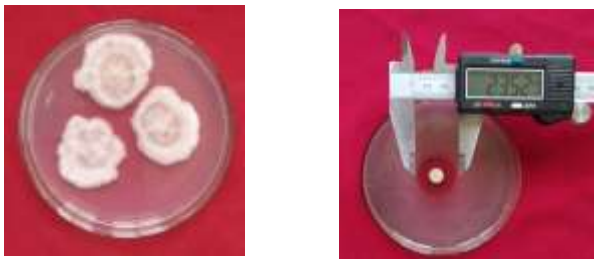


Figure 4 Antibacterial Activity of Isolated Fungus FE-01against *Escherichia coli*

Figure 5 Antibacterial Activity of Isolated Fungus FE-04 against *Escherichia coli*

Figure 6 Antibacterial Activity of Isolated Fungus FE-05 against *Escherichia coli*

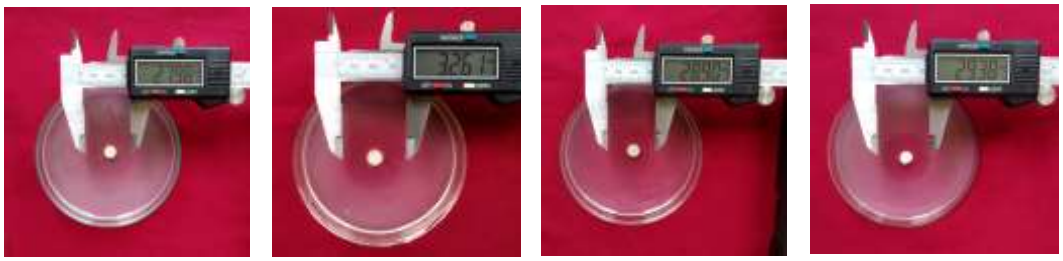
Effect of Fermentation Medium on the Antibacterial Activity of Secondary Metabolite

To investigate the effect of fermentation medium, the antibacterial activity of secondary metabolite produced from the selected isolated fungus FE- 05 was studied in four fermentation broths (Endo and Inaba, 2004). In this study, Potato Glucose Yeast Extract Medium showed the maximum inhabitation zone of 32.61 ±0.35 mm among the rest of other fermentation media (Table 3, Figure 7).

Table 3 Antibacterial Activity of Secondary Metabolite Produced from Selected Fungus FE – 05 on the Four Kinds of Fermentation Medium

Fermentation medium	Inhibitation Zone (mm)
I. Glucose Yeast Extract Medium	27.98 ±0.34 mm
II. Potato Glucose Yeast Extract Medium	32.61 ±0.35 mm
III. Glucose Malt Extract Medium	28.90 ±0.35 mm
IV. Glucose Malt Extract Peptone Medium	29.38 ±0.35 mm

± standard deviation (SD)



(I) (II) (III) (IV)

Figure7 Antibacterial Activity of Secondary Metabolite Produced from Selected Fungus FE – 05 on the Four Kinds of Fermentation Medium

Effect of pH on the Antibacterial Activity of Secondary Metabolite

The optimization of pH of the fermentation media on the antibacterial activity of secondary metabolite was done by carrying out the fermentation study at five different pH values 5.0, 5.5, 6.0, 6.5 and 7.0. In this study, maximum inhabitation zone of 32.33 ±0.27 mm was observed at pH value of 6.5 (Table 4, Figure 8).

Table 4 Antibacterial Activity of Secondary Metabolite Produced from Selected Fungus FE – 05 on Five Different Kinds of pH Value

pH Value	Inhibitation Zone (mm)
pH 5.0	27.42 ±0.38 mm
pH 5.5	29.55 ±0.35 mm
pH 6.0	32.26 ±0.34 mm
pH 6.5	32.33 ±0.27 mm
pH 7.0	29.84 ±0.37 mm

± standard deviation (SD)

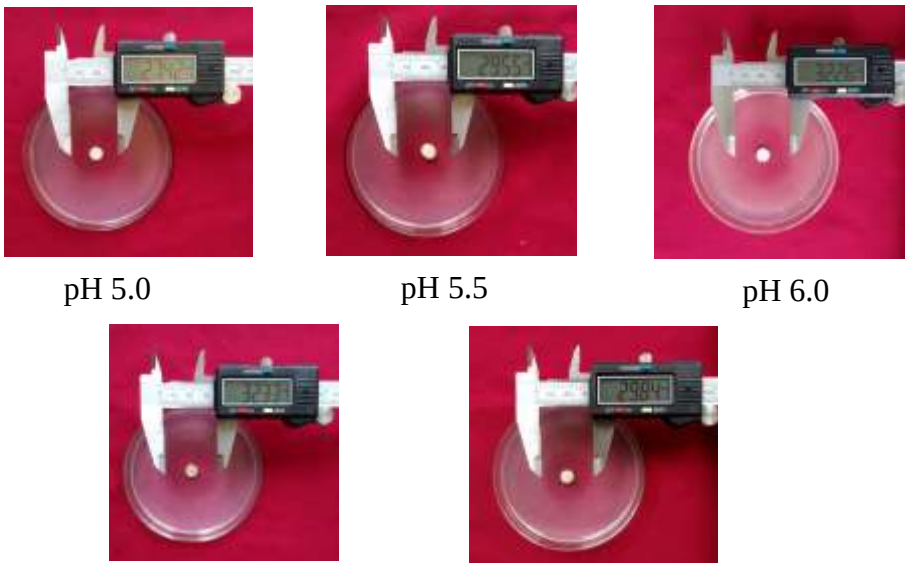


Figure 8 Antibacterial Activity of Secondary Metabolite Produced from Selected Fungus FE – 05 on Five Different Kinds of pH Value

Identification of endophytic fungus FE - 05

Identification of selected endophytic fungus which showed the clear zone of inhabitation against *Escherichia coli* was done according to Ando and Inaba (2004).

Macroscopic Features of endophytic fungus FE - 05

Colonies are slow growth. Texture is velvety to powdery. The color is olivaceous green to olivaceous brown.

Microscopic Features of endophytic fungus FE - 05

Colonies produce septate hyphae. Hyphae, conidiophores and conidia are pigmented. Conidiophores are distinct from vegetative hyphae, erect and straight, mostly unbranched. Conidia are produced in branched acropetal chains, consist of one to two-celled, and have a distinct hilum. Conidia are close to the conidiophore where the chain branched and forming the "shield-shaped" appearance. According to these external morphology and microscopic results, fungus FE-05 was identified as *Cladosporium* sp. (Figure 9).

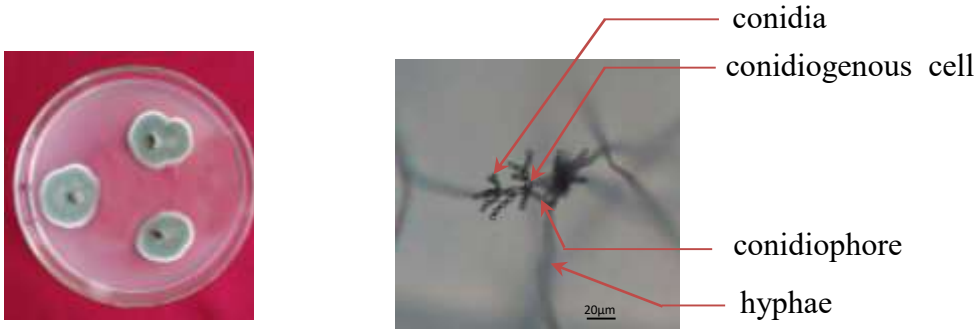


Figure 9 Morphology and photomicrograph (800 X) of fungus FE-05

Discussion

Marine derived fungi were known as the important colonizers of a large variety of organic substrates including sponges, corals, mollusks, seagrasses, seaweeds and other invertebrates. They also act as endophytes, saprobes, parasites and pathogens in the marine ecosystem (Loque et *al*., 2010). In the recent years, several bioactive compounds have been isolated from various marine microbes. These bioactive natural compounds represent the new resources for the development of medically useful compounds (Anand et *al.*, 2006).

In the present study, one of the seagrass species, *Enhalus acoroides* collected from Shwe-Thaung-Yan coastal area were employed for the isolation of marine endophytic fungi. In this study, only six endophytic fungi were isolated from *Enhalus acoroides*, two fungi from rhizome and four from leaf (Table 1). Antibacterial activities of all isolates against three test organisms were carried out by paper disc diffusion assay method. In the present investigation, endophytic fungus FE-05 showed the maximum inhabitation zone against *Escherichia coli* (Table 2 and Figure 6). All of isolated fungi did not show any antibacterial activity on the other test organisms, *Staphylococcus aureus* and *Pseudomonas fluorescence*. However, Than Than Aye (2019) described that five endophytic fungi isolated from three seagrasses species have been shown the antibacterial activity on *Pseudomonas fluorescence*.

In this research work, four fermentation broths were used in the investigation of effects of fermentation medium and it is found that Potato Glucose Yeast Medium showed the maximum inhabitation zone. Moreover, the effects of pH on the fermentation medium were also studied with five different pH values. In this research work, the maximum inhabitation zone was observed at pH value of 6.5. This result is closely related with the environmental condition of intertidal zone in which seagrass species grown very well. By the microscopic observations, fungus FE-05 was identified as *Cladosporium* sp. (Figure 9).

Conclusion

Although seagrass plants have shown the bioactive potential for various natural products, the microbial studies on seagrass species are very rare. For this reason, the present study was carried out on the screening of antibacterial activity of marine microbes associated with seagrass plants in the coastal region of Myanmar. But there is still a need for the extensive study of other environmental parameters such as temperature, salinity, alkalinity, etc. and these parameters greatly influence on the growth and metabolic production of marine microbes.

Acknowledgements

The author greatly appreciates Rector and Pro-rectors of Pathein University for their kind permission to do this research work. Special thank goes to Dr. Cherry Aung, Professor and Head of Marine Science Department, Pathein University for her supporting and giving valuable suggestions. The author would like to express sincere thanks to Dr Moe Moe Aye, Associate Professor of Botany Department, Magway University for her valuable suggestions, guidance and literature provided.

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COMPARISON ON THE SEAGRASS COMMUNITY AT THE THREE SITES NEAR DAWEI, TANINTHARYI REGION, MYANMAR

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Abstract

The seagrass meadows, three sites namely, Wa Maw Aw, Myin Khyar Aw and Ta Yaw Kam (North Bay) in the Dawei Township, Tanintharyi Division, were studied during December, 2018. A total of 5 species of seagrasses were recorded including *Cymodocea rotundata, Halodule uninervis, Halophila decipiens, H. ovalis* and *Syringodium isoetifolium* from the study areas. The dominant seagrass species of Wa Maw Aw was *Halophila ovalis*; *Halophila decipiens* in Myin Khyar Aw and *Cymodocea rotundata* in Ta Yaw Kam. Four species of seagrass were observed in Ta Yaw Kam however two species in Wa Maw Aw and only one species in Myint Khar Aw. The highest percentage coverage, 53.75% was observed at Wa Maw Aw however the lowest was at Myin Khyar Aw with 35.28% whereas Ta Yaw Kam with 44.56%. At Ta Yaw Kam, the seagrass biomass, most wet weight 11.31 gm wet. wt m^{-2} (Above ground) and 46.59 gm wet.wt.m^{-2} (Below ground) and the most dry weight of seagrass 3.28 gm dry.wt m^{-2} (Above ground) and 21.47 gm dry.wt m^{-2} were found in the below ground due to the abundance of *Cymodocea rotundata.* However the lowest wet weight (0.59 gm wet. wt m^{-2}) in the above ground and dry weight (0.32 gm dry. wt m^{-2}) in the above ground were observed at Myin Khyar Aw d ue to *Halophila decipiens*. The highest sand-muddy 98.67% was found in Wa Maw Aw but the lowest 90.67% in Myin Khyar Aw. The most transparency 6.10 m was recorded in Wa Maw Aw however the lowest one 2.3 m in Myin Khyar Aw.

Keywords: Diversity, dominant, highest, lowest and seagrass.

Introduction

Seagrass meadows play a significant role in the processes and resources of near shore coastal ecosystems, as they have physical, chemical and biological effects on habitats. Many fish and shellfish species, including those of commercial interest, are attracted to seagrass habitats for foraging and shelter, especially during their juvenile life stages (Gullström *et al*. 2002). Eleven species of seagrasses have been described in Myanmar (Soe-Htun *et al*. 2017). Seagrasses grow in soft sediments, from the low water mark to the depths of about 3-5 m and are inhabited by a rich associated biota.

Seagrass meadows are valuable habitats having economic and ecological importance in coastal ecosystem. Seagrasses represent one of the important and highly productive ecosystems of the world, which supports a variety of life forms ranging from microbes to marine mammals like dugongs. The objectives of this research are 1) to identify the morphotaxonomy of seagrass species in study areas; 2) to know biodiversity of seagrass; 3) to understand the abundance and distribution of seagrass; 4) to recognize the coverage of seagrass and biomass; 5) to know the different types of soil texture that growing the seagrass in the study areas.

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Materials and Methods

Seagrasses sample were collected from 3 study sites (Ta Yaw Kam at Lat 13° 40' 45.803" N, Long 98° 7' 34.630" E; Wa Maw Aw at Lat 13° 38' 1.994" N, Long 98° 7' 9.643" E; Myin Khyar Aw at Lat 13° 32' 56.588" N, Long 98° 8' 49.594" E) by uprooting the seagrasses with a small trowel During December, 2018 (Fig. 1). The collections were initially washed, cleaned and preserved in 5% formalin in seawater. Samples of seagrasses were examined mainly on the vegetative characters with a dissecting microscope, and then pressed on herbarium sheets to prepare as voucher specimens for each locality. Moreover, some water parameters namely, temperature, salinity, pH and transparency were measured in the field. This study has followed the SeagrassNet protocol (2006), consisting of three fixed, parallel, 50 m cross-transects referred to as cross-transects A, B and C, with cross-transect A closest to shore and C most seaward; B, midpoint of these cross-transects were established on a transect laid out seaward, perpendicular to the shore. In addition, the soil texture (clay, silt and sand- muddy) were also measured.

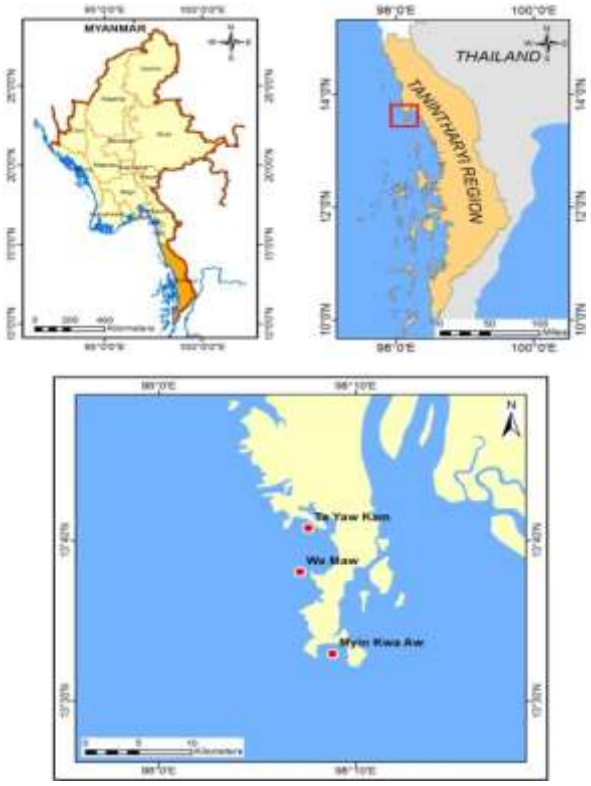


Figure 1 Map showing the survey sites of seagrasses in Dawei Township, Tanintharyi Region.

Results

A total of five species belonging to four genera from two families of seagrasses are collected from three stations in the Dawei. These are *Cymodocea rotundata*, *Halodule uninervis*, *Syringodium isoetifolium*, *Halophila decipiens* and *H. ovalis*. *C. rotundata* is the most dominant species in Ta Yaw Kam however *H. ovalis* is the most abundant species in Wa Maw Aw and *H. decipiens* is only dominant in Myin Khyar Aw. Four species, *C. rotundata*, *S. isoetifolium*, *Halodule uninervis* and *Halophila ovalis* are observed in Ta Yaw Kam but only one species is found in Myin Khyar Aw and two species, *Halophila ovalis* and *Halodule uninervis* are distributed in Wa Maw Aw (Fig. 2).

1. Coverage of seagrass

In Ta Yaw Kam Station showed in the average percentage coverage of seagrass 44.56%. Among four species, *C. rotudata* 34.66% is most commonly observed in this station. In Wa Maw Aw Station recorded the average coverage of seagrass 53.75%. In this station, *Halophila ovalis* 47.65% is dominantly found. However, the average coverage of seagrass 35.28% is observed in Myin Khyar Aw Station. In this station, one specific species, *Halophila decipiens* 35.28% is dominantly recorded.

2. Biomass of seagrass

The biomass of seagrass was measured as two parts, above ground (photosynthesis part) and below ground (non-photosynthesis part). It displays the variation of the wet weight in above-ground (5.59-16.58 gm wet.wt m^{-2}) and below-ground (36.05-65.93 gm wet.wt m^{-2}) of Ta Yaw Kam however above-ground (5.4-18.89 gm wet.wt m^{-2}) and below-ground (18.7-78.08 gm wet.wt m^{-2}) in Wa Maw Aw; above-ground (0.19-1.18 gm wet.wt m^{-2}) and below-ground (1.35-4.11 gm wet.wt m^{-2}) in Myin Khyar Aw.

Although, the above-ground biomass of seagrass in the dry weight range the above-ground (0.77-4.82 gm dry.wt m^{-2}) and below-ground (16.58-30.66 gm dry.wt m^{-2}) in Ta Yaw Kam; above-ground (0.58-5.4 gm dry.wt m^{-2}) and below-ground (2.32-22.17 gm dry.wt m^{-2}) in Wa Maw Aw however above-ground (0.19-0.39 gm dry.wt m^{-2}) and below-ground (0.25-0.79 gm dry.wt m^{-2}) in Myin Khyar Aw are found (Figs. 3-4).

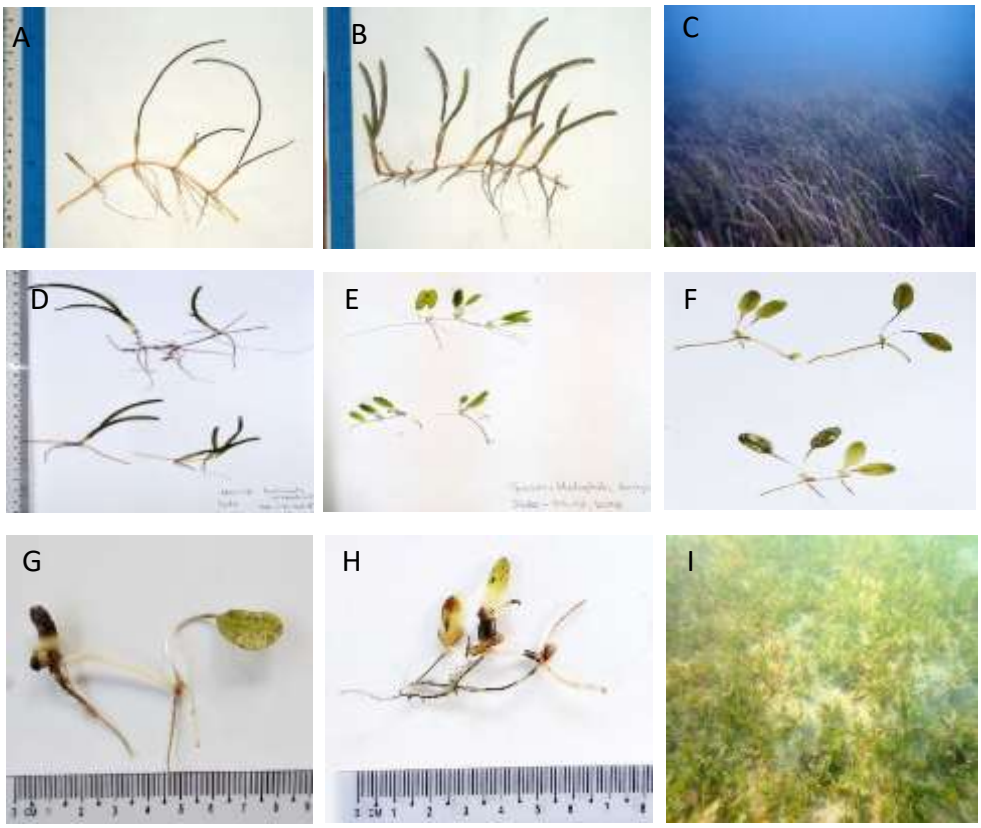


Figure 2 A-I) A) *Syringodium isoetifolium* (Ascherson) Danty; B) *Cymodocea rotundata* Ehrenberg et Hemprich ex Ascherson; C) Meadow of *C. rotundata*; D) *Halodule uninervis* (Forsskal) Ascherson; E) *Halophila decipiens* Ostenfeld; F) *Halophila ovalis;* G and H) The seed of *H ovalis*; I) Meadow of *H ovalis*.

The seed of *Halophila ovalis* is observed in Wa Maw Aw. Seagrass grow in a range of sediment types and depend on several abiotic factors. The more clay (1.67%) and silt (7.67%) are observed in Myin Khyar Aw than the other stations. The most sand-muddy (98.67%) is found in Wa Maw Aw among them.

Moreover some water parameters of Ta Yaw Kam are recorded as temperature 28°C, salinity 32‰, transparency 4.88m, pH 5.3; Wa Maw Aw with temperature 30°C, salinity 32‰, transparency 6.10m, pH 5.2 and Myin Khyar Aw with temperature 31°C, salinity 30‰, transparency 2.3m, and pH 5.7.

Discussion

In the present study, the number of seagrass species (5 species) was found in the Dawei including of three stations namely, Ta Yaw Kam, Wa Maw Aw and Myin Khyar Aw. Among 5 species, only 4 species, *Syringodium isoetifolium, Cymodocea rotudata, Halodule uninervis* and *Halophila ovalis,* were found in Ta Yaw Kam and 2 species, *H. uninervis, H. ovalis* in Wa Maw Aw; only one species, *Halophila decipiens* in Myin Khyar Aw (Fig. 2).

In comparison, the most percentage covers of seagrass recorded in this study were 53.75% in Wa Maw Aw however lowest 35.28% in Myin Khyar Aw. *C. rotudata* 34.66% was most commonly observed in Ta Yaw Kam Station however *H. ovalis* 47.65% was dominantly found in Wa Maw Aw Station and *H. decipiens* 35.28% was dominantly recorded in Myin Khyar Aw Station.

In Ta Yaw Kam Station, *C. rotudata* was most abundance at 3 cross transects but *H. uninervis* was not observed at transect C line. In Wa Maw Aw Station, *H. ovalis* was the most abundant at all transects but *H. uninervis* was the most dominant at the transect C line. In Myin Khyar Aw Station, *H. decipiens* was common observed at all transects (Fig. 5). Seagrasses growing in Wa Maw Aw was associated with the seaweed species, *Padina.* In Ta Yaw Kam Station, the seagrass bed was observed together with *Dictyota* and *Padina*.

The most wet weight of seagrass (65.93 gm wet.wt m^{-2}) and the most dry weight of seagrass (30.66 gm dry.wt m^{-2}) were found in the below ground at Ta Yaw Kam however the lowest wet weight and dry weight (0.19 gm wet.wt m^{-2}) in the above ground at Myin Khyar Aw (Figs. 3-4). Because of the size of plant was larger in Ta Yaw Kam than Myin Khyar Aw′specimens.

In the present study, the most transparency 6.10 m was recorded in Wa Maw Aw however the lowest 2.3 m in Myin Khyar Aw. Moreover the most temperature 31 °C was observed in Myin Khyar Aw however the lowest 28 °C in Ta Yaw Kam. The salinity was observed as 32 ‰ in both Ta Yaw Kam and Wa Maw Aw Yaw Kam however 30 ‰ was found in Myin Khyar Aw. The most value of pH 5.7 was observed in Myin Khyar Aw but the lowest 5.2 in Wa Maw Aw.

In the present study, the sediment types of seagrass, the most clay 1.67 % and the most silt 7.67% were observed in Myin Khyar Aw however the lowest 0.67% in Wa Maw Aw. The highest sand-muddy 98.67% was found in Wa Maw Aw but the lowest 90.67% in Myin Khyar Aw. In the present study, there were the difference types of habitats of seagrasses: the intertidal habitats of the rocky and sandy platforms at Wa Maw Aw however no the rocky in Ta Yaw Kam. In the present study, the substrate types of seagrasses between Myin Khyar Aw where more clay & silt and Wa Maw Aw where more sand, were also found to be differed with seagrass meadows in the Dawei, Tanintharyi Coastal Region of Myanmar.

Gullström *et al*. (2002) reported *Halophila ovalis, Cymodocea rotundata, Cymodocea serrulata, Syringodium isoetifolium* were very common in the Western Indian Ocean. This result was similar to the present study. Hemminga and Duarte (2000) described the decline in seagrass species richness with increasing silt content in South East Asian seagrass meadows. This result

was similar to the present study. Soe-Htun *et al*. (2001) described the total of nine species of seagrasses from the three coastal regions of Myanmar and the more species than the present result.

Soe-Htun *et al*. (2009) described *C. rotundata* distributed only in the Tanintharyi Coastal Region. This result was similar to the present study. Prathep (2010) reported the five species of seagrass found at Koh Tha Rai: *Enhalus acoroides, C. rotundata, Thalassia hemprichii, H. ovalis* and *H. uninervis*. This result was a little similar to the present study. Pierre (2012) described the dominant species for the Ifaty sites were *S. isotifolium* and *T. hemprichii* however Ta Yaw Kam was dominated by *C. rotundata* and Wa Maw Aw with *H. ovalis.*

Osathanunkul *et al*. (2015) described *Halophila ovalis* obtained from Tungkhen Bay, Phuket Province, Thailand and this result was similar to the present study. Soe-Htun *et al*. (2017) described 11 species of seagrasses were recorded from the Myeik Archipelago and Rakhine Coastal Areas however 5 species were observed in the present study. Moe Lwin Lwin *et al*. (2019) described *C. rotundata* was dominant species in Bo Cho Island and Nyaung Pin Aw. This result was similar to the present study.

Govindasamy *et al*. (2013) described the dominant seagrass species of Palk Bay, Bay of Bengal, India was *S. isoetifolium* and this result was similar to the present study. De la Torre-Castro (2006) described the seagrass meadows provide social ecological resilience. The goods and services associated with seagrass ecosystems and also appreciated by locals were fishing.

Management actions should be required ensuring these habitats are not lost for the sustainable development of fishery. The current study was allowed for long-term

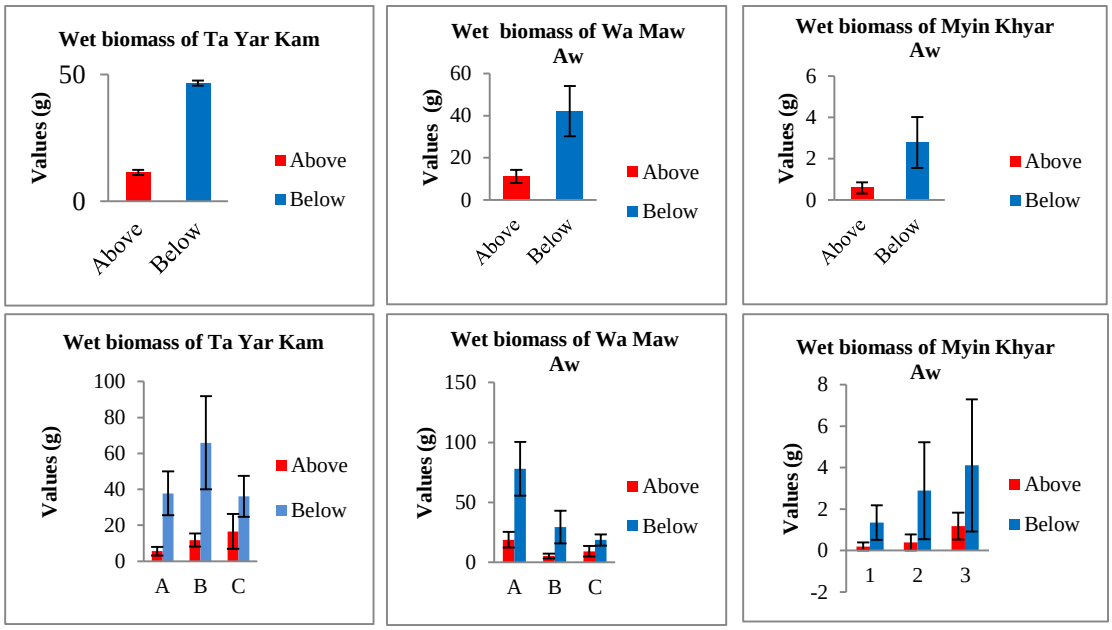
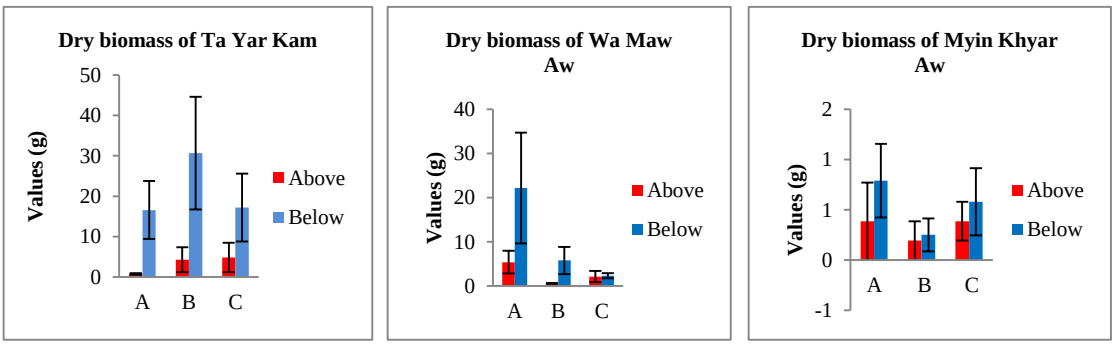


Figure 3 Comparison of the wet biomass of seagrass at 3 different stations during the present study.



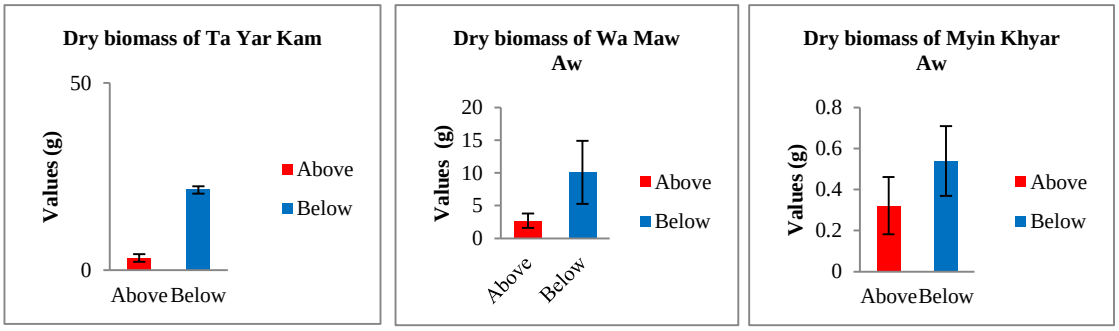


Figure 4 Comparison of the dry biomass of seagrass at 3 different stations during the present study.

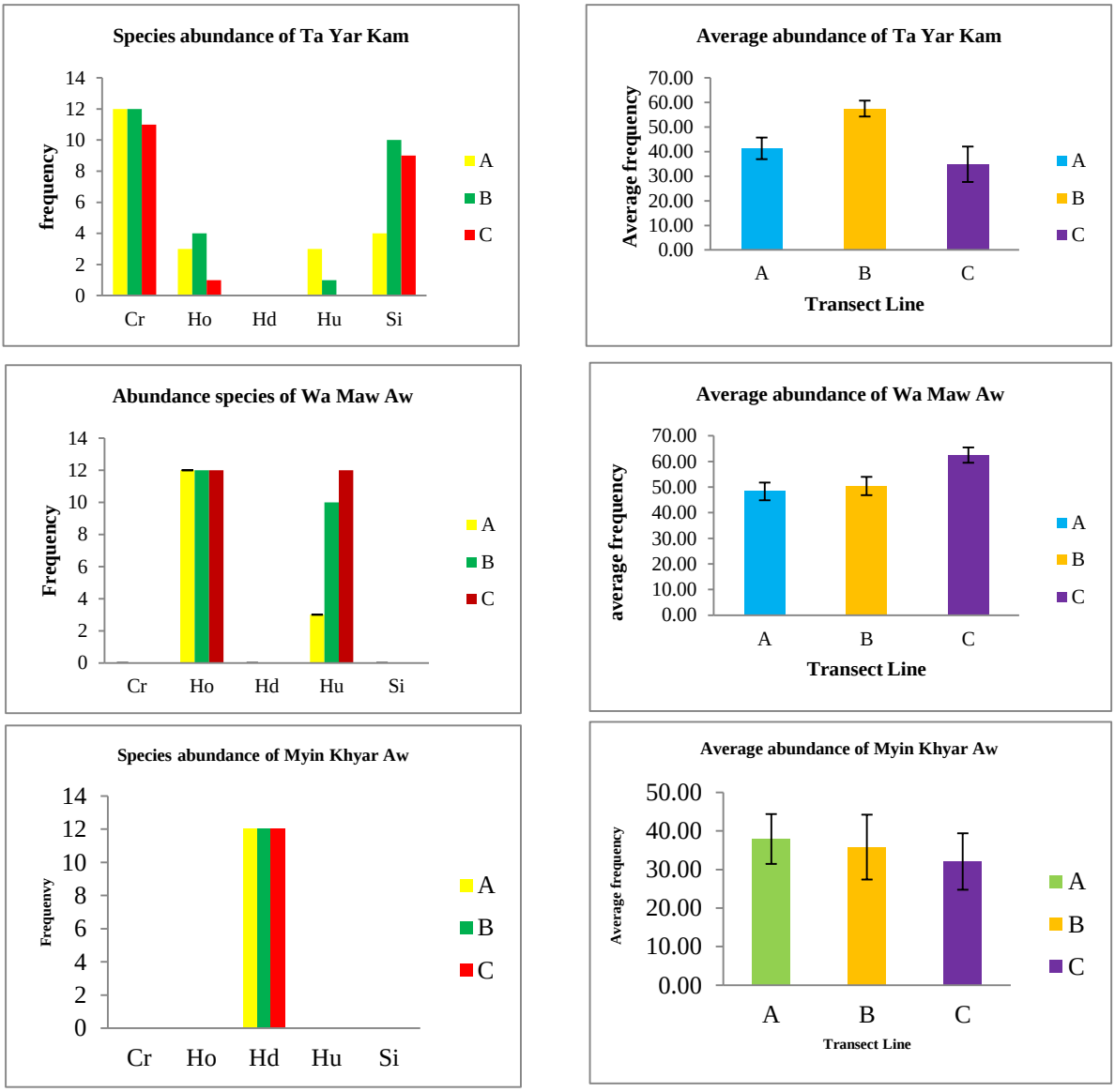


Figure 5 Comparison of the species abundance of seagrass at 3 different stations during the present study.

monitoring of seagrass beds and provides the ability to quantitatively measure the impact of management interventions aimed at seagrass conservation.

Conclusions

In the present study, the seagrasses meadow was larger in Wa Maw Aw and Ta Yaw Kan than Myin Khyar Aw. The seagrasses biomass depends on the species. *Halophila decipiens* favour the mud whereas *Cymodocea rotundata* and *H. ovalis* more grow in the sandy-mud. The salinity and transparency effect to seagrass ecosystem. From the ecological point of view of seagrasses communities were important marine resources due to the nursery and feeding grounds for the marine organisms. This study contributes a baseline result for further study of seagrasses in Myanmar Coastal Areas.

Acknowledgements

We are very grateful to Dr Nyo Nyo Tun, Professor and Head, Department of Marine Science, Myeik University for her permission to do this research. We also thank to Woodside Energy Ltd. and Fauna & Flora International for funding during this work.

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ZOOPLANKTON DIVERSITY AND DISTRIBUTION IN THE WATERS OFF TANINTHARYI REGION, MYANMAR

Zin Lin Khine[1], Khin May Chit Maung[2], Pann Mo Mo[3], Zaw Myo Hein[4], Si Thu Hein[2] and Nyo Nyo Tun[5]

Abstract

The zooplankton samples from 23 stations in the waters off Tanintharyi Region were collected by R/V DR. Fridtjof Nansen (August – September 2018). The 209 species and 35 taxa of zooplankton were observed in the present study area. Copepods were the most abundant and dominant species at all stations. *Acrocalanus gibber, Paracalanus parvus, P. aculeatus, Nanocalanus minor, Undinula vulgaris, Acrocalanus longicornis, Corycaeus andrewsi*, and *Canthocalanus pauper* were very common in the study waters. The highest species composition was recorded (141 occurrences) at St 854 and the most abundant (6622 no/m^3) at St 796. Zooplankton diversity of (H' > 3.9) were represented high values in three fish spawning grounds of the Tanintharyi Region. The species diversity of H', D' and E' values were usually high at stations close to the coast.

Keywords: Abundance, composition, copepods, Tanintharyi Region, zooplankton.

Introduction

Plankton are composed of the phytoplankton and zooplankton found near the surface in aquatic environments. There are two groups used to classify zooplankton by their development stages: meroplankton (temporary plankton) and holoplankton (permanent plankton). Zooplankton are central components of marine ecosystems, forming the base of most marine food webs. The important zooplankton organisms, including copepods, cladocerans, decapod larvae, rotifers, ciliates, artemia, and copepods are the food for fish larvae (Santhosh and Anil, 2014). Calanoid and cyclopoid copepods were the principal prey for fry fish, and harpacticoid copepods were the essential invertebrate food items (Whitfield, 1985).

The three surveys (2013-2018) of R/V DR. Fridtjof Nansen conducted plankton sampling, hydrographic parameters (CTD), pelagic trawl and bottom- trawl sampling and benthos sampling in Rakhine, Ayeyarwaddy and Tanintharyi Waters. The scientific data, including the distribution and diversity of different species at various trophic levels (Phyto- and zooplankton, fish eggs, and larvae), played a vital role in sustainable fisheries management Myanmar waters.

The objectives of the present study were - to observe the composition and abundance of zooplankton, to illustrate the distribution of zooplankton species, to determine the zooplankton abundance related to environmental parameters and to evaluate the diversity of zooplankton.

Materials and Methods

Marine ecosystem survey in Myanmar waters was carried onboard R/V DR. Fridtjof Nansen for six weeks (August–September 2018). Twenty-three zooplankton sampling stations, including three fish spawning grounds (spawning triangles) in Tanintharyi Coastal Waters, were designated (Figure 1). The WP2-net (56 cm diameter and mesh size 180 µm) was hauled vertically at a speed of ~0.5 ms-1 at the water depth of 30m for each station. The sample was preserved in seawater with a solution of 4% formaldehyde buffered with borax and was deposited Marine

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Science Laboratory for species identification and quantification. Zooplankton was identified up to species levels with references to Kasturirangan (1963), Borror (1973), Newell and Newell (1973), Arvin(1977), Wells (1984), Conway and White (2003), Conway (2003), Boxshall (2004), Mulyadi (1997a,b), Al-Yamani (2011a,b) and Conway (2012a, b). The subsamples of zooplankton were counted under a binocular microscope and calculated for abundance (no/m^3). The Jaccard similarity index JI = j / (a + b-j) was used to analyze potential similarities/dissimilarities of zooplankton species composition between stations (Shamsudin and Yasin, 1996). Zooplankton species diversity (*H´*), evenness (*E´*), and richness (*D´*) were calculated as follows, using the formula of Shannon and Weaver, 1963 and Pielou, 1966. $H' = -\sum$ Pi *ln Pi , $E' = H'$ / ln S, D' = S-1 / ln N (Ludwig and Renylods,1998). All statistics data were analyzed by the R program.

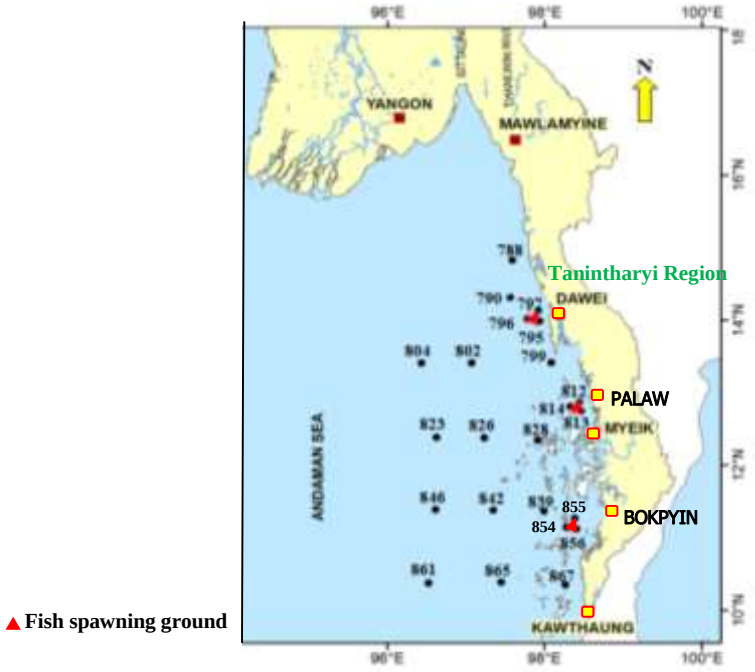


Figure 1 Zooplankton sampling stations in the waters off Tanintharyi Region

Results

Composition

Zooplankton communities comprised of 209 species under 11 phylum and 35 taxa (Table 1). Zooplankton including 7 species of Protozoa, 4 species of Foraminifera, 5 species of Radiozoa, 12 species of Cnidaria, 1 species of Ctenophora, 7 species of Annelida 13 species of Chaetognatha, 13 species of Mollusca, 4 species of Cladocera, 7 species of Ostracoda, 66 species of Calanoida, 24 species of Cyclopoida, 9 species of Harpacticoida, 10 species of Amphipoda, 1 species of Isopoda, 3 species of Mysidae, 4 species of Euphausidae, 3 species of Decapoda and 16 species of Appendicularia were identified up to species level. The 35 taxa of meroplankton which were not identified to generic or species levels incorporated the larvae of Anthozoa, Bryozoa, Polychaeta, Mollusca, Decapoda, Copepoda and Echinodermata. Copepoda was the most diverse group containing the highest number of species (102), followed by Chaetognatha (32), Mollusca (13), Cnidaria (12) and Amphipoda (10). The highest zooplankton species composition of (141) was found at St 854 (near Owen Island) whiles the lowest species composition of (32) at St 828 (near Thayawthahangyi Island) (Figure 2). The clusters of sampling stations associated with zooplankton species composition were analyzed by Jaccard Index (JI).

Dendrogram illustrated that the dissimilarity of the groups or clusters of sampling stations ranged between 0.4 and 0.9 (Figure 3).

Among calanoid copepods, *Acrocalanus gibber, A. longicorni, Paracalanus parvus, P. aculeatus, Nanocalanus minor, Undinula vulgaris, Corycaeus andrewsi and Canthocalanus pauper* ranged between 96 -87% composition was very common in the present study waters. The percent occurrence of *Corycaeus speciosus, C.catus, C.latus, Oithona nana O.attenuata O. similis, Oncaea venusta, O.clevei, Oikopleura longicauda, O.fusiformis, Euterpina acutifrons,* and *Flaccisagitta enflata* represented 87-74% composition was common in the present study area. *Metacalanus aurivilli Calanopia elliptica, C.aurivilli, C.minor C.thompsoni, Labidocera kroyeri L.euchaeta Pontellina plumata, P.spinipes, P.valida, P.fera, Tigriopus sp, Tortanus forcipatus, T.barbatus,* Ctenophora, Bryozoa, Cladocera and Gastropoda larvae were rare (less than 9 % occurrence) in the present study area. Chaetognatha, Decapoda, Euphausidae, Mysidae, Isopoda, Appendicularia, Cnidaria and Polychaeta larvae were more common at St 854 than other stations.

Abundance and distribution

The zooplankton abundance ranged from (515 no/m^3) at St 802 to (6622 no/m^3) at St 796 (Figure 2). The densities of zooplankton were higher at nearshore stations than offshore stations. Copepods were the most abundant species accounting for 79.26% of total zooplankton abundance. The densities of calanoid copepods were 53.94% of total copepods followed by cyclopoids (10.32%), poecilostomatoids (9.33%), and harpacticoids (5.66%) in respective order. Chaetognatha was the second most abundant group after copepods, comprising 7.14% of total zooplankton density. Other major groups observed were Appendicularia (9.24%) and Cnidaria (1%). The rest of the groups contributing <1% of the total density included Euphausiadae, Mollusca, Decapoda, Protozoa, Foraminifera, Chordata, Ostracoda, Annelida, Mysidae, Cladocera, Actinopoda, Radiolaria, Amphipoda, Isopoda, and Ctenophora.

The station-wise abundance and distribution of different zooplankton groups and the dominant copepod species were illustrated in Figure 4A- B. The first fish spawning ground (around Launglong – Bok, Moscos and Maungmagan Islands) was represented as high zooplankton densities of (3787 no/m^3 at St 795), (6222 no/m^3 at 796) and (4599 no/m^3 at St 797) respectively. The most dominant species of *Undinula, Nanocalanus, Paracalanus, Acrocalanus, Acartia, Candacia, Oithona, Oncaea,* and *Microsetella* ranged in cell densities from 40 to 51 no/m^3 in the first spawning ground (Figure 4A). The zooplankton abundance with the moderate numbers ranging (from 1353 to 2435 no/m^3) were observed at St 812, 813 and 814 in the second fish spawning ground (near Thamihla, Anyin-pho-Anyin-ma, and Kawdwe Islands). *Nanocalanus, Paracalanus, Acrocalanus, Eucalanus, Euchaeta, Acratia,* and *Lucicutia* were the significant components of copepods (ranging from 53-23 no/m^3) in the second fish spawning ground. The high numbers of zooplankton were observed between 2976 no/m^3 and 4599 no/m^3 at St 854, 855 and 856 in the third fish spawning ground (near Owen, Aleman and Kawye Islands). The key copepod species including *Nanocalanus, Paracalanus, Undinula, Acrocalanus,* and *Centropages* were in high abundance (ranging from 53-30 no/m^3) in the third fish spawning ground. Among fishing grounds, the highest zooplankton abundance was found in the first fish spawning ground. The twenty zooplankton groups except Copepoda were regarded as (<15%) of the total zooplankton abundance in all fish spawning grounds (Figure 4A-B).

Table 1 Inventory list of zooplankton species and larvae recorded in the present study area

Sr.No	Species Name	Sr.No	Species Name	Sr.No	Species Name
	Protozoa	46	*F. robusta*	92	*Euchaeta concinna*
1	*Tintinnopsis gracilis*	47	*Mesosagitta minima*	93	*E. elongatus*
2	*T. radix*	48	*Zonosagitta pulchra*	94	*E. wolfendeni*
3	*T. ampla*	49	*Z. bedoti*	95	*Scolecithrix danae*
4	*T. tubulosa*		**Mollusca**	96	*S.bradyi*
5	*T. directa*	50	*Cresis clava*	97	*Centropages furcatus*
6	*T. butchlii*	51	*C. virgule*	98	*C. yamadai*
7	*Undella columbiana*	52	*Hyalocylis striata*	99	*C. orsinii*
	Foraminifera	53	*Styliola sp*	100	*C. tenuirenis*
8	*Globigerina bulloides*	54	*Cilo sp.1*	101	*C. elongatus*
9	*G. rubesuns*	55	*Cavolinia sp.1*	102	*C. gracilis*
10	*Globorotalia inflata*	56	*Limacina trochiformis*	103	*C.dorsipinatus*
11	*Globoquadrina dutertrei*	57	*L.bulimoides*	104	*Lucicutia flavicornis*
	Radiozoa	58	*Desmopterus papilio*	105	*L.ovalis*
12	*Acanthometron sp.*	59	*Atlanta inflata*	106	*Metacalanus aurivilli*
13	*Acanthochiasma dichontoma*	60	*A. brunnea*	107	*Pseudodiaptomus aurivilli*
14	*Acanthochiasma rubescens*	61	*A. peroni*	108	*P.mertoni*
15	*Hexacontium sp.*	62	*A. lesueurii*	109	*Candacia bradyi*
16	*Acrosphaera spinulosa*		**Arthropoda**	110	*C.catula*
	Cnidaria		**Cladocera**	111	*C.discaudata*
17	*Liriope tetraphylla*	63	*Evadne nordmanni*	112	*C.pachydactyla*
18	*Aglaura hemistoma*	64	*Pseudevadne tergestina*	113	*Acartia erythraea*
19	*Solmundella bitentaculata*	65	*Penilia avirostris*	114	*A.spinicauda*
20	*Euphysa sp.*	66	*Penilia sp.*	115	*A.pacifica*
21	*Diphyes dispar*		**Ostracoda**	116	*A.negligens*
22	*D. chamissonis*	67	*Cypridina sinuosa*	117	*A. danae*
23	*Diphyes sp.1*	68	*Cypridina sp.1*	118	*A.centrula*
24	*Diphyes sp.2*	69	*Cypridina sp.2*	119	*A. sewelli*
25	*Lensia conoidea*	70	*Cypridinodes asymmetrica*	120	*Calanopia elliptica*
26	*L.multicristata*	71	*Pyrocypris sp.1*	121	*C.aurivilli*
27	*Abyla leuckarti*	72	*Pyrocypris sp.2*	122	*C.minor*
28	*A. haeckcli*	73	*Conchoecia elegans*	123	*C.thompsoni*
	Ctenophora		**Copepoda**	124	*Labidocera acuta*
29	*Beroe ovata*		**Calanoida**	125	*L.pectinata*
	Annelida	74	*Nanocalanus minor*	126	*L.minuta*
30	*Callizona sp*	75	*Canthocalanus pauper*	127	*L.pavo*
31	*Vanadis sp.1*	76	*Undinula vulgaris*	128	*L.kroyeri*
32	*Tomopteris elegans*	77	*U. caroli*	129	*L.euchaeta*
33	*T. pacifica*	78	*U. darwini*	130	*Pontellina plumata*
34	*Sagitella kowalewskii*	79	*Acrocalanus gibber*	131	*Pontella danae*
35	*Pelagobia longicerrata*	80	*A. longicornis*	132	*P.spinipes*
36	*Lopadorrhynchus appendiculatus*	81	*A. gracilis*	133	*P.valida*
	Chaetognatha	82	*A. similis*	134	*P.fera*
37	*Aidanosagitta crassa*	83	*A. inermis*	135	*Tortanus forcipatus*
38	*A. regularis*	84	*Paracalanus parvus*	136	*T.barbatus*
39	*A. neglecta*	85	*P. aculeatus*	137	*Temora turbinate*
40	*Flaccisagitta enflata*	86	*P.dubia*	138	*T.discaudata*
41	*F.hexaptera*	87	*P.crassiostris*	139	*T.stylifera*
42	*Pseudosagitta lyra*	88	*Calocalanus pavo*		
43	*Sagitta bipunctata*	89	*E. subcrassus*		**Cyclopoida**
44	*Ferosagitta ferox*	90	*E.monachus*	140	*Oithona nana*
45	*F. hispida*	91	*E.attenuatus*	141	*O.attenuata*
41	*F.hexaptera*	87	*P.crassiostris*	139	*T.stylifera*
42	*Pseudosagitta lyra*	88	*Calocalanus pavo*		
43	*Sagitta bipunctata*	89	*E. subcrassus*		**Cyclopoida**

Sr.No	Species Name	Sr.No	Species Name	Sr.No	Species Name
44	*Ferosagitta ferox*	90	*E.monachus*	140	*Oithona nana*
45	*F. hispida*	91	*E.attenuatus*	141	*O.attenuata*
142	*O. similis*	179	*Tulbergella sp.*		
143	*O.spinirostris*	180	*Rhabdosoma brevicaudatum*		**Zooplankton larvae (Meroplankton)**
144	*O.rigida*	181	*Rhabdosoma sp.*	1	Tentaculate larva of Arachnactis
145	*O.plumifera*	182	*Oxycephalus sp.*	2	Cyphonautes larva of bryozoa
146	*O.brevicornis*		**Isopoda**	3	Nectochaete larva of eulalid
147	*O.setigera*	183	*Idotea emarginata*	4	Young Autolytus
148	*O.simplex*		**Mysidae**	5	Trochophore larva of nereid
149	*Oncaea venusta*	184	*Siriella affinis*	6	Nectochaete larva of nereid
150	*O.conifera*	185	*Promysis orientalis*	7	Young Sagitella
151	*O.clevei*	186	*Mesopodopsis orientalis*	8	Larvae of nepthyid polychaetes
152	*Corycaeus speciosus*		**Euphausidae**	9	Larvae of spionid polychaete
153	*C.latus*	187	*Pseudeuphausia latifrons*	10	Nectochaete of Glycera
154	*C.andrewsi*	188	*Stylocheiron carinatum*	11	Larva of polynoinid polychaete
155	*C.catus*	189	*S.insularis*	12	Syllid polychaete
156	*C.conifera*	190	*S. affinis*	13	Phyllodocid polychaete
157	*C.asiaticus*		**Decapoda**	14	Trochophore larva of sabellarid
158	*Farranula gibbula*	191	*Acetes indicus*	15	young Platynereis
159	*Sapphirina nigromaculata*	192	*A. japonicus*	16	larva of Disoma
160	*S.ovatolanceolata*	193	*Lucifer penicillifer*	17	Nauplius larvae of penaeid prawn
161	*S.stellata*		**Appendicularia**	18	Zoea larvae of penaeid prawn
162	*S.angusta*	194	*Fritillaria pellucida*	19	Mysis larvae of penaeid prawn
163	*Copilia quadrata*	195	*F.formica*	20	Late larva of Acetes
	Order Harpacticoida	196	*Oikopeura cophocerca*	21	Phyllosoma larva of Palinurus
164	*Microsetella norvegica*	197	*O.fusiformis*	22	Larva of Brachyura
165	*M. rosea*	198	*O. longicauda*	23	Zoea larvae of brachyura
166	*Macrosetella gracilis*	199	*O. dioica*	24	Megalopa larvae of brachyura
167	*Miracia efferatia*	200	*O. rufescens*	25	Alima larva of stomatopoda
168	*Euterpina acutifrons*	201	*Stegosoma magnum*	26	Trochophore larva of Mollusca
169	*Longipedia weberi*	202	*Doliolum denticulatum*	27	Veliger larva of janthinid gastropod
170	*Clytemnestra rostrata*	203	*D. gegenbauri*	28	Veliger larva of atlantid gastropod
171	*C.scutellata*	204	*Dolium sp.*	29	Veliger larva of Echinospira
172	*Tigriopus sp.*	205	*D.nationalis*	30	Veliger larvae of gastropod
	Order Amphipoda	206	*Salpa fusiformis*	31	Veliger larvae of bivalve
173	*Phronimella elongata*	207	*S. maxima*	32	Ophiopluteus larva of ophiuroid
174	*Hyperia sp.*	208	*S. cylindrical*	33	late ophiopluteus larva
175	*Lestrigonus sp.*	209	*Iasis zonaris*	34	Echinopluteus larva of echinoid
176	*Phrosina semilunata*			35	Bipinnaria larvae of asteroid
177	*Brachyscelus sp.*				
178	*Glossocephalus milne-edwardsi*				

Diversity

The Shannon diversity index was analyzed based on zooplankton abundance and species composition. The diversity index H' values ranged from 3.23 to 4.56 were usually high values in the coastal stations (Figure 5). Most stations showed the diversity index values $H' \geq 4$ but only

one station revealed 3.23. The richness index *D'* values based on species richness were ranging between 5.2 and 19.5. As evenness *E' obtained over* 0.8 in the present study area, the high index values showed no difference among the stations. It exhibited a balanced community of the study waters. The index of diversity showed *H'* ranging from 3.9 to 4.6, richness *D'* (6.8 -18.8) and Evenness *E'* (0.9 - 0.98) respectively in three fish spawning grounds of the study waters. It indicated higher zooplankton diversity and the well-balanced system of the zooplankton community in these fishing grounds.

Environmental Conditions

More uniform temperatures were represented in the present study area, ranging from 28.7 to 27.9 ºC and the mean temperature was 28.3ºC (± 0.21) (Figure 6-7). The correlation of seawater temperature with zooplankton abundance was small negative (r = -0.03). Higher salinity values of seawater (32-33ppt) were recorded in the offshore areas (St 802, 823, 846, 861 and 865) while the lower values of (<30.39 ppt) were observed at in the nearshore stations including three fish spawning grounds (Figure 7). The average salinity value occurred at 30.6 ppt (±1.5) (Figure 6). Zooplankton density showed a medium negative correlation (r = -0.5) with the seawater salinity. In general, the zooplankton abundance was higher in the nearshore stations with low salinity values than offshore stations with high salinity values (Figure 7).

Dissolved oxygen concentration in seawater varied from higher levels found in the offshore stations (St 842, 826 and 861) to lower levels observed in the nearshore stations such as St 797 (near Maungmagan Island) and St 799 (near Dawei Point-Shin Maw) (Figure 7). The average dissolved oxygen concentration was recorded at 3.9 ml/l (± 0.7) (Figure 6). The oxygen minimum zone (OMZ) did not occur in the present study waters and dissolved oxygen well saturated at all stations. The changes in dissolved oxygen concentrations and zooplankton abundance occurred in a small negative correlation (r = -0.3).

Discussion

According to the assessment of zooplankton species composition from R/V DR. Fridtjof Nansen Ecosystem Survey in the Southern Myanmar Waters (2013-2018), 212 species and 39 taxa in 2013 (Zin Lin Khine, 2014), and 209 species and 35 taxa in 2018 (the present result, 2020) were recorded. It indicated that the zooplankton species were widely distributed in the present study waters. Copepoda was the most critical group and followed by Chaetognatha, Mollusca, Cnidaria, Amphipoda, and Appendicularia were generally distributed in the present study waters. From the previous results, Copepoda, Protozoa, Chaetognatha, Cnidaria, Isopoda, and Appendicularia were ubiquitous in the southern Myanmar waters (Saw Han Shein 1975, Zin Lin Khine, 2009, 2013, 2014, and Jitlang, *et. al.* 2012).

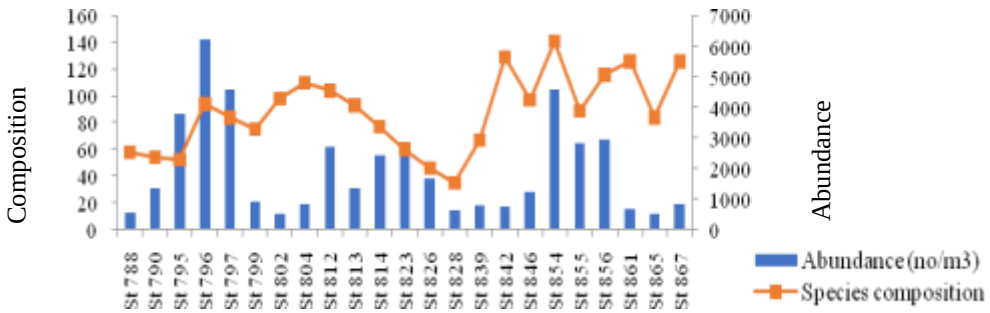


Figure 2 Station wise zooplankton abundance and composition in the present study area

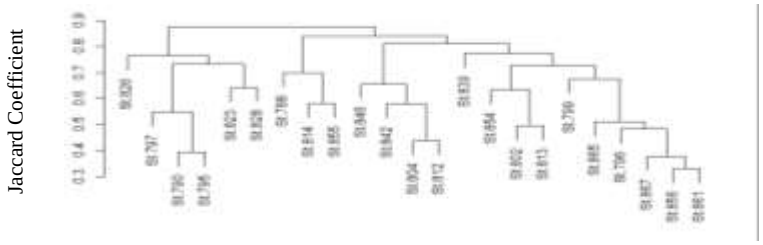
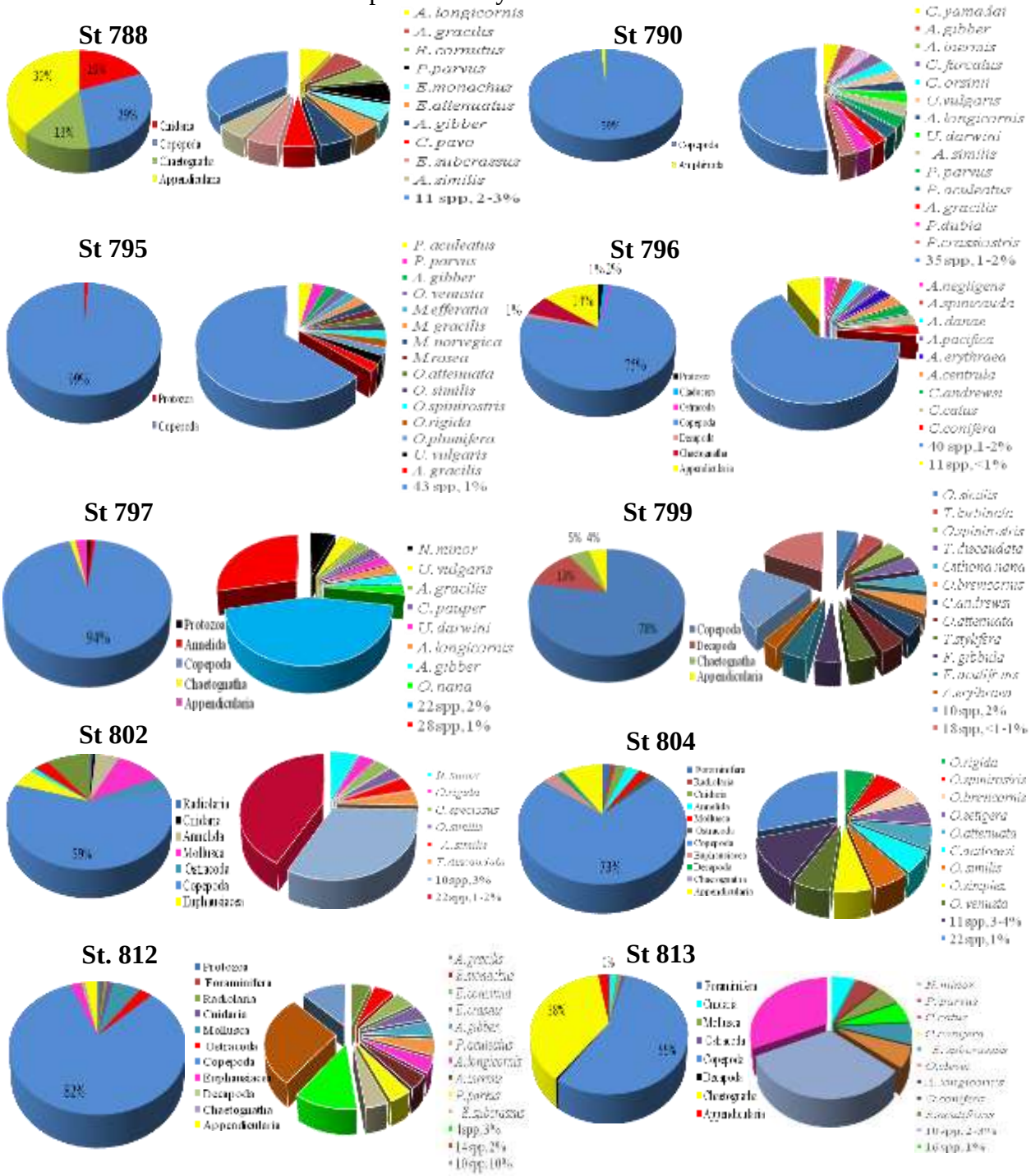


Figure 3 Dendrogram showing the Jaccard dissimilarity of zooplankton species composition found 23 stations of the present study area



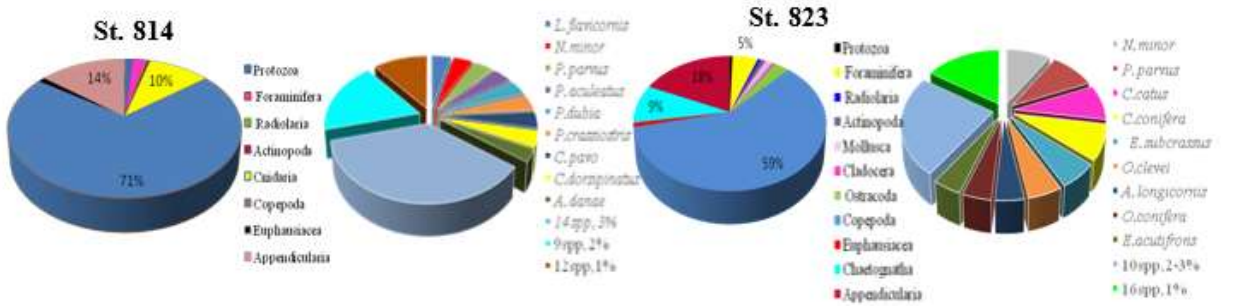
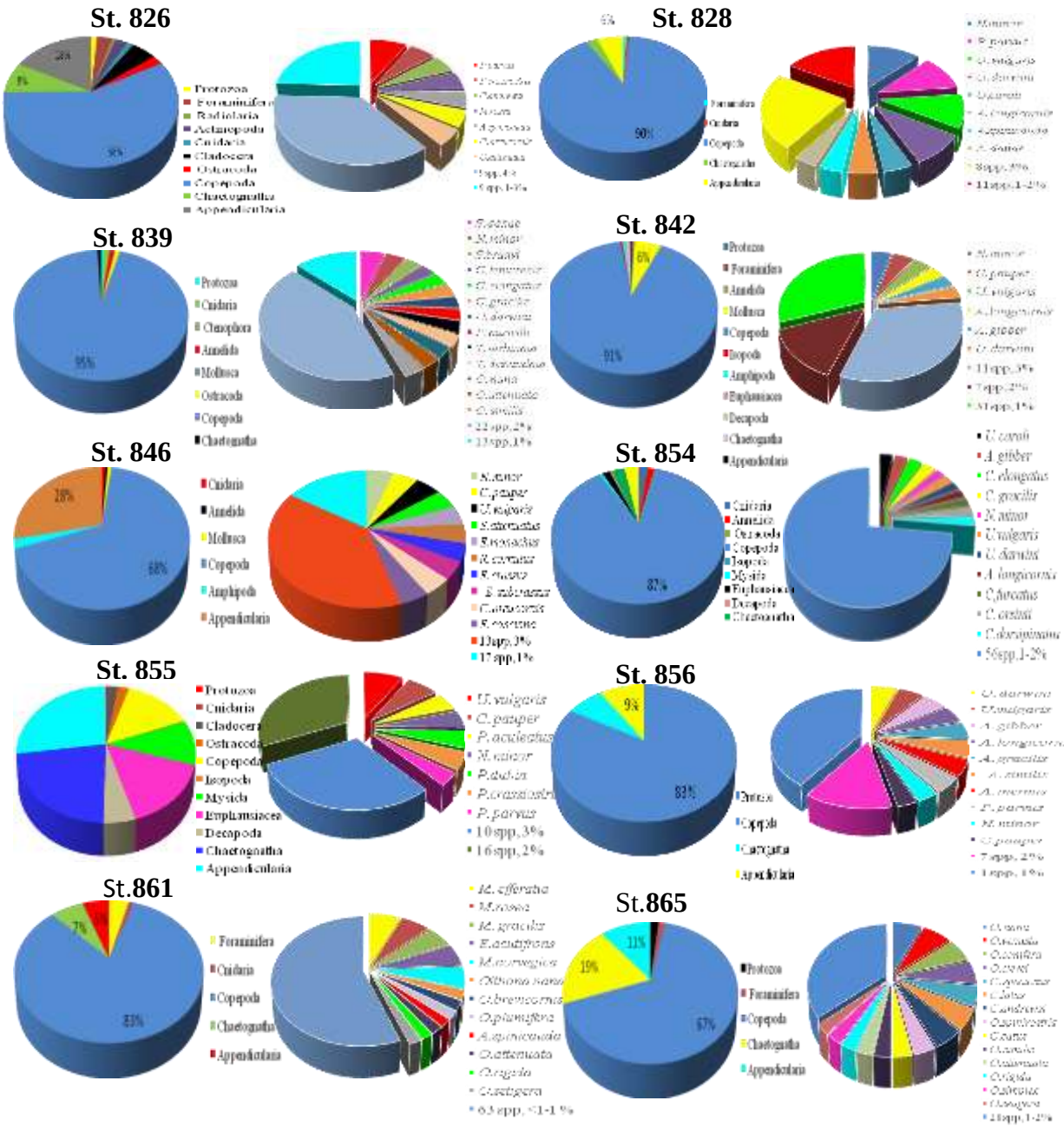


Figure 4 A. Relative abundance of main zooplankton groups (%) and dominant copepods found at each station in the present study area



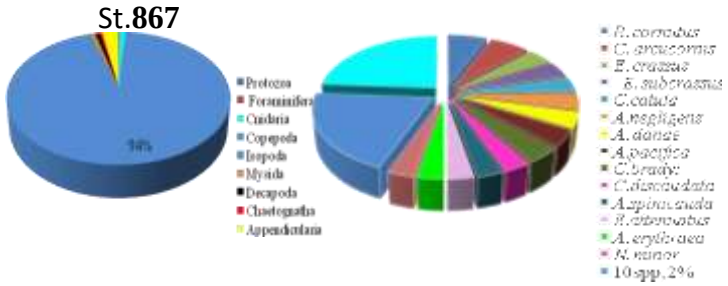


Figure 4 B Relative abundance of main zooplankton groups (%) and dominant copepods found at each station in the present study area

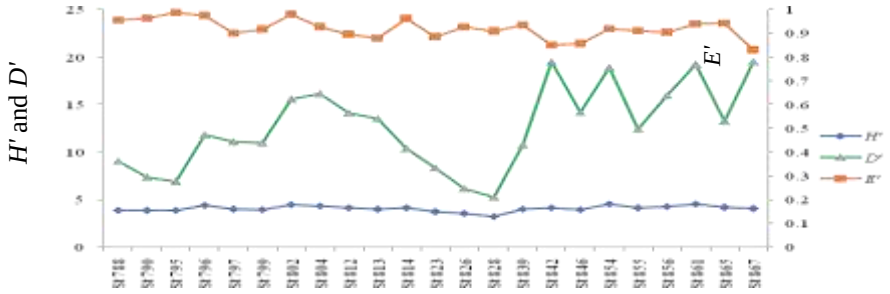


Figure 5 Zooplankton diversity indices by station in the present study area

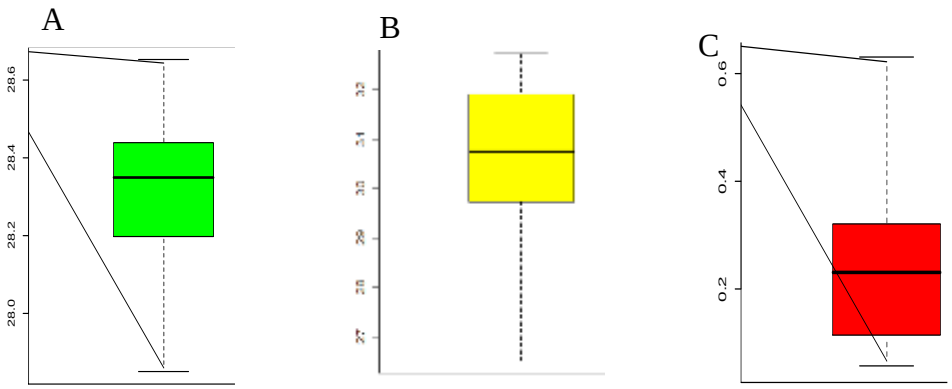


Figure 6 (A-C). Box plot showing the variables for Temperature (ºC), Salinity (ppt), and Oxygen (mg/l) in the present study area

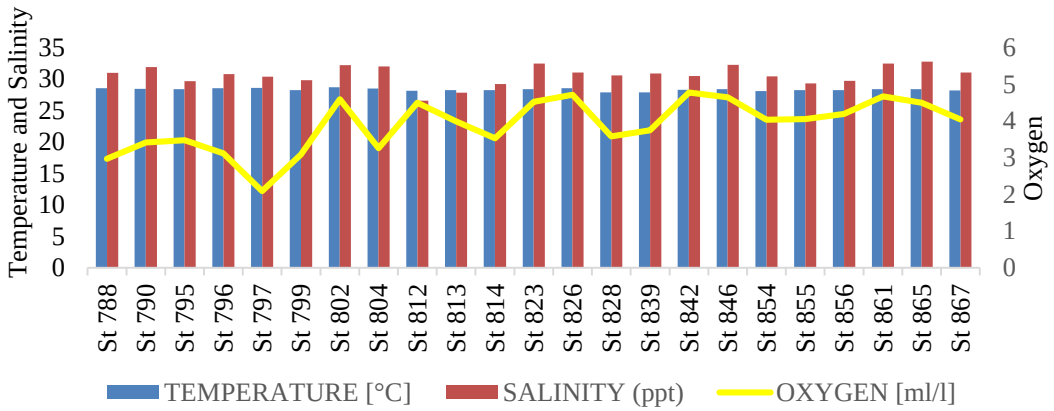


Figure 7 Seawater temperature, salinity and oxygen by stations

Copepods, Protozoa, Cladocera, Crustacea and Mollusca larvae were the important prey for larval fish (Nagasawa, 1993 and Anderson, 1994). The previous and present observations in the study waters proved that the main zooplankton groups supported fish larvae's food.

The previous results (Saw Han Shein 1975, Zin Lin Khine, 2009 and 2014) and present observations (2020) pointed out the major food items including *Undinula, Nanocalanus, Paracalanus, Acrocalanus, Acartia, Candacia, Oithona, Oncaea* and *Microsetella* were the dominant and abundant species in Myanmar waters. Moreover, *Tintinnopsis*, *Globigerina, Acetes* and shrimp larvae were generally distributed in the present study areas including fish spawning grounds. Therefore, it was apparent that these above species could improve secondary producers in the present study waters' marine food chain. As *Calanus, Paracalanus, Acartia*, and *Oithona* were the important prey of fish larvae and planktivores, they were significant links in marine food webs and provided to sustain commercial fish stocks (Turner,2004). *Tintinnopsis, Globige rina, Paracalanus,Oithona, Microsetella, Euterpina* and *Acetes* were the main food items for *Sardinella* spp. and *Rastrelliger* spp. (Nyo Nyo Tun, 2013 and Lett Wai Nwe, 2014).

Among copepods, *Acratia*, *Paracalanus, Acrocalanus*, and *Oithona* were extensively distributed during several environmental parameters at almost all stations in the present study area. Likewise, Vineetha *et al*. (2015) observed that *Acratia*, *Paracalanus* and *Oithona* were very common in different hydrological parameters. The present results proved the physicochemical parameters of seawater affecting the abundance of zooplankton. Zooplankton abundance correlated negatively with temperature, salinity and dissolved oxygen. Similarly, Puelles (2019) stated that zooplankton abundance showed a negative correlation with dissolved oxygen concentration. Salinity was the major factor determining the variability of zooplankton abundance while the temperature was the minor factor (Sribyatta, 1996).

In the present observation, zooplankton abundance and diversity were very high in neritic areas including fishing grounds. Zooplankton abundance and diversity played an essential role in the bio-productivity potential of inshore and offshore waters. Different zooplankton as the significant prey could provide fish larvae to sustain fishery stocks in the study waters. It was apparent that the zooplankton community could indicate to assess the status of fisheries resources in the present waters. Jivaluk (1999) recognized that the highest abundance of zooplankton coincided with denser concentrations of fish larvae and the catch of pelagic fish (anchovies and sardines) and demersal fish were high to correspond with the peaks of zooplankton.

Conclusions

Qualitative and quantitative zooplankton studies were conducted to determine the composition, abundance, distribution, diversity, and dissimilarity coefficient values of zooplankton communities in the waters off Tanintharyi Region. Copepods were usually the dominant members of the zooplankton and the main food prey for fish larvae. Zooplankton abundance was increased in the first fishing ground (around Launglong – Bok, Moscos and Maungmagan Islands) and the third one (near Thamihla, Anyin-pho-Anyin-ma, and Kawdwe Islands). As zooplankton abundance changes depend on the physical parameters of seawater, higher zooplankton abundance occurred in the nearshore stations coinciding with low salinity. The overall result mentioned that Shannon diversity values were high in the present study waters. It was an essential point to assess the water fertility of the present study area. As the most extraordinary zooplankton diversity provided the water productivity plentifully, the present study waters could be assessed as high productive area.

Acknowledgments

We are grateful to Dr. Ni Ni Oo, Rector, and Dr. Win Win Than, Pro-rector of Myeik University, for their support in preparing this work. **We appreciate the EAF-Nansen Programme, Norad, IMR, BOBLME, FAO, and DoF- Myanmar for giving the research opportunities.** We would like to express our gratitude to Kathrine Michalsen, Stamatina Isari, Jens-Otto Krakstad, Institute of Marine Research, Bergen, Norway and Dr. Tun Thein, DoF-Myanmar. We would like to gratefulness our colleagues for their cooperation.

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SPAWNING PERIOD OF BLOOD COCKLE *TEGILLARCA GRANOSA* (LINNAEUS, 1758) IN MYEIK COASTAL AREAS

Khin May Chit Maung[1], Ei Thal Phyu[2] & Nyo Nyo Tun[3]

Abstract

The spawning period of blood cockle *Tegillarca granosa* was studied by using the histological analysis of the gonads. Five maturity stages of gonads were noticed as the immature, developing, mature, spawning, and spent stages. The spawning period was from July to February with high in November and December. Males and females attain maturity in the same period. The observed mean length at first maturity was 29mm total length in males and 31mm in females. The number of females was predominant than males. Sexual dimorphism could not be noticed in species of *Tegillarca granosa.*

Keywords: blood cockle, histological analysis, maturity stages, *Tegillarca granosa*

Introduction

The blood cockle *Tegillarca granosa* (locally known as Gyin) is a commercially exploited bivalve species for human consumption. They belong to the Family Arcidae that is thick and solid shelled marine bivalves. It is commonly known as blood cockle because of the presence of haemoglobin that enables it to colonize habitats of low oxygen concentration (Poutiers 1998). The diagnostic characteristics of *T. granosa* is the presence of strong nodules on the radial ribs, about 18 radial ribs with wide interstices at both the left and the right shell valves, slightly longer shell than high and hinge area composed of small teeth (Souji and Radhakrishnan 2015). They are widely distributed in the Indo-West Pacific region and inhabit intertidal and sub-tidal mudflats areas and seaward of mangrove forests (Poutiers 1998).

Blood cockles are harvested from a wide population and caught as by hand or drag during the low tide from the mudflat. Nowadays, the sowing culture of blood cockle is very popular in Myeik coastal areas in which blood cockle spats obtained from the natural are used for seeds to sow culture. Therefore it is important to get a stable supply of spats for sustainable blood cockle aquaculture. Overexploitation will lead to diminishing the supply of natural spats. Thus, the understanding of the reproductive period of this species is essential for cultivation, management, and conservation strategy on their resources. The present study aimed to analyze and find out the maturation period and size at first maturity of blood cockle. It is also tend to fill the gap of the biological information in the literature about the blood cockle of Myeik coastal areas.

Materials and Methods

The monthly collections of blood cockles were made from the landing site of Myeik from January to December 2018 (Figure 1). Total length (maximum distance along with the anterior and posterior valves) and total weight were measured to the nearest 0.01mm using vernier calipers and to an accuracy of 0.01g by using the digital balance respectively. Identification of blood cockle was followed on the classification systems of Poutiers 1998, and Souji and Radhakrishnan 2015.

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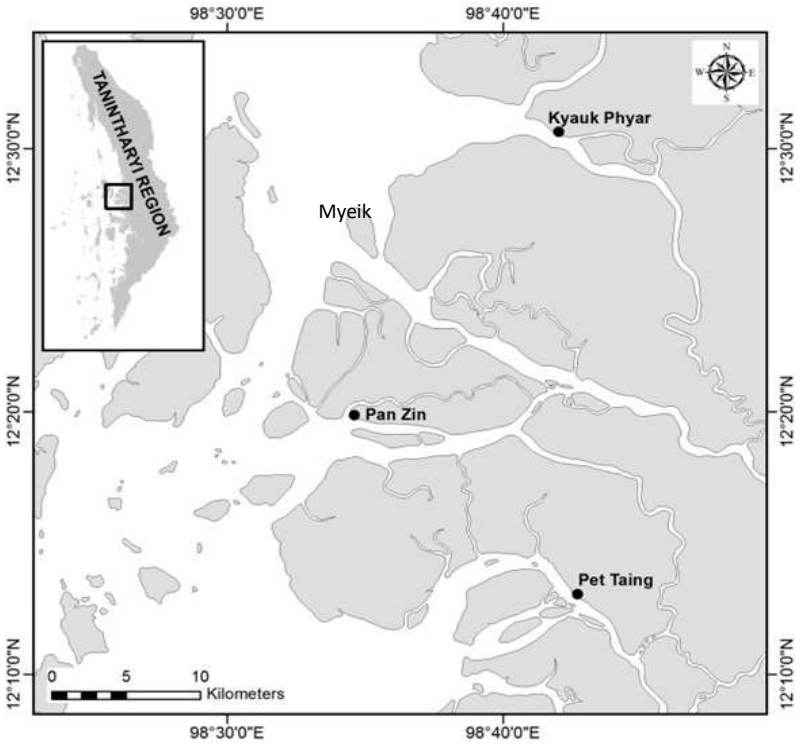

Figure 1. Map showing the sample collecting area

A total of 240 blood cockles samples with the range of 26-35mm shell length and 8.1-33 g shell weight were histologically examined for the determination of sex and stages of gonad maturity. For histological analysis, the whole soft tissues were removed from the shell and the flesh containing most parts of the gonads were fixed in formalin, dehydrated in upgraded series of ethanol, and cleared in xylene. After that the fixed tissue was embedded in paraffin, cut seven micrometer thick sections with a rotary microtome, stained using hematoxylin and eosin procedure, mounted on the microscopic glass side, and examined under the microscope. The classification of the gonad stages was followed by the systems used by Yurimoto *et al*. (2014). Five maturation stages of gonadal development: immature (Stage I), developing (Stage II), mature (Stage III), spawning (Stage IV), and spent (Stage V) stages were distinguished and presented in Table 1.

The sex of cockles was confirmed by the color of gonad and histological examination of the gonads. Sex ratio was tested for the expected ratio of 1:1 by chi-square (χ^2) analysis according to the formula:

$$\chi^2 = \frac{\sum(O-E)^2}{E}$$

where O= observed frequency of males or females, E= expected frequency of males or females

The length attains sexual maturity L50 was estimated by fitting the point where the total length of cockle (X-axis) and 50% level of maturity (Y-axis) are met.

To obtain a quantitative value that represents the reproductive activity, the gonad index was calculated as follows: GI= (sum of specimens number ascribed to each category*category score)/total number of cockles according to Ceballos-Vazquez *et al.* 2000 by using a numerical grading system. Three categories were assigned according to the development of the gonad, with 1=spawning and spent, 2= developing and 3= mature.

Table 1 Description and criteria for the gonadal stages of *Tegillarca granosa*

No.	Gonadal stages	Histological characteristics
1	Immature	Sex is indistinguishable in the gonads of immature stage and difficult to observe germ cells in the gonads. The wall is mainly occupied by connective tissue or an empty genital tube (Fig. 3A)
2	Developing	Male- Spermatogonia appear along the wall of the follicle wall. Spermatocytes and few spermatids can be seen. (Fig. 3B)
		Female- Oogonia appear along the wall of the follicle wall. Immature oocytes are attached to the tube wall. (Fig. 3F)
3	Mature	Male- Follicles are full of spermatozoa with their tails pointing towards the center of the tube. (Fig. 3C)
		Female- Follicles are full of mature oocytes that are irregular or polygonal shapes with the oval nucleus. (Fig. 3G)
4	Spawning	Male- Some spermatozoa are released that causing the empty space in the follicle wall. Many spermatozoa still remain in the genital tube. Some of the genital tubes are contracted and partially collapsed. (Fig. 3D)
		Female- Mature oocytes decrease in number causing the space in the follicle wall. Many oocytes with late-developing mature stages still remain in the genital tube. Mature oocytes exhibit nuclear disappearance because of germinal vesicle breakdown. (Fig. 3H)
5	Spent	Male- The genital tube is deformed and devoid of spermatocytes, which have completely spawned. Some spermatozoa still remain. (Fig. 3E)
		Female- The genital tube is deformed and degenerated. Much of the tube is empty. (Fig. 3I)

Results

The gonad is situated in the basal region of the body and envelops the dark green digestive gland. The morphology of the cockle gonad shows that the area of the gonad increases according to the increased levels of gonad maturity. The coloration of the gonad tissue layer in the blood cockle varies from orange-red to pale orange in females and from white to grayish-white in males for different maturity stages (Fig 2). The histological changes of the gonad in males and females during the reproductive cycle are illustrated in Figure 3.

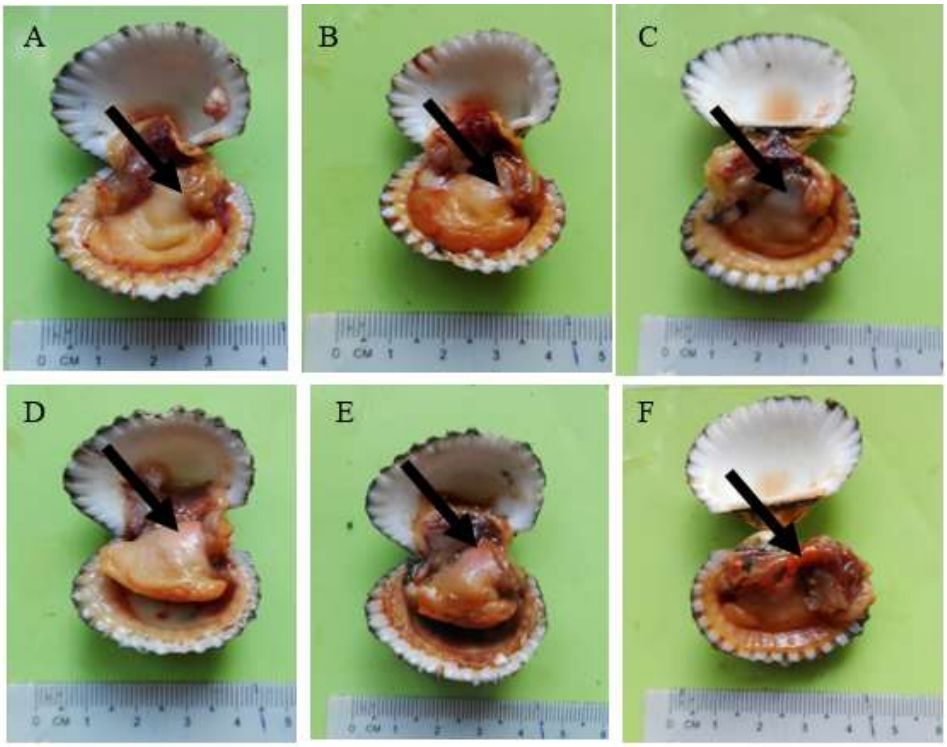

Figure 2. The coloration of *Tegillarca granosa* gonad morphology A-C) Males and D-F) Females

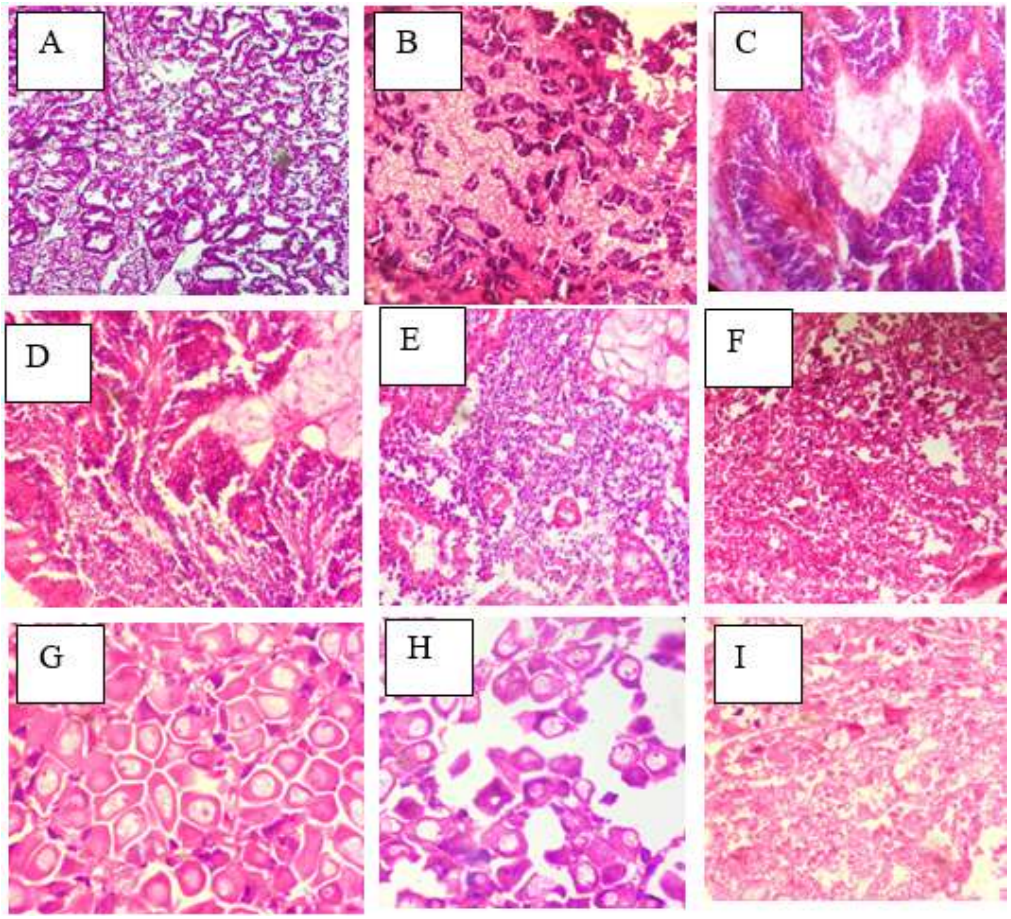

Figure 3 Gonadal stages of *Tegillarca granosa* A) Immature, B-E) Males and F-I) Females, (B, F) developing, (C, G) mature, (D, H) spawning and (E, I) spent, Scale bar=100µm

Occurrence of different maturity stages

The occurrence number and percentage of males and females in different maturity stages were recorded and described in Table 2 and Figure 4. Stage I (immature) occurred in all months except April, May, June, and September, comprising the range of 5% to 15% of all monthly samples. Immature cockles (Stage II) were recorded from January to June for males and from January to July for females. The percentage of immature males and females was high in March, April, and May. Males and females cockles with mature gonads (Stage III) were noticed from March to October. Percentages were varied from the lowest 5.5% (August) to the highest 40% (June) for mature males and from 10% (September) to 50% (June) for mature females. The spawning stage of males and females (Stage IV) occurred in January, February, and July to December. The highest percentage of spawning stage cockles were recorded in November and December, accounting for 44.4% and 47.3% for males and 38.9% and 42.1% for females respectively. The percentage of spent stage cockles (Stage V) was high in February and low in October for males and high in January and low in November and December for females. No spent stage of males and females were observed in April, May, June, and July.

Table 2 Monthly occurrence number of different maturity stages in *Tegillarca granosa*

Month	I	Males				Females			
		II	III	IV	V	II	III	IV	V
Jan	3	2		3	3	1		3	5
Feb	3	1		3	5	1		4	3
March	1	4	3		1	5	4		2
April		4	4			5	7		
May		5	6			3	6		
June		1	8			1	10		
July	2		4	3		2	6	3	
Aug	2		1	4	3		3	3	4
Sep			2	5	4		2	4	3
Oct	1		3	5	1		3	4	3
Nov	2			8	2			7	1
Dec	1			9	1			8	1

I: Immature, II: Developing, III: Mature, IV: Spawning, V: Spent

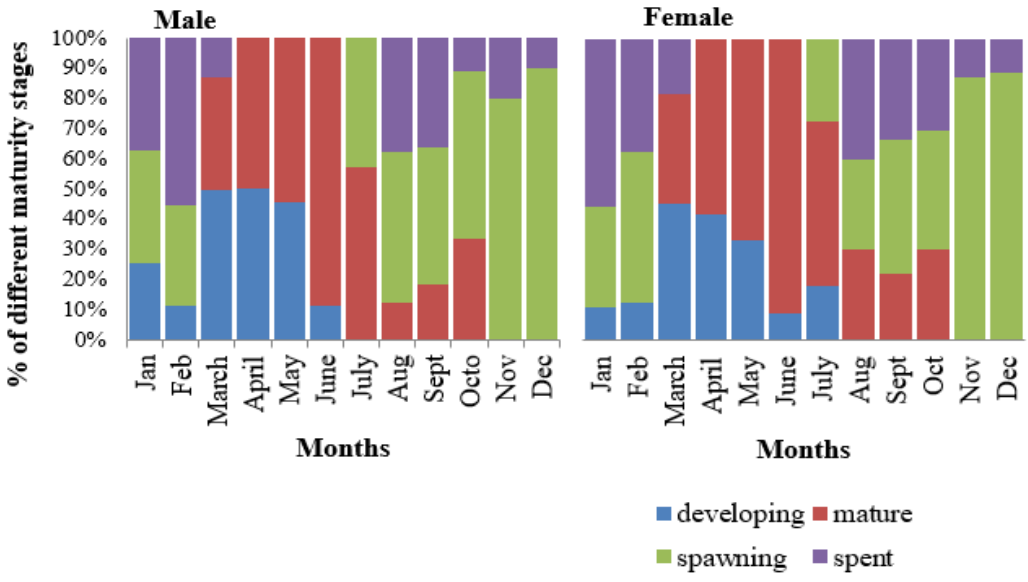

Figure 4 Percentage occurrence of different maturity stages in *Tegillarca granosa*

Length at first maturity

Examination of the percentage maturity stages of different size groups revealed that the percentage of mature cockles increased with the increase of length for both males and females. The percent of 28 in males and 25 in females were mature at 26-28 mm length, 55% males and 40% female at 28-30 mm length, 56.2% males and 57% females at 30-23 mm length, 65.5% males and 62.5% females at 32-34 mm length, 80% males and 78.2% females at 34-36 mm length, 87.5% males and 90.3% females at 36-38 mm length, 90% males and 97% females at 38-40 mm length and 100% for males and females at 40-42 mm length. Thus, the mean size group at first maturity (50%) was considered as 29 mm for males and 31 mm for females (Fig 5).

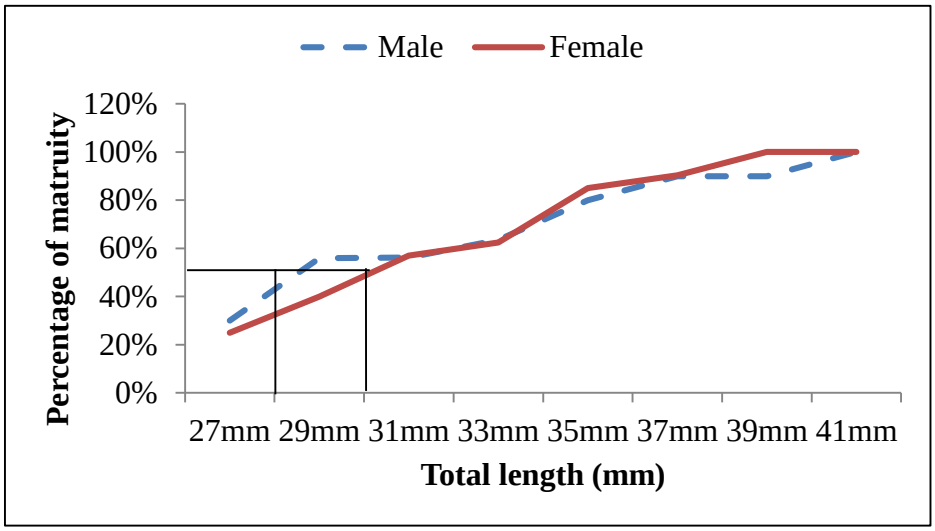

Figure 5 Length at first sexual maturity of males and females

Sex ratio

The sex was determined based on the gonad color and histological analysis of the gonad. The overall result of the sex ratio determined from the histological slides was 0.9 males: 1 female. Males were more abundant in January, March, April, June, July, August, and October (Table 3). The range of chi-square values (0.04 to 0.8) showed that there was no significant difference in males and females for all months from the expected 1: 1 ratio.

Table 3 The monthly sex ratio of *Tegillarca granosa*

Month	Male: Female	Chi-square (χ^2)	Month	Male: Female	Chi-square (χ^2)
Jan	0.9:1	0.06	July	0.6:1	0.8
Feb	1.1:1	0.06	Aug	0.8:1	0.2
March	0.7:1	0.5	Sep	1.2:1	0.2
April	0.7:1	0.8	Oct	0.9:1	0.04
May	1.2:1	0.2	Nov	1.25:1	0.2
June	0.8:1	0.2	Dec	1.1:1	0.04

Gonad index

Monthly quantitative assessments of histological reproductive condition (GI values) were varied from 1 to 2.8 for males and from 1 to 2.9 for females (Fig 6). High GI values occurred in March, April, May, and June coinciding with the occurrence of mature cockles. Low GI values were observed in November, December, January, and February, coinciding with the spawning activity. The average GI values obtained for males and females were 1.7 and 1.8 respectively.

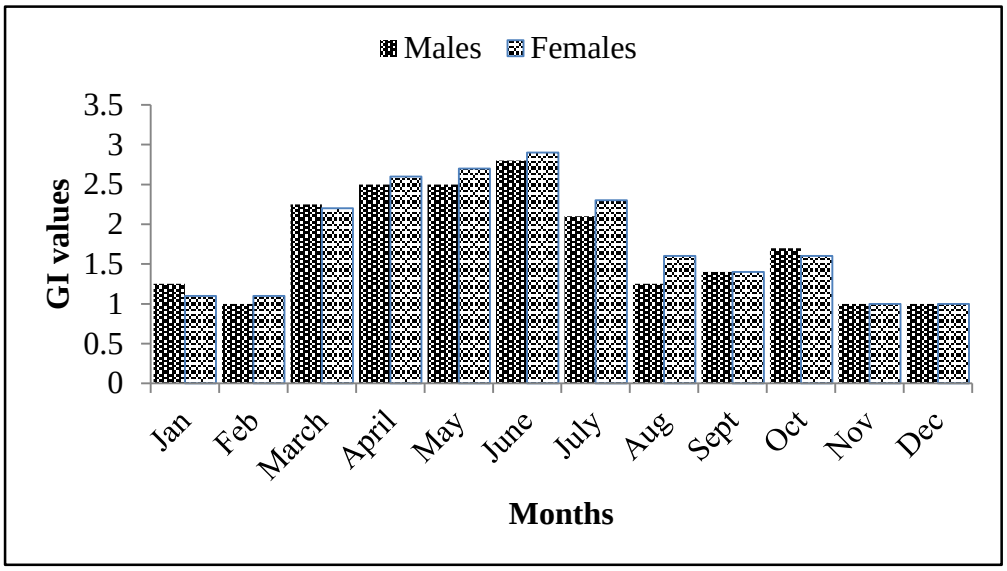

Figure 6 Monthly gonad index (GI) values of males and females *Tegillarca granosa*

Discussion

Tegillarca granosa (formerly known as *Anadara granosa*) belonged to the family Arcidae is a commercially valuable species due to human consumption and market demand for exportation. They are exploited from both natural populations and as a sowing culture. To sustain long-term exploitation and protection, the knowledge of the reproductive cycle of the blood cockle is essential. Gonad maturation and sexuality in the population of *Tegillarca granosa* can be studied through the macroscopic examination of visceral mass and microscopic examination of gonad by histological technique. The most precise results of the stage of the gonad can be obtained through histological examinations.

Little is known about the sex-determining mechanism of bivalves and mollusks in general. So far it is known that there are no morphologically distinguishable chromosomes (Afiati 2007). Different schemes of classifications of gonadal maturity are available in bivalves. There are five maturity stages of blood cockle gonad identified as immature, developing, mature, spawning, and spent stages in the present study. The identification of maturity stages was followed by Yurimoto *et al* (2014). Four stages of gametogenesis: developing, ripe, spawned out, resorbing were identified in male and female of *Anadara antiquata* from Pakistan by Jahangir *et al.* 2014.

During the present study, no hermaphrodites were found in any of the gonads of *Tegillarca granosa* section examined. There is no sexual dimorphism could be noticed in *Anadara senilis* from west African by Yankson (1982), *Anadara rhombea* from Porto Novo coast by Natarajan and John (1983), in *Anadara antiquatea* from Philippine by Toral-Barza and Gomez (1985), and *Anadara antiquata* from the northern Arabian Sea by Jahangir *et al.* (2014). But some hermaphrodite individuals were encountered in *Anadara granosa* from central Java, Indonesia (Brotohadikusumo 1994). Afiati (2007) also reported that hermaphrodites occurred in *Anadara granosa* and *A. antiquatea* with a percentage of 1.43% and 1.45% in the specimens of Central Java respectively.

Based on histological analysis of gonad and observed GI values, spawning stages of males and females cockles were observed from July to February with a peak in November and December during the present study. Yurimoto *et al.* (2014) stated that the spawning season of the blood cockle in the tropical region is very long. Different spawning period of the blood cockle from the different regions was reported by various authors. Spawning of cockle *Anadara antiquata* occurred

continuously throughout the year, with peak spawning from July to September (Toral-Barza and Gomez 1985). According to the four-year study on the spawning behavior of *Anadara granosa* in Kakinada bay, Narasimham (1988) described that the blood clam spawns throughout the year and their major spawning months vary between years. Muthiah 2004 also stated that *Anadara granosa* breeds throughout the year with peak spawning from January to April in Malaysia. Suwanjarat *et al.* (2009) stated the breeding season of *Anadara granosa* in Pattani Bay was mainly from July to August.

The present result of the spawning period that occurred in blood cockle *Tegillarca granosa* from Myeik areas was nearly similar to the report of Khalil (2013), Yurimoto *et al.* (2014), Jahangir *et al.* (2014), and Saputra *et al.* (2019). Khalil (2013) reported that the spawning period of blood cockle *Anadara granosa* was continuous throughout the year in the northern Straits of Malacca, peak spawning from October until January. Yurimoto *et al.* (2014) reported that the spawning period of *Anadara granosa* along the Selangor coast, Peninsular Malaysia was from November to February. Jahangir *et al.* (2014) and Saputra *et al.* (2019) also stated the dominant of the spawning stage of blood cockle in December.

Determination on first maturity length is the basic requirement for the protection and sustainable exploitation of the stock. *Tegillarca granosa* mature first at a mean length group of 29 mm in males and 31 mm in females in the present study. The observed size at first maturity of the present study was smaller than that of Saputra *et al.* (2019) in which the estimated length at first maturity of blood cockle from Banjar Kemuning river as 35.73mm in males and 37.21mm in females and larger than that of Narasimham (1988) in which male cockles from Kakinada bay attain the first maturity at 20mm and female at 24mm in length.

Sex ratio studies show information on the proportion of male to female fish in a population and are expected to be 1: 1 in nature. Any deviation from this ratio may indicate the dominance of one sex over the other. Pathansali and Soong 1958 reported that the equal sex number of *Anadara granosa* in the culture of Malaysia. The present study showed that the occurrence number of females was high than males. However, the analysis of the chi-square method showed that there was no significant difference at the 5% probability level. So, it can be concluded that the sex ratio of male and female cockle based on the one year study period is in a balanced condition. Females were also dominant in the population of blood cockle *Anadara inaequivalvis* in the southeastern Black Sea Coast (Sahin *et al.* 2006) and the population of *Anadara antiquatea* from the northern Arabian Sea (Jahangir *et al.* 2014). However, Natarajan and John (1983) and Brotohadikusumo (1994) indicated the predominance of males in the population of *Anadara rhombea* from the backwaters of Porto Novo and the population of *Anadara granosa* from Indonesia respectively.

Conclusion

According to the histological observation on the gonad, it would be concluded that the spawning period of *Tegillarca granosa* was high in November and December. Thus, intensive harvesting of blood cockles should be avoided during this time to protect the production of spat and to fulfill the demand for human consumption from year to year. Yet the present result still provides important biological information to produce the artificial blood cockle spat. More studies are still needed to do research on breeding biology for the long term period and to assess the stock of this area for successful aquaculture and management.

Acknowledgments

We would like to express our special thanks to Dr. Ni Ni Oo, Rector of Myeik University for her permission to carry out this research. We also wish to express our gratitude to Dr. Tatsuya Yurimoto and Dr. Hajime Saito, scientist researchers from Japan International Research Center for Agricultural Sciences (JIRCAS) for providing technical support for the histological analysis of bivalve gonad and supporting the laboratory equipment.

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ABUNDANCE OF THE GENUS *CONUS* LINNAEUS 1758 (GASTROPODA: CONIDAE) FROM ANDREW BAY IN RAKHINE COASTAL REGION

Naung Naung Oo*

Abstract

Andrew Bay is ecologically and biologically significant marine area in Rakhine Coastal Region. This research is the first attempt to photo-document, record, and determine the relative abundance of *Conus* species in Rakhine Coastal Region. This study was carried out in three study sites of Andrew Bay in 2014. There were a total of 879 cone shells collected encompassing 30 known species. Most of the number of cone shells was found in Pearl Island which constitutes about 60.5% of the entire collection. The most abundant species are *C. arestophanes*, *C. vinctus*, and *C. mustelinus*. While the rare species include *C.* marmoreus, *C. betulinus*, *C. ferrugineus*, *C. vexillum*, and *C. nussatella*, and the rarest among those species are *C. miles*, *C. quercinus*, *C. tessulatus*, and *C. virgo* with only one specimen collected. Moreover, the diversity of cone shells found along the world oceans were also described.

Keywords: *Conus* species, relative abundance, Andrew Bay, Rakhine Coastal Region.

Introduction

In Myanmar, there were 40 species of *Conus*, of which 30 species namely *C. imperialis* Linnaeus, 1758; *C. zonatus* Bruguière, 1792; *C. litteratus* Linnaeus, 1758; *C. eburneus* Bruguière, 1792; *C. leopardus* Rödiing, 1798; *C. crassus* Sowerby, 1857; *C. ebraeus* Linnaeus, 1758; *C. prytanis* Sowerby, 1882; *C. achatinus* Gmelin, 1791; *C. monile* Bruguière, 1792; *C. vitulinus* Bruguière, 1792; *C. miles* Linnaeus, 1758; *C. vexillum* Gmelin, 1791; *C. flavidus* Lamarck, 1810; *C. geographus* Linnaeus, 1758; *C. aulicus* Linnaeus, 1758; *C. episcopus* Bruguière, 1792; *C. omaria* Bruguière, 1792; *C. textile* Linnaeus, 1758; *C. textile verriculum* Reeve, 1848; *C. gloriamaris* Chemnitz, 1777; *C. tigrinus* Sowerby, 1858; *C. betulinus* Linnaeus, 1758; *C. figulinus* Linnaeus, 1758; *C. quercinus* Solander, 1786; *C. hyaena* Hwass, 1792; *C. blanfordianus* Crosse, 1867; *C. striatus* Linnaeus, 1758; *C. tulipa* Linnaeus, 1758 and *C. terebra* Linnaeus, 1758 were identified but 10 species were unidentified up to species level by Soe Thu (1980).

The family Conidae is one of the major groups of gastropod animals which are mainly characterized by the possession of intense venom apparatus and highly predacious and nocturnal in feed and feeding habits (Gugulothu, Raveender, Shah and Koteswar 2017). They normally have a toxicant sting, which they use for their predatory activity on their prey, such as polychaete annelids, echiurans, small fishes, and other gastropods (Kohn et.al 1999) as mentioned by Lee and Park (2014). Moreover, cones are characterized by the possession of a venom gland and a highly modified radular tooth that they use as a harpoon to inject venom into their prey (Díaz et.al 2005; Puillandre, Duda, Meyer, Olivera and Bouchet 2015).

The genus *Conus* Linnaeus 1758, is considered to be the most species-rich modern marine genus, with more than 500 extant and several hundred extinct species (Rockel, Korn and Kohn 1995; Duda, Kohn and Palumbi 2001) as cited in Harasewych (2014). However, Tucker and Tenorio (2009) in (Lee and Park 2014) stated that it contains more than 600 extant species. But, as of 2014, from 30 valid species known to Linnaeus, the recent accepted number of species stands at 803 based from World Register of Marine Species (WoRMS).

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They occur mainly in the tropical zone of all world oceans, although the majority of species concentrate in the Indo-Pacific and western Pacific regions (Keen 1971; Walls 1979; Duda *et.al* 2008) as cited by (Lee and Park 2014). These live in the tropical and warm temperate seas of the world, from the intertidal to depths of almost 1,000 m (Puillandre *et.al* 2015). Cone shells inhabit various types of sea bottom substrates, including rocky grounds, sand or mud plains, coral reefs and seagrass beds, and reach from the intertidal zone to more than 1,000 m of depth (Dance and Cosel 1977) in (Díaz *et.al* 2005).

Conidae is a very popular family among gastropods due to its rarity and beautiful color. Many fishermen families among coastal villages are actively engaged in the collection of these shells and also in the shell handicraft industry. They are widely used for making dolls, fantasy flower sculptures of gods, etc (Sary *et.al* 2014).

They are undoubtedly one of the most unmistakable groups due to its characteristic conic shell and variety of colors. Cone shells are the most popular collectable shells among amateur and professional conchologists, and the shells of rare species may reach exorbitant prices (Díaz *et.al* 2005). The striking shell patterns have long attracted a large cadre of collectors. They are also economically important because shell collectors and commercial traders have established active markets for their shells globally (Cabrera 1984). In the seventeenth century, cone shells were among the most valuable of natural history objects (Olivera and Corneli 2014). Nowadays, several *Conus* peptides are widely used research tools in neuroscience and some other potential therapeutic agents. Its venom becomes increasingly important in medicine and neurobiology (Craig, Bandyopadhyay and Olivera 1999).

Cone snails are therefore important to biodiversity since they have evolved into one of the largest of all marine genera. Likewise to biopharmaceutics because they offered unparalleled opportunities in the development of novel drugs. Lastly, to economics since their shells provide income to poor fishing communities through sales to tourists, traders and a global business in the specimen shell trade (Peters, Leary, Hawkins, Carpenter and Roberts 2013).

The Rakhine Coastal Region has a coastline stretching about 740 km facing the Bay of Bengal which possesses marine fishery resources. Andrew Bay (Lat. 18˚25′ N, Long. 94˚15′ E) is one of the unique ecosystem in Rakhine Coast where is a huge habitat with rich biodiversity. However, no studies were conducted yet, nor published research about the diversity of cone shells in Andrew Bay. But there's already evidence of over gleaning and exploitation of coastal resources in the bay. Therefore, studying and documenting them is very important before they become extinct. The objectives of current study are 1) to identify the diversified species of cone shells; and 2) to investigate the species abundance of cone shells population in their natural habit. Establishing baseline data about this species is also necessary for future researches.

Materials and Methods

This study on the relative abundance of *Conus* species along Andrew Bay was carried out in 2014. The collection of specimen was conducted from the three sampling sites of Andrew Bay, namely; Pearl Island, Tha-byu Gyaing and Maung-shwe-lay Gyaing (Fig. 1). Specimens were collected from the tidal flats, seagrass beds, rocky intertidal areas, sandy shores and beaches through reef walking and gleaning or hand picking. Live and dead *Conus* species were gathered. Species collected were photo documented and recorded. In the identification process, the book entitled "FAO Species Identification Guide for Fishery Purposes: The Living Marine Resources of the Western Central Pacific (Volume 1) Seaweeds, Corals, Bivalves and Gastropods" was utilized. Other supplementary websites were also visited for its verification such as www.gastropods.com, www.bily.com, indopacific seashells.com, conchylinet.com and the World Register of Marine

Species (WoRMS). To determine the relative abundance of each species, the scale shown in Table 1 which was adopted from Slimming and Jarrett (1970) and Jackson (1995) as indicated in (Agombar, Dugdale and Hawkswell 2003) was utilized.

Table 1 Scale used to record the relative abundance of shells collected in Andrew Bay over the period of study

Scale	Relative abundance	Number of specimens found during the period
1	Rare	1 to 4
2	Uncommon	5 to 8
3	Occasional	9 to 20
4	Fairly common	21 to 30
5	Common	31 to 99
6	Abundant	100 or more

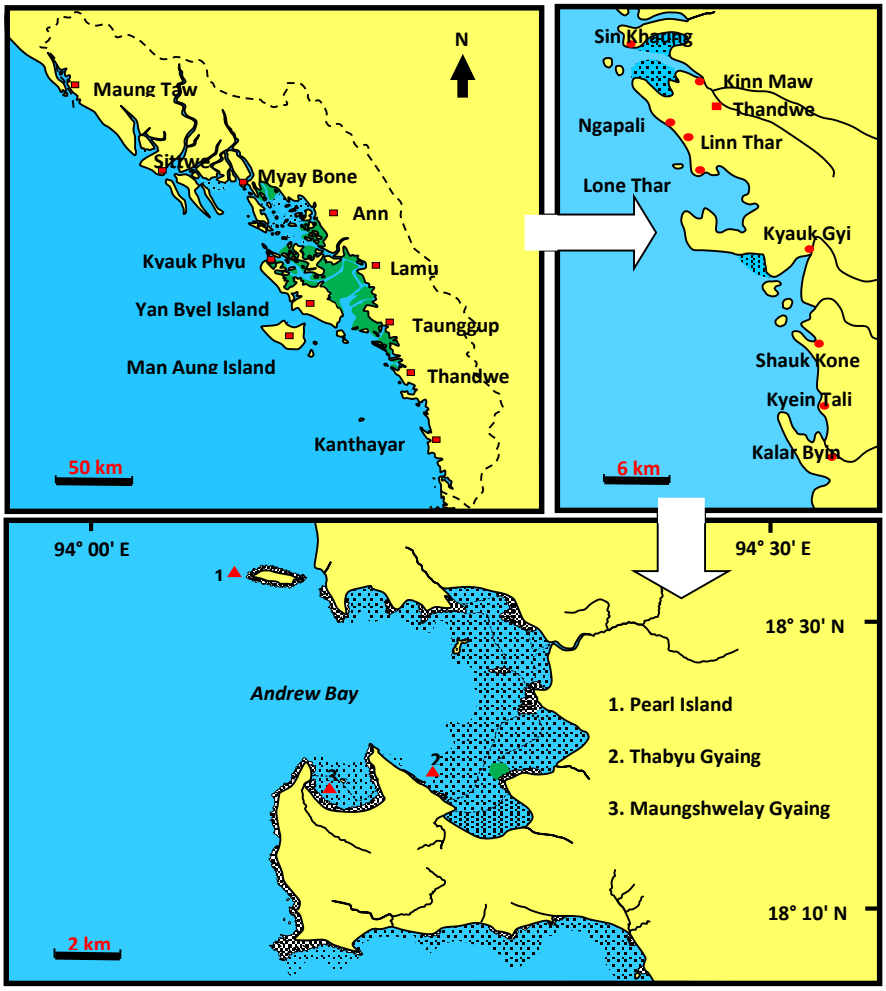

Figure 1 Map showing the sampling sites of cone shells in Andrew Bay

Results and Discussion

A total of 879 cone shells were collected belonging to 30 different *Conus* species. Of which 532 individual cones or 60.5% were found in Pearl Island, 217 or 24.7% came from Tha-byu Gyaing and 130 or 14.8% came from Maung-shwe-lay Gyaing. From the 30 different species

recorded, there were 25 species found in Pearl Island, 23 from Tha-byu Gyaing, and 22 from Maung-shwe-lay Gyaing. Based on the relative abundance, three species were classified as abundant namely; *C. arestophanes*, *C. vinctus*, and *C. mustelinus*. Six species were categorized as common which include *C. ebraeus, C. rattus, C. striatus, C. stercusmuscarum, C. capitaneus, and C. monachus*. Only one species was categorized as fairly common which is the *C. glans*. Seven species of cones were categorized as occasional. These are *C. lividus, C. muriculatus, C. omaria, C. textile, C. varius, C. thalassiarchus, and C. zeylanicus*. Four species were classified as uncommon, which include *C. imperialis, C. eburneus, C. terebra, and C. virgo*. Lastly, nine species were considered as rare which include *C. marmoreus, C. betulinus, C. ferrugineus, C. vexillum, C. nussatella, C. miles, C. quercinus, C. tessulatus,* and *C. virgo* (Table 2 and Fig. 2 to 5).

Table 2 Relative abundance of *Conus* species in Andrew Bay

No.	Species	Sampling site			Total count	Relative abundance description
		St. 1	St. 2	St. 3		
1	*Conus arestophanes* (Sowerby II, 1857)	63	71	0	134	A
2	*Conus betulinus* (Linnaeus, 1758)	0	0	3	3	R
3	*Conus capitaneus* (Linnaeus, 1758)	16	7	9	32	C
4	*Conus ebraeus* (Linnaeus, 1758)	49	11	20	80	C
5	*Conus eburneus* (Hwass in Bruguiere, 1792)	0	1	7	8	U
6	*Conus ferrugineus* (Hwass in Bruguière, 1792)	2	0	1	3	R
7	*Conus glans* (Hwass in Bruguiere, 1792)	12	6	5	23	F
8	*Conus imperialis* (Linnaeus, 1758)	6	2	1	9	U
9	*Conus lividus* Hwass in Bruguière, 1792	7	6	4	17	O
10	*Conus marmoreus* (Linnaeus, 1758)	1	2	1	4	R
11	*Conus miles* (Linnaeus, 1758)	0	0	1	1	R
12	*Conus monachus* (Linnaeus, 1758)	26	2	3	31	C
13	*Conus muriculatus* (Sowerby II, 1833)	10	6	0	16	O
14	*Conus mustelinus* (Hwass in Bruguière, 1792)	82	20	16	118	A
15	*Conus nussatella* (Linnaeus, 1758)	0	3	0	3	R
16	*Conus omaria* (Hwass in Bruguière, 1792	8	2	5	15	O
17	*Conus pulicarius* (Hwass in Bruguière, 1792)	2	0	0	2	R
18	*Conus quercinus* (Lightfoot, 1786)	0	0	1	1	R
19	*Conus rattus* (Hwass in Bruguiere, 1792)	55	5	10	70	C
20	*Conus stercusmuscarum* (Linnaeus, 1758)	26	17	10	53	C
21	*Conus striatus* (Linnaeus, 1758)	39	8	15	62	C
22	*Conus tessulatus* (Born, 1778)	1	0	0	1	R
23	*Conus textile* (Linnaeus, 1758)	9	3	2	14	O
24	*Conus thalassiarchus* (Sowerby II, 1834)	2	6	4	12	O
25	*Conus terebra* (Born, 1778)	4	0	2	6	U
26	*Conus varius* (Linnaeus, 1758)	5	8	0	13	O
27	*Conus vinctus* (Adams, 1855)	102	22	7	131	A
28	*Conus virgo* (Linnaeus, 1758)	2	1	3	6	U
29	*Conus vexillum* (Gmelin, 1791)	1	1	0	2	R
30	*Conus zeylanicus* (Gmelin, 1791)	2	7	0	9	O
	Total	**532**	**217**	**130**	**879**	

Symbol: St. 1 = Pearl Island; St. 2 = Tha-byu Gyaing; St. 3 = Maung-shwe-lay Gyaing; A = Abundance; C = Common; F = Fairly common; O = Occasional; R = Rare; U = Uncommon.

Andrew Bay in Rakhine Coastal Region is a haven of a rich diversity of marine organisms. Unraveling this diversity had posed a tremendous challenge. Marine mollusc studies are still among those that are overseen by many researchers. To date, there is still a lack of basic information such as diversity and species checklist that make it impossible to assess the rate of population lost among existing marine molluscs (Tabugo, Pattuinan, Sespene and Jamasali 2013).

This study was able to record 879 total count of cone shells collected from the coastal areas of the Andrew Bay and initially identified 30 different species. The abundant species were *C. arestophanes*, *C. vinctus* and *C. mustelinus* while the rare species were *C. miles*, *C. quercinus*, *C. tessulatus* and *C. virgo*. Lastly, the rarest *Conus* species recorded were *C. miles*, *C. quercinus*, *C. tessulatus* and *C. virgo* with only one specimen collected throughout the duration of the study. The sampling site of Pearl Island has the most number of *Conus* species observed and collected Maung-shwe-lay Gyaing has the least. There's a great possibility that more species will still be discovered especially in deeper waters and with exhaustive sampling and method used in gathering shells.

There were several studies conducted by different authors from different localities about the diversity of this species. Kohn (1978) reported 70 *Conus* species from Sri Lanka and 64 species in Maldives and Chagos; Subba Rao (1991) reviewed the Conids of Andaman and Nicobar Islands, recorded about 45 *Conus* species; Rockel *et.al* (1995) gave a detailed note on world living *Conus* and documented 316 valid species along with several subspecies and forms from the tropical Indo-Pacific region; Richmond (1999) documented 198 species of Conidae from Western Indian Ocean; Nguyen (2005) recognized 122 species in Vietnamese waters and the Conidae documented from the Philippines was 287 species (Poppe 2008) as mentioned by (Gugulothu, Raveender, Shah and Koteswar 2017). This is summarized in Table 3.

Table 3 Summary of the studies conducted about the diversity of *Conus* species in different places around the world

Authors	Place	No. of Cone Species	Year
Kohn	Sri-Lanka, Maldives and Chagos	134	1978
Subba Rao	Andaman and Nicobar Islands	45	1991
Rockel *et.al*	Indo-Pacific Region	316	1995
Richmond	Western Indian Ocean	198	1999
Nguyen	Vietnamese Waters	122	2005
Poppe	Philippines	287	2008
Dolorosa *et.al*	Tubbataha Reef, Palawan	21	2015
Present study	Andrew Bay, Rakhine Coastal Region	30	2014

Conclusion

The present study initially listed 30 species of cone shells (Family Conidae) in Andrew Bay, Rakhine Coastal Region. The most number and kinds of *Conus* species observed in Pearl Island. From those species, the most abundant are *C. arestophanes*, *C. vinctus* and *C. mustelinus*. While the rare species include *C. marmoreus*, *C. betulinus*, *C. ferrugineus*, *C. vexillum* and *C. nussatella* and the rarest among those species are *C. miles*, *C. quercinus*, *C. tessulatus* and *C. virgo* with only one specimen collected. This initial record may increase with future researches, especially in deeper waters. Thus further study is recommended to enrich it. The diversity of *Conus* species observed suggests that it could be a good biodiversity indicator, thus awareness and conservation may be done.

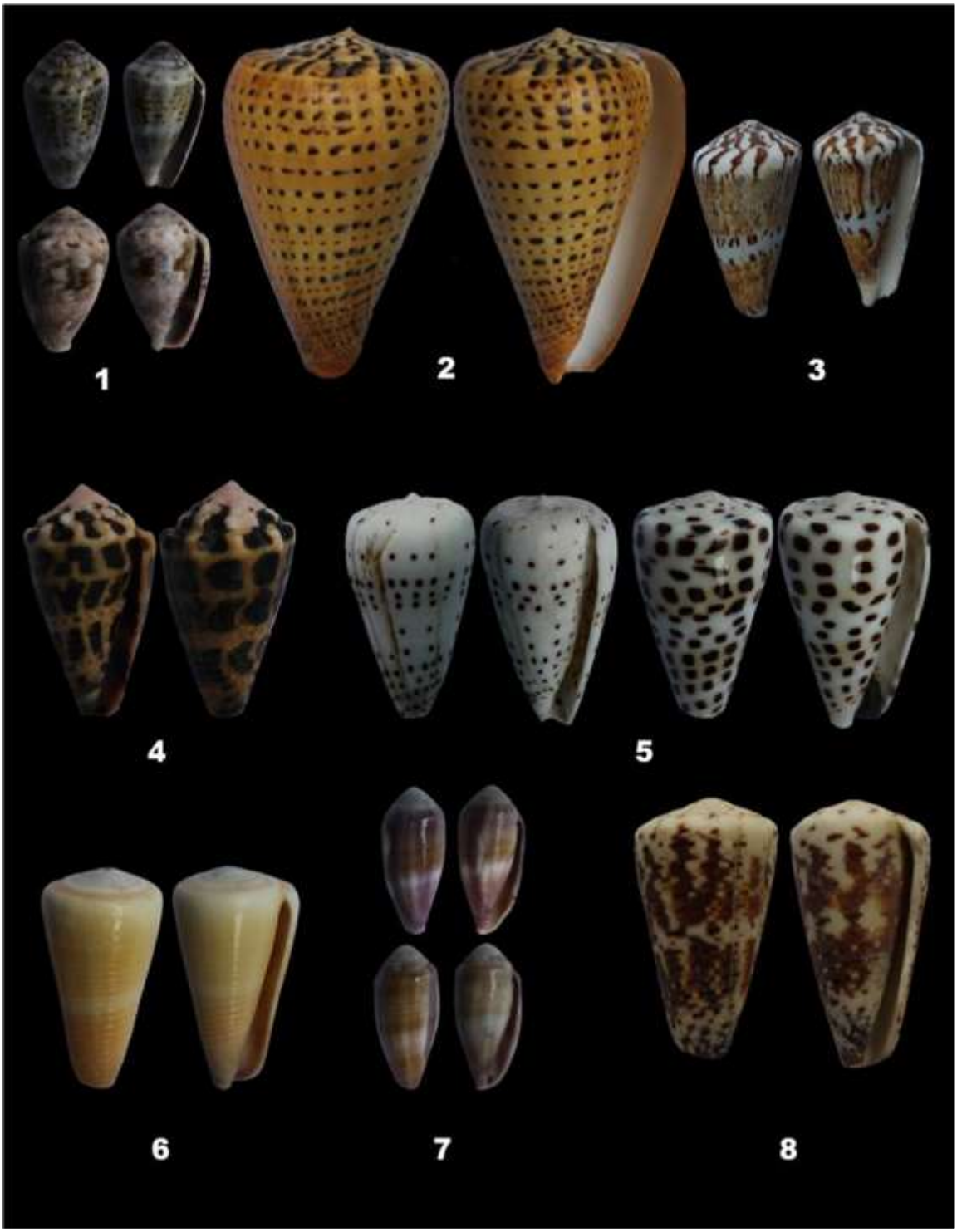

Figure 2 (1-8): *Conus* species found in Andrew Bay. 1) *Conus arestophanes*; 2) *C. betulinus*; 3) *C. capitaneus*; 4) *C. ebraeus*; 5) *C. eburneus*: 6) *C. ferrugineus*; 7) *C. glans*; 8) *C. imperialis*.

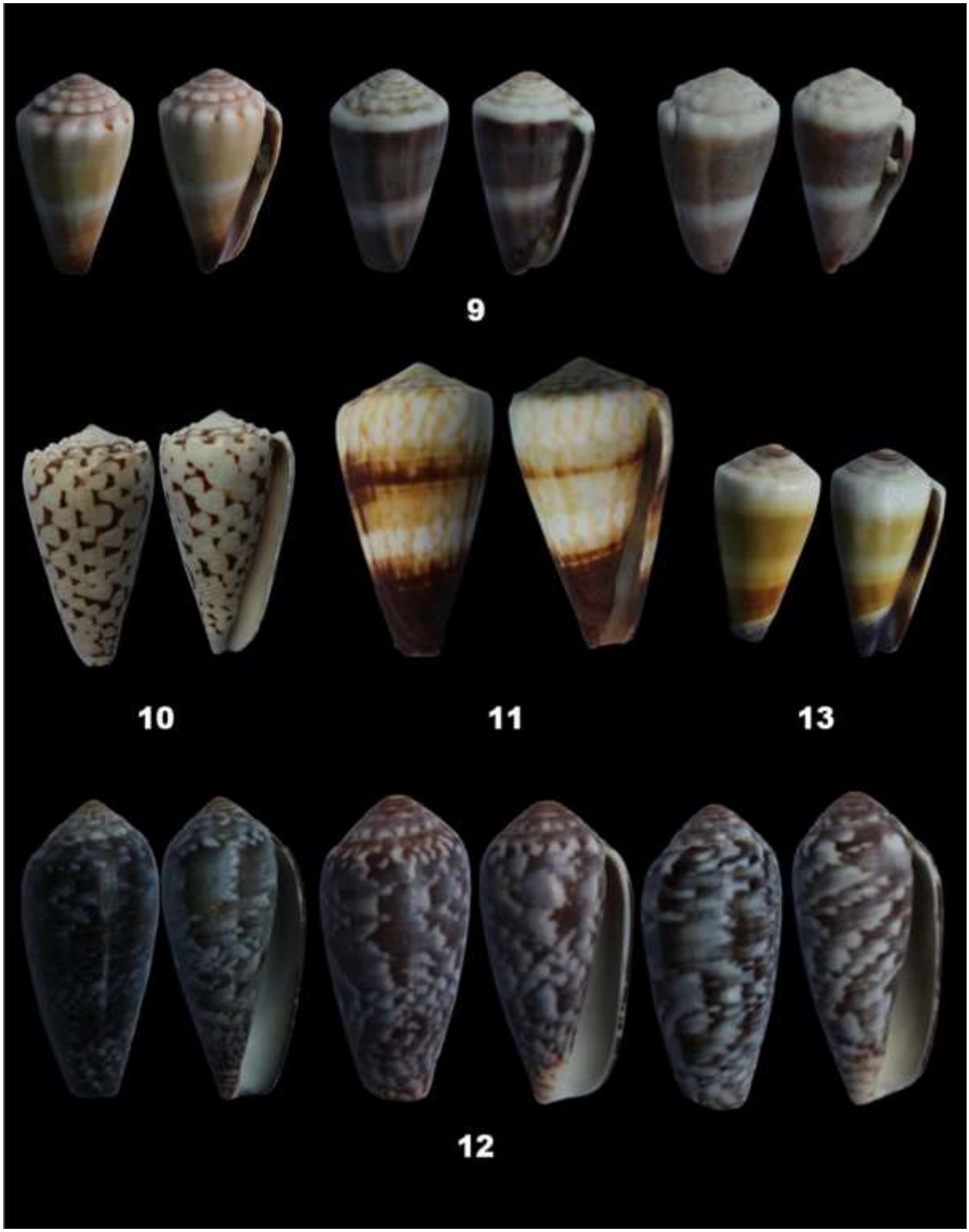

Figure 3 (9-13): *Conus* species found in Andrew Bay. 9) *Conus lividus*; 10) *C. marmoreus*; 11) *C. miles*; 12) *C. monachus*; 13) *C. muriculatus*.

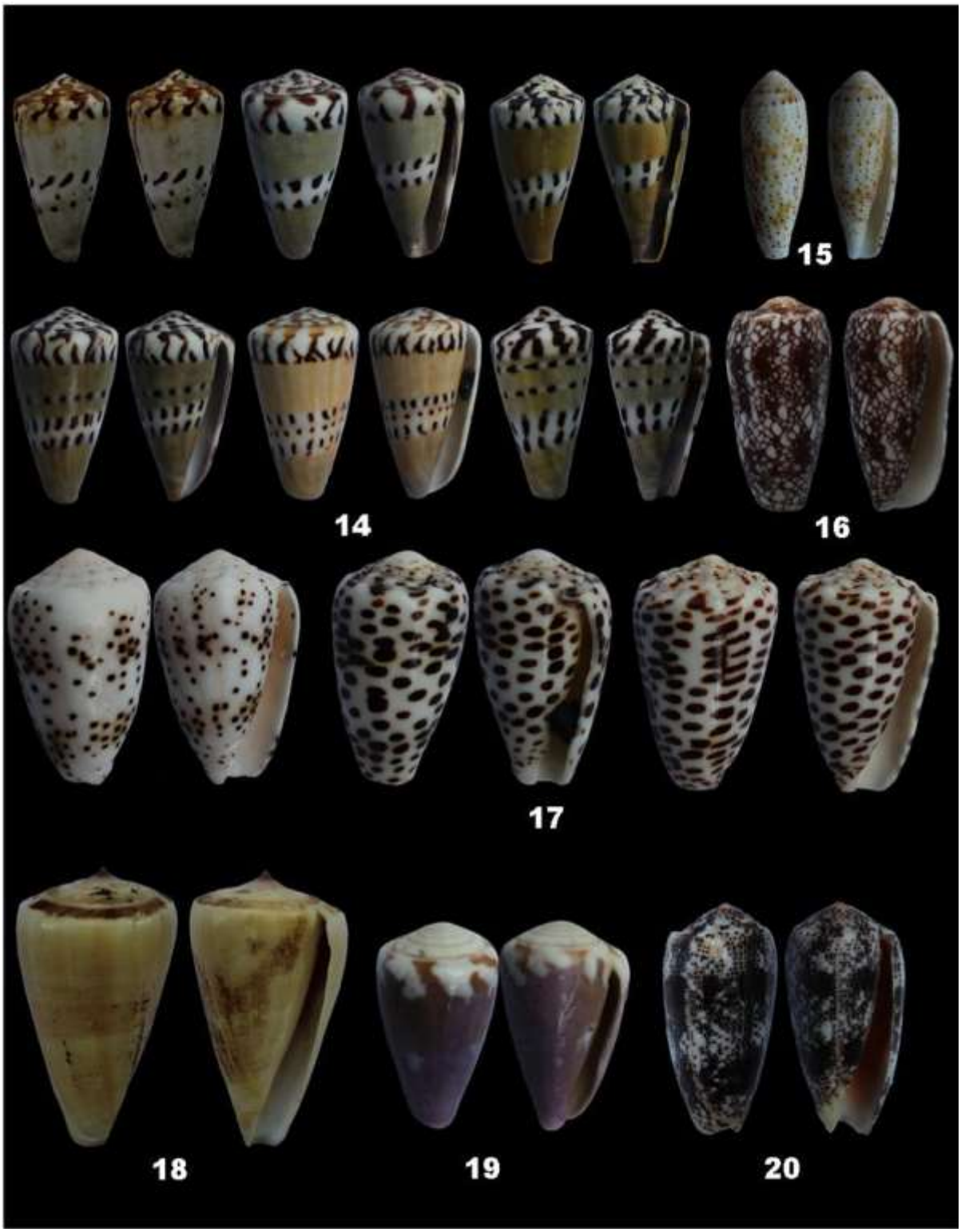

Figure 4 (14-20): *Conus* species found in Andrew Bay. 14) *Conus mustelinus*; 15) *C. nussatella*; 16) *C. omaria*; 17) *C. pulicarius*; 18) *C. quercinus*; 19) *C. rattus*; 20) *C. stercusmuscarum*.

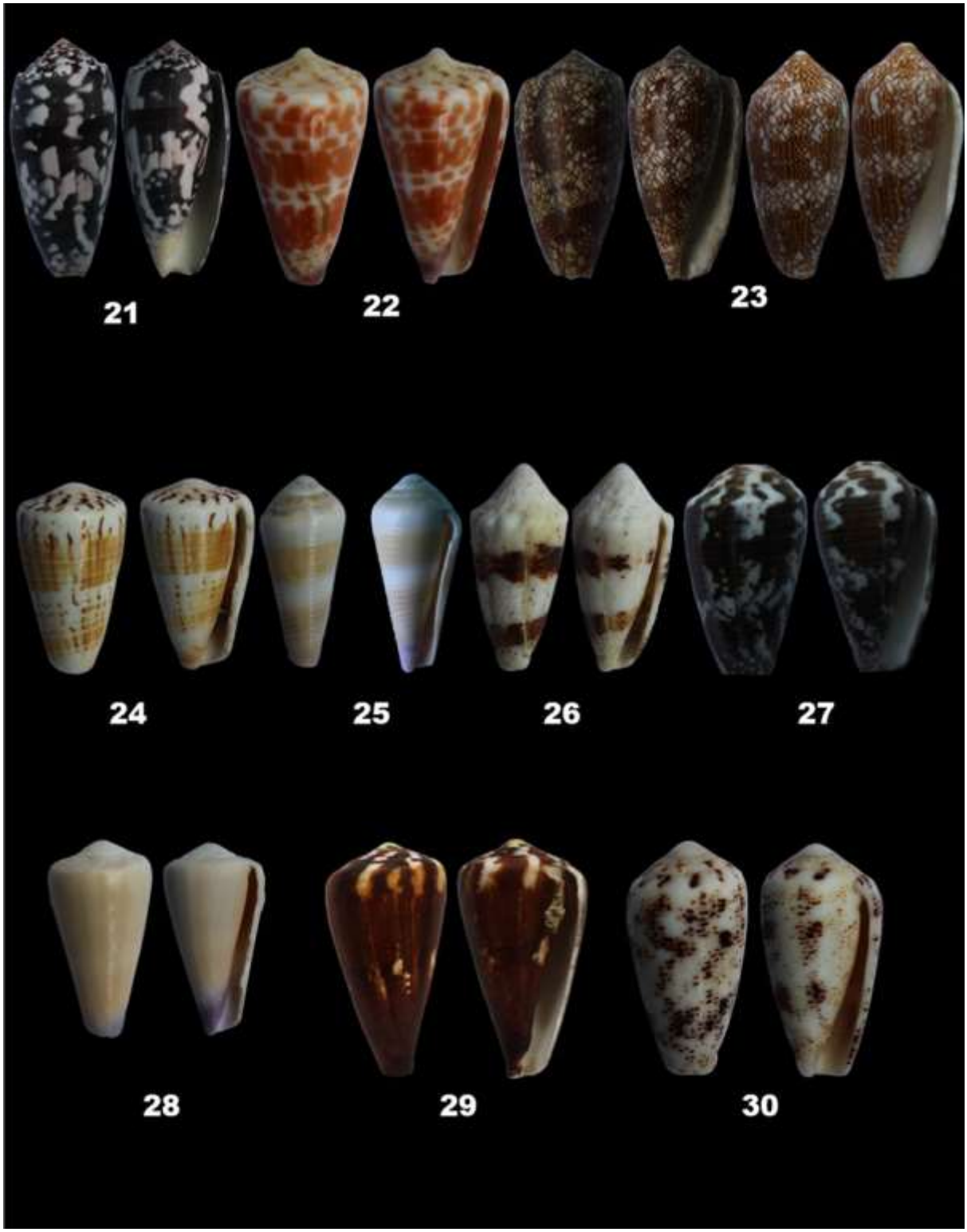

Figure 5 (21-30): *Conus* species found in Andrew Bay. 21) *Conus striatus*; 22) *C. tessulatus*; 23) *C. textile*; 24) *C. thalassiarchus*; 25) *C. terebra*; 26) *C. varius*; 27) *C. vinctus*; 28) *C. virgo*; 29) *C. vexillum*; 30) *C. zeylanicus*.

Acknowledgements

I would like to express my gratitude to Dr. Win Naing, Rector of Sittway University; Dr Khin Maung Zaw, Pro-rector of Sittway University for their permission to undertake this research work. I would like to express my sincere thanks to Dr. Mya Kyawt Wai, Associate Professor and Head of Department of Marine Science in Sittway University for her advices and needful assistance. My final thank goes to local people from my study areas in Rakhine Coastal Region, for their assistance in the sample collections and Daw Lwin Lwin who has put in back breaking hours studying seashell but more importantly has kept me focused.

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STUDY THE VEGETATIVE STRUCTURE OF MANGROVE FROM UTO TIDAL CREEK, SHWE -THAUNG-YAN AREA BY USING LINE TRANSECT METHOD

Khin Thandar Linn[1] , Cherry Aung[2] , S Aung Myo Htay[3]

Abstract

The vegetative structure of mangrove from Uto tidal creek which is situated at the Shwe -Thaung-Yan coastal area has been conducted by using the Line Transect Method (English *et al.*, (1997). The mangrove area at Shwe- Thaung- Yan coastal area is about 2000 ha and comprised a total of 17 true mangrove species. The study area is based the Uto tidal creek which is favorable for the establishment of mangrove. Six transect lines which are perpendicular to the shore line were laid along the tidal creek to determine the vegetative structure of the mangroves. Each transect line is 90m long and three sample points of 30m×10m were intercepted on all transect lines. The visual observation of number of mangrove plants, number of species, number of new shoots, and number of cut branches were recorded. The highest and the smallest plants of different species within each sample points were also identified and measured its height and circumference. All the recorded data were then used to estimate the vegetative structure of the area. The environmental parameters such as soil type and salinity were also then measured. A total of nine true mangrove species such as *Rhizophora mucronata, R. apiculata, Ceriops decandra, Bruguiera gymnorhiza, B.cylindrica, Xylocarpus granatum, Lumnitzera littorea, Aegiceras corniculatum* and *Nypa fruticans* were recorded. Among the recorded species of within the sample points, *Ceriops decandra* and *Bruguiera gymnorhiza* are represented as the dominant species.

Keywords: *Bruguiera gymnorhiza*, *Ceriops decandra*, Line Transect Method, mangrove plants, vegetative structure.

Introduction

Mangrove is a kind of forest virtually confined in the tropics. They are dicotyledonous shrubs or trees growing along tidal mudflats and on shallow coastal water frequently consisting of mono specific patches or belt (Hogarth 2015). The diverse flora and fauna associated with mangrove ecosystems can also provide opportunities for nature education, tourism and scientific study, thereby providing additional social and economic benefits (Guebas *et al*., 2005). Many ecological surveys were carried out using the transect methods in sampling in particular to estimate the population abundance and status of vegetative structure. There are several types of transect which are especially used in any types of forest and the terrestrial and marine biodiversity as well. The common used methods are Line Transect Methods and Belt Transect Method. Line transects are used to illustrate a particular gradient or linear pattern along which communities of plants and, or animals changes. They provide a good way of being able to clearly visualize the changes taking place along the line. A belt transect will supply more data than a line transect. It will give data on the abundance of individual species at different points along the line, as well as on their range (Gates 1979).

The vegetative structure can be ascertained from systematic sampling at fixed intervals along a transect line by graphing the number of species recorded, as a function of the number of samples examined. A transect is a path along which one counts and records occurrences of the objects of study e.g. the plants in the forest or vegetation. It can be made using a nylon rope or measuring tape marked and numbered at with regular intervals, all the way along its length. This is laid across the area of study. The position of the transect line is very important and it depends

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on the direction of the environmental gradient which to be study. The precise position placed end up the clear results for a particular habitat (Burnham *et al*., 1980).

Mangroves, nowadays, are becoming vulnerable to degradation and loss in different areas of the world (Erftemeijer, P. L. A., & Hamerlynck, O. 2005). The number of mangroves has been alarming due to anthropogenic activities that pose a big threat to destruction of the ecosystem and diversity of life. Coastal development, aquaculture, pollution and overharvesting have led to loss of mangroves globally. There are problems that the mangrove ecosystems faced such as habitat destruction, invasive species, over population, over exploitation, and pollution (Spalding. M *et al.*, 2010). The assessment of mangroves plays a critical role in the conservation and protection of the habitat and biodiversity. Estimating the community structure of mangroves is becomes an important consideration in the rehabilitation and monitoring of mangroves especially in the degraded marine ecosystem. (Mcleod,E.,& Salm,R.V.2006). The present study aimed to know the vegetative structure of tidal creek mangrove by using the Line Transect Method (English *et al.*, 1997) and to assess the status of forest type whether vulnerable or grow up which need to conserve for the benefit of coastal biodiversity.

Materials and Methods

An assessment of vegetative structure of Uto tidal creek mangrove at the Shwe -Thaung-Yan coastal area has been carried out by using the Line Transect Method (English *et al.*,1997) in July 2019. The mangrove formation in the area is related with the meandering of the tidal creeks which is intruding to the inner region. Total six transect lines which are perpendicular to the shore line were laid along the tidal creek to determine the vegetative structure of the mangroves. The location of the transect lines has been shown in Figure 1. In this method, each transect line is about 90 m long, on which three 30m×10m sample plots were intercepted by using the measuring tapes. The visual counting of total mangrove plants, total number of species, total new shoots and cuts branches within each of the sample plots were recorded. Then each of 30m point transect, identification of the plant species and recorded their shape, measured their height and circumference of the highest and smallest ones. The data of species composition, number of plants, number of cut branches, number of new shoots, mean height and mean circumferences for all sample plots (3x6 plots) were then used to estimate the vegetative structure. The soil type and texture were also noted. The recorded species were identified according to Hundley and Chit Ko Ko (1961) and John Kress *et al.,* (2004) and Wim Giesen, Stephan Wulffratt, Max Zieren and Liesbeth Scholten, (2006). The location of study areas are

1. Transect 1. Lat N. 17 ˚03′ 30″ Long E.094 ˚27 ′18″
2. Transect 2. Lat N. 17 ˚03′ 55″ Long E.094 ˚27 ′31″
3. Transect 3. Lat N. 17 ˚05′ 03″ Long E.094 ˚29 ′23″
4. Transect 4. Lat N. 17 ˚ 03′ 59″ Long E.094 ˚27′ 10″
5. Transect 5. Lat N. 17 ˚ 04′ 00″ Long E.094 ˚27′ 14″
6. Transect 6. Lat N. 17 ˚ 04′ 37″ Long E.094 ˚27′ 04″

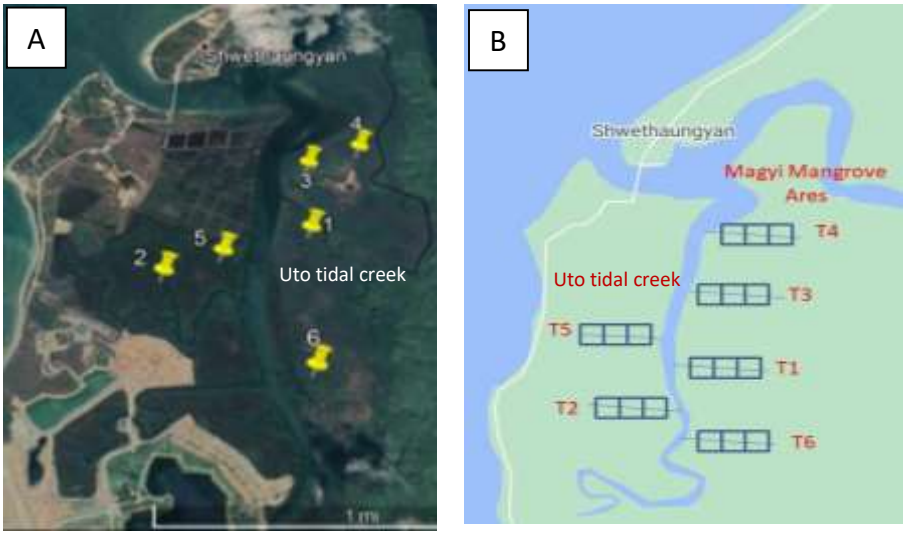

Figure 1 A) Location of the mangrove at Uto tidal creek; B) Sketch map showing the location of the transect.

Results

In the present study, six transect lines were laid for the vegetative structure assessment in the Uto tidal creek, Shwe Thaung Yan coastal area. The tidal creek is meandering into the inner region of the land and favor for good establishment of mangrove plants. The salinity ranges are 10‰ in transect 1, 10 ‰ in transect 2, 12 ‰ in transect 3, 15 ‰ in transect 4, 10‰ in transect 5, 5‰ in transect 6 and most of the substrate is clay-loam. Only 9 true mangrove species were recorded and which will be used to calculate and estimate the vegetative structure within the transect lines. The recorded species were *Rhizophora mucronata, R. apiculata, Ceriops decandra, Bruguiera gymnorhiza, B. cylindrica, Xylocarpus granatum, Lumnitzera littorea, Aegiceras corniculatum* and *Nypa fruticans*. The species composition and the total of individual plants were presented in Table 1. From the table, *Ceriops decandra* showed the highest abundance followed by the *Bruguiera gymnorhiza* and *Rhizophora apiculata* in the area. Transect 2, 3 and 4 represented as the highest number of mangrove composition.

Table 1 Total number of plants and composition of species in the transects

No	Species	T 1	T 2	T 3	T 4	T 5	T 6	Total individuals
1	*C. decandra*	23	31	100	81	50	-	285
2	*R. mucronata*	-	1	-	8	1	-	10
3	*R. apiculata*	32	29	2	5	16	26	110
4	*B. gymnorhiza*	13	97	28	23	31	-	192
5	*B. cylindrica*	-	-	-	-	-	2	2
6	*X. granatum*	-	1	-	-	8	-	9
7	*L. littorea*	-	-	1	-	-	-	1
8	*A. coniculatum*	-	-	-	-	-	1	1
9	*N. fruticans*	-	-	-	-	-	1	1
	Total	**68**	**159**	**131**	**117**	**106**	**30**	

The composition structure of various types of mangrove parameters according to each of the sample plots were presented in Table 2. According to this, the highest composition of species were recorded in transect 2, plot A.

Table 2 The composition structure of plants according to sample plots

T	Plots	No. of plants	No .of new shoot	No. of species	No. of Cut branches	Tallest plant	Smallest plant
1	A	7	10	1	30	1	1
	B	32	27	3	40	3	3
	C	29	50	2	40	2	2
2	A	36	2	5	40	5	5
	B	41	30	3	60	3	3
	C	82	25	3	30	3	3
3	A	37	23	2	60	2	2
	B	70	17	2	30	2	2
	C	24	8	4	50	4	4
4	A	39	11	3	12	3	3
	B	42	15	4	6	4	4
	C	36	10	3	8	3	3
5	A	36	24	4	1	4	4
	B	39	5	3	20	3	3
	C	31	13	3	0	3	3
6	A	16	30	1	0	1	1
	B	7	25	1	0	1	1
	C	7	40	4	0	4	4

T=Transect

The vegetative data including number of recorded species, mean height and mean circumstance of tallest and smallest plants of each of the species, percentage number of plants, percentage of cut branches and percentage of new shoots in all sample plots were presented in Table 3.

Table 3 The vegetative data of mangrove according to transect

T	Plots	Recorded species	Mean Height (m)	Mean Circumference (m)	No. of plants %	No.of new shoot %	No. of Cut branches %
1	A	*R.apiculata*	4.42	1.3	10.29	11.49	27.27
	B	*C.decandra*	1.67	0.85	16.17	31.03	36.36
		B.gymnorhiza	0.48	0.95	19.11		
		R.apiculata	1.67	0.9	11.76		
	C	*R.apiculata*	1.61	1	25	57.47	36.36
		C.decandra	1.17	0.85	17.64		
2	A	*C.decandra*	2.54	1.9	3.77	3.5	30.76
		B.gymnorhiza	1.58	0.95	16.98		
		R.apiculata	3.35	1.5	0.62		
		X.granatum	4.27	1.8	0.62		
		R. mucronata	4.11	1.7	0.62		
	B	*B.gymnorhiza*	1.58	1.05	15.72	52.63	46.15
		R.apiculata	2.44	1.25	5.03		
		C.decandra	1.37	0.8	5.03		
	C	*R.apiculata*	1.83	0.85	12.57	43.85	23.07
		B.gymnorhiza	1.35	0.7	28.30		
		C.decandra	0.96	0.65	10.69		
3	A	*C.decandra*	2.03	1	21.37	47.91	42.85
		B.gymnorhiza	1.52	0.6	6.87		
	B	*C.decandra*	1.75	0.75	45.8	35.41	21.42
		B.gymnorhiza	2.19	1.25	7.63		
	C	*L .littorea*	3.66	2.2	0.76	16.66	35.71
		C.decandra	2.74	1.15	9.16		
		B.gymnorhiza	2.97	1.15	6.87		
		R.apiculata	2.97	1.15	1.52		
4	A	*C.decandra*	2.04	1.2	13.67	30.55	46.15
		B.gymnorhiza	2.28	1.55	16.23		
		R.mucronata	2.01	1.1	3.41		
	B	*C.decandra*	1.67	0.85	29.91	41.66	23.07
		B.gymnorhiza	2.44	1.4	0.85		
		R.apiculata	1.91	0.95	4.27		
		R.mucronata	3.05	1.2	0.85		
	C	*C.decandra*	1.44	0.8	25.64	27.77	30.76
		B.gymnorhiza	2.59	1	2.56		
		R.mucronata	2.59	1.25	2.56		
5	A	*C.decandra*	2.28	1.3	15.09	57.14	4.76
		B.gymnorhiza	3.18	1.95	10.37		
		R.mucronata	6.1	2.1	0.94		
		X.granatum	3.43	1.4	7.54		
	B	*C.decandra*	1.37	1	23.58	11.9	95.23
		B.gymnorhiza	2.44	1.5	7.55		
		R.apiculata	3.41	1.3	5.66		
	C	*C.decandra*	2.28	0.8	8.49	30.95	0
		B.gymnorhiza	2.71	0.9	11.32		
		R.apiculata	3.35	1.65	9.43		
6	A	*R.apiculata*	3.05	1.3	53.33	31.57	0
	B	*R.apiculata*	1.98	0.8	23.33	26.31	0
	C	*B.cylindrica*	2.28	1.35	6.67	42.10	0
		R.apiculata	4.57	1.8	10		
		N.frutican	3.35	25	3.33		
		A.corniculatum	1.73	0.9	3.33		

T=Transect

The percentage compositions of mangrove plants according to the species were showed in Figure 2. According to the studied of total percentage number of species, 99.97%in transect 1, 99.95% in transect 2, 99.98% in transect 3, 99.95% in transect 4, 99.97% in transect 5 and 99.99% in transect 6. In transect 1, three species were recorded and *R.apiculata* representing the highest abundance. In transect 2, five species are distributed and *B. gymnorrhiza* recorded as the highest abundance. In transect 3 and 4, the condition is the same in which four species are recorded and *C. decandra* representing the highest abundance. In transect 5, five species are recorded and *C. decandra* showed as the highest abundance. In transect 6, four species are distributed and *R. apiculata* are recorded as the highest abundance.

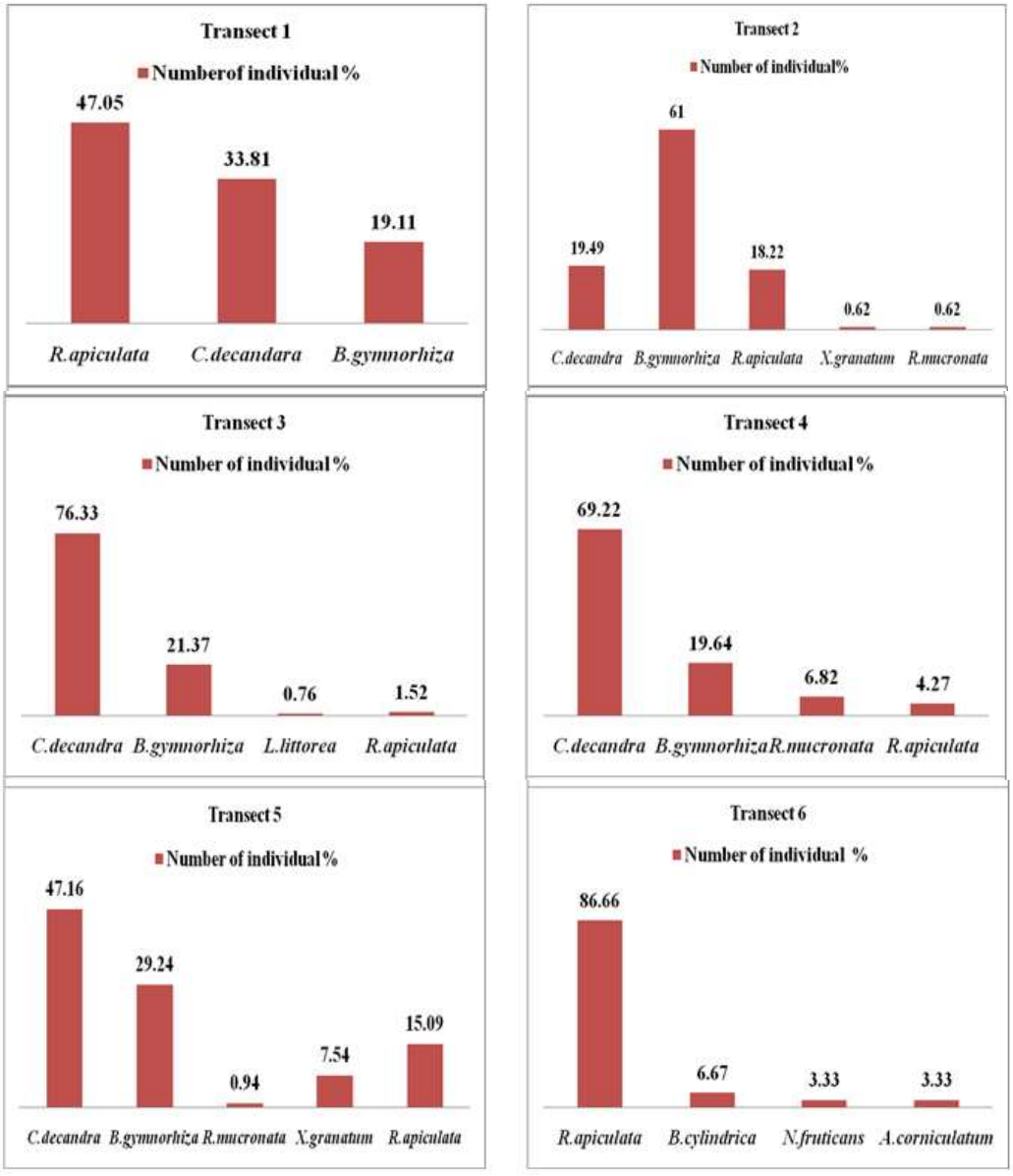

Figure 2 Percentage number of species from six transects point

In this study, the new shoots were also counted to determine the status of survival of recruitment. The highest abundance of recruit plants was observed at transect 6 representing 26% and the lowest was at transect 4 representing 10%. The results were showed in Figure3.

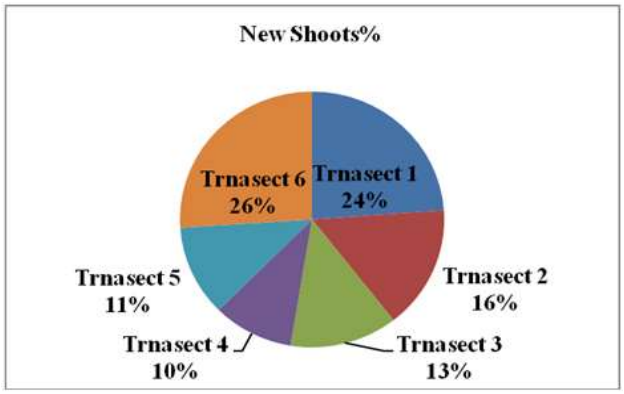

Figure 3 New Shoots percentage of six transects point

Within the transect, the cut branches plants were also counted to determine the status of human interference. Almost all the transect were observed to be encountered the human impacts that cut the plants except transect 6 which was observed to be no cut branches of the plants. The results were showed in Figure4.

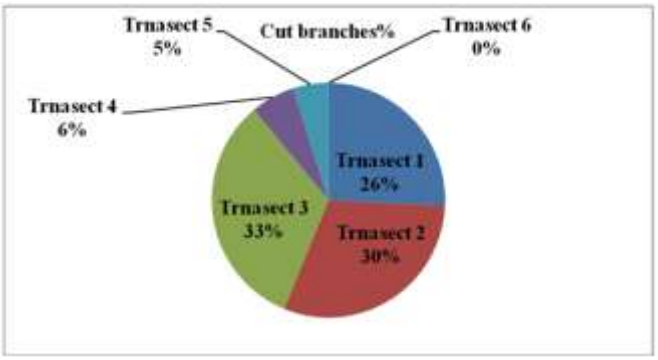

Figure 4 Cut branches percentage of six transects point

According to the recorded species, *Ceriop decandra* is representing the highest abundance followed by *B. gymnorrihiza* and *R. apiculata*. Among the recorded species, *N.fruticans, A.corniculatum, L .littorea, B.cylindrica, R.mucronata* and *X.granatum* are rarely found. The results were showed in Figure 5.

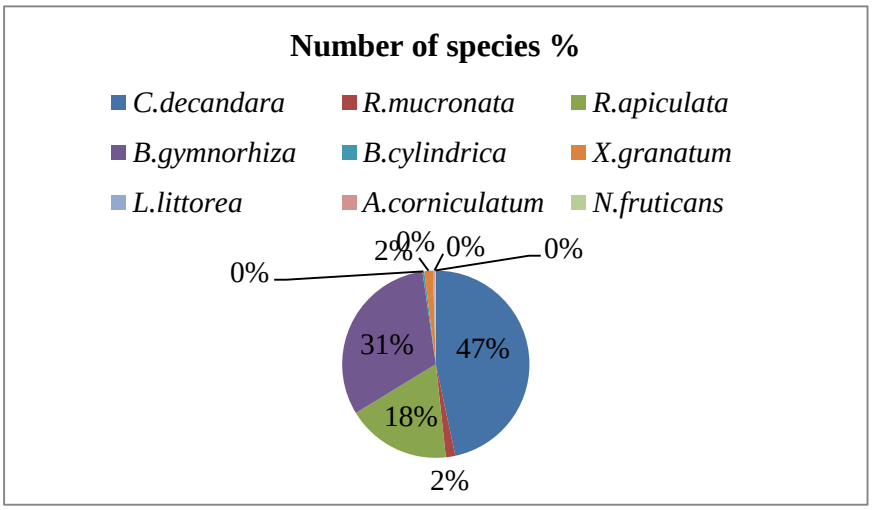

Figure 5 Number of species percentage from six transects point

Table 4 List of plant species recorded in study area

Family	Species	Local name
Rhizophoraceae	*Rhizophora mucronata* Lamk.	Byu-che-dauk ywet-wyine
	Rhizophora apiculata Blume.	Byu-che-dauk ywet-chun
	Ceriops decandra (Griff.)Ding Hou	Madama
	Bruguiera gymnorhiza (L.) Lamk.	Byu-oat-saung
	Bruguiera cylindrica (L.)Bl.	Byu-kyet-tet
Meliaceae	*Xylocarpus granatum* Konig	Pinle-ohn
Combritaceae	*Lumnitzera Littorea* (Jack)Voigt.	Eit-ma-thwe-pwint-ni
Myrsinaceae	*Aegiceras Corniculatum* (L.)Blanco	Yae-Kayar
Arecaceae	*Nypa fruticans* Wurmb.	Dani

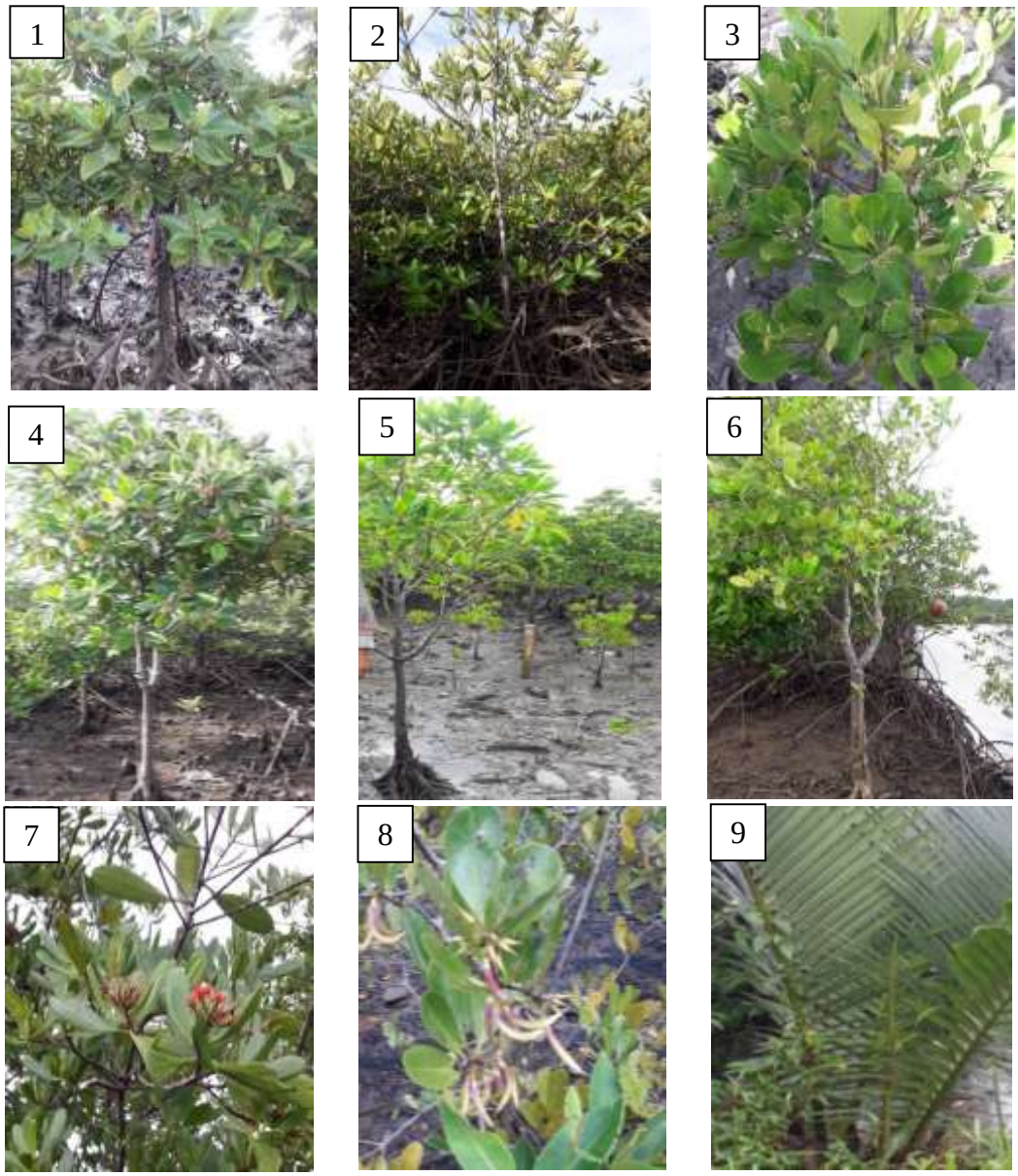

Figure 6. 1)*R. mucronata;* 2)*R.apiculata;* 3)C.*decandra;* 4) *B.gymnorhiza* ; 5)*B.cylindrica;* 6) *X.granatum;* 7) *L .littorea;* 8) *A.corniculatum;* 9) *N.fruticans*

Discussion

Mangrove is tropical maritime trees that have special aerial roots and salt-filtering tap roots. There are several species of mangrove trees found all over the world. Some prefer more salinity, while others like to be very close to a large fresh water source such as river. Some prefer areas that are shelter for waves and some species have their roots cover with sea water every day during high tide. Others are more sensitive to salinity, and grow closer to the shore and other species grow on dry land (Tri *et al*.,1998). In Philippines, majority of the common genera are *Rhizophora*, *Avicennia*, *Bruguiera* and Sonneratia (Calumpong & Menez 1996) Approximately 60 to 70 mangrove and associated mangrove species from 26 families are found in the Philippines. An estimated forty species are considered true mangroves which can be defined as those which are restricted to the mangrove community while associated species may also grow in other habitats (Melana & Gonzales 1996). In Malaysia there are million ha of mangrove forest reserves and the country has rich plant diversity. In terms of mangrove forest cover, Sarawak has the second largest area of mangrove coverage in Malaysia, accounting for 26% of the total (126,400 ha), but only 48% of this is protected as permanent forest reserves (Latiff and FaridahHanum 2014).

In Myanmar, there are found 33 mangrove species and over 100 mangroves and associate. They occurred in southern and south western coastlines but a large number of mangroves species under the threat of extirpation. The dominant species can be found in Myanmar are *Avicennia spp., Rhizophora spp., Bruguiera spp., Ceriops spp., Sonneratia spp., Xylocarpus spp.*, (U Win Maung, 2012). From the recorded of Zockler.C and Aung. C (2019), there is 32 species in Rakhine, 29 species in Ayeyarwady and 43 species Taninthary region.

The present study was implemented to know the status of forest type of an inner mangrove of Uto tidal creek by using the transect method. This method is the simplest ecological sampling method and useful to examine the vegetative structure or community structure of any types biodiversity. In this study area, five families of nine true mangroves species were recorded. The families are Rhizophoraceae, Meliaceae, Combritaceae, Myrsinaceae, Arecaceae and the dominant species Rhizophoraceae found in all six transects points.

Regarding the tallest plants in each species, the tallest *R.mucronata* species was found in transect 5 (height 6.1m/circumference 2.1m); the tallest *R.apiculata* species was found in transect 6 (height 5.5m/ circumference 2.5m); the tallest *C. decandra* species was found in transect 2 (height 3.9m/circumference 2.2m); the tallest *B.gymnorhiza* species was found in transect 5 (height 5.5m/circumference 2.7m); the tallest *B. cylindrica* species was found in transect 6 (height 2.5m/circumference 1.4m); the tallest *X. granatum* species was found in transect 5 (height 4.6m/circumference 1.8m); the tallest *L .littorea* species was found in transect 3 (height 3.7m/circumference 2.2m); the tallest *A. corniculatum* species was found in transect 6 (height 1.7m/circumference 0.9m) and the tallest *N. fruticans* species was found in transect 6 (height 3.4m/circumference 25m). In the study, the new shoots were also counted to determine the status of survival of recruitment. The highest abundance of recruit plants was observed at transect 6 representing (95) individuals and the lowest was at transect 4 representing (36) individuals. Within the transect, the cut branches plants were also counted to determine the status of human interference. Almost all the transect were observed to be encountered the human impacts that cut the plants except transect 6 which was observed to be no cut branches of the plants.

Conclusion

Based on the findings of the study, it was concluded that the plants species are tolerate salinity fluctuation. In summertime, the salinity of this area is nearly 30‰ but in this sample collecting time, the salinity ranges are 5-15‰. The recorded salinity range are 10‰ in transect line 1,2 and 5, the transect 3 has salinity 12 ‰, the transect 4 has salinity 15 ‰, the transect 6 has

salinity 5 ‰ and most of the substrate is clay-loam. There were nine species collected such as *R.mucronata, R.apiculata* C.*decandra* , *B.gymnorhiza* ,*B.cylindrica, X.granatum, L .littorea, A.corniculatum* and *N.fruticans*. Different mangrove vegetation types are followed according to high tide to low tide; upstream to downstream; and soil characters as well. In the present study there is no significant different in vegetation dominant type within the sample plots and transects. The highest total number of plants were recorded in transect 2,3,4 and the lowest at transect 6. The highest percentage of new shoots were observed at transect 6 and the lowest at transect 4. Almost all the transect were representing the highest cut branches but rarely recorded that there is no cut branches at transect 6. Totally about 611 individual plants were recorded within six transects which can represent as the moderately dense area of mangrove. The most dominant species in the study area are C. *decandra*, followed by *B. gymnorhiza* and *R. apiculata.* The rare species in this area are *N.fruticans, A.corniculatum, L .littorea , B.cylindrica, R.mucronata* and *X.granatum.*

Acknowledgements

The author greatly appreciates Rector and Pro-rectors of Pathein University for their kind permission to do this research work. Special thank goes to Dr. Cherry Aung, Professor and Head of Marine Science Department, Pathein University for her valuable suggestions, guidance and literature provided. The author would like to express sincere thanks to Dr Htay Aung (Retired) Professor and Head of Marine Science Department, Pathein University for his critical reviews and suggestions. Also sincere thanks go to Dr. Min Oo, Associate Professor of Marine Science Department, Pathein University for his supporting and giving valuable suggestions.

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GROWTH OF *PERNA VIRIDIS* (LINNAEUS, 1758) FROM EXPERIMENTAL RACK CULTURE IN SITAW, YE ESTUARY, SOUTHERN MON COASTAL AREA

Win Win Nwe[1], Naung Naung Oo[2], Aung Aung Aye[3]

Abstract

The study on the experimental rack culture of *Perna viridis* (Linnaeus, 1758) was conducted in Sitaw, Ye estuary (Lat. 15º 11' N, Long. 97º 48' E), Southern Mon coastal area. The mussel seeds (8.64 ± 0.73) were collected from the rocky shore of Sitaw and planted in "Plastic baskets" to evaluate their growth and survival rate between May 2016 and April 2017. The result showed that the seeded mussels attained 90.03±11.46 mm in length within 1 year with a mean growth rate of 7.28 mm/month. The length frequency data of 60 individuals of green mussels were analyzed using the latest version of the FAO-ICLARM Fish Stock Assessment Tools (FiSAT II). The estimated asymptotic lengths (L_∞) and growth coefficient (k) of the cultivated Asian green mussel in Ye estuary were relatively high at 120.23 mm and 1.3 year^{-1}. The environmental parameters of Ye estuary seem to favor for the growth and high survival rate (>90%) of mussel. The optimum sizes of around 10-25 mm mussels should be seeded and the culture period of around 5 months was optimum in a favorable season. The information provided here will certainly make small scale farming of mussels more popular and generate greater employment opportunities and income in the future.

Keywords: *Perna viridis*, Growth, Southern Mon coastal area, Survival, Ye estuary.

Introduction

The green mussel, *Perna viridis* (Linnaeus, 1758) belongs to the Phylum Mollusca, Class Bivalvia and the Family Mytilidae. This mussel is under Order Mytiloida and Genus *Perna*. Despite *P. viridis* distributes in its native tropical waters of the Indo-Pacific region of Asia (Siddall, 1980), it had been recently introduced to the other regions and finally to South Africa (Micklem, 2014). In Myanmar, *P. viridis* distributes along three coastal areas and this species is locally called Yawt Twar and Gone Sein in Rakhine Coastal Region, Be-wun in Mon Coastal Region, and Kha-yu-nyo in Taninthayi Coastal Region.

P. viridis is extensively cultured due to its high productivity, high tolerance to a wide range of environmental conditions and requiring less farm management (McFarland *et al*., 2013). Commercial cultivation of marine mussel *Perna* is extensively carried out in several countries (FAO, 2017) especially in the Southeast Asian region. Thailand and Philippines are the major green mussel producers followed by India, Malaysia and Singapore (Saraya, 1982; Vakily, 1989; Sasikumar, 2007). Nearly two hundred and eighty two thousand tonnes of *P. viridis* is produced worldwide per year through culture (FAO, 2009). The tropical and subtropical marine mussel *P. viridis* achieves marketable size relatively within a short culture period of about 6 months (Sivalingam, 1977; Smaal, 1991; Rajagopal *et al*., 1998a)

The consuming of green mussel in Mon coastal area is only dependent on the natural mussel beds. Rich green mussel beds are in the subtidal rocky substrates and stretches up to in Ye River. Mussels are removed from natural bed, mainly for local consumption as food and for local fishermen income. The mussel fishing is done as off times occupation by the fishermen mainly during 7 a.m to 9 a.m. They collect mussels from intertidal rocks using iron implements like chisels with or without wooden handle or a knife during low tide. Some of them collect the mussels from

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the submerged mussel bed by diving with the aid of air compressor. The green mussel fishery, however, is self-regulated in Sitaw, Ye estuary. The mussel pickers stop mussel picking during the southwest monsoon season because of turbulence and strong current in this season. The peak mussel picking occur during the post monsoon season (September to May) after the self-imposed control on mussel picking during the monsoon months. After fishing, they dispose them off to the customers in fresh condition. In recent years the fishermen exploit mussels from the natural beds even before the mussels reach the harvestable size and the population of mussels on this area is, therefore, being reduced.

Perna viridis meat is one of the chief protein sources for human consumption from marine resources and it is popular for its delicious taste, containing high nutritional values (Taib *et al*., 2016). Some aspects of their biology relevant to fishery and culture have, therefore, been studied by several workers around the world. There are only few researches on the biology and culture of *P. viridis* in Myanmar. Tin Nu (1985) studied the spatfall of *P. viridis* in the present study area. She investigated the monthly growth rate, survival and mortality of *P. viridis* and discussed their relationship with the environmental parameters. Myo Nandar Myint (2014) studied on the spat fall of green mussel *P. viridis* in Myeik coastal waters. She stated the general diagnostic characters of this species. The techniques for mass culture of *P. viridis* are required to fulfill the food requirement for local people and their employment at Myanmar coastal areas. This study aims to investigate the growth performance and survival of *P. viridis* cultivated on rack system in Ye estuary and to initiate the green mussel farming in coastal waters of Myanmar.

Materials and Methods

Study area: The study on the experimental culture of green mussel was conducted at Sitaw, Ye estuary (Lat. 15º 11' N, Long. 97º 48' E), about three-fourth mile to the southeast across the mouth of Ye River, from Zeephyuthaung Village, Ye Township, southern Mon coastal area (Fig. 1). Sitaw is an intertidal rocky shore area and it is known for a daily level fishery of fish, gastropods, green mussel and oyster. The river is mostly rain-fed and the flow is closely related to the seasonal rainfall. During the monsoon season (June-September), the river frequently overflows while during the dry months (November-April), the river often experience periods of reduced flow. During low water spring tide, sand bars are formed in the mouth of river.

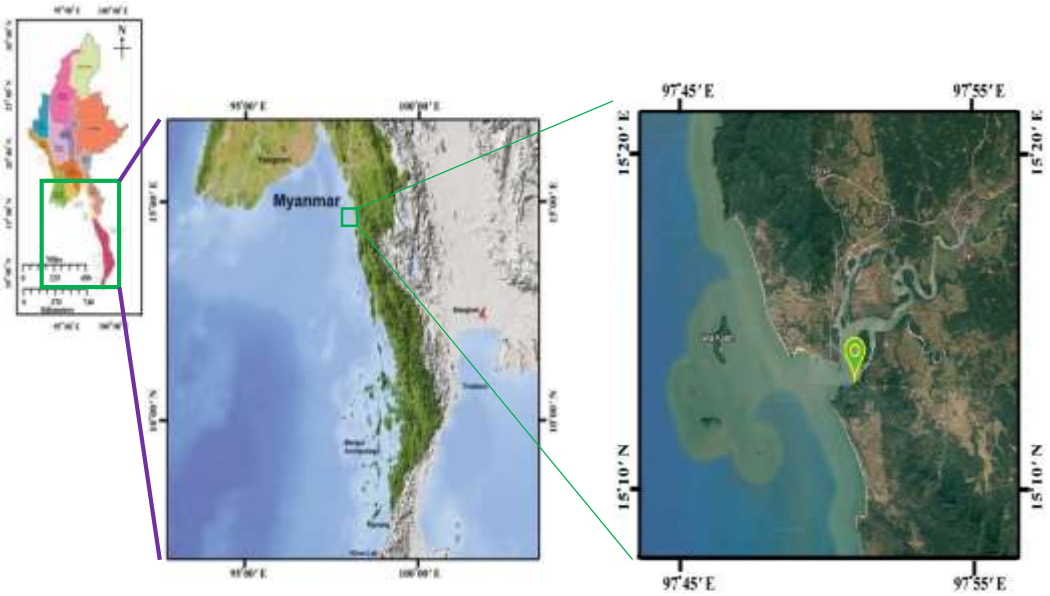

Figure 1 Map showing the experimental rack culture site in Sitaw (Source: Google Maps).

Rack structure and mussel seeding: For studying the mussel growth, a wooden rack (4 m x 2.5 m) was constructed in the subtidal zone of Sitaw, Ye estuary (Fig. 2 A) where water depth was about 2 m at low water spring tide and about 7 m at high water spring tide. Mussel seeds of length about 10 mm (Fig. 2B) collected by using a chisel from their natural habitat in the intertidal rocky shore of Sitaw were cleaned and placed into the cages. The baskets (2ft length ×1.5ft length × 1ft height) were used as the cages for mussel seeding. Then the seeded cages were tied with nylon ropes and suspended in the water column from the rack with the bottom of the baskets about 1 m above the sea bed to study growth rate and survival rate of the mussels.

Figure 2 A-C. Experimental rack culture of *Perna viridis*: **A**) Rack for mussel seeding; **B**) Mussel seeds on the rocky shore; **C**) Measuring shell length.

Envirionmental Parameters: The mean values of salinity, temperature and pH were recorded by using Horiba water monitor, refractometer and pH meter at monthly interval. There was marked fluctuations in the salinity during monsoon seasons. The average salinity of the surface water varied from 6.54‰ in August 2016 to 31.4 ‰ in March 2016 and it also varied from 6.38‰ in June 2017 to 30.50‰ in March 2017. Surface water temperature ranged from 27.5°C to 32.2°C in 2016 and from 27.0°C to 32.6°C in 2017. During the experiment, the pH range of the surface seawater was narrow, from 6.4 to 7.6 in 2016 and from 6.2 to 7.4 in 2017. Seasonal variations in environmental parameters of Sitaw are primarily influenced by the prevailing monsoon regime. During the study period, southwest monsoon commenced by the last week of May and the highest rainfall 1210.8 mm was recorded in August 2016 and 1628.14 mm in July 2017.

Data Analysis

Growth Rate: For the analysis on growth rate of *P. viridis* cultured at Ye estuary, the shell length of each specimen was monthly measured to the nearest 0.01 mm with Vernier Calipers (Fig. 2C) and the length data was divided into 8 mm length classes. As no sexual dimorphism could be discerned externally, no effort was made to study the growth related to sex. The data were analyzed using FiSAT II software by length-frequency data analysis (Gayanilo *et al*., 1996).

Asymptotic length (L_∞) and growth co-efficient (k) of the von Bertalanffy Growth Formula (VBGF) were estimated by means of ELEFAN-1 (Pauly and David, 1981). The emphirical growth curve was fitted to the total length data using the von Bertalanffy's growth equation: $L_t = L_\infty [1\text{-}e^{-k(t\text{-}t_0)}]$ where, L_t = length at time 't'; L_∞= length at infinity (asymptotic length); e = base of the natural logarithm; k = growth coefficient; t = time of observation; and t_0 = arbitrary origin of growth.The growth coefficient (k) was estimated by the least square method following

Bal and Rao (1994) as b=e* where b is the slope of the equation l_t =1 = a + bl_t, and 'e' is the base of natural logarithm.

Specific growth rates for each month were calculated from the mean monthly length attained by the animal according to the formula of Bal and Jones (1960):

$$\mathbf{SGR} = \frac{\mathrm{Log_e}\, L_2 - \mathrm{Log_e}\, L_1}{T_2 - T_1} \times 100$$

Where, $\mathrm{Log_e}$ = natural logarithm, L_2 and L_1 = the shell length at time T_2 and T_1 respectively and the growth was expressed in percentage per month.

Survival Rate: To determine the survival rate of mussels, every individual of each cage were counted for the number of alive mussel. Any mussel having its valve open or having the smell of decomposition was treated as a dead one. The survival of the mussel was expressed in percentage and was calculated using the formula (Vural *et al*., 2015):

$$S=n/N \times 100.$$

Where, S= the survival rate (%) for each sampling, n = the number live mussels for each sampling and N = the initial number stocking. Finally, at the close of experiment, the entire stock of mussels was collected and survival rates (%) were computed from the initial and final data.

Results and Discussion

Growth Rate: The mean monthly length of *Perna viridis* in this study suggests that the animal attained a length of 90.03 mm in a year and the growth rate was 7.28 mm/month (Fig. 3A & Table1). The highest growth rates in *P. viridis* were reported with a maximum growth rate of 9.3 mm/month in the Parasan coastal waters of Indonesia, (Noor *et al*., 2019) and 10 mm/month on the Philippine seashore (Yap *et al*., 1979). Kamal and Khan (1998) reported that the growth of green mussels inhabiting the Moheshkhali jetty, Bangladesh attained a length of 88.12 mm in a year and the growth rate was 7.34 mm/month. The monthly growth rate of 7.2 mm, 6 mm and 5 mm was observed by Sreenivasan *et al*., (1989), Rao *et al*., (1975), and Qasim *et al*., (1977), respectively, for *P. viridis* by the end of the first year, from Indian natural environments. The growth rate of *P. viridis* in the Turpialito Hydrological Station located in the Gulf of Cariaco, north eastern Venezuela was reported as 7.1 mm/month (Urbano *et al*., 2005). The lowest growth rate in *P. viridis* was reported in Hong Kong waters, with a maximum growth rate of 5.1 mm/month (Cheung, 1933). This comparison reveals that the growth of *P. viridis* cultured in Sitaw waters grows faster than those inhabiting some Indian waters and Hong Kong water while this result was slower than those of Indonesia waters and Bangladesh waters.

Figure 3 **A**-C. Experimental grow out culture at Sitaw: A) Growing mussels; B) Accumulation of silt and invertebrate worms in the cage; C) Attachment of oysters on the cage.

Table 1 Average observed length, growth increment, specific growth rate and the length as determined by the von Bertalanffy's growth equation of *Perna viridis* in Ye River Estuary.

Month	Average observed length (mm) (Mean ±S.D.)	Growth increment (mm)	Specific growth rate(%)	Length determined by growth equation $L_t = L_\infty[1-e^{-k(t-t_0)}]$
May. 2016	8.64±0.73	-	90.56	14.26
Jun.	21.37±1.94	11.37	37.76	25.14
Jul.	31.17±1.88	9.80	25.36	34.91
Aug.	40.17±2.02	9.00	19.40	43.66
Sept.	48.77±3.37	8.60	14.00	51.52
Oct.	56.10±4.92	7.33	13.38	58.58
Nov.	64.13±6.34	8.03	8.47	64.91
Dec.	69.80±7.81	5.67	8.03	70.59
Jan. 2017	75.63±8.85	5.83	7.96	75.69
Feb.	81.90±10.55	6.27	6.92	80.26
Mar.	87.77±11.55	5.87	2.54	84.36
Apr.	90.03±11.46	2.26	_	88.05
Mean		7.28		

The tropical *P. viridis* farming has a harvesting phase commences when the mussels reach minimum commercial size. An optimal harvest of marketable size is achieved after a culture period of 6 months (Sivalingam, 1977; Rivonkar *et al*., 1993), while Mohamed (2015), stated the harvestable size reached within 4-6 months. The size of *P. viridis* in Pasaran island waters, Indonesia, can reach 57 mm in six months and 79.8 mm in 11 months cultivation (Noor *et al*., 2019). Cheung 1993 reported that *P. viridis* from Hong Kong waters is only able to reach a size of 60 mm/year due to the contaminated and unhealthy water in the cultivation area. Qasim *et al*., (1977) reported that mussels attained marketable size (60-64 mm) in 5 months by rope culture in natural beds at Goa, India. Rao *et al*., (1975) found that mussels reach 60 mm in length in 6 months on floating buoys at Vengurla Bay in the Arabian Sea. Kamal and Khan (1998) also observed that this species grow to 65 mm in length in 6 months on poles of Moheshkhali jetty at Moheshkhali channel, Bangladesh.

Jakate *et al*., (2009) reported green mussels from initial length of 31.2 mm attained the marketable size shell lengths of 63.3 mm and 62.3 mm on nylon rope and nylon strip substrata, respectively, within 5 months. After 5 months, the growth rate was reduced due to freshwater influx, resulting in final average shell length of 67.3 mm for the nylon rope and 65.5 mm for the nylon strip rens, in 7 months. However, Hickman (1992) reported that the marketable size of *P. viridis* can be achieved only after 12-24 months cultivation period in subtropical mussel farming. In the present investigation, mussels attained 64.13 mm within 6 months (Table 1) that shows the potentiality of the present study site as a culture ground.

The growth increment and the specific growth rate of *P. viridis* were faster at the early age than in the later stages (Table 1). The decrease in growth rate and specific growth rate was observed with increase in shell length. These results were agreed with the findings of several workers for *P. viridis* and similar reduction in growth rates were observed in older mussels (Rivonkar *et al*., 1993; Kamal and Khan, 1998; Rajagopal *et al*., 1998a; Kripa *et al*., 2009; Awan *et al*., 2012). The reason for this reduced growth rate may be the reduction in metabolic activity with age, which accounts for decreased growth rate as seen from reduction in specific growth rate.

Growth performance: The values estimated for the parameters of the von Bertalanffy's growth equation are shown in table 1. Substituting these values in the von Bertalanffy's growth equation, the equations for rack culture could be expressed as $L_t=120.23[1-e^{-1.3(t+0.01381)}]$. From the theoretical growth curves, it can be observed from the rack culture that animal attains a length of 88.05 mm, 111.46 mm, and 117.84 mm at the age of 1, 2 and 3 years, respectively (Fig. 4). The computed growth curve with these parameters has been shown over the restructured length distribution in figure 4.

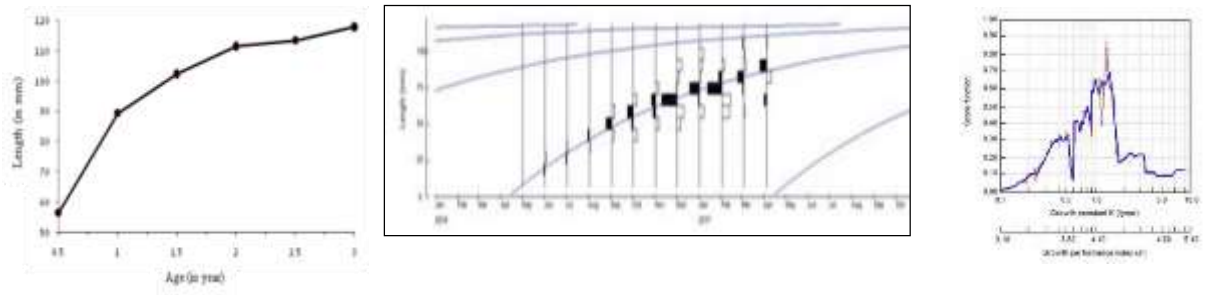

Figure 4 Theoretical growth curve and Von Bertalanffy's growth curves (L_∞= 120.23mm and k= 1.3 year^{-1} for *Perna viridis* superimposed on restructured length-frequency histograms. Black and white bars = positive and negative deviation from 'weighted' moving average of length classes. Number of sample = 60 individuals.)

The asymptotic length (L_∞) suggests the maximum theoretical length of an organism can attain under given rate of growth. The present estimates of L_∞ =120.23 mm is likely to be more accurate estimates for *P. viridis* because the mean monthly length of the mussels varied from 8.64 mm to 90.03 mm (Table 1). In addition, the minimum and maximum length observed from the present rack culture in a year was 62 mm and 115 mm, respectively. This is future supported by the maximum recorded sizes (L_{max} = 115 mm) of the mussels which was very near the estimated values of (120.23 mm). Though variations in L_∞ are related to feeding efficiency, but L_{max}/ L_∞ is constant for a given species.

In the present study, the asymptotic length (L_∞ =120.23 mm) of the cultivated green mussel in Sitaw is closed to those reported in Myeik coastal warters, Southern Myanmar at 118.13 mm (Myo Nandar Myint, 2014) and also closed to those reported in other Asian countries particularly in Malaysia and Thailand at 113.4 mm and 112.0 mm (Taib *et al*., 2016; Tuaycharoen *et al*., 1988). This result also resemble to those reported Bangladesh at 124.6 mm (Kamel and Khan, 1998). This result is higher than those reported at 41.9 mm in India (Rivonkar *et al*., 1993), at 102.4 mm in Malaysia (Al-Barwani *et al*., 2007) and at 101.9 mm in Hongkong (Lee, 1985) while it is lower than those reported at 136.9 mm and 184 mm from India waters (Narasimham, 1981; Hemachandra *et al*., 2017) and at 136.5 mm from offshore waters of Naf River Coast, Bangladesh (Khan *et al*., 2010).

Hemachandra *et al*., (2017) stated that the coefficient "k" (the rate at which the animal approaches the theoretical maximum) can be used to compare between the growth of related species or same species in varied habitats. The "k" value (1.3 yr^{-1}) of the mussel in Sitaw, Ye estuary, was higher compared to those in Myeik coastal waters at 0.37 yr^{-1} (Myo Nandar Myint, 2014), in India waters at 0.25 yr^{-1}, 0.10 yr^{-1} , 0.11 yr^{-1} respectively (Narasimham, 1981; Rivonkar *et al*., 1993; Hemachandra *et al*., 2017), in Thailand at 1.00 yr^{-1} (Tuaycharoen *et al*., 1988), in Hong Kong at 0.30 yr^{-1} (Lee, 1985), and in Moheshkhali channel, Bay of Bengal, Bangladesh at 0.11 yr^{-1} (Kamel and Khan, 1998). The present "k" value (1.3 yr^{-1}) was closed to the "k" values (1.5 yr^{-1} and 1.7 yr^{-1}) from Malaysia waters (Al-Barwani *et al*., 2007; Taib *et al*., 2016). Interestingly, this value was similar to that of *P. viridis* reported from offshore waters of Naf River Coast, Bangladesh (Khan *et al*., 2010).

During the experimental rack culture period, only one dead mussel was observed from 60 seeded mussels in August although the accumulation of silt was occurred throughout the culture period. Therefore, 98.33% survival rate was observed in August and 100% survival was investigated in the other months. The high survival rate of *P. viridis* in Sitaw waters may be due to the environmental conditions of the study site and due to the tolerance of this species. The green mussel is reported to have tolerance for reduced salinities (Segnini *et al.,* 1998; Sallih, 2005). It can grow in water salinity ranging from 5.2‰ to 39.8 ‰ (Rajagopal *et al.,* 1998b). Green mussels in the Indo-Pacific region experience an average annual water temperature range between 12 and 32 ºC (Rajagopal *et al*., 2006), with an optimal range between 26-32ºC (Power *et al*., 2004). The acceptable pH range for most finfish and shellfish species is reported as 6.0-8.2 (Sivalingam, 1977) and 6.8-8.5 (Sasikumar, 2007). Segnini *et al*., (1998) stated that *P. viridis* can colonize even muddy sediments, point to the high level of tolerance of green mussels to high suspended particulate matter. Shin *et al*., (2002) also stated that *P. viridis* can tolerate a high level of suspended particulate matter (up to 1200 mg l).

The fouling organisms such as shrimps, young fishs, oysters, polychaetes, barnacles and gastropods were found on the rack and cages during the present study (Fig. 3B & 3C). The observation of fouling organisms was also reported by Jayalakshmy *et al.,* (2013), Myo Nandar Myint (2014) and Anil *et al*., (2017). The epibiotic barnacles were observed throughout the culture period in Sitaw. Qasim *et al*., (1977), Soundarajan *et al*., (1988) and Karayucel *et al*., (2002) described that fouling of mussels by barnacles was heavy over the culture materials. Bell (2013) reported that there is no negative impact of epibiotic barnacles on the growth of mussels and barnacle epibionts create a new interface between the mussel and its environment and this interaction can affect other members of the community.

The present study area is regarded as a suitable site for mussel fariming because they ranged within the suggested good conditions for mussels (Rajagopal *et al.,* 1998b; Rajagopal *et al*., 2006; Power *et al*., 2004; Sivalingam, 1977; Sasikumar, 2007). The absence of diffuse agricultural input, domestic and industrial discharges in the study area seem to observe the healthy mussels from this area. The ideal values for a suitable culture site are 27 to 35‰ for salinity; 27 to 30 ºC for water temperature; and 7.7 to 8.4 for pH (Aypa; Soon and Ransangan, 2016). Appukuttan *et al*., (2000) observed that the growth of mussels was much faster in the open sea than in estuaries. However, due to lack of security of farm structure and farmed stock in the open sea (Kripa and Mohamed, 2008), estuaries are the preferred locations for mussel farming and 250 estuarine mussel farms are existing in India and Thailand (Mohamed *et al*., 2003; Prakoon *et al*., 2010). Sitaw, Ye estuary, therefore, should be selected for the culture of *P. viridis* and the optimum sizes of about 10-25 mm should be seeded and the culture period of around 5 months was optimum in a favorable season.

Conclusion

The growth rate (1.3 $year^{-1}$) of *P. viridis* observed from the present study is faster than that of reported for the same species from Indian, Thailand, Hong Kong, Moheshkhali channel, Bangladesh and Myeik waters (Myanmar) and a little slower than that of Malaysia waters. However the growth rate was the same with offshore waters of Naf River Coast, Bangladesh. This variation of mussel's growth rate is significantly influenced by initial seeded mussel size, geographic region, and different cultivation methods. The high survival rate (>90%), increase in the lenght of the shell, high asymptotic lenght and growth coefficient value observed from the present study may be due to the environmental conditions of the study site and due to the tolerance of this species. These reults also indicate that Sitaw, Ye estuary is regarded as a suitable site for mussel farming. The information provided here will certainly make small scale farming of mussels more popular and generate greater employment opportunities and income in the future. However,

the natural seed resource on the mussel beds in Sitaw, Ye estuary, cannot support mussel culture industry of some magnitude. Therefore, induced spawning, larval rearing and seed production should be carried out in the hatchery to initiate the mussel farming in Myanmar.

Acknowledgements

I would like to express my gratitude to Dr. Win Naing, Rector of Sittway University and Dr. Khin Maung Zaw, Pro-rector of Sittway University for their permission to undertake this research work. I would like to express my sincere thanks to Professor Dr. San Tha Tun, Head of Marine Science Department, Mawlamyine University, for his valuable guidance, close supervision and for providing with the departmental facilities during this study. I am very grateful to Dr. Mra Kyawt Wai, Professor, Head of Marine Science Department, Sittway University, for her encouragement during preparing the manuscript. I sincerely extend my thankful appreciation to the anonymous reviewers of the manuscript for the valuable comments and constructive criticisms. My final thank goes to U Luu Pyan and local people from Zeephyuthaung Village, for their selfless help throughout the study period.

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ENVIRONMENTAL CONTROL OF SEASONAL VARIATIONS IN THE PHYTOPLANKTON COMMUNITY STRUCTURE ALONG THE COAST OF TANINTHARYI, SOUTHERN MYANMAR

Khin Khin Gyi[1], Wint Thuzar Nwe[2], Myo Min Tun[3], Sein Moh Moh Khaing[4]

Abstract

The seasonal fluctuations in phytoplankton assemblages and the controlling environmental factors were investigated for 24 months along the coast of Tanintharyi from June 2013 to June 2015. A wide fluctuation in cell abundance 72,450-714,396 cells l^{-1} at Kawthaung, 47,416-947,501 cells l^{-1} at Myeik, 8,930-28,439 cells l^{-1} at Kampani, 8,976-17,888 cells l^{-1} at Ye and 5,162-16,986 cells l^{-1} at Setse were noted during the study period. Amongst, Kawthaung and Myeik stations had the highest phytoplankton abundance whereas Ye and Setse stations showed remarkably lower abundance. A clear seasonal trend was found at all five stations with a sharp peak in the pre-monsoon months and a gradual decrease in the monsoon and post-monsoon periods. Phosphate concentration was relatively high at Myeik and Kawthaung stations, and also the increase of water temperature and salinity in the pre-monsoon months may probably stimulate a high abundance of phytoplankton. On the other hand, a decrease of phytoplankton abundance was found in the monsoon season which was characterized by low water temperature, low salinity, and low level of nutrients.

Keywords: Abundance, monsoon, nutrients, phosphate, pre-monsoon, salinity, water temperature

Introduction

Phytoplankton community is an important biological component of the aquatic ecosystem, constituting a central role in aquatic food webs. They constitute not only half of the global primary production (Field et al. 1998) but they also play an important role in nutrients cycling, such as nitrogen, phosphorous, and carbon. Moreover, through diverse strategies for nutrient uptake, phytoplankton community composition affects rates and fluxes of elements in the ecosystem (Falkowski et al. 1998). Phytoplankton communities are regulated by both bottom-up and top-down processes (Alpine and Cloern 1992), interactions among phytoplankton taxa likely also affect community structure and function (Griffiths et al. 2016). Structural changes in the phytoplankton community are also strongly related to the physical, chemical, and biological features of water bodies and are expected to be highly sensitive to ongoing environmental changes. Variations of abiotic water conditions occur naturally throughout the day and over the seasons of the year (Gast et al. 2014). Therefore, the investigation of structural aspects focuses on the whole phytoplankton community is an important approach for understanding the aquatic ecosystems.

Concerning the phytoplankton community, there have been reported by several studies focused on the species distribution, occurrence and abundance (Si Thu Hein 2010, Khin Yu Nwe 2011, Lett Wai Nwe 2011, Tin Tin Kyu 2012, Zin Mar Aye 2012, Aung Myo Hsan 2013, Thida Nyunt 2013, Zarni Ko Ko 2014 and 2018, Yin Yin Htay et al. 2019). However, seasonal influences of environmental parameters on the variations of the phytoplankton community have not been reported yet. Here in the present study, I analyzed the environmental factors that control phytoplankton variability through 2-year study periods along the coast of Tanintharyi, southern Myanmar.

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Materials and Methods

Sampling site

Phytoplankton samples were collected at the five stations along the Tanintharyi coastline, namely, Kawthaung (Lat. 9º 58. 204′ N, Long. 98º 33. 701′ E), Myeik (Lat. 12º 26. 186′ N, Long. 98º 35. 461′ E), Kampani (Lat. 14º 05. 288′ N, Long. 98º 04. 143′ E), Ye (Lat. 15º 11. 585′ N, Long. 97º 47. 518′ E) and Setse (Lat. 15º 56. 965′ N, Long. 97º 36. 330′ E) from June 2013 to June 2015 (Fig.1). The sampling areas were influenced by the monsoon season. Therefore, a calendar year was divided into three seasons for ecological purposes. The division for three seasons was based on changes in the temperature in the annual cycle of the region. The seasons were recognized as the pre-monsoon period (February to May), the monsoon period (June to September) and the post-monsoon period (October to January).

Sample collection

A small-mesh phytoplankton net of 20 µm is used in the sample collection. The phytoplankton samples of the surface water were collected by a plastic bucket of known volume water, 60L. Then, the water passed through the mesh fixed to the bottom of a plastic cylinder. Care was taken to wash all the cells off the sieve. Samples were preserved immediately with a 1% formaldehyde solution. In the laboratory, samples were analyzed for species identification and counting. While collecting the water samples, temperature, salinity, and pH were measured in-situ with the help of a digital thermometer, salinity refractometer, and portable Hanna pH meter. Water samples for nutrients were also collected at each station. For nutrient analyses, water samples were filtered with 0.2 µm pore sized Millipore filter and frozen for later analysis of nitrate (NO_3-N), phosphate (PO_4-P), and ammonia (NH_4-N). Then, samples were sent to the Ministry of Fishery and Livestock in Yangon for analysis.

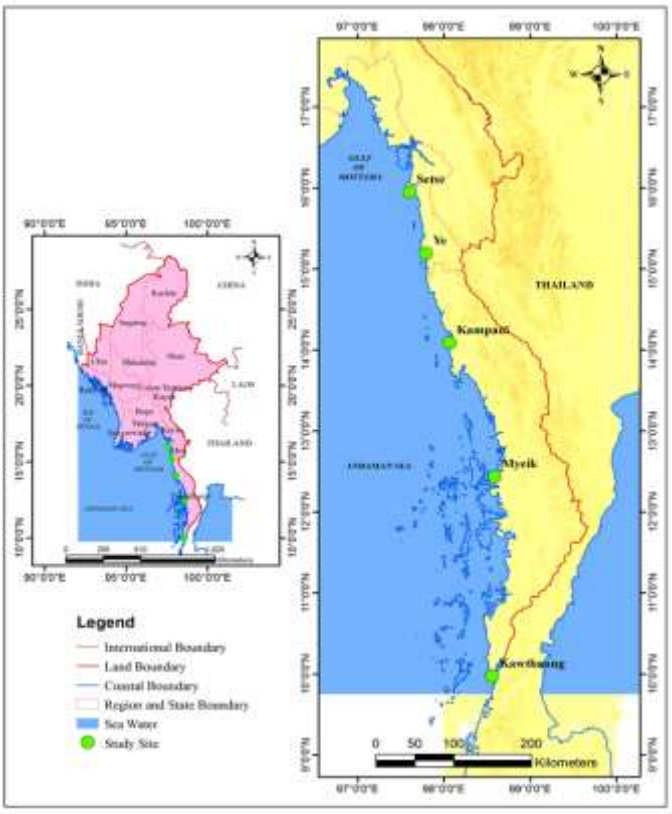

Figure 1 Map showing the sample collection sites in Tanintharyi coastal waters.

Results and Discussion

Environmental parameters

The fluctuations of temperature, 24-33°C were recorded at the five stations during the study period. Water temperature was high during the pre-monsoon period (February to May) and low in the monsoon season (June to September) and again an increasing trend in the post-monsoon period (October to January). The surface salinity had a broad range 14-34‰ with a maximum in the pre-monsoon period and a minimum in the monsoon months when precipitation increased. pH value varied between 6.5-7.8 during the observation period, with a high value in the pre-monsoon months and low from June to January (Fig. 2).

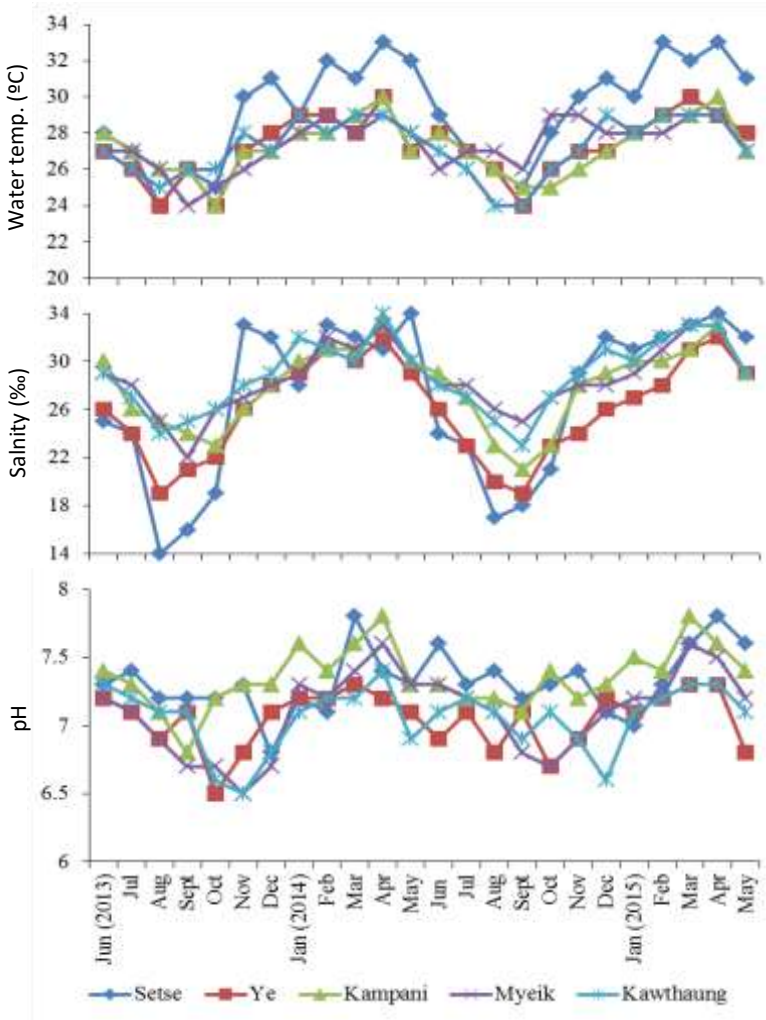

Figure 2 Seasonal variations of water temperature (ºC), salinity (‰), and pH from June 2013 to May 2015.

Nutrients

Fig. 3 shows the temporal variations in nutrient concentrations at the five sampling stations. Nitrate, phosphate, and ammonia concentrations varied between 0.01 and 0.06 mg l^{-1}, between 0.01 and 1.5 mg l^{-1}, and between 0.01 and 0.11 mg l^{-1}, respectively, during the study period. Nitrate and ammonia showed little fluctuations in concentration throughout the study period. The concentrations of phosphate at Myeik and Kawthaung stations were relatively high in the pre-monsoon period, especially in April but in other months, there was no significant variation in concentration at the five stations.

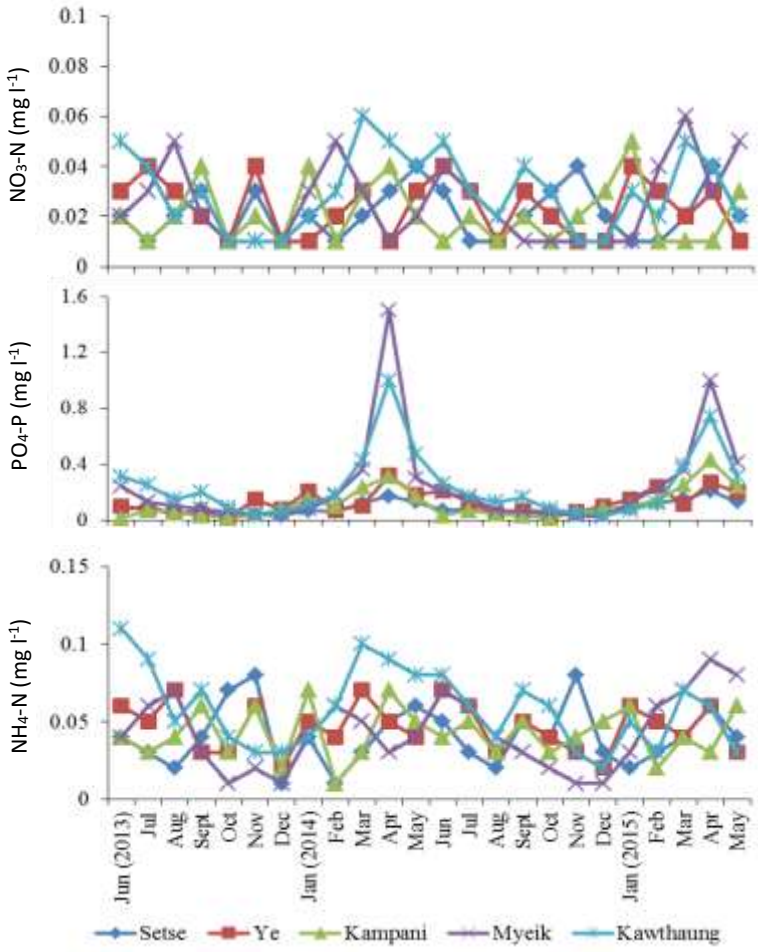

Figure 3 Seasonal variations of nitrate (NO_3-N, mg l^{-1}), phosphate (PO_4-P, mg l^{-1}), and ammonium (NH_4-N, mg l^{-1}) concentrations from June 2013 to May 2015.

Phytoplankton abundance and species composition

Phytoplankton abundance ranged from 72,450-714,396 cells l^{-1} at Kawthaung, 47,416-947,501 cells l^{-1} at Myeik, 8,930-28,439 cells l^{-1} at Kampani, 8,976-17,888 cells l^{-1} at Ye and 5,162 to 16,986 cells l^{-1} at Setse, respectively (Fig. 4). The phytoplankton community was dominated by diatoms (Bacillariophyceae) 155 species accounting on average, for 78% of the total abundance of phytoplankton. Dinoflagellates, a total of 42 species of Dinophyceae, comprised 21% of the phytoplankton community. Additionally, 2 species of Dictyochophyceae were also recorded, accounted for only 1% of the total phytoplankton abundance.

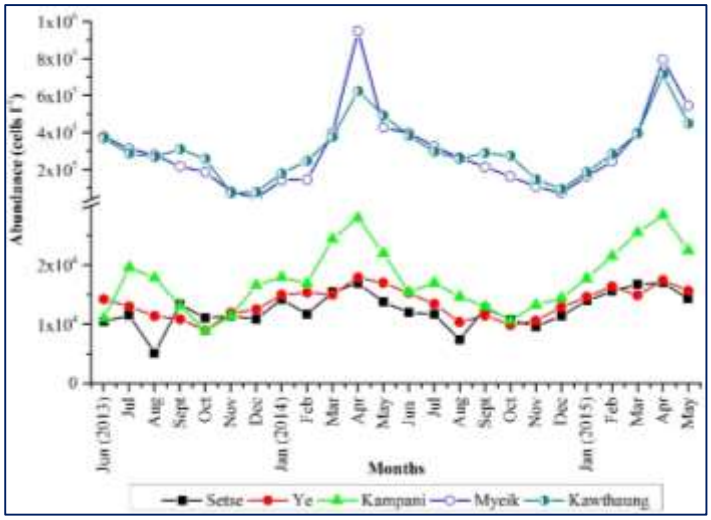

Figure 4 Seasonal variations of phytoplankton abundance (cells l^{-1}) at the five stations from June 2013 to May 2015.

Seasonal variations in the cell abundance of phytoplankton at each station are presented in Fig. A clear seasonal trend in phytoplankton abundance was seen at all five stations with a sharp peak in the pre-monsoon period, especially in April, after which a gradual decrease in cell abundance was found in the monsoon and post-monsoon periods. A similar result was also noted in the literature reported by Aung Myo Hsan (2013), Thida Nyunt (2013), and Yin Yin Htay (2014) who described the high phytoplankton abundance in the pre-monsoon months.

Microscopic observations revealed 42 species such as *Melosira nummuloides, M. borreri, Thalassiosira subtilis, T. rotula, Skeletonema costatum, Paralia sulcata, Cyclotella striata, Lauderia annulata, Coscinodiscus lineatus, C. radiatus, Odontella sinensis, O. mobiliensis, Eucampia cornuta, Hemiaulus sinensis, Bellerochea horologicalis, Ditylum sol, Rhizosolenia imbricata, R.setigera, Proboscia alata, Guinardia striata, G. flaccida, Bacteriastrum delicatulum, B. hyalinum, Chaetoceros curvisetus, C. diversus, C. densus, C. lorenzianus, C. subtilis, Asterionellopsis glacialis, Fragilaria crotonensis, F. capucina, Tabellaria fenestrata, Climacosphenia moniligera, Thalassionema nitzschioides, T. frauenfeldii, Nitzschia sigma, N. longissimia, N. seriata, Cylindrotheca closterium, Prorocentrum micans, Dinophysis caudata,* and *Ceratium furca* were noted as the dominant phytoplankton species in Tanintharyi coastal water. Taylor (1975) reported *Guinardia flaccida* was noted as the predominant diatom species in Thailand which was in agreement with the present result found at Kawthaung station. *Thalassionema nitzschioides* was abundantly collected at Tanintharyi waters throughout the observation period, which agrees well with the finding of Kamba and Yuki (1980). Moreover, the present findings of dominant phytoplankton species in Tanintharyi coastal waters were similar to the results reported by Khin Yu Nwe (2011), Yin Yin Htay (2014, 2019), and Zarni Ko Ko (2014, 2018).

Phytoplankton succession in relation to environmental parameters

Temperature and salinity

The relationships of water temperature and salinity to the cell abundance of phytoplankton species that appeared with high frequencies during the study period are shown in Fig. 5. *Skeletonema costatum* and *Asterionellopsis glacialis* were found over a wide range of salinity, 15-34‰. These species seem to have the possibility of salinity tolerance. Balzano et al. (2011) reported *S. costatum* showed growth between salinity of 0 and 35‰. *Thalassiosira subtilis, Lauderia*

annulata, *Paralia sulcata*, and *Fragilaria crotonensis* were commonly observed in the salinity range of 20-34‰. The species that were most abundant within the salinity range between 25 and 34‰ were *Rhizosolenia setigera*, *Bacteriastrum hyalinum*,

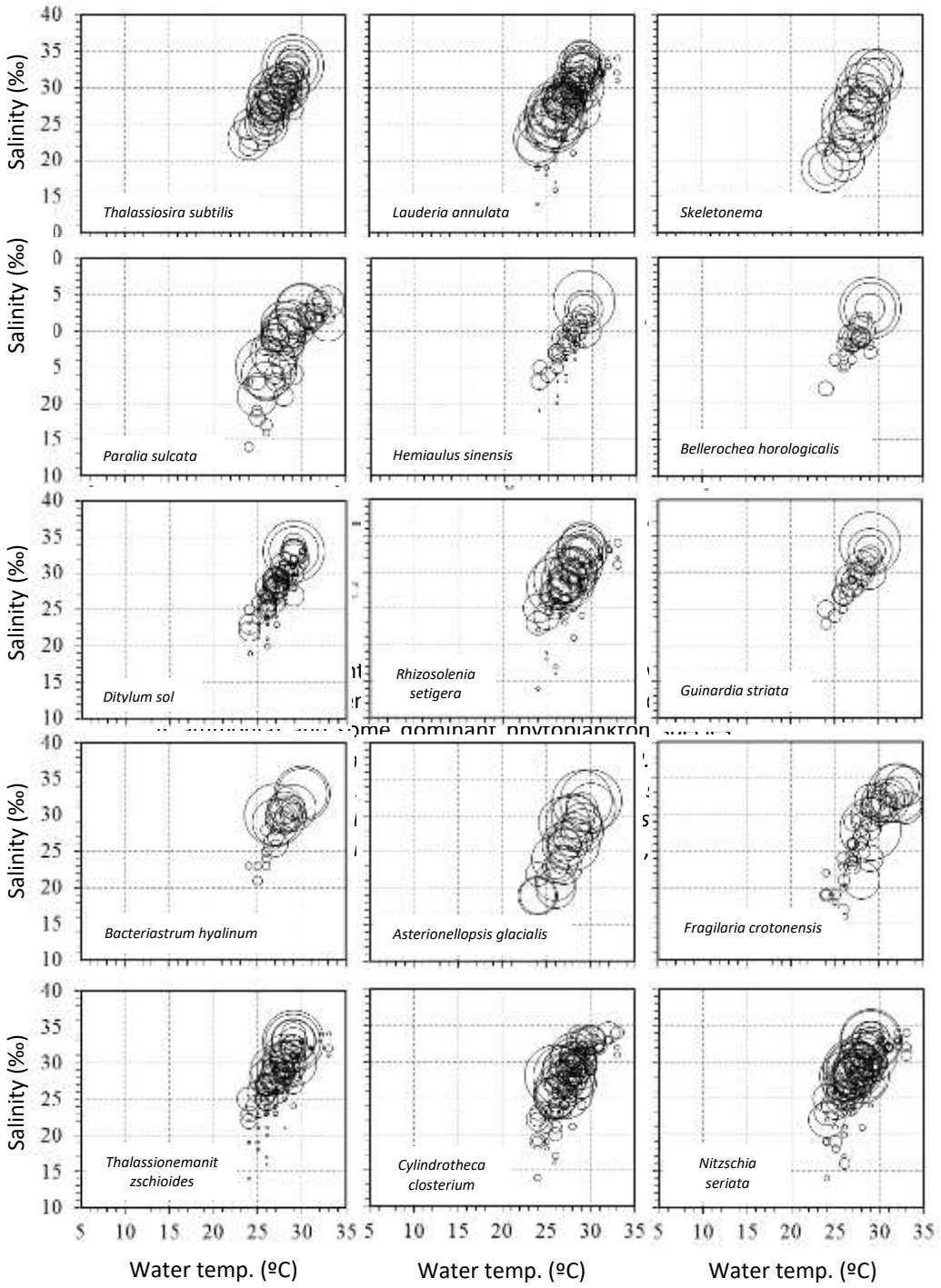

Figure 5 Temperature-salinity plots for abundance of the dominant phytoplankton species.

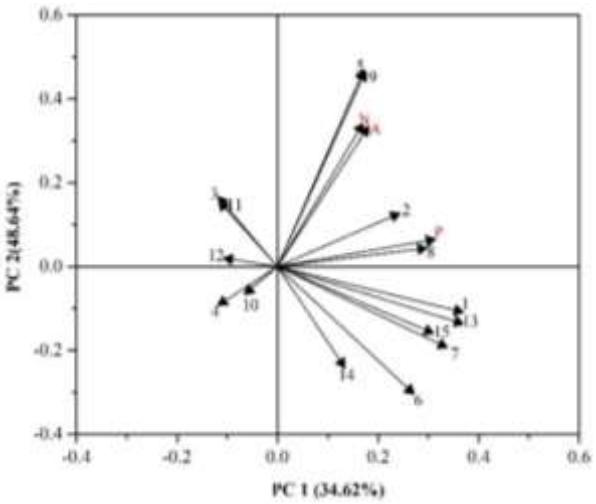

Figure 6 Principal component analysis (PCA) biplot showing the relationship between nutrients (N=nitrate, P= phosphate, A=ammonia) and some dominant phytoplankton species (1= *T. subtilis*, 2= *L. annulata*, 3= *S. costatum*, 4= *P. sulcata*, 5= *H. sinensis*, 6= *B. horologicalis*, 7= *D. sol*, 8= *R. setigera*, 9= *G. striata*, 10= *B. hyalinum*, 11= *A. glacialis*, 12= *F. crotonensis*, 13= *T. nitzschioides*, 14= *C. closterium*, and 15= *N. seriata*).

Thalassionema nitzschioides, and *Nitzschia seriata*. *Hemiaulus sinensis, Bellerochea horologicalis, Ditylum sol,* and *Guinardia striata* were common in high salinity water (over 30‰). *Cylindrotheca closterium* had higher cell abundance in the salinity range between 25 and 30‰. Concerning the surface water temperature and phytoplankton abundance, most species were plentifully collected in the temperature range of 24-33°C. The present results reflect many diatom species have a well-defined ecological preference.

Nutrient concentrations

Principal Component Analysis (PCA) biplot of dominant phyto-plankton species and nutrient concentrations was presented in Fig. 6. Lines in the PCA biplot pointing in the same directions are positively correlated, while lines pointing in opposite directions are negatively correlated. *Ditylum sol, Bellerochea horologicalis, Thalassionema nitzschioides, Thalassiosira subtilis, Rhizosolenia setigera, Hemiaulus sinensis, Guinardia striata, Cylindrotheca closterium, Nitzschia seriata, Rhizosolenia setigera,* and *Lauderia annulata* had a positive correlation with phosphate except for 5 species *Paralia sulcata, Skeletonema costatum, Bacteriastrum hyalinum, Asterionellopsis glacialis,* and *Fragilaria crotonensis* which were negatively correlated with phosphate concentration. The correlation of dominant phytoplankton with nitrate and ammonia showed a similar pattern to those of phosphate. According to the literature (Brockmann and Kattner 1997), phosphate concentrations are higher in summer months. Thus, the enrichment of nutrients may probably stimulate high cell abundance of phytoplankton during the summer months of the observation period.

Conclusion

In this study, the seasonal fluctuations of phytoplankton assemblages were examined with response to the controlling environmental variables at the five stations along the Tanintharyi coastline. At all stations, the abundance was higher in the pre-monsoon months, probably due to high water temperature, high salinity, and the increase of nutrient concentrations. On the other hand, the decrease in cell number of phytoplankton was noted in the monsoon period which may likely due to low water temperature, low salinity, and a decrease in the nutrient levels. Regarding the seasonal abundance of phytoplankton, Kawthaung and Myeik had higher numbers of

phytoplankton among five stations because of more diverse kinds and frequently occurring phytoplankton species at these water columns. It was further considered that structural changes in the phytoplankton community are a good indicator of water quality and aquatic ecological status as they show the complexity and rapid responses to the fluctuations of environmental parameters.

Acknowledgments

The author deeply indebted to Dr. Aung Myat Kyaw Sein, Rector of Mawlamyine University, and Dr. Mie Mie Sein and Dr. San San Aye, Pro-Rectors of Mawlamyine University, for their permission to undertake this research. I wish to express my sincere thanks to Dr. Khin Maung Cho, Pro-Rector (Retd.), Mawlamyine University, for his kind suggestions in preparing the manuscript. Special thanks are to Dr. San Tha Tun, Professor, and Head of the Department of Marine Science, Mawlamyine University for providing lab facilities.

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THE CULTURE STUDIES ON THE FORMATION AND GROWTH OF THE SECONDARY BRANCHES AND REPRODUCTIVE STRUCTURES OF *HYPNEA SPINELLA* (C. AGARDH) KÜTZING (GIGARTINALES, RHODOPHYTA) FROM SETSE COASTAL AREA

Sein Moh Moh Khaing[1], Myo Min Tun[2], Khin Khin Gyi[3] & Wint Thuzar Nwe[4]

Abstract

The plants of *Hypnea spinella* (C. Agardh) Kützing collected from the tidal pools in the upper intertidal zone of Setse coastal area (Lat. 15° 52' N, Long. 97° 35' E) had been culture under the laboratory conditions. It was carried out to investigate the formation and growth of *H. spinella* (C. Agardh) Kützing based on the early stages of secondary branches and reproductive structures in culture. In this study, the maximum growth was found in the salinity 25‰. After five days, a new filamentous sprout grew and seven days later, a single apical cell which develops at terminate of the branches and branchlets. After ten days, laterals and new branch filaments arised and after fifteen days initiating, short branchlets which gradually grow up on the branches like spiny outgrowth. Moreover, tetrasporangial form observed at the basal, middle and upper portion of the branchlets encircling the entire surface were described.

Keywords: branches, culture, *Hypnea spinella*, laboratory, salinity, Setse.

Introduction

Red algae are found from the intertidal to the deep limits of the photic zone, displaying a wide variety of morphologies including unicells, filaments, crusts, sacs, blades, and finely branched forms. They are most abundant in tropical waters and most of them are benthic and some are epiphytic on other seaweeds and seagrasses. Red algae are the largest in numbers of species among the marine algae. Many are microscopic but most are macroscopic in multicellular forms. Moreover, some members of the red algae are calcareous. The red algae contain photosynthetic pigments, chlorophyll a and c, carotenes, xanthophylls and phycobilins especially r-phycoerythrin which causes the red coloration. Their cell walls are composed of cellulose and pectic compounds such as agar, carrageenan, and furcellaran (Guiry and Guiry 2017).

In red algae, *Hypnea* species are found as economically and medically important seaweeds and also can be recorded as a part of livelihood resource for many countries. The morphotaxonomy and utilization of *H. spinella* (C. Agardh) Kützing of the Asian, American, British, African and Austalian coasts has been studied by several researchers (Tanaka 1941, Taylor 1950, Krishnamurthy and Joshi 1970, Lewmanomont and Ogawa 1995, Jha *et al.* 2009, Coppejans *et al.* 2009, 2010, Pham *et al.* 2011, Guiry and Guiry 2017). Similar studies have received from three coastal regions of Myanmar by Aung Myint 1975, Soe-Htun *et al*. 2009 a, Soe-Htun *et al*. 2009 b, Hlaing Hlaing Htoon 2009 and Sein Moh Moh Khaing 2017.

In the present study, *H. spinella* (C. Agardh) Kützing collected from the Setse coastal area had been cultured under laboratory conditions. The stages of development of branches, branchlets, and reproductive structures of this species were presented in the present research. The main objectives of this study are: 1) to investigate the formation of secondary branches of *H. spinella* (C. Agardh) Kützing, and 2) to know the early stages of reproductive structures of *H. spinella* (C. Agardh) Kützing under the laboratory conditions.

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Materials and Methods

The plants of *H. spinella* (C. Agardh) Kützing were collected from the tidal pools in the upper intertidal zone of Setse coastal area (Lat. 15° 52' N, Long. 97° 35' E) in Thanpyuzayat Township, Mon State, from May 2016 to February 2017. The collected plants were kept in ice-box and brought to the laboratory immediately after collection. Morphological studies were mainly based on the fresh and the herbarium specimens deposited in the herbarium of Marine Science Department.

In the laboratory, the fresh and healthy specimens were thoroughly washed with painting brushes in the sterile seawater to remove epiphytes and some contaminants. The culture apparatus (glass bottles, cover slices, Petri dishes, forceps and brushes) were cleaned with tap water, and then they were sterilized with boiling water. Natural sea water was collected from Setse coast, and stored it in plastic drums placed in laboratory. For the culture experiments, seawater was filtered with the Whatman No. 1 filter papers. Natural seawater was heated for hypersalinities and diluted with distilled water to reduce salinity. Sterile seawater was adjusted to salinities (20‰, 25‰, and 30‰) using a refractometer.

For the experiments on the vegetative growth of secondary branches, fresh and healthy plants were selected and used as seed materials. The plants were cut into pieces of 5 mm in length by hand using double-edged razor blades. The five pieces of healthy plants were inoculated onto cover slices in the Petri dishes, 25 ml of seawater of Provasoli's Enriched Seawater (PES) medium (Provasoli 1968) (Table. 1) in 20‰, 25‰ and 30‰ salinities. The medium was replenished every 3 days intervals. Cultures were maintained on the culture shelf at room temperature (28°C±1) under continuous light for 30 days. The formation and growth of the secondary branches were examined every day under compound microscope (Olympus CO11 Japan) throughout the experimental period. Microscopic measurements were recorded by micrometer (µm) using ocular meter. The important characteristics of this study were photographed under the light microscope with a Sony DSC-WX80 digital camera and the results assembled from digital photographs are processed by Adobe Photoshop CS4. This study followed the classification system of Matinfar *et al*. (2013).

Table 1 Provasoli's Enriched Seawater Medium (PES).

Stock solutions (Each in 100 ml water)		Millimeters of stock solutions to be added
$NaNO_3$	35 g	10
Na_2 glycerophosphate	5 mg	10
Vitamin B_{12}	1 mg	10
Thiamine	50 mg	10
Biotin	0.5 mg	10
Tri buffer		-
Fe (as EDTA 1:1 molar)		250
Fe $(NH_4)_2$ $(SO_4).6H_2O$, + Na_2 EDTA,	351 mg 300 mg/ 500 ml	
P II trace metals		250

Add 20 ml of the above stock solution mixture to 1000 ml of filtered seawater to prepare full-strength medium

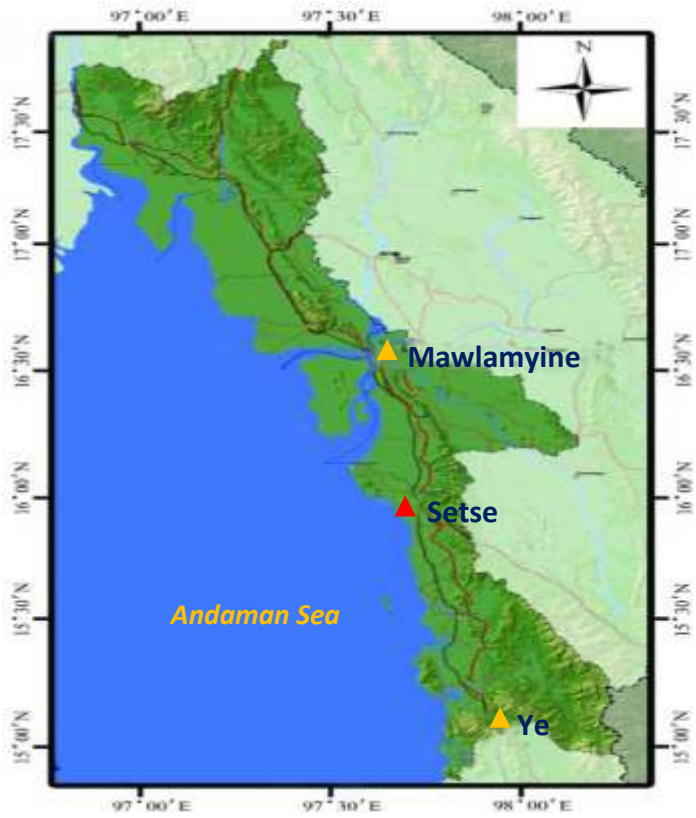

Figure 1 Map showing the collection site of the *Hypnea spinella* (C. Agardh) Kützing from Setse coastal area.

Results

A classification system of the *Hypnea spinella* (C. Agardh) Kützing

Phylum : Rhodophyta

Class : Florideophyceae

Order : Gigartinales

Family : Cystocloniaceae Kützing 1843

Genus : *Hypnea* J. V. Lamouroux 1813

Species : *Hypnea spinella* (C. Agardh) Kützing

General morphology of *Hypnea spinella* (C. Agardh) Kützing

References.- Taylor 1950:135; Krishnamurthy and Joshi 1970: 22; Aung Myint 1975: 52-57, figs. 7-10, 44; Coppejans *et al.* 2009: 182, fig. 150; Hlaing Hlaing Htoon 2009: 45-46, figs. 86-88; Soe-Htun *et al*. 2009a: 148-149, fig. 5; Soe-Htun *et al*. 2009b: 296; Coppejans *et al* . 2010: 198; fig. 123; Myo Min Tun 2013:101, figs. 113-115.

Plant purplish green to red in color, 2-3 cm in height, occuring as intricate cushion, pulviniformis thallus, terete, texture firm fleshy, alternately and dichotomously branches are freely erect, 5-7 mm long and 0.5 mm broad branches form prostrate with tip rising upwards by many accessory holdfasts. Thallus decorated with small spines or branchlets about 1-3 mm long, acutes originated from the primary branches, more frequent in the middle and basal parts of the plants. Spherical tetrasporangial sori decorated out from the surface of the frond, especially at the upper and middle regions of the branchlets.

Development of secondary branches and reproductive structures of *Hypnea spinella* (C. Agardh) Kützing

In this study, the maximum growth was found in the salinity 25‰. The secondary branches of *H. spinella* grew well at room temperature and this salinity regime. The color of secondary branches was purplish red under fluorescent light tested.

After five days, a new filamentous sprout grew about 50-60 µm long (Fig. 5). In the young filamentous sprout pericentral and central cells were formed. Seven days later, a single apical cell which develops at the terminate of the branches and branchlets, cuts off a daughter cell (sub-apical cell) which later give rise to an axial filament which inturn cut off lateral pseudoparenchymatous tissue, consisting of large and elongate periaxial cells (Fig. 6).

H. spinella have a distinct apical cell which is generally ovoid to obovoid, sometimes oblong to round or tholus-shaped (Fig. 3). It can be seen prominent and rarely submerged at the tip of the thallus under microscope. Two dividing surfaces are apparent and usually a sub-apical cell present under it (Fig. 19). After that, the young filamentous sprouts become polysiphonous. The elongation of sprout continues, measuring about 150 µm long and 30 µm broad. Ten days later, laterals and new branch filaments arised (Fig. 7). Branch was up to 1.3-1.8 mm in length with acute pieces and dichotomized branched. After that, thallus with successive branches, cell divisions and branching pattern with alternate branching. Prostrate branches on the cover slice in the present study were terete and like a rod; straight, long and thin. Terminal portion of branches are gradually tapering towards the apex or the tips of the branches may be acute and straight, but some of them from the small accessory discs or modified hooks for secondary attachment.

In this stage, accessory holdfasts emerge from the upper, middle and basal portions of secondary branches (Fig. 8). Occurrence of numerous accessory disc-like holdfasts is a common feature in this study. Holdfasts are generally hemisphere in shape, 40-50 µm in diameter and these are modified by further division of cortical cells (Figs. 9, 10). And then, the formation of accessory discs were tested by pull out small branches gently from cover slice using forcep.

Fifteen days later, short branchlets which gradually grow up on the branches like spiny outgrowth seemingly determinate are called branchlets measuring about 180 µm long and 40 µm broad (Figs. 14-16). Frequently, these can be mistaken with short undeterminate branches, especially at the upper portion of the branches. Branchlets are important parts for tetrasporangial sori that occur on them.

Twenty days later, tetrasporangia form at the basal and upper portion of the branchlets encircling the entire surface, 60-80 µm in diameter (Figs. 30, 35). Nevertheless, some tetrasporangial sori occur in the middle region of the branchlets (Figs. 31, 32). Tetrasporangia occur on the swollen surfaces of fertile branchlets. External appearance of the tetrasporangial sori can be distinguished by their darkly colored spots which occur abundantly and visible under the microscope.

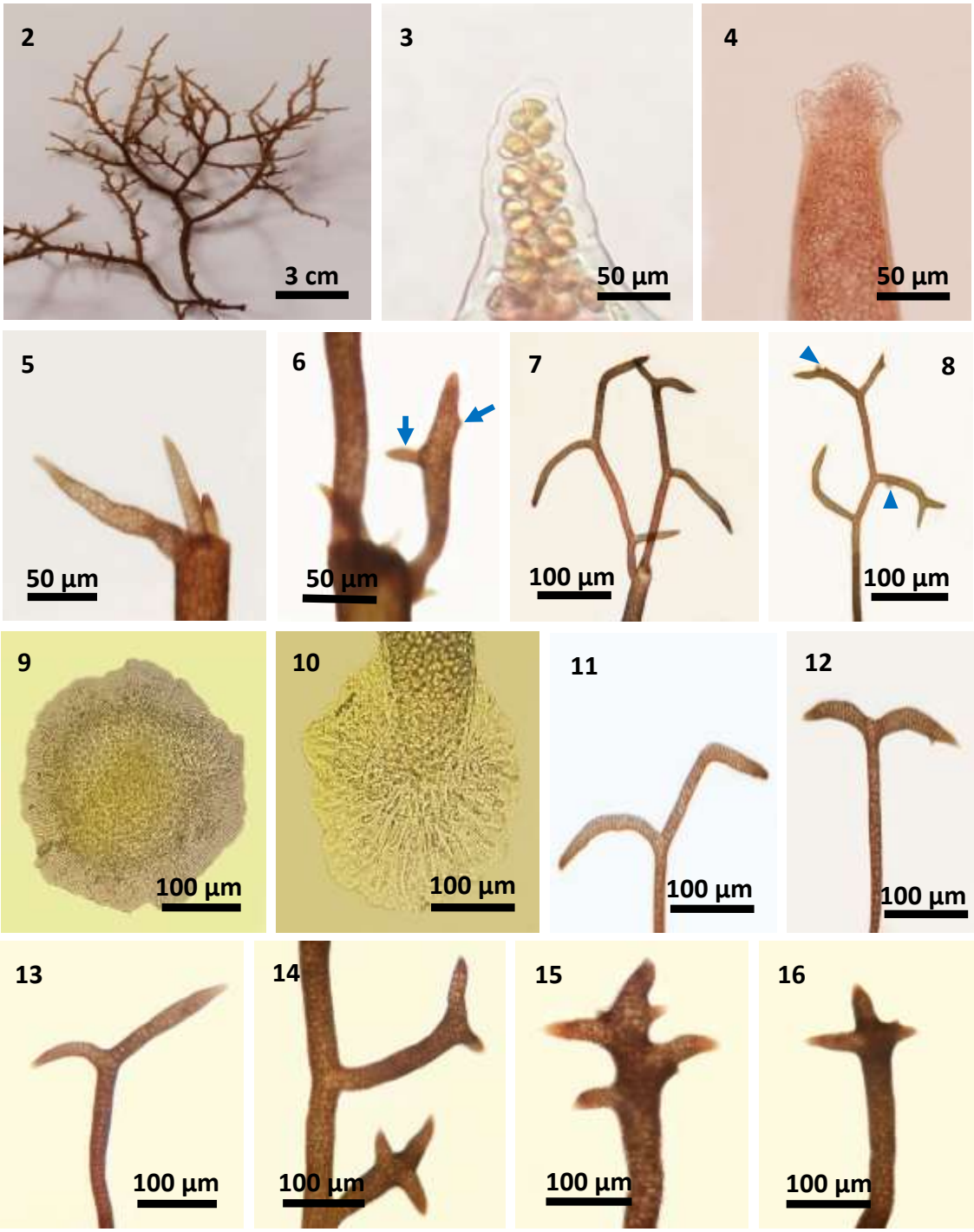

Figure 2-16. The formation and growth of the secondary branches of *H. spinella* (C. Agardh) Kützing: 2) Habit of *H. spinella* (C. Agardh) Kützing ; 3) Ovoid or oboviod apical cell; 4) Apical portion of new filament; 5) New filamentous sprouts on main branch; 6) Small branchlet and new shoot on new branch (arrows); 7) Laterals and new branch filaments; 8) Accessory holdfasts at the upper and basal portions of secondary branches (arrowheads); 9) Accessory holdfast on the apical portion of branch and composed with divisions of cortical cells; 10) Circular-shaped accessory holdfast; 11-12) Different secondary branches; 13-16) Two to seven times spinulose branchlets on the upper portion of the small branches.

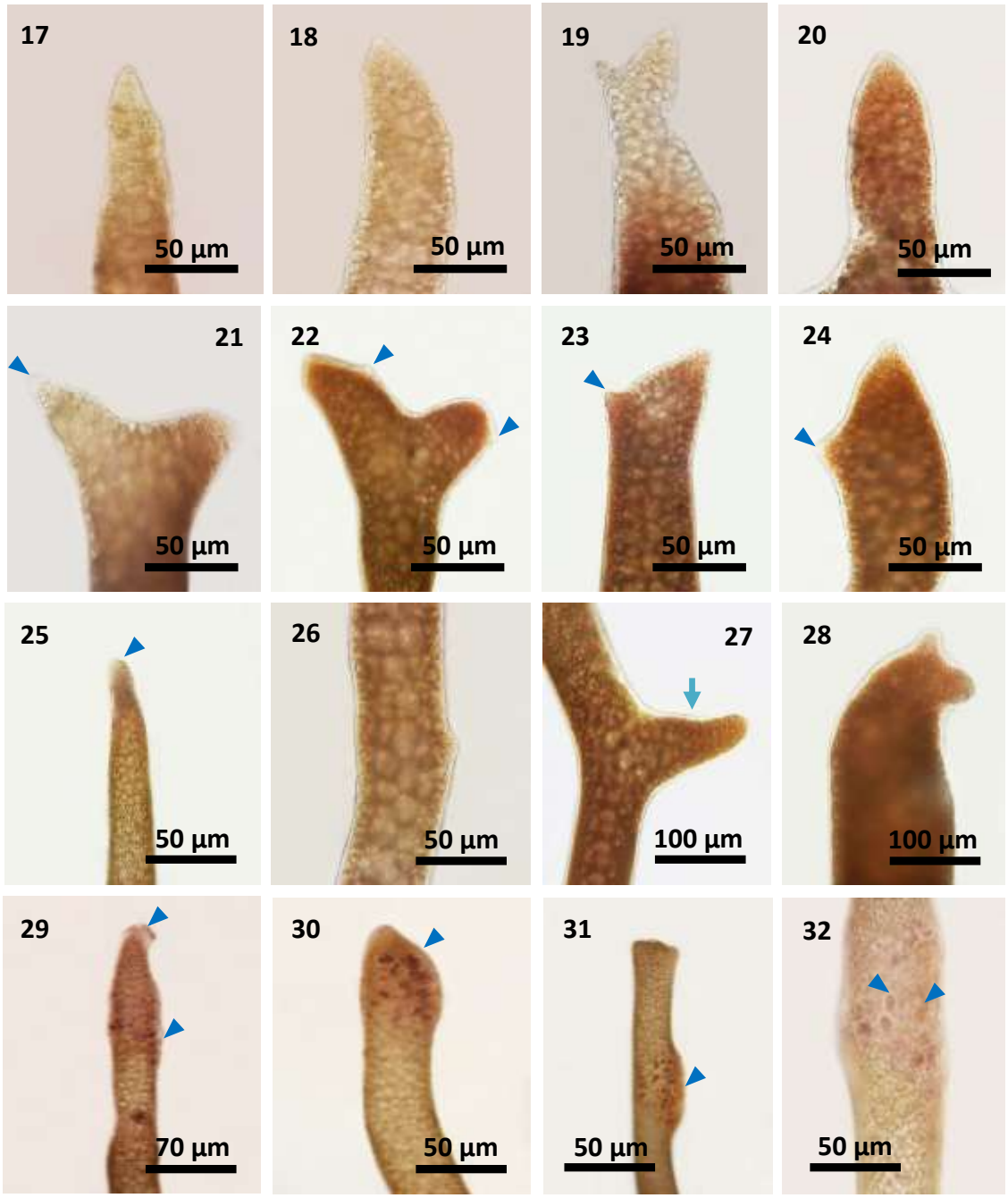

Figure 17-32. The formation and growth of branchlets and reproductive structures of *H. spinella* (C. Agardh) Kützing: 17-18) Apical portions of new branchlets; 19) Cell division at the apical portion of branchlet; 20) Apical portion of branchlet; 21-24) Different new shoots on the apical portion of branchlets (arrowheads); 25) New accessory disc at the apical portion of branchlet (arrowhead); 26) Small shoot at the margin portion of branch; 27) Branchlet at the middle portion of branch (arrow); 28) Two new shoots at the apical portion of branchlet; 29) Tetrasporangial and accessory disc at the upper portion of branchlets encircling the entire surface (arrowheads); 30) Tetrasporangial at the apical portion of branchlets (arrowhead); 31-32) Tetrasporangial at the middle portion of branchlet (arrowheads).

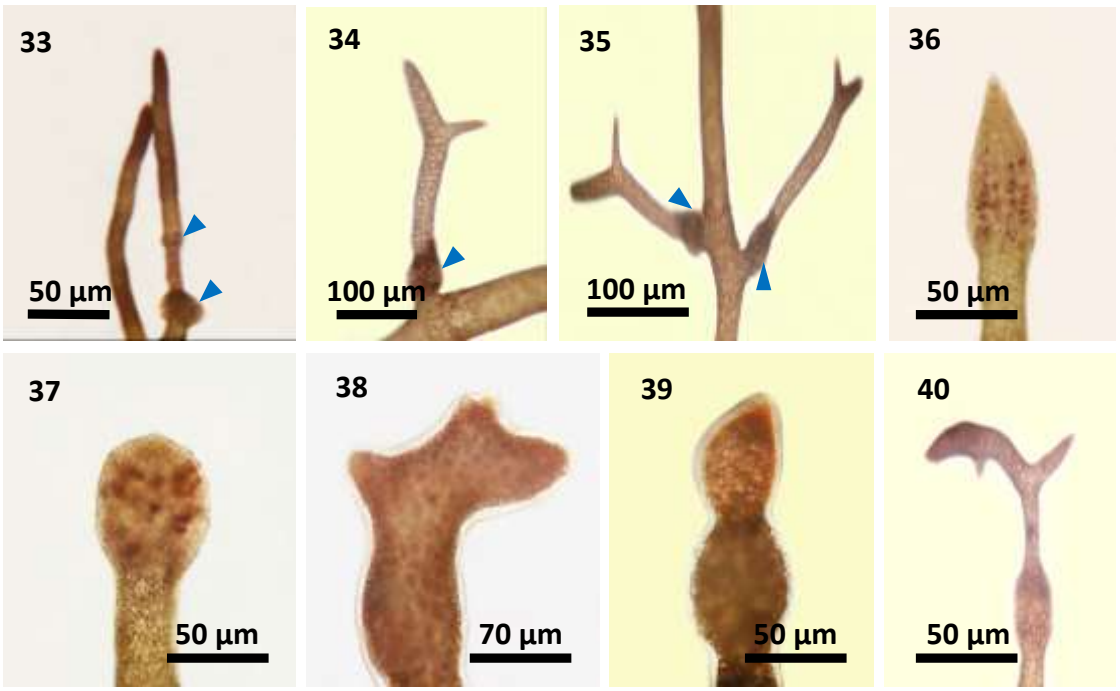

Figure 33-40 The formation and growth of branchlets and reproductive structures of *H. spinella* (C. Agardh) Kützing: 33) Tetrasporangial on the middle and basal portions of branchlets (arrowheads); 34-35) Tetrasporangial on the basal portion of branchlets (arrowheads); 36-37) Tetrasporangial at the apical portions of branchlets; 38-40) Different formation of tetrasporangial sori.

Discussion

In culture study, the maximum growth was found in the salinity 25‰. However, the growth of secondary branches was slow in the salinity 20‰ and 30‰. The secondary branches of *H. spinella* (C. Agardh) Kützing grew well at room temperature and this salinity regime. The light intensity was one of the important factors, which controlled the growth of the *H. spinella* (C. Agardh) Kützing, rather than light quality. The result of this study coincides with the growth of *H. spinella* (C. Agardh) Kützing which commonly occur in the tidal pools at the upper intertidal zone.

Teoh *et al*. (2010) studied temperature tolerance and temperature optimum of algae isolated from different habitats. They stated that temperature optimum of *H. cenomyce* J. Agardh and *H. spinella* (C. Agardh) Kützing was 30° C and, temperature tolerance of these species was 15-30° C respectively. Ding *et al*. (2013) observed the growth rates and changes of several photosynthetic pigments in *H. cervicornis* by setting up different ranges of salinity. The growth rate first increase then decrease as the temperature increases, while growth tends to decline as salinity increases. The optimum salinity and temperature conditions for growth are 25 ‰ and 25° C, respectively. And then, salinity and temperature have significant effects on photosynthetic pigments in this species.

Yokoya *et al*. (2007) studied growth responses and photosynthetic characteristics of wild and phycoerythrin-deficient strains of *H. musciformis* (Wulfen) J. V. Lamouroux. They described that the growth responses to irradiance, photoperiod and temperature variations, pigment contents, and photosynthetic characteristics of the brown and green strains of *H. musciformis* (Wulfen) J. V. Lamouroux. The result showed that growth rates increased as a function of irradiance, but with further increase in irradiance, become light saturated and remained almost unchanged. The highest

growth rates of the brown and green strains were observed in temperature of 20-25° C under long and short photoperiods.

Ribeiro *et al*. (2013) studied effects of nitrogen and phosphorous availabilities on growth, pigment, and protein contents in *H. cervicornis*. They advised the selection of seaweed species for use as biofilters should be based on the knowledge of their nutrient requirements and tolerance to wide variations of nutrients concentrations.

Treatments were composed of sterilized seawater enrich with 25 % von Stosch solution (without nitrogen and phosphorous), and nitrate or ammonium and phosphate were added in a combination of 100:1 and 10:1 nitrogen/phosphorous (N/P). Growth rates of *H. cervicornis* increased linearly with addition of ammonium, but with nitrate addition, growth varied following saturation kinetic, and the highest growth rate (14.45% d^{-1}) was observed in N/P ratio of 10:1. An excess of nutrients was accumulated as proteins and phycobiliproteins (mainly as allophycocyanin and phycoerythrin) at higher phosphate availability (N/P ratio of 10:1), and *H. cervicornis* tolerated the highest ammonium and nitrate concentrations. These physiological responses suggest that this species could be used as biofilter for nutrient removal in eutrophicated seawater and could be cultivated in integrated multitrophic aquaculture system.

In the present study, nitrogen, phosphate, and ammonium were contained in Provasoli's Enriched Seawater (PES) medium, but not adjusted increase and decrease ratio of these concentrations. However, the effects of these elements for the growth of branches of *H. spinella* (C. Agardh) Kützing were studied in culture period. Phosphorous is vital to seaweed growth and it involved in many functions, including photosynthesis, nutrient movement within this plant, important in cell division and development of new cells.

The growth rate of all branches gradually decreased after 25 days. Results of this study indicate that replenishment of medium and changes of Petri dishes were important for the growth of this species. If Petri dishes were should be change in time-lag of culture period, unseen contamination can occur in these dishes.

In this study, partial view of habitat and variation of characters in thallus and branches formation were recorded monthly in the field. Detailed studies indicate that *H. spinella* (C. Agardh) Kützing mainly grows in the tidal pools at the upper intertidal zone of Setse coastal area. Maximum development of this species generally occurs during rainy season in this area. *Gracilaria canaliculata* are the important substrata for some *H. spinella.* Moreover, seasonal variations of these marine algae and associated seaweeds seemed to be quantitatively rare in numbers due to the turbidity by the heavy rate of sedimentation in May and developing of the crop attains its peak from June to October.

In this research, a gradual degradation of this species commences in November and most of this species in this area are disappear from December to April. Nevertheless, seasonal variations of the genus *Hypnea* in Rakhine coastal region demonstrate that regeneration commences in December and developing of the crop attains its peak from January to March. From this study, commencement and declination periods are not entirely uniform for three coastal regions and each year. Similar observation was given by Aung Myint (1975) who had studied on the genus *Hypnea.*

Conclusion

Cultural studies on the secondary branches and reproductive structures of *H. spinella* (C. Agardh) Kützing were carried out in order to know the formation and growth stages under the laboratory conditions. In this study, the maximum growth was found in the salinity 25‰. The secondary branches of were grew well at room temperature and this salinity regime. The color of secondary branches was purplish red under fluorescent light intensity tested. In this study, twenty

days later, tetrasporangia formed at the basal and upper portion of the branchlets encircling the entire swollen surfaces of fertile branchlets.

Acknowledgements

I am indebted to Dr. Aung Myat Kyaw Sein, Rector of Mawlamyine University and Dr. San San Aye, Pro-Rector of Mawlamyine University, for their encouragement and supports in preparing this work. I am very grateful to Dr. San Tha Tun, Professor and Head of the Department of Marine Science, Mawlamyine University, for his valuable suggestions and constructive criticisms on this study. I would like to thanks all staffs of Aquaculture Research Centre, Setse for their support which facilitated smooth execution of the Setse field. I would like to thank my beloved parents, U Myo Chit and Daw Mya San Yi, for their physical, moral and financial supports throughout this study.

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STOMACH CONTENTS OF THE SPADENOSE SHARK *SCOLIODON LATICAUDUS* MULLER & HENLE 1838 IN MON COASTAL WATER

Myo Min Tun[1], Khin Khin Gyi[2], Sein Moh Moh Khaing[3] & Wint Thuzar Nwe[4]

Abstract

Stomach contents of the spadenose shark in Mon coastal water were studied by using the samples collected from the fish landing sites during October 2017 to September 2018. The present study revealed that the stomach contents of *Scoliodon laticaudus* were categorized by cephalopods (26.4%), fish (26.3%), shrimps (25.7%) and other crustaceans (21.6%) in the study period. According to the number and frequency occurrence, *S. laticaudus* is a cephalopods feeder and their feeding rate is not different in stations of the present study. The least food composition is other crustaceans for *S. laticaudus.* Seven species of fishes such as *Rastrelliger brachysoma*, *Nemipterus japonicus*, *Harpodon nehereus*, *Johnius coitor*, *Polynemus paradiseus*, *Coilia dussumieri* and *Trichiurus lepturus*, two species of cephalopods namely *Sepia* sp. and *Loligo* sp., three species of shrimps including *Penaeus indicus*, *Acetes indicus* and *Metapenaeus brevicornsis* in the stomach of this species were examined. In the stomach of *S. laticaudus*, 14 phytoplankton species and 16 zooplankton species were examined.

Keywords: food, feeding, *Scoliodon laticaudus*, shark, stomach contents, Mon coastal water

Introduction

Food and feeding habit studies of fish based upon analysis of stomach contents can provide the information in the fishery biology. Feeding is the dominant activity during the entire life cycle of fish. Food is the basic requirements for growth, development, reproduction, survival and existence of all organisms. The distribution and fluctuation of the food may affect the migration, shoaling and spawning behavior of fish stock and even the fishery. The knowledge on the relationship between the fishes and food organisms, feeding habits in relation to sexual cycle, condition of food and selectivity in feeding is an important aspect of fisheries managements (Osuna-Peralta *et al.* 2014). The spadenose shark *Scoliodon laticaudus* is an active carnivore with a mixed diet composing of small sized prawns, squilla and molluscs.

In the study area, the fish is an essential part of the diet and the main role of the fishery sector. The aim of the present study is to investigate the food items consumed by the studied fish species and to know the feeding of shark in different seasons along the Mon coastal areas including Ahlayt, Sebalar, Kyaikkhami, Setse and Zeephyuthaung.

Materials and Methods

The spadenose shark was collected from five fish landing sites of Mon coastal water during October 2017 to September 2018 (Fig. 2). Samples of *Scoliodon laticaudus* were taken from Mon coastal water to understand the food and stomach content. In the laboratory, total length and the body weight for each specimen was measured in fresh condition. The belly of fish was cut open. The methods of stomach content analysis are examined by Devadoss (1989), Atkinson and Percy (1991), Joyce *et al*. (2002) and Lopez *et al.* (2010). The stomach of each individual was removed and preserved in 5% formaldehyde-seawater solution. Then all of the contents were carefully taken out and identified under the microscope. The identification of food items were based on the

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classification system used by Allen and Cupp (1930), Aung Kyi (1976), Fischer and Whitehead (1981), Carpenter (1988), Davis (1955), Han Shein (1975), Motomura (2004), Htay Htay Mon (2009), Su Su Hlaing (2010), and Thida Nyunt (2013). After that, food items were categorized and then identified to the genus or species level. Residual liquids of stomach contents were identified for phytoplankton and zooplankton.

Figure 1 Selected species the spadenose shark *Scoliodon laticaudus*

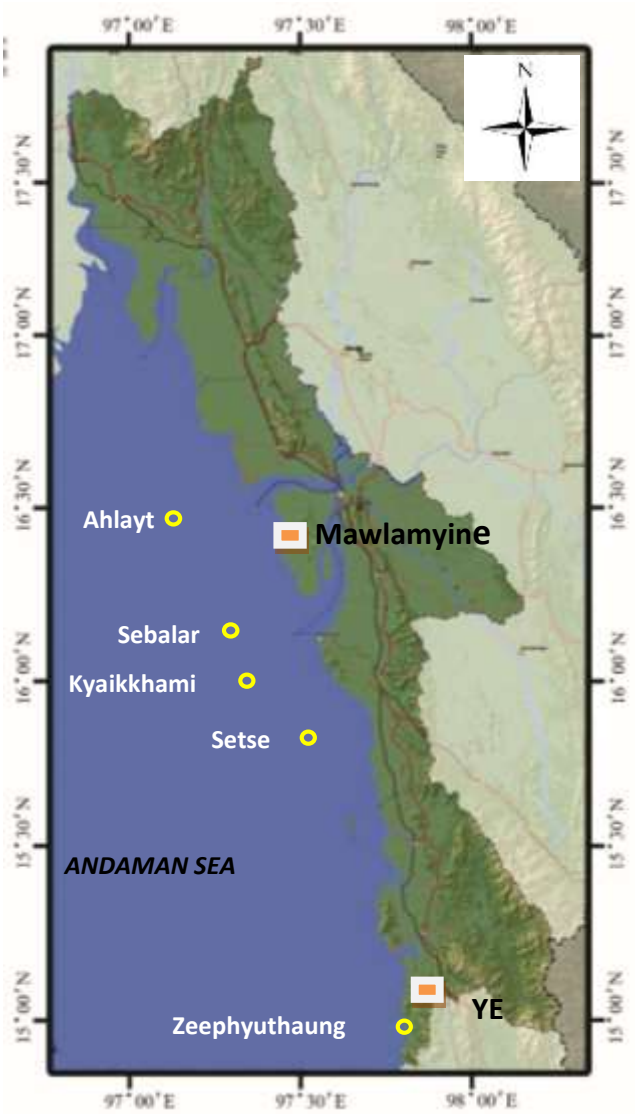

Figure 2 Map showing the sample collection sites of the study areas.

Results and Discussion

Food and stomach contents of the spadenose shark *Scoliodon laticaudus* in Mon coastal water

The composition of the foods of *S. laticaudus* was shown in Figure 3. Fish, shrimp, cephalopods and other crustaceans formed major part of the diet. Fish belonging to seven species could be identified such as *Rastrelliger brachysoma*, *Nemipterus japonicus*, *Harpodon nehereus*, *Johnius coitor*, *Polynemus paradiseus*, *Coilia dussumieri* and *Trichiurus lepturus*. Among crustaceans, prawns belonging to three species could be identified such as *Penaeus indicus*, *Acetes indicus* and *Metapenaeus brevicornsis*. Squilla and crabs formed a good portion of the diet. The molluscs were represented by species of *Sepia* and *Loligo* in good numbers and occasionally by a few molluscan shells.

Crustaceans were dominant in the diet of this shark throughout the year. Prawn, *Penaeus indicus* was found dominant. In addition to prawns, other crustaceans such as small crabs and *Squilla* spp. were represented in the diet in all months. The young sharks preferred prawn diet rather than fish and mollusks as they grow up, the feeding preference is gradually shifted to fishes and fast moving mollusks like the squids and cuttle fishes (Cordova- Zavaleta *et al.* 2018).

A total of 30 species of plankton were identified from the stomach contents of the spadenose shark *S. laticaudus* as shown in Figure 4 and 5. There are thirteen species of diatoms including *Bacteriastrum hyalina*, *Biddulphia mobilliensis*, *Chaetoceros diversus*, *Coscinodiscus lineatus*, *Ditylum sol*, *Hemidiscus cuneformic*, *Lauderia borealis*, *Nitzchia lanceolate*, *Odontella sp.*, *Pleurosigma angulatum*, *Rhizosolenia stol*, *Thalassionema nitzchoid* and *Triceratium favus*; one species of dinoflagellates such as *Ceratium furca*; one species of protozoa namely *Calcarina* sp.; five species of copepods including *Eucalanus subcrassus*, *Paracalanus purvus*, *Acartia erythrae*, *Oithona nana* and *Oithona similis*; three species of ctenophores including *Beroe cucunus*,*Sagitella sp* and *Spionoid* larva; one species of Chaetognatha namely *Sagitta crassa*; one species of Annelida; one species of amphipods namely *Elasmopus* sp; four species of other zooplankton larvae including *Mesopodopsis orientalis*, *Acetes* sp, *Lucifer penicillifer* and *Squilla alima* larva. *Acetes* sp., larvae of bivalve and gastropods were encountered in small quantities. Fish pieces, molluscan shell pieces, scale and eggs contributed in the diet of this shark.

The stomach contents of *S. laticaudus* consist of different food items and were grouped into main four categories: shrimps, fishes, cephalopods and other crustaceans. Cephalopods including octopus, *Sepia* and *Loligo* were the most dominant food items, comprising of 26.4 % of total food items, followed by fish (26.3%), shrimps (25.7%) and other crustaceans (21.6%). Table 1 showed that the variation in monthly percentage in number of main different food groups of *S. laticaudus*. The percentage in number of shrimps ranged from the minimum of 20.5% (March) to the maximum of 29.5% (August) of total items. The range of the composition of fish was lowest in July with 21.1% of total food items and highest in November with 30% of total food items. The cephalopods were found to be minimum 15.9% in August and maximum 31.7% in March. Other crustaceans constituted the lowest composition in 18.4% with July and highest in 25.1% in August.

The spadenose shark, *S. laticaudus* locally called Nga-man-tha-leik is one of the smallest carcharhinids inhabiting the shallow waters of the continental shelf of the Indo-Pacific region (Horn *et al.* 2013). The spadenose shark, *S. laticaudus* is found abundantly in the coastal waters of Mon State throughout the year. It was observed that in the juveniles (7 to 20 cm) and adolescent sharks (21 to 30 cm) and adult sharks (greater than 30 cm) were well fed.

Table 1 Monthly diet composition (%) of *Scoliodon laticaudus* in the study area

Months	Food composition (%)			
	Shrimps	**Fish**	**Cephalopods**	**Other crustaceans**
October (2017)	28.2	28.2	20.5	23.1
November	25	30	25	20
December	27.5	27.5	25	20
January (2018)	27	27	27	19
February	27.1	24.3	24.3	24.3
March	20.5	27.3	31.7	20.5
April	27.7	23.4	25.5	23.4
May	21.4	23.8	31	23.8
June	20.5	25.6	30.8	23.1
July	28.9	21.1	31.6	18.4
August	29.5	29.5	15.9	25.1
September	25.6	27.9	27.9	18.6
Average	25.7	26.3	26.4	21.6

The type of food eaten indicates normally the place where the sharks forage and the nature of its habitat. It is popularly believed that sharks swallow all that come their way. In the present study, it is seen that *S. laticaudus* exhibits a preference for a particular diet during different facets of its life history. Incidental fishing for sharks coincides with the appearance of pelagic fishes on the Mon coastal water. Driggers III *et al.* (2012) described that they found the presence of sardines in the stomach of *S. laticaudus* in the western North Atlantic Ocean during September- March period proved that they prefer to feed on these fast moving pelagic fishes but in the present study, the sardine fishes are not found in the stomach of this species.Preti, Smith and Ramon (2001) studied the feeding habits of the common thresher shark (*Alopias vulpinus*) sampled from California-based drift gill net. Their report mentioned the foods in the diet of thresher shark (big shark) but not the same of the foods of the spadenose shark in the present study.

Likewise during their early growing period after parturition when they could not move fast, they seek to bottom living fishes like small soles, silverbellies and crustaceans like shrimps and small crabs. When they grow up and have gained enough strength, they migrate to the pelagic zone and start actively preying on the pelagic fishes like *Coilia*, Bombayduck and fast moving molluscs like squid and cuttlefish (Veras *et al*. 2009). *S. laticaudus* is an active carnivore with a mixed diet composing of small sized fish, shrimps, other crustaceans and molluscs (Plumlee and Wells, 2016). Cephalopods were frequently found on all species.

In the present study, there were seven species of fishes, two species of cephalopods, threee species of shrimps in stomachs of *S. laticaudus* were examined. The small proportions of two groups: phytoplankton and zooplankton were identified. In *S. laticaudus*, 14 phytoplankton species and 16 zooplankton species were examined. Among phytoplankton observed in the diet of *Coscinodiscus* spp. and *Pleurosigma* sp. were the most dominant species in almost all months. In addition, other phytoplankton species were found occasionally in small portions of the diets. The groups of zooplankton larvae, gastropod larvae, bivalve larvae, fish larvae, Juvenile shrimp and *Acetes* sp. were also recorded in the diet. All planktons formed the minor portion of the diet of *S. laticaudus*. The small molluscan shell pieces and fish remains occurred in the diet for all months.

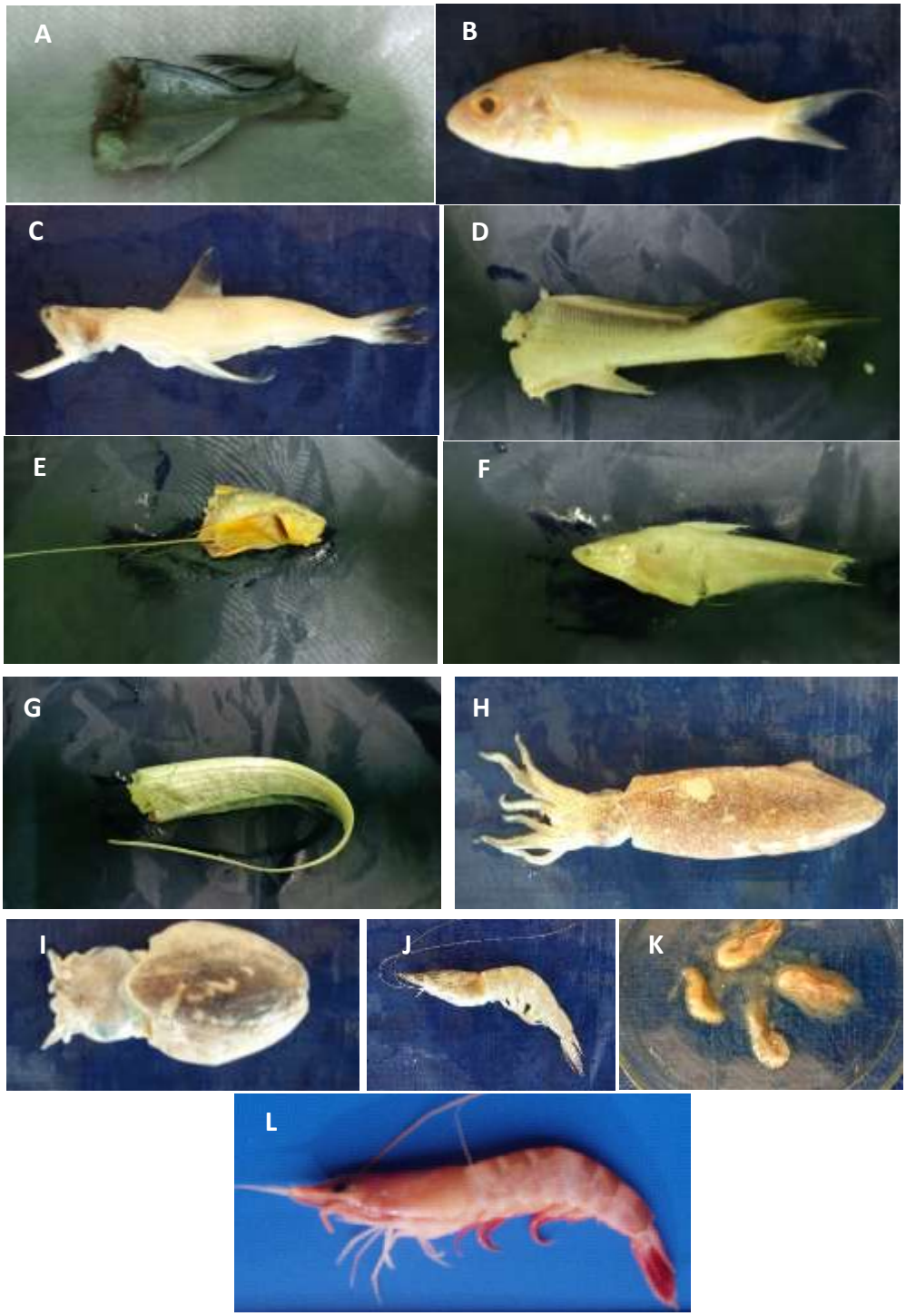

Figure 3 A-L. Food species of the spadenose shark *Scoliodon laticaudus* in study period. A) *Rastrelliger brachysoma*; B) *Nemipterus japonicus*; C) *Harpodon nehereus*; D) *Johnius coitor*; E) *Polynemus paradiseus*; F) *Coilia dussumieri*; G) *Trichiurus lepturus*; H) *Loligo* sp.; I) *Sepia* sp.; J) *Penaeus indicus*; K) *Acetes indicus* and L) *Metapenaeus brevicornsis*.

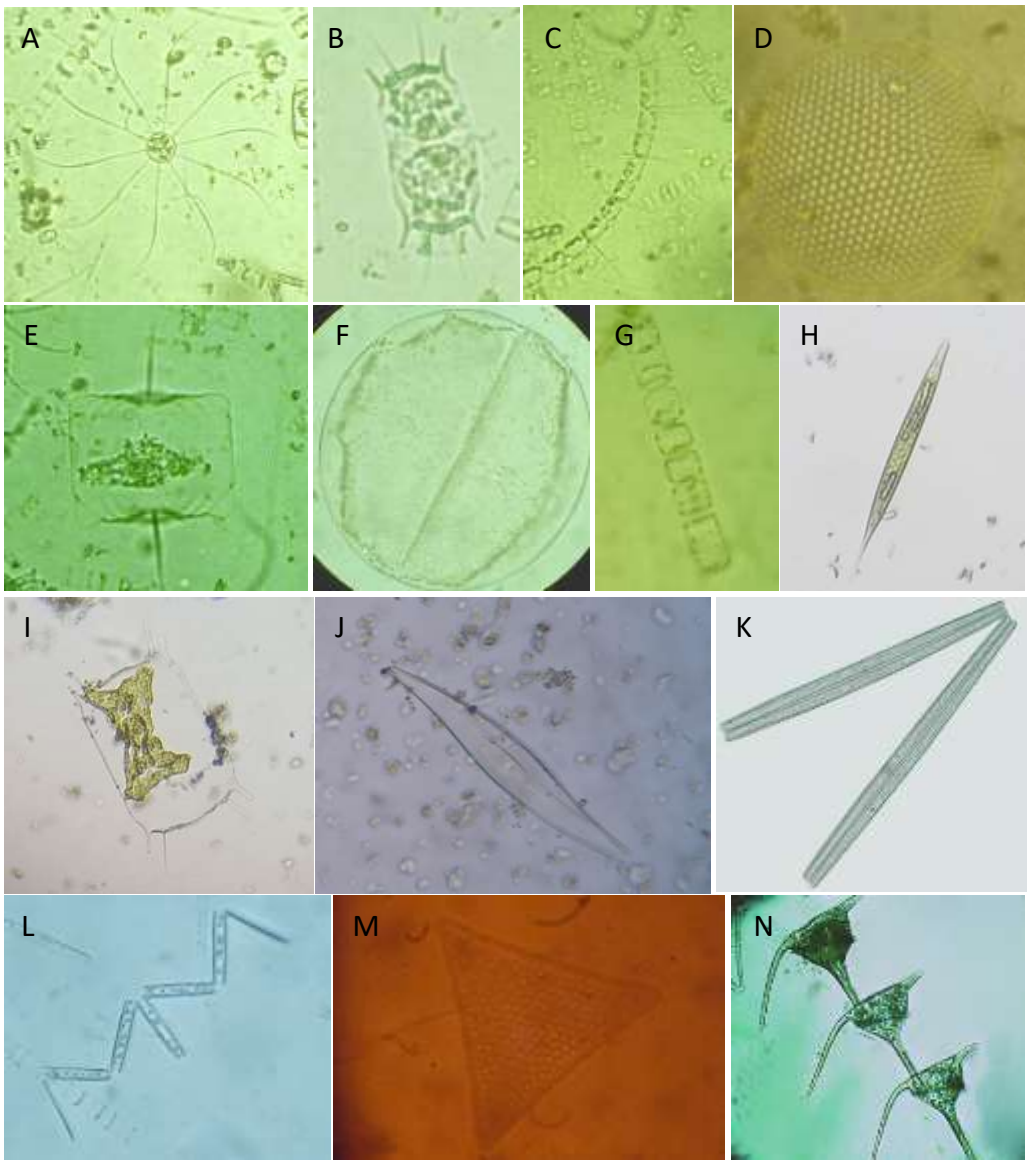

Figure 4 A-N. Some food items of phytoplankton species in the stomach of the spadenose shark *Scoliodon laticaudus*: A) *Bacteriastrum hyalina*, B) *Biddulphia mobilliensis*, C) *Chaetoceros diversus*, D) *Coscinodiscus lineatus*, E) *Ditylum sol*, F) *Hemidiscus cuneformic*, G) *Lauderia borealis*, H) *Nitzchia lanceolate*, I) *Odontella sp.*, J) *Pleurosigma angulatum*, K) *Rhizosolenia stol*, L) *Thalassionema nitzchoid*, M) *Triceratium favus* and N) *Ceratium furca.*

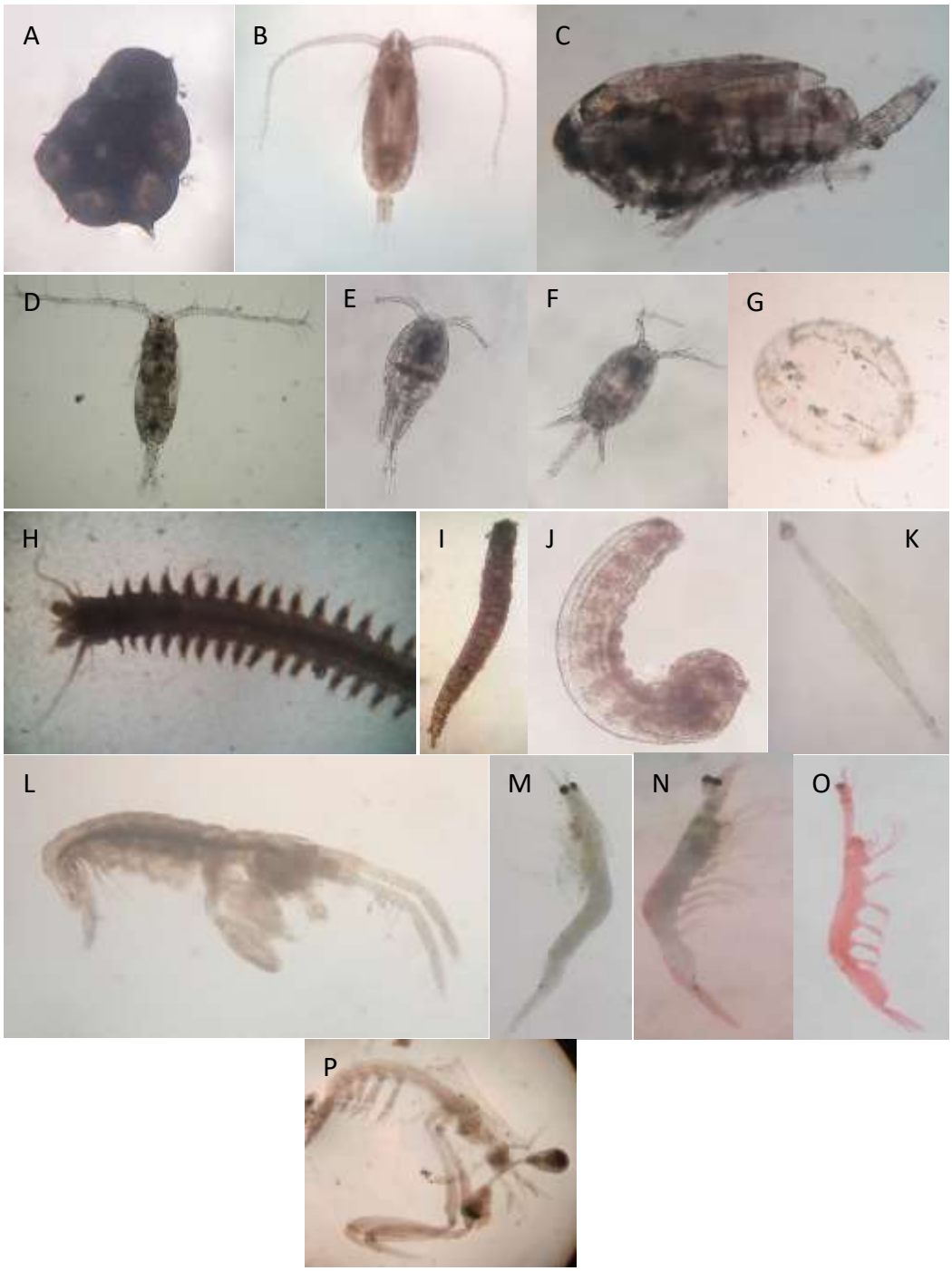

Figure 5 a-p. some food items of zooplankton species in the stomach of the spadenose shark *scoliodon laticaudus*: a) *calcarina* sp., b) *eucalanus subcrassus*, c) *paracalanus purvus*, d) *acartia erythrae*, e) *oithona nana*, f) *oithona similis*, g) *beroe cucunus*, h) annelida sp., i) *sagitella sp.*, j) *spionoid* larva, k) *sagitta enflata*, l) *elasmopus* sp., m) *mesopodopsis orientalis*, n) *acetes* sp, o) *lucifer penicillifer* and p) *squilla alima* larva.

Conclusion

Study on the feeding biology of the spadenose shark *Scoliodon laticaudus* showed that the species was the pelagic feeder. The analyses of *Scoliodon laticaudus* indicated that the feeding intensity varied with seasonality and fish size. The investigation on the diet of species showed that

most frequent food items were *Rastrelliger brachysoma*, *Nemipterus japonicus*, *Harpodon nehereus*, *Coilia dussumieri*, *Sepia* and *Loligo* in the stomachs, it may be said that this species *Trichiurus lepturus*, *Penaeus indicus*, *Acetes indicus*, *Metapenaeus brevicornsis* was pelagic and benthic carnivores. This report is the first reference of stomach contents study of sharks in Myanmar.

Acknowledgements

I am indebted to Dr. Aung Myat Kyaw Sein, Rector and Dr. San San Aye, Pro-Rector of Mawlamyine University, for their encouragement and supports in preparing this work. I am very grateful to Dr San Tha Tun, Professor and Head of the Department of Marine Science, Mawlamyine University, for his valuable suggestions and constructive criticisms on this study. I would like to express my sincere thanks to my students, Department of Marine Science, Mawlamyine University, for their kindly help me in many ways during field trips. Many thanks go to Professor Dr. Tint Swe, Retired Head of the Department of Marine Science, Mawlamyine University, for his assistance in preparations of the manuscript. I would like to thank my beloved parents, U Thein Win and Daw Kyi Aye, for their physical, moral and financial supports throughout this study.

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SPECIES COMPOSITION AND ABUNDANCE OF *TENUALOSA* SPECIES IN NGA YOKE KAUNG COASTAL AREA

Soe Thaw Thaw Tun[1], Lwin Mar Aung[2]

Abstract

This study was conducted on the species composition and abundance of *Tenualosa* spp. in Nga Yoke Kaung coastal area from June 2018 to February 2019. Two species of *Tenualosa*, namely *Tenualosa ilisha* and *Tenualosa toli* were observed in study sites. The species composition of *Tenualosa* spp. and other fishes caught by drift gill net using mesh size of 2.5 – 3.5 inches are described monthly in this study. The catch composition showed that the high species composition of *Tenualosa ilisha* was found in Sin Ma during June to September and in Nga Yoke Kaung during June to August. In *Tenualosa toli*, the highest composition was found in Sin Ma in October and also in Nga Yoke Kaung in December. In order to estimate the abundance of *Tenualosa* spp. in study areas, catch per unit effort of each species were calculated from the catch weight (kg) during study periods. The best production of *T. ilisha* was June to September in both study sites and the maximum CPUE value was observed in August in Nga Yoke Kaung. *T. toli* was more abundant during September to February in study areas. The fishing areas and fishing gears used for Hilsa fishery were recorded in this study.

Keywords: *Tenualosa ilisha, Tenualosa toli*, species composition, CPUE, fishing gear

Introduction

With a coastline of nearly 3, 000 km, several large estuarine, delta systems and numerous offshore islands, Myanmar possesses a large diversity of coastal habitats, including coral reefs, mangroves, sandy beaches and mudflats (FAO, 2003). These vast areas are natural habitats and nursery ground for shell fish and fin fishes which are of economically importance in Myanmar fisheries. The present study was conducted in Sin Ma and Nga Yoke Kaung coastal areas which are situated in lower Rakhine coast. Sin Ma village is located at Shwe Thaung Yan Township, Pathein District and is about 9 miles far from Ngwe Saung Beach. Nga Yoke Kaung is situated at Ayeyaewady Region, Pathein District, Nga Pu Taw Township the west coast of Myanmar. Nga Yoke Kaung Bay is very close to Gaw Yan Gyi Island that is famous for new potential site of eco-tourism destination at present. Both Sin Ma and Nga Yoke Kaung coastal areas are famous for fishing activity and people who live in these areas depend on fisheries and its products for their livelihoods.

Tenualosa spp. is fishes belonging to the Family Clupeidae in all temperate and tropical coastal waters. This family consists of herrings, sardines, menhadens, shads, and their relatives. They have a worldwide distribution, inhabiting marine and brackish waters. The Hilsa shad, *T. ilisha* (Hamilton, 1822), locally known as "Nga Tha Lauk" in Myanmar, is a major contributor to fish consumption in Myanmar east of India and Bangladesh. The species is also found in Iran, Iraq, Kuwait, Malaysia, Oman, Pakistan, Qatar, Saudi Arabia, Sri Lanka, Thailand, United Arab Emirates and Viet Nam (Freyhof, 2014). The fish is extremely rich in amino acids, minerals and lipids, especially essential and poly-unsaturated fatty acids (Alam et al., 2012). This euryhaline anadromous species can be found in marine, coastal and freshwater environments and often demonstrates schooling behavior in coastal waters.

T.toli is also known as, "Nga Tha Lauk Yauk Pha" in Myanmar and occur in the estuaries and adjacent coastal areas of India to Java and to the South China Sea. This species is found along the coastal waters of countries in the Bay of Bengal Large Marine Ecosystem (BOBLME) region

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such as India, Sri Lanka, Bangladesh, Myanmar, Malaysia, Thailand and Indonesia except for Maldives (Preston, 2004). Among these two species T. toli was not recorded from the rivers and it may be strictly marine form. Only one species, *T. ilisha* was found to occur along the river as it is known to be a migratory species (Khaing Myat Myat Htwe, 2012).

According to Fisheries Department of Myanmar, livestock and fisheries sector contributed to 8% of national GDP in the 2017-2018 fiscal year with the total production 5.87 million metric tons of fish. Of which, 2.72 million metric tons that accounted for 46% of the total fish production was from freshwater fisheries, and 3.15 million metric tons accounted for 54% of the total production of fish was from marine fisheries. The amount of 11379.95 metric tons of hilsa was exported to 27 different countries worldwide, with 17 in Asia, six each in the Middle East and Europe, with the value at US$ 32.17 million, in 2017 – 2018. Hilsa has a large market demand and is currently among the top five fish export products in Myanmar.

Materials and Methods

Study sites and study period

The fish landed by commercial catch from Sin Ma and Nga Yoke Kaung landing sites are collected during June 2018 to February 2019. The study areas are shown in figure (1). The locations of sampling sites were:

(1) Sin Ma (Lat 16º 43′N Long 94º 22′ E) (Shwe Thaung Yan Township)

(2) Nga Yoke Kaung (Lat 16º 31′ N Long 94º17′ E) (Nga Pu Taw Township)

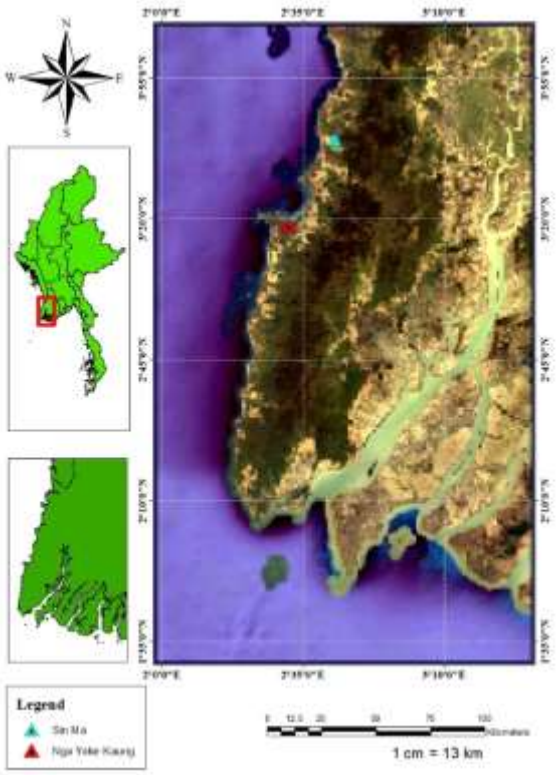

Figure 1 Map showing the study sites

Sample collection

Sample collections were conducted on commercial landing sites of study areas. Colour patterns and measurements of the samples were recorded immediately after collections. Also for later studies, specimens were photographed, using digital camera and then preserved in 10 percent formaldehyde solution. Monthly catch weight (kg/boat) of study fishes was taken from Sin Ma and Nga Yoke Kaung fish landing sites during June 2018 to February 2019. The fishing gears, species composition, volume of catch, fishing areas were recorded. Species composition was calculated in terms of percentages by weight.

Identification

The identification of this study followed the references of Day (1878), Munro (1955), FAO species identification sheets (1974), Jayaran (1981), Whitehead (1985), FAO species identification sheets (1985), Talwar and Jhingran (1991), De Bruin et al. (1995), Mya Than Tun (2001), Hla Win *et al*. (2008) and Khine Myat Myat Htwe (2012).

Catch Per Unit Effort

Abundance was estimated from the weight (kg) of the total catch of each site for each species over the study period. Catch per unit effort was calculated as: Where, C_i is the catch size (either in the number or mass of fish) per gear i,

$$CPUE = \Sigma \frac{(\frac{Ci}{Ei})}{n}$$

E_i is the effort expended by gear i and

n is the number of gears used (Pollock et al., 1994).

Effort was calculated by multiplying the total fishing hours by the length of net expressed in 100 m units. Effort units were standardized to 100 m net·hr^{-1}

Results

General description

Tenualosa ilisha: Body moderately deep, compressed and fairly elongated in shape, brilliant silvery, dark bluewish green on dorsal; A dark blotch behind gill opening, followed by a series of small spots along flank in juveniles but present or absent spots in the adults. Abdominal edge keeled with a row of scutes. Head moderate, compressed and scaleless. Mouth is terminal; upper jaw with a distinct median notch. Opercle smooth, without bony striae. Gill rakers fine and numerous. Eyes large. Dorsal-fin origin at midpoint of body; anal fin base fairly short and caudal fin deeply forked. Scales moderate, regularly arranged and cycloid type.

Tenualosa toli: Body is fusiform, compressed and fairly elongated in shape, silvery in colour and golden shot on flanks when fresh; dark greenish blue on back and on snout; a diffuse dark mark behind gill opening, but no other spots on flank. Abdomen rounded scutes. Head short, compressed. Mouth is terminal; upper jaw with distinct median notch Opercle smooth without bony striae. Gill rakers fine but not numerous; eyes large and covered by broad adipose lids. Dorsal-fin origin slightly anterior to midpoint of body; last dorsal-fin ray not elongated. Anal-fin base short; the caudal fin deeply forked and longer than the head length, its lobes are equal. Scales moderate and fairly thick, cycloid type.

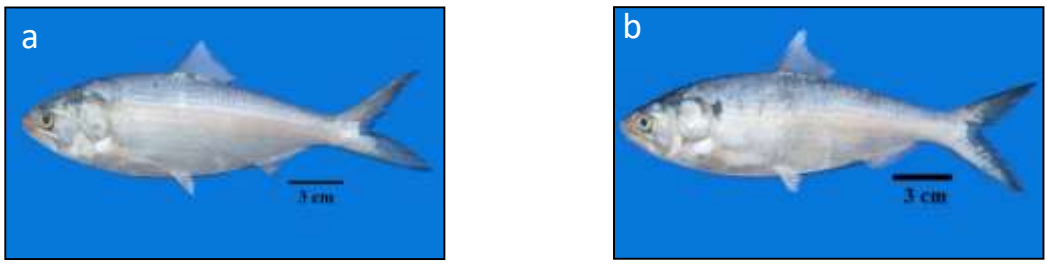

Figure 2 (a) *Tenualosa ilisha*, (b) *Tenualosa toli*

Species composition of *Tenualosa* spp. in study sites

The species composition of *Tenualosa* spp. caught by drift gill net using mesh size of 2.5 – 3.5 inches was observed. Species composition was calculated in terms of percentages by weight. Monthly species composition of *Tenualosa* spp. in Sin Ma was shown in figure. 3 and also in Nga Yoke Kaung as described in figure 4. In Sin Ma landing site, the high catch composition of *Tenualosa ilisha* was observed from June to September, with the peak percentage (62.12%) in July and the low amount in composition was found during the remaining months. *T. toli* was the highest account in composition of total catch in October among the study months. In Nga Yoke Kaung, the highest composition of *T.ilisha* was recorded during June to August as 75.07% in August, 73.69% in June and 63.95% in July. In the case of *T. toli*, the high catch composition was dominant as recorded from August to February.

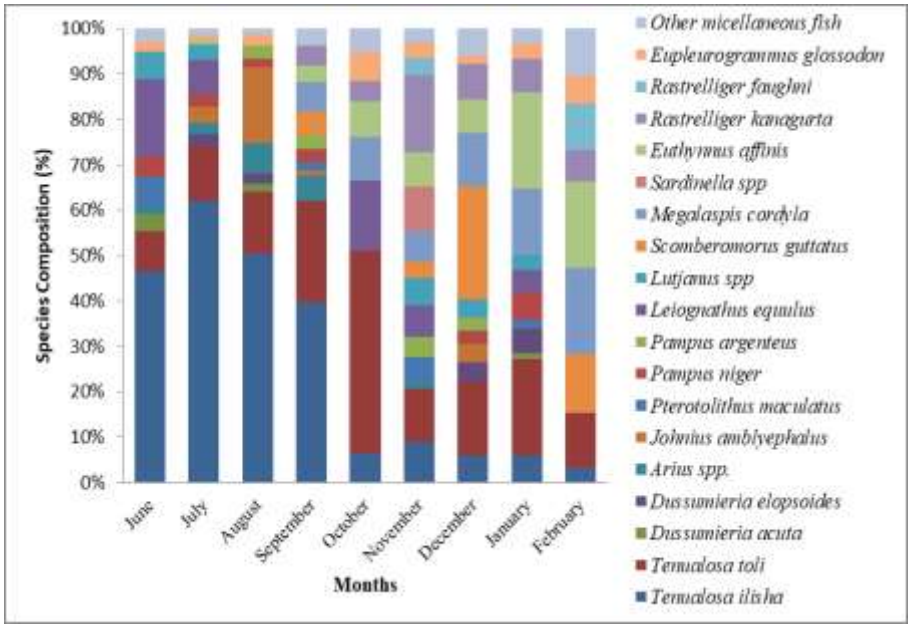

Figure 3 Monthly species composition of *Tenualosa* spp. and other fish caught by drift gill net in Sin Ma

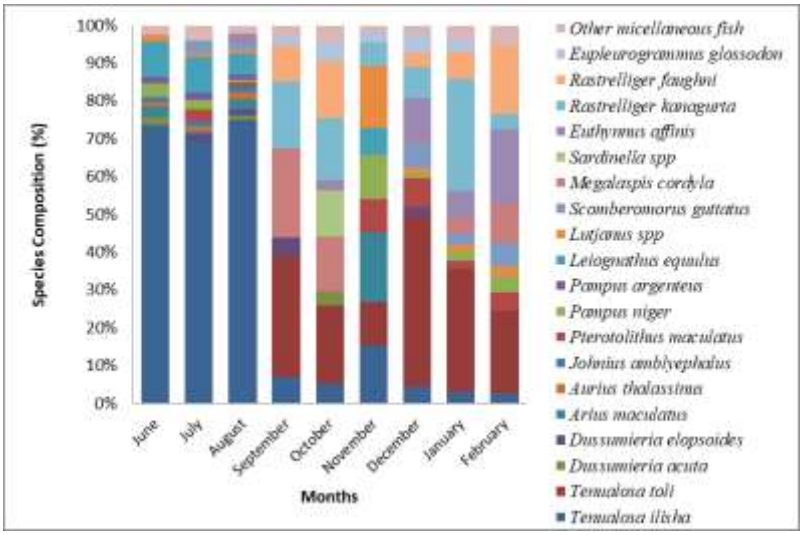

Figure 4 Monthly species composition of *Tenualosa spp*. and other fish caught by drift gill net in Nga Yoke Kaung

Abundance of *Tenualosa* spp. in study areas

Average catch weight (kg/boat) of *Tenualosa* spp. were randomly collected at the study areas as shown in figure 5 and 6. At Sin Ma landing site, the fishing time of *T. ilisha* was observed from June to September, which is the good production of the year for the catch of this species and the catch weight was high in July and August, amounted to 1100.75 kg/boat (25.94%) and 1256.77 kg/boat (29.61%) respectively and the size of fish caught in these months are usually large and marketable size (>0.65 kg) for export. The lowest catch was found in October and February.

In Nga Yoke Kaung, it was found that the good production of *T. ilisha* had been recorded from June to August, with peak in August, amounted to 1714.58 kg/boat (34.20 %). The size of *T. ilisha* caught in these months are recorded as large as marketable for local and export. The low catch weights were occurred from December and February. In the case of *T.toli*, the highest catch weights are recorded in September, amounting to 458.55 kg/boat (19.07%), and in October, amounting to 450.87 kg/boat (18.75%) in Sin Ma. In Nga Yoke Kaung, the high production was found in December and January, 547.35 kg/boat (22.52%) and 723.87 kg/boat (29.78%) respectively. In order to estimate the abundance of *Tenualosa* spp. in study sites, catch per unit effort of each species were calculated from the catch weight (kg) during study periods. The value of catch per unit effort of *Tenualosa* spp. for both study sites was shown in Table.1 and 2.

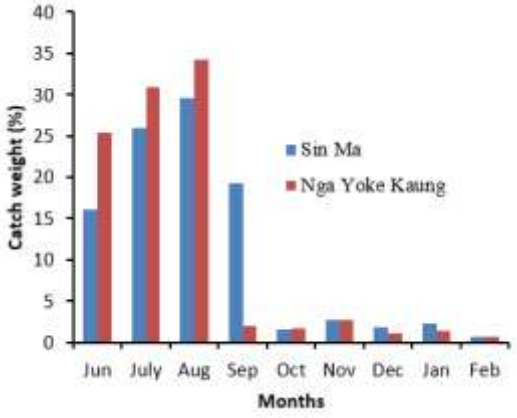

Figure 5 Monthly catch weight of *T. ilisha* at Sin Ma and Nga Yoke Kaung

Table 1 Monthly catch per unit effort (kg/100 m-net^{-1}·hr^{-1}) of *T.ilisha* in the study areas.

Month	Jun	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Sin Ma	1.76	2.85	3.25	2.11	0.17	0.30	0.20	0.26	0.08
Nga Yoke Kaung	5.37	6.55	7.24	0.41	0.34	0.59	0.22	0.29	0.15

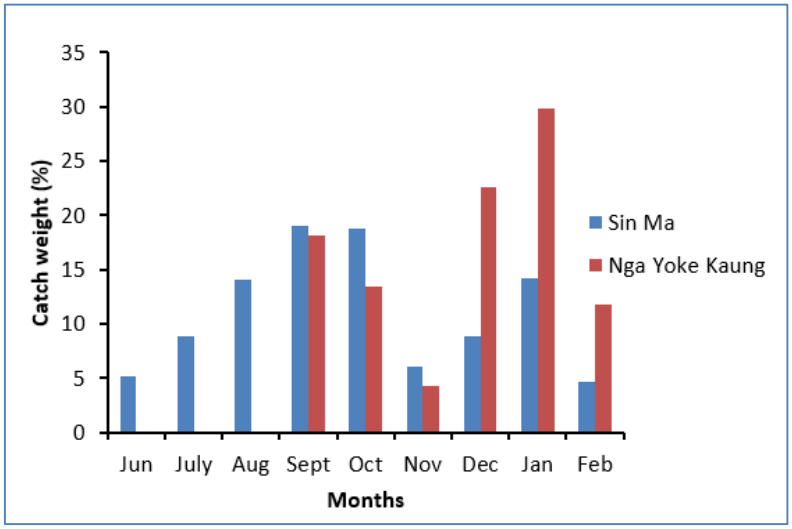

Figure 6 Monthy catch weight *T. toli* at Sin Ma and Nga Yoke Kaung

Table 2 Monthly catch per unit effort (kg/100 m-net^{-1}·hr^{-1}) of *T. toli* in the study areas.

Month	Jun	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Sin Ma	0.32	0.55	0.88	1.19	1.17	0.38	0.55	0.89	0.29
Nga Yoke Kaung	0	0	0	1.86	1.38	0.44	2.31	3.05	1.21

Fishing gear and fishing area

Figure 7 (a) hilsa fishing net mesh size; (b) Lead sinker; and (c-e) Some floats used in the head rope; (f) fishing area (WCS, Myanmar)

Discussion

Species and composition is an indicator of the habitat of a particular fish species in a certain aquatic environment. In present study, monthly species composition of *Tenualosa* spp. and other fishes caught by drift gill net were recorded in Sin Ma and Nga Yoke Kaung landing sites. According to the result, the species composition of *T. ilisha* in Sin Ma was the highest value found from June to September, greater than one third of the total catch (>35%), it was dropped to the lowest from October to February, less than (<10%) of total species composition. In Nga Yoke Kaung, the high composition of *T. ilisha* was found in June to August, the highest value was recorded in August, constituted to 75.09% of the total composition and the catch composition was declined noticeably in all remaining months. In 2012, Khaing Myat Myat Htwe recorded that *Tenualosa ilisha* was found in Hyeingyi Kyun and Pathein, and remarked this species as a migratory one, and *T. toli* was found as dominant species in open sea (saline water) and was not observed in river. She also discussed that the fishing season for *T. ilisha* was from September to November, with peak in November in Ngawun River Areas, Pathein.

In Myanmar, hilsa are mainly spread along the Ayeyarwady Delta, migrating from marine areas though brackish waters to freshwater areas. Baran *et al*. (2015) proposed this migration takes place through three main routes: the Pathein River, the Ayeyarwady River in the central dry zone and the Ayeyarwady River in the delta. They identified two periods of upstream migration: one in the wet or southwest monsoon season (August to October or even December), and one in the dry season around March.

Merayo *el at* (2020) described on the migratory patterns of hilsa shad in the Myanmar Ayeyarwady delta. They stated that mature hilsa in saline areas of the Pathein route are most

abundant in summer (June–August) and in October–November and largest fish were more abundant in July and September. According to the finding of Bladon (2019), hilsa specimens caught in fresh water were significantly smaller and lighter than those caught in brackish and saline water. This finding showed that hilsa spawn in the freshwater zone, which also provides a nursery area for juveniles before they migrate towards the coast, where they reach maturity. Eighty percent of mature fish were found more abundant in coastal waters than immature fish, while in fresh water, immature fish dominated. In October, the catch weight of *T. ilisha* dropped to the lowest and in November, the smaller fish (<0.65 kg) of this species were landed in small amount in study site during the present. For marine capture, it was concluded that the good production time for *T.ilisha* was June to September in both Sin Ma and Nga Yoke Kaung.

According to BOBLME (2015) stock assessment *Tenualosa ilisha* in Myanmar, the fishing season for inland fishery started from October to February in Nga Pu Taw, Pyapon and Pathein. As *T.ilisha* was the anadromous species, they have to migrate to river for breeding and spawning. In the case of *T. toli,* the species composition was found higher in both study sites during September to February 2019. The highest value was recorded in Sin Ma in October (44.6%) and in Nga Yoke Kaung in December (45.23%) of total catch.

By the comparison of CPUE value of *T. ilisha* for both study sites, it was higher in Nga Yoke Kaung than Sin Ma in June, July and August. As the maximum value was found in August in Nga Yoke Kaung (7.24), it was concluded *T.ilisha* was more abundant in Nga Yoke Kaung than in Sin Ma. Both study sites were famous and commercially important for hilsa shad fishery for local and especially export to other countries.

The comparison of CPUE value in Sin Ma and Nga Yoke Kaung showed that there was no significantly difference, so that the estimation of abundance of *T. toli* is high during September to February in both landing sites. By comparison of *Tenualosa ilisha* with *T. toli, T. ilisha* was found at both study sites during the study period, and both the catch volume and the size of landed fish of *T. ilisha* was greater than those of *T. toli.* Khin Swe Mya el (2019) at reported that the high catch weight of *T. ilisha* was found in Pyanmalot river, one of the Ayeyarwady Delta areas in January and February.

According to the data on the observation of local fishermen, the production of two *Tenualosa* species is declining than the previous years. Catch weight of hilsa for study sites was low in October to February. Local fishermen mentioned that the amount of catches during fishing time of *Tenualosa* species is varied depending on the weather. Heavy rainfalls also decrease the catch and disturb the fishing operation. Climate change, including increasingly variability in weather patterns, was a major threat to fishing activity.

The fishing gear used in catching hilsa fishes was mainly on monofilament gill net. The choice of net for hilsa fishery operation in different areas and different seasons depends on the current velocity, the nature of the catch, and to a large amount, on their financial condition. The effective fishing gear for hilsa was drift gill net (hilsa net) as shown in fig 7(a-e). It has an average length of 1316 – 4389 m and the height of it depends on the target species and has an average length of 4.57 – 6.8 m. The mesh sizes are from 2.5 – 3.5 inches. It is made of monofilament nylon. It is set at the depth of (40 – 60 m). As hilsa fishery of Sin Ma and Nga Yoke Kaung Coastal Areas constituted to only marine capture fishery, the fishing areas could not be determined exactly because of migration of fish information gathering rather than survey had to be done. Information was obtained from questionnaires or interviews with local fishermen during study periods. Fig. 7(f) represented the fishing areas of drift gill net, estimated to 1487 km^2 in Sin Ma coastal area and Nga Yoke Kaung coastal areas.

Conclusion

The present study focused on the species composition occurrence of *Tenualosa* spp. and their monthly catch weights and CPUE values. The result has clearly shown the peak fishing season of *Tenualosa* species in study landing sites throughout the study period. Thus, a ban should be taken on catches of adult hilsa fish in July and August in study sites, when the largest brood mature fish are found in both the Ayeyarwady and Pathein route areas. This would provide protection for mature hilsa, particularly the large, older females predominantly in the coastal zone.

Acknowledgements

I am thankful to Rector Dr. Si Si Hla Buu, Pathein University for her permission accepting the research. I am greatly indebted to Dr Cherry Aung, Professor and Head of Marine Science Department, Pathein University. I am also greatly obligated to Dr Htay Aung, Professor (Retired) Pathein University, for his invaluable guidance and suggestions.

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SOME BOLOGICAL ASPECTS OF *OTOLITHOIDES PAMA* (HAM-BUCH, 1822) FROM PHYAN YAY KYAW VILLAGE, NGA PU TAW TOWNSHIP, AYEYAWADY RGION

Lwin Mar Aung[1], Soe Thaw Thaw Tun[2], Myat Thu[3]

Abstract

Study on some aspects of biology of *Otolithoides pama*, (Family: Sciaenidae) was studied in the Phyan Yay Kyaw village, Nga Wun River, Nga Pu Taw Township, Ayeyawady Region between June 2015 to May 2016. Fifty percent of male matures at the average length of 21.5cm and that of female at 17.5 cm TL. The overall sex ratio was 1male:1.2females (X^2=1.8). The highest gonadosomatic index value was observed in June and July for both sexes. Spawning season was from December to July and with the peak spawning months was June and July. Length-weight relationship of *O.pama* was negatively allometric growth (b<3). There was significanty difference between the slopes at 1% level; a combined relationship of male and females was obtained.

Keywords: Gonadosomatic index, spawning season, sex ratio, length-weight relationship

Introduction

In Myanmar Sciaenid fish are harvested by bottom gill nets, set bagnets and otter board trawlers. Sciaenids form a commercially important group of fishes in the catches of both the powered and non-powered craft. Ayeyawaddy region lies between north latitude 15˚40′ and 18˚30′ approximately and between east longitude 94˚15′ and 96˚15′ and has an area of 13,566 sq-miles (http:// myanmartravelinformation.com).

Length at first maturity is use for the potential spawners lost from the stock by fishing. The gonadosomatic index which is an index of gonad of eggs is determined size relative to fish size is a good indicator of gonadal development of fish (Dadzie and Wangila, 1980). The percentage of body weight of fish that is used for production by the gonadosomatic index. The slight variation in spawning time in different regions are primarily because of differening environmental parameters such as temperature, light, salinity which cause changes in physiological activities and subsequently spawning time (King, 1995).

Information on sex ratio is necessary for the assessment of the relationship between individuals the environment and the state of the population. In nature, the sex ratio showed the population of males and females proportion and could be expected to be 1:1 and any deviation from this ratio can examine the dominance of one sex over the other.

Length-weight relationship can be used to predict weight from length measurements made in the yield assessment (Pauly, 1993). Length-weight relationship is important in fisheries science, to raise length frequency samples to total catch, or to estimate biomass from underwater length observations. Fish can attain either isometric growth, negative allometric growth or positive allometric growth. Isometric growth is associated with no change of body shape as an organism grows.

Phyan Yay Kyaw village is situated near the Nga Pu Taw Township, located along the Nga Wun River in Southern part of Pathein. In this study area, the major catching fish was *Tenualosa sp*. and *Otolithoides pama* is the second fishing. The fishermen in this area were using man power motorized vessels and bottom-set gillnets for catching of this species. This fish is highly esteemed as food and the swimbladder of these fishes may be extensively used for making isinglass (Bhuiyan, 1964). Thus, the present study was conducted to assess size at first maturity, sex ratio,

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gonadosomatic index (GSI), spawning season and to estimate the length-weight relationship of *Otolithoides pama*

Materials and Methods

Study area and study periods

Nga Wun River which is a western branch of Ayeyawady River. Nga Wun River is also called the Pathein River. A total of (50) fishing boats from Phyan Yay Kyaw Village was fishing in the Nga wun River. Of the caught species, the average (70) kg of *Otolithoides pama* fishes was normally caught a boat per day. The specimens was conducted to Phyan Yay Kyaw Village (Lat. 16° 36′ N, Long. 94° 43′ E), Nga Pu Taw Township, Pathein District, Ayeyawady Region from June, 2015 to May, 2016.

Data collection

The fish samples were collected monthly from two fishing boats based on fish landing areas of Phyan Yay Kyaw village. The studied fishes are caught by bottom –set gill net (4.5 cm x 13.5 cm). The collected samples were then measured the body length (TL) (from the snout to the end of caudal fin) in centimeter by using fish measuring board and total wet weight (TW) in gram by using electronic balance. Identification was followed after FAO (1972, Vol-III)

Size at first maturity

The size at first maturity of the species was determined on the basis of the percentage occurrence of mature fishes in divided size groups for the purpose of determining the minimum length at first maturity in females and males.

Sex ratio

The sex of each specimens was identified by examination of the gonads. The proportion of the two sexes relative to one another was used to calculate the sex ratio. Sex ratio was calculated and tested for the expected ratio 1:1 by chi square(X^2) analysis according to the formula;

$$X^2 = \sum(O-E)^2/E$$

Where O= observed frequency of males or females, E=expected frequency of males or females

Gonadosomatic Index (GSI)

To examine, changes in the gonads as a means for estimating the spawning season of this species the gonadosomatic index (GSI) for females was computed according to Bichy (2004) as follow;

$$GSI = [OW / (BW – OW)] \times 100$$

Where OW = ovary weight (g) and BW = body weight (g).

Length–weight relationship

The length-weight relationship was calculated by the method of least squares using the parabolic equation:

$$W = aL^b \text{ (Le Cren, 1951)}$$

and after logarithmic transformation has the form of Log W = Log a + b Log L, Where W is the total weight of fishes in gram, L is the total length of fishes in cm, ‘a’ is a coefficient related to body form, and ‘b’ is an exponent indicating allometric growth when unequal to 3. The parameters a and b were estimated by linear regression of log-transformed weight and length.

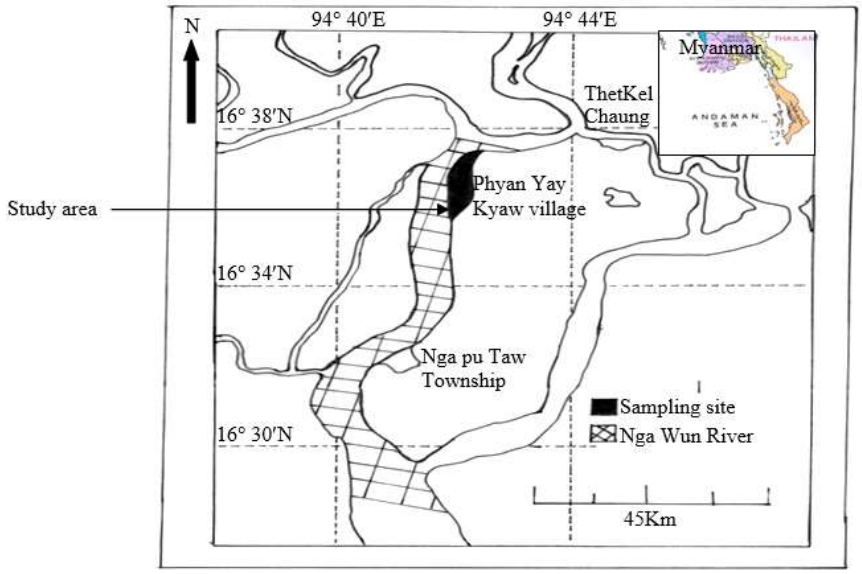

Figure 1 Map showing the study area

Results

Classification of *Otolithoides pama* (Ham-Buch., 1822)

Phylum	Chordata
Class	Acctinopter
Order	Perciformers
Family	Sciaenidae
Genus	*Otolithoides* Fowler, 1933
Species	*Otolithoides pama* (Ham-Buch., 1822)

Descriptive account on *Otolithoides pama* (Ham-Buch., 1822)

Synonyms **:** *Bola pama* Ham-Buch., 1822

Sciaenoides pama: Day, 1876

Pama pama: Fowler, 1993; Weber & de Beaufort, 1936

FAO name: Pama croaker.

Local name: Nga-poke-thin.

Fin Formula: B. VII; D.X/I/40-45; P.I.20; VI /7; A.II/8; C.16; LI. (54-57)

Diagnostic features: A large species with a conical snout and large terminal mouth; upper jaw reaching back beyond eye. Body oblong-elongate in shape. Teeth enlarged in both jaws, with 1or 2 pairs of canines in upper jaw and sometimes a pair of large teeth at tip of lower jaw. Lower gill rakers 11 to 14, long at joint of arch, shorter in front. Swimbladder carrot-shaped, with a single pair of appendages, arising from posterior end of bladder and running forward beside it to enter the head where they branch under the skull. Dorsal fin with 10 spines, followed by a low notch, second part of the fin with 1 spine and 40 to 45 soft rays. Pectoral fin as long as or longer than head; anal fin with 2 spines and 7soft rays, the 2nd spine short and weak. Caudal fin pointed. Scales cycloid

(smooth) on head and ctenoid (rough to touch) on body, very small above anterior part of lateral line; lateral line scales reaching to tip of caudal fin. Colour: no distinctive markings; light brownish along back and white beneath; head shot with gold and purple. Fins are yellowish.

Local occurrence: Rakhine Coast, Ayeyawady Delta and and Tanintharyi Coast.

Geographical distribution: Bay of Bengal, Myanmar, Malay Peninsula; India.

Main fishing gear: Caught with bottom gill nets, set bag nets and otter board trawls.

Figure 2 Study species, A) Otolithoides pama B) Swimbladder

Size at first maturity

Size of *Otolithoides pama* at first maturity was determined the average size at which 50 percentage of occurrence in total length. The total length ranging (7.5-31.5 cm) for female and (15.5-31.5 cm) for male were observed during the study period. Appearance of sexual maturity was observed at 19.5-23.5 cm length group in male and 15.5-19.5 cm length group in female. Fifty percent of male matures at the average length of 21.5cm and that of female at 17.5 cm indicating that females mature at smaller than their male counterparts.

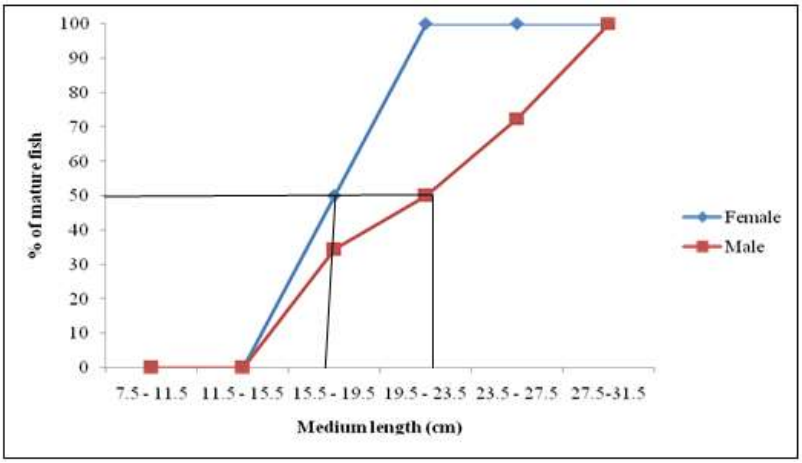

Figure 3 Size at first maturity of male and female *Otolithoides pama*

Sex ratio

A total of 780 specimens of *Otolithoides pama*, 353 males and 427 females were examined for sex ratio. The sex ratio for monthly variation was shown in Table1. Females were abundantly dominance than males during the study period except the month of July, November and January. The overall sex ratio was 1male:1.2females (X^2=1.8). Therefore, chi square test of the average ratio shows that the sex ratio was not significantly difference from the excepted 1:1.

Table1 Monthly sex ratio of *Otolithoides pama*

Months	M:F	X^2	Months	M:F	X^2
June	1:1.1	0.04	December	1:2.3	4.6
July	1:0.6	1.1	January	1:0.8	0.7
August	1:2.4	8.4	February	1:0.7	1.6
September	1:1.8	2.6	March	1:0.8	2.2
October	1:1.4	1.1	April	1:0.7	2.1
Nov	1:0.9	0.1	May	1:0.8	1.1

Gonadosomatic Index (GSI)

Gonadosomatic index was used to corroborate the spawning period of study species. In male *Otolithoides pama*, the high GSI value was observed in June and July. The GSI value decreased from August to December and then gradually increased from January to July. In femal *O. pama*, the peak GSI was observed in July. The GSI value was gradually decreased from August to November and then increased from December to May. The trends of GSI values in both sexes were similar. Gonadosomatic index of females were found remarkably higher in values over males. The peaks of GSI values showed that it closely related to the peaks of spawning period.

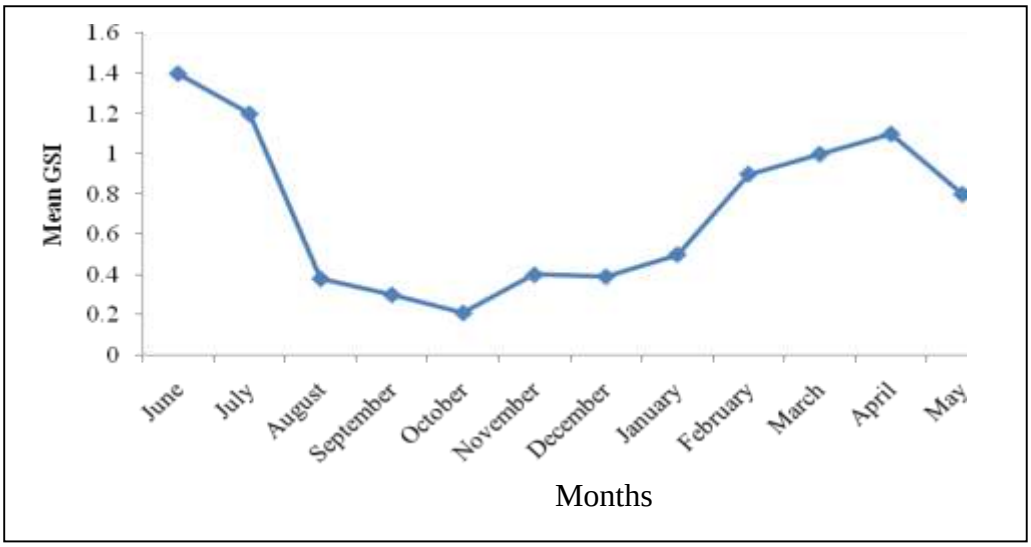

Figure 4 Monthly mean GSI of male *Otolithoides pama*

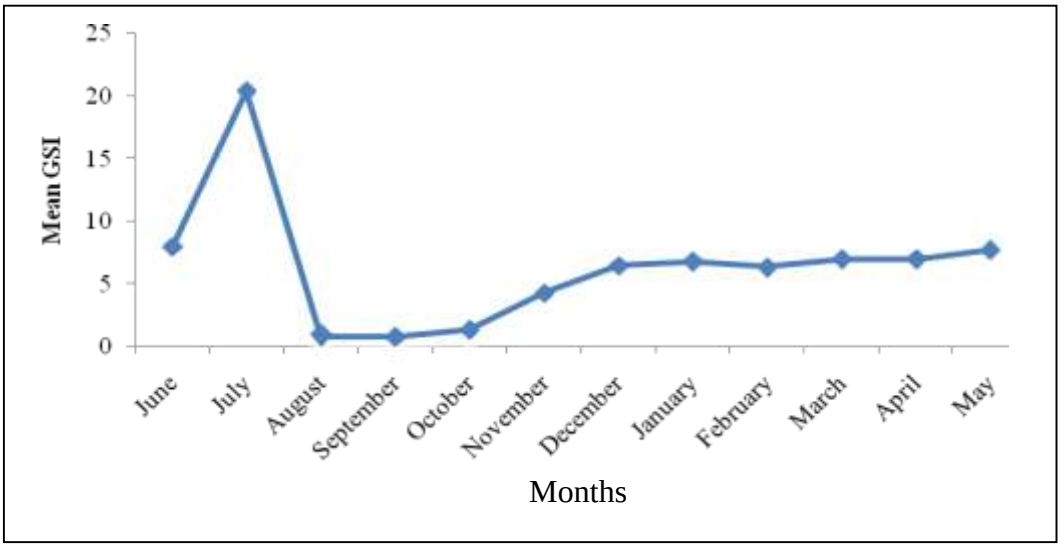

Figure 5 Monthly mean GSI of female *Otolithoides pama*

Spawning season

The GSI values of gonad maturation of the males and females indicated the presence of mature and spent stages of *Otolithoides pama*, with higher GSI values indicating the reproductive period of *O. pama*. Spawning season to be formed December to July with the peak spawning months was June and July.

Length-weight relationship

As fish grow in length, they increase in weight. A total of 182 females ranging from 7.5 to 31.5cm length with weighing from 21.7 to 156.1g and 166 males ranging from 15.5 to 31.5cm length and weighing from 34.7 to 181.4g, were used to calculate the length-weight relationship. The relationship between length and weight for males, females and pooled sexes were shown respectively.

In male: Log W= 2.803Log TL - 1.939

In female: Log W=2.075TL - 0.984

The data of sexes were pooled and a common relationship calculated by the equation: Log W=2.403TL - 1.406.

According to "b" value, male represented the highest (b=2.803), followed by b=2.403 in combined sex and b=2.075 in female. The specimens of *O. pama* were observed less than 3. The value of the slope b was lower than 3 (isometric growth) indicating that the growth of *O. pama* was negatively allometric growth and the weight increased slowly as compared to the cube of length. However, the b values in male showed nearly isometric growth compared to the other sexes. The length and weight of male was greater than the female. There was significantly difference between the slopes at 1% level; a combined relationship of male and females was obtained.

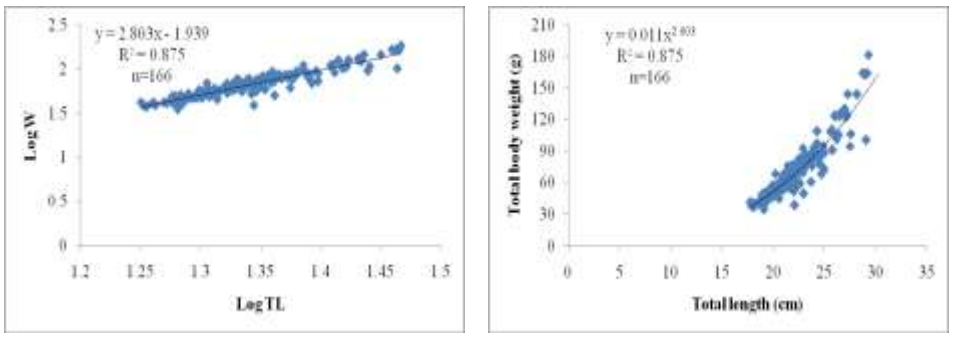

Figure 6 Length-weight relationship in male of *Otolithoides pama*

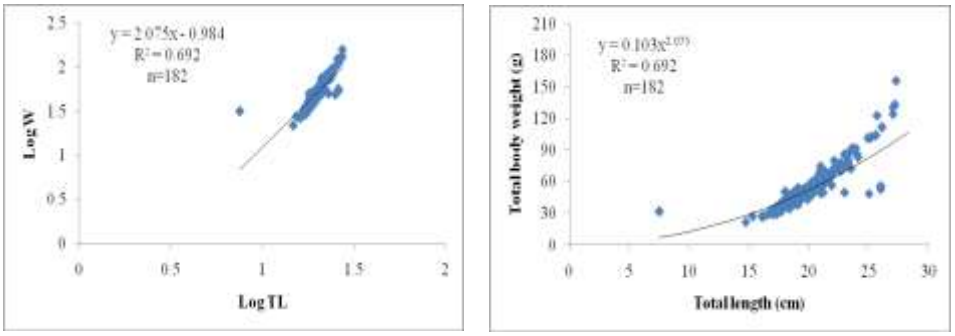

Figure 7 Length-weight relationship in female of *Otolithoides pama*

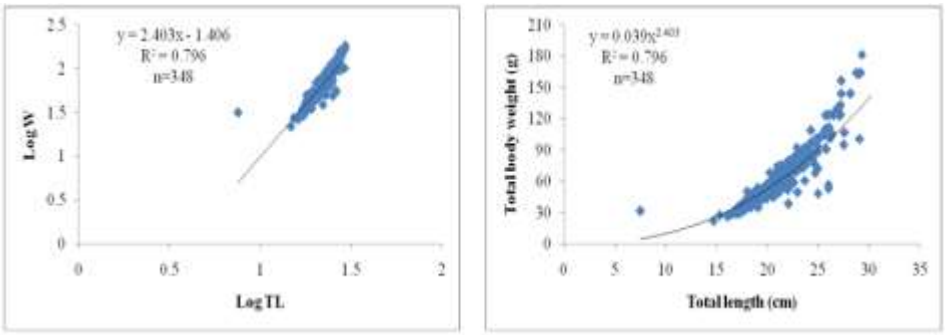

Figure 8 Length-weight relationship in pooled sexes of *Otolithoides pama*

Discussion

Size at first maturity is considered as a mark to assess the minimum catch size of study species. Min Oo (1995) reported that the minimum size at first maturity in *Otolithoides pama* can be taken as 145-170mm size groups. In the present study, female *Otolithoides pama* mature between size group15.5-19.5 cm and male mature between size group19.5-23.5 cm that was a little variation above the author.

The first sexual maturation is an important point in the animal's life history and must be taken into account for successful fish management. In the present study, appearance of sexual maturity at a mean total body length of 17.5cm in female and 21.5cm in males indicated that females mature at smaller size than their male counterparts in study area.

. Hashmi (1966) reported that the male to female ratio of *O.argenteus* was 1:2 and Ghosh (2008) also stated that male to female ratio was 1:1.09 in *O.biauritus*. Tin Hnin Wai (2010) studied the reproductive biology of *Otolithoides pama* in Pathein River, the sex ratio of males to females

was 1:2 in her study. San San Myaing (2010) described the sex ratio of *O.pama* was 1male:5.98females. In the present study, the overall sex ratio of males to females was 1:1.2 and according to the monthlty sex ratio, females were more abundantly dominance than males throughout the study period except the month of July, November and January. And so, these findings agree with above author.

Tin Hnin Wai (2010) reported that the high values of GSI were observed during the period from January to May with a peak in March. In the present study, high values of GSI were recorded in June and July for both male and female of *O. pama*, which suggested that the spawning period of *O.pama* was June and July. Osman (2011) stated that the highest value of GSI was in June and minimum value was in May. This suggestion was agreeing with the present study.

In the present study, the value of GSI was high in June and July and decreased from August to November and then increased from December to May, which show a longer spawning period of this species. Ohnmar Min (2013) assumed that the individuals of the *O.pama* population in Mon Coastal waters are asynchronous spawners. The present findings were nearly similar to those of Ohnmar Min (2013).

Ghosh (2008) stated that the peak breeding season of *Otolithoides biauritus* was in the monsoon season from May to August as evidenced by maturity stages and gonadosomatic index. San San Myaing (2010) described the spawning season of *Otolithoides pama* to be from January to December with the peak spawning months were reported February, March and September. In this present study, spawning season to be formed December to July with the peak spawning months was June and July.

Ghosh *et al*., (2008) stated that the length-weight relationship of *Otolithoides biauritus* showed that growths allometric and there was no significant difference between sexes and this was not agreed with the present finding. The length and weight of male was greater than female in this study. There was significanty difference between the slopes at 1% level; a combined relationship of male and females was obtained. The length –weight relationship of *O.pama* , the b value was lower than 3 in the study of Min Oo (1995), Ohnmar Min (2013) and Zaw Myo Hein (2014).

In the present study, the "b" value for male represented the highest (b=2.803), followed by b=2.403 in combined sex and b=2.075 in female. The specimens of *Otolithoides pama* were observed less than 3. The value of the slope b was lower than 3 (isometric growth) indicating that the growth of *O.pama* was negatively allometric growth and the weight increased slowly as compared to the cube of length. The present results were thus agreement with the above author.

Conclusion

The present study shows that sexual maturity at a mean total body length of 17.5cm in female and 21cm in male indicate that females mature earlier than males. The spawning season of *Otolithoides pama* to be formed December to July with the peak spawning months was June and July. In male and female sex ratio, females were more abundantly observed than males throughout the study period. Moreover, the present study could conclude that some biological aspects *O.pama* from Phyan Yay Kyaw village, Nga Pu Taw Township, Ayeyawady region shows similar to those found for this species in other regions.

Acknowledgement

I wish to express to my special thanks Dr.Cherry Aung, Head of Department of Marine Science, Pathein University. I would like to express my sincere gratitude to Dr Pwint Thu Aye, Lecturer, Department of Zoology, University of Mandalay for her close supervision, guidance and encouragement throughout the work.

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SPECIES COMPOSITION AND ABUNDANCE OF HYTOPLANKTON IN DAWEI COASTAL WATERS

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Abstract

Species composition and abundance of phytoplankton were studied bimonthly at Dawei coastal waters in Myanmar during the period from June 2019 to January 2020. Samples were collected at the four stations, namely, Maungmagan (station 1), Kampani (station 2), Nyawpyin (station 3), and Kyaukmatak (station 4). A total of 148 species in which 6 classes are of 36 orders, 48 families, and 65 genera was recorded in the present study. Centric diatoms contributed a maximum percentage composition (80% in total) followed by the pennate diatoms (18%), whereas dinoflagellates comprised a minimum percentage composition, with an average of 2 %. Silicoflagellates were rarely observed. Among the four stations, the highest cell abundance (3139 cells/mL) was observed at station 1, with a 43% in total, which was followed by station 3 (1667 cells/mL) and station 2 (1573 cells/mL), with a percentage of 23% and 22% in total, respectively. The least cell abundance (889 cells/mL) was found in station 4 with a percentage of 12% in total. Phytoplankton abundance was high in the post-monsoon period but the low cell abundance in the monsoon season. *Skeletonema costatum, Leptocylindrus danicus, Proboscia alata, Bacteriastrum delicatulum, Chaetoceros compressus, Thalassionema frauenfeldii, Chaetoceros lorenzianus, Thalassionema nitzschioides* were dominant species along Dawei coastal waters.

Keywords: Abundance, centric, Dawei, dinoflagellates, pennate, species composition

Introduction

Phytoplankton, also known as microalgae, are similar to terrestrial plants in that they contain chlorophyll and require sunlight to live and grow. Most phytoplankton are buoyant and float in the upper part of the ocean, where sunlight penetrates the water. There are two main classes of phytoplankton which are known as diatoms and dinoflagellates. (NOAA) About 90% of the total production in the marine ecosystem is contributed by plankton (Achary *et al.* 2010).

Phytoplankton is very important because they form the base of the aquatic food web (Reynolds, 1984). Phytoplankton is the pioneer of an aquatic food chain. Biological production can be used as an index of trophic status and fisheries resource potential in any aquatic body (Jhingran, 1992).

Because of its importance in the marine ecosystem, many previous studies from various aspects of ecological approaches have been reported by several researchers related to its primary production, species composition, and abundance (Guo *et al*. 2014; Simo-Matchim *et al*. 2016). In this study, I have conducted species composition and abundance of phytoplankton in the four study areas of Dawei coastal waters. The main objective of the study is to know the species composition and abundance of phytoplankton in Dawei coastal areas.

Materials and Methods

Phytoplankton samples were collected at the four stations of Dawei coastal areas such as Maungmagan (Lat 14° 14′ N long 98° 02′ E), Kampani (Lat. 14° 04′ N, Long.98° 04′ E), Nyawpyin (Lat. 13° 38′ N, Long 98° 08′ E) and Kyaukmatak (Lat 13° 38′ N, Long 98° 08′ E), from July 2019 to January 2020.

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Phytoplankton samples were collected using a standard plankton net. A known volume of 50 L surface water was scooped with the bucket for quantitative sampling, then sieved to the final volume of 200 mL. After that samples were fixed with formalin (final concentration 1%) and also preserved with Lugol's neutralized iodine solution. Then sampling bottles were transported to the laboratory at the Department of Marine Science, Mawlamyine University. The sampled bottles were settled for 24 hours to the final volume of 50 mL for phytoplankton cell counting and identification. Species identification was mainly based on Cupp 1943, Hasle *et al.* 1997, Tomas 1997, Taylor *et al.* (2007), Al-Kandari *et al.* (2009), and Al-Yamani and Saburova (2019). Cell count and identification were made by using a light microscope (Nikon Eclipse E200) and Sedgewick-Rafter counting chamber, followed the methods reported by LeGresly and McDermott (2010). The counting unit of phytoplankton is expressed as cells/mL.

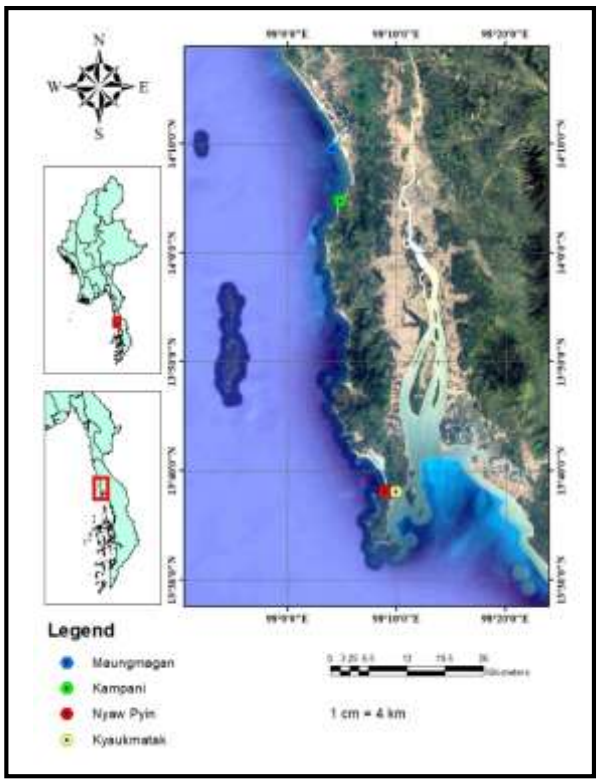

Figure 1 Map showing the sample collection sites in Dawei coastal waters

Results and Discussion

Phytoplankton species composition and abundance

A total of 148 species in which 28 species are of 8 orders, 11 families, 15 genera of Class: Coscinodiscophyceae, 50 species, 8 orders, 10 families, 15 genera of Class: Mediophyceae, 7 species, 4 orders, 4 families, 6 genera of Class: Fragillariophyceae, 33 species of 8 orders, 11 families, 16 genera of Class: Bacillariophyceae, 29 species, 7 orders, 11 families, 12 genera of Class: Dinophyceae, and 1 species, 1 orders, 1 family, 1 genus of Class: Dictyochophyceae was noted in this study.

Regarding the species occurrence of phytoplankton in Dawei coastal area, station 1 has the highest species occurrence (91 species) followed by station 4 (87 species), station 3 (80 species), and station 2 (78 species), respectively. Concerning the percentage composition of the different phytoplankton groups, centric diatoms 83%, pennate diatoms 15%, and dinoflagellates 2% were recorded in station 1. In station 2, centric diatoms 74%, pennate diatoms 22%, dinoflagellates 4%

and centric diatoms 83%, pennate diatoms 16%, dinoflagellates 1% were observed as station 3. Besides centric diatoms, 71%, pennate diatoms 26%, dinoflagellates 3% were found in station 4. The phytoplankton abundance ranged from 558-947 cells/mL in station 1, 184-630 cells/mL in station 2, 114-890 cells/mL in station 3, and 63-624 cells/mL in station 4.

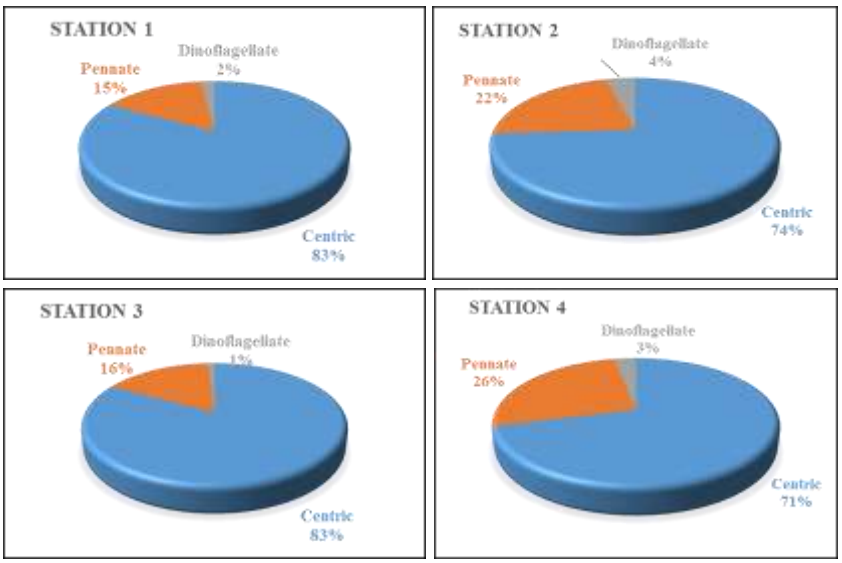

Figure 2 Species composition of phytoplankton in four study areas.

Species composition and occurrence of phytoplankton in four study areas

Centric diatoms	**St 1**	**St 2**	**St 3**	**St 4**
Hyalodiscus subtalis	+	+	_	+
Thalassiosira hyalina	+	_	+	+
Thalassiosira rotula	+	_	_	_
Thalassiosira subtilis	+	+	+	+
Thalassiosira osterupii	_	_	+	_
Planktonilla sol	_	+	+	+
Cyclotella striata	+	+	+	+
Lauderia annulata	+	+	+	+
Skeletonema costatum	+	+	+	+
Coscnodiscus centralis	_	_	+	+
Coscinodiscus granii	+	_	_	+
Coscinodiscus marginatus	+	+	_	+
Coscinodiscus oculus	+	_	_	+
Coscinodiscus radiatus	+	+	+	+
Coscinodiscus wailesii	_	_	+	+
Palmeria hardmaniana	_	+	_	_
Paralia sulcata	+	+	+	+
Asteromphalus cleveanus	+	_	_	_
Asterompjalus flabellatus	_	+	+	_
Bddulphia alternans	+	_	_	_
Bddulphia buddulphiana	_	+	_	_
Bddulphiia rhombus	_	+	+	_

Centric diatoms	**St 1**	**St 2**	**St 3**	**St 4**
Odontella granulata	+	–	–	–
Trieres mobiliensis	+	–	+	+
Trieres sinensis	–	+	+	+
Triceratium favus	–	+	+	–
Teiceratium reticulum	–	–	+	–
Stephanopyxis nipponica	–	–	–	+
Eucamppia zoodiacus	+	–	–	+
Eucamppia cornuta	+	+	+	+
Hemiaulus hauckii	+	+	+	–
Hemiaulus membranceus	+	–	–	+
Hemiaulus sinensis	+	+	+	+
Climacodium biconcavum	+	+	+	+
Climacodium frauenfeldianum	–	–	–	+
Bellerochae horologicalis	–	–	–	+
Helicotheca tamesis	–	–	–	+
Streptotheca indica	+	–	–	+
Ditylum brightwellii	+	+	+	+
Ditylum sol	+	+	+	+
Leptocylindrus danicus	+	+	+	+
Leptocylindrus mediterranius	+	–	–	–
Dactyliosolen fragillissimus	–	–	+	–
Rhizosolenia bergonii	+	+	+	–
Rhizosolenia cochlea	–	+	–	+
Rhizosolenia hyalina	+	–	+	+
Rhizosolenia imbricata	+	+	+	+
Rhizosolenia robusta	–	–	–	+
Rhizosolenia setigera	+	+	+	+
Rhizosolenia shrubsolei	–	+	–	+
Proboscia alata	+	+	+	+
Proboscia indica	+	–	–	+
Guinardia cylindrus	+	–	–	–
Guinardia flaccida	+	+	+	+
Guinardia striata	+	+	+	+
Bacteriastrum comosum	+	+	+	+
Bacteriastrum curvetum	–	+	–	–
Bacteriastrum delicatulum	+	+	+	+
Bacteriastrum furcatum	+	–	+	–
Bacteriastrum hyalinum	+	+	+	+
Bacteriastrum varians	–	+	+	–
Chaetoceros affinis	+	+	+	+
Chaetoceros coarctatus	+	–	+	–
Chaetoceros compressus	+	+	+	+
Chaetoceros constrictus	+	+	+	–

Centric diatoms	**St 1**	**St 2**	**St 3**	**St 4**
Chaetoceros costatus	–	–	+	–
Chaetoceros curvisetus	+	+	+	+
Chaetoceros danicus	+	+	+	+
Chaetoceros decipiens	+	–	+	–
Chaetoceros denticulatus	+	+	+	–
Chaetoceros diversus	+	+	+	+
Chaetoceros didymus	–	–	+	+
Chaetoceros laciniosus	+	+	+	–
Chaetoceros lorenzianus	+	+	+	+
Chaetoceros laevis	+	+	+	–
Chaetoceros messanensis	+	+	+	+
Chaetoceros peruvianus	–	+	+	+
Corethrom criophilum	+	+	–	–
Pennate Diatoms				
Asterionellopsis glacialis	+	–	+	–
Fragilaria capucina	+	+	+	+
Centronella reichelti	–	–	+	+
Thalassionema frauenfeldii	+	+	+	+
Thalassionema nitzschioides	+	+	+	+
Tabellaria fenestrata	–	+	–	–
Climacosphenia moniligera	+	–	–	–
Grammatophora marina	–	–	+	–
Lyrella lyra	–	–	+	–
Achnanthes fimbriata	–	–	+	–
Cocconeis guttata	+	–	+	+
Pinnularia trevelyana	–	+	–	–
Diploneis chersonensis	+	–	–	–
Diploneis smithii	+	+	+	+
Diploneis splendica	–	–	+	–
Diploneis nitescens	–	–	+	–
Diploneis finnica	–	–	+	–
Diplonies weissflogii	–	–	+	–
Meuniera membranacea	–	+	–	–
Navicula deliculata	+	+	+	+
Navicula granii	+	+	+	+
Haslea trompii	–	–	+	–
Pleurosigma aestuarii	+	+	–	+
Pleurosigma angulatum	–	–	–	+
Pleurosigma elongatum	–	+	–	–
Pleurosigma pelagicum	–	–	–	–
Pleurosigma normanii	+	+	–	+
Amphora angusta	+	–	–	–
Amphora linceolata	+	+	–	–

Centric diatoms	**St 1**	**St 2**	**St 3**	**St 4**
Bacillaria paxillifera	+	_	_	+
Cylindrotheca closterium	+	+	_	+
Nitzschia longissima	+	+	_	+
Nitzschia lorenziana	_	_	_	+
Nitzschia frigida	_	_	+	_
Nitzschia seriata	+	+	+	+
Nitzschia sigma	_	+	_	_
Nitzschia panduriformis	_	_	_	+
Tryblionella coarctata	_	+	+	_
Surirella gemma	_	+	_	_
Surirella ovalis	+	_	_	+
Dinoflagellates				
Prorocentrum micans	+	+	+	+
Prorocentrum rhathymum	+	_	_	+
Prorocentrum sigmoides	+	_	+	+
Dinophysis caudata	+	+	+	+
Dinophysis parvula	_	+	_	_
Dinophysis miles	+	_	_	+
Ornithocercus magnificus	_	_	_	+
Ornithocercus steinii	_	+	_	_
Akashiwo snaguinea	_	+	_	+
Ceratium furca	+	+	+	+
Ceratium fusus	+	+	+	+
Ceratium furocoides	_	_	_	+
Ceratium gibberum	_	+	_	_
Ceratium macroceros	+	_	+	+
Ceratium trichochoceros	+	_	_	_
Ceratium breve	_	_	_	+
Pyrocystis lunula	_	_	+	+
Cladopyxis hemibranchiata	+	_	_	_
Gonyaulax spinifera	+	_	_	_
Pyrophacus horologicum	+	_	_	_
Pyrophacus steinii	+	_	_	_
Diplopsalis lenticula	+	_	_	+
Protoperidinium compressum	+	+	_	_
Protoperidinium divergens	+	_	_	_
Protoperidinium oblongum	_	+	_	+
Protoperidinium oceanicum	_	+	_	+
Protoperidinium pentagonum	_	_	_	+
Protoperidinium venustum	+	_	_	_
Noctiluca scintillans	+	_	_	+
Silicoflagellates				
Dictyocha fibula	+	+	+	+
Total	**91**	**78**	**80**	**87**

Present = +, Absent = -

In the present study, six major classes were composed in the phytoplankton community such as Coscinodiscophyceae, Mediophyceae, Fragilariophyceae, Bacillariophyceae, Dinophyceae, and Dictyocho-phyceae. In a total of 148 species, Coscinodiscophyceae 19%, Medio-phyceae 34%, Fragilariophyceae 5%, Bacillariophyceae 22%, Dinophyceae 19%, and Dictyochophyceae 1% were observed.

Regarding the different phytoplankton groups in four study areas were observed centric diatoms are the most contributive and followed by pennate diatoms, dinoflagellates, and silicoflagellates. In our study, centric and pennate diatoms showed an overall coverage of 80 %. A similar condition was also reported in the Bay of Bangle where diatoms accounted for more than 95% of the phytoplankton population (Shenoy et al., 2006).

Skeletonema costatum, Leptocylindrus danicus, Proboscia alata, Bacteriastrum delicatulum, Chaetoceros compressus, Thalassionema frauenfeldii, Chaetoceros lorenzianus, Thalassionema nitzschioides were common during the present study which agrees well with the findings of Sarojini and Sarma (2001) who reported *Nitzschia, Chaetoceros* and *Rhizosolenia* were dominant in the Bay of Bengal. Moreover, a similar finding was noted in Raji et al. (2012) who stated the above phytoplankton species were common in the coastal water of Port Blair, South Andaman Island.

Concerning the abundance of phytoplankton, monthly variations were observed at Dawei coastal waters which were probably related to the seasonal environmental variables. The highest cell abundance was recorded in January 2020 (post-monsoon period) whereas the lowest was observed in July 2019 (monsoon period) (Fig.3). According to the literature (Muraleedharan et al. 2010, Badsi et al. 2012), high light intensity, and nutrient concentrations during pre-and post-monsoon periods promoting the increase of phytoplankton abundance. In contrast, heavy rainfall and river discharge loaded heavy tons of sediments in the monsoon months may reduce the water clarity, then, in turn, influenced the low cell density of phytoplankton.

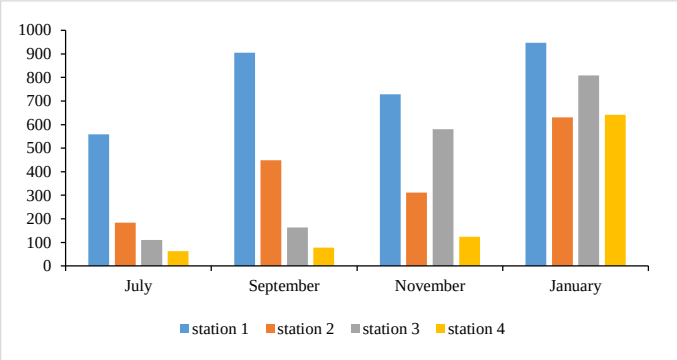

Figure 3 Phytoplankton abundance from July 2019 to January 2020.

The cluster analysis for the phytoplankton abundance among the four study months identified November and January had the high phytoplankton abundance, with over 90% similarity level.

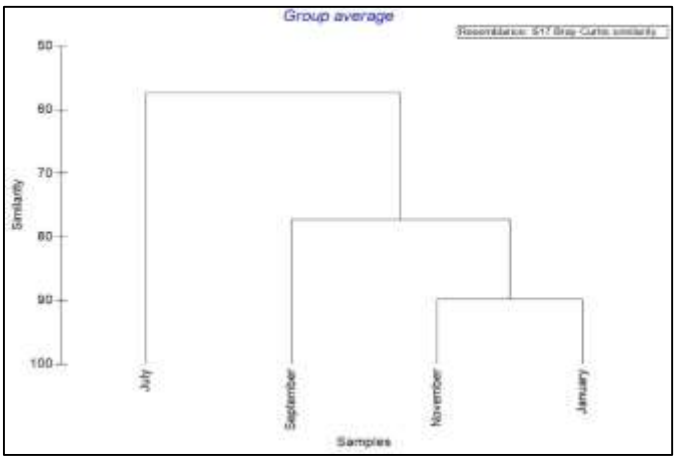

Figure 4 Dendrogram produced by clustering the abundance of different phytoplankton groups during the four study months.

Conclusion

Concerning the species composition of phytoplankton, centric diatom contributed the maximum percentage composition in Dawei coastal waters. During the study period, phytoplankton composition and abundance showed noticeable variations at the four stations. The resulted differences are likely due to the local forcing environmental parameters such as water temperature, salinity, and nutrient concentration, etc. Therefore, further study needs to be continued for the understanding of environmental forcing on the phytoplankton community.

Acknowledgements

I would like to express my sincere thanks to Dr. Aung Myat Kyaw Sein, Rector of Mawlamyine University, and Dr. San San Aye, Pro-Rectors of Mawlamyine University, for their permission to do this research. Many thanks are especially to Dr. San Tha Tun, Professor, and Head of the Department of Marine Science, Mawlamyine University, for his permission to use the departmental facilities. Many thanks are also to Dr. Khin Khin Gyi, Lecturer of the Department of Marine Science, Mawlamyine University for her help concerned with the academic literature.

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STUDY ON THE MORPHOLOGY, DAILY GROWTH FORM AND LIFE CYCLE OF *ARTEMIA* SP (BRINE SHRIMP) USING DIFFERENT KINDS OF FEEDING

Wint Yee Paing[1]

Abstract

The morphology, daily growth form and life cycle of *Artemia* were observed under different kinds of feeding such as S*pirulina* dry powder, yeast, and rice bran and formulated diet (shrimp meal) in the laboratory culture conditions. The optimum survival rate for *Artemia* was observed in the feeding of rice bran. And as well the second were observed in the feeding of *Spirulina* dry powder, yeast and formulated diet (shrimp meal) receptively but their high fraction of water soluble components which cannot be ingested by the brine shrimp that interferes with the water quality of the culture medium.

Keywords: *Artemia*, Brine shrimp, *Spirulia* powder.

Introduction

Artemia is the genus of aquatic crustaceans known as brine shrimp. and the only genus in the family Artemiidae. It lives in high saline waters. It is widely distributed throughout the world. It is the most important live feed organism. The genus *Artemia* is comprised of both bisexual and parthenogenetic strains (Stappen,1996). *Artemia* is a typical primitive arthropod with a segmented body to which is attached broad leaf-like appendages. *Artemia* is a continuous, nonselective, particle filtering organism (Coutteau and Sorgeloos, 1989). The coupling of propulsion, respiration, and filtration by the thaoracopods results in a practically continuous filter feeding (Coutteau and et.al, 1992). *Artemia* becomes an important input for the success of aquaculture, which has tremendously developed in recent years. As aquaculture is likely to develop manifold in future, the demand for *Artemia* would likely to increase in conjunction with its phased development (Coutteau and Sorgeloos, 1989). At present, the availability of *Artemia* from its natural resources is very much limited. To meet the ever increasing demand for *Artemia* biomass and cysts, culture of *Artemia* in the ponds is as an alternative source of their availability. The present study was also focused on the morphology and life cycle of *Artemia* and to know the optimum growth rate of *Artemia* with different kinds of foods. And also to develop the basic knowledge of *Artemia* culture in laboratory conditions.

Materials and Methods

Dry *Artemia* cysts 0.1g were soaked in freshwater for 30minutes under continuous illumination at room temperature in a cylindrical tank. Generally, all the nauplii hatch out within 48 hours. After completion of all cysts, the container has to be covered with a dark cloth. Light source has to be provided at the bottom of the container. Positive phototactic behavior of the *Artemia* nauplii is exploited for separating the hatched nauplii from empty and unhatched cysts. Nauplii swam towards the lighted bottom of the culture container and accumulate from where they have to be collected by siphoning through the provision in the bottom. In the present study, the types of feeds for *Artemia* are *Spirulina* powder, yeast, rice bran and formulated diet (shrimp meal). For one crop culture, there was taken a period of over 14 days. In one culture crop, two different kinds of feeds are introduced into two tanks and tested for one kind of feed in three times. The experiment was carried out at the laboratory of Department of Marine Science, Pathein University.

[1] Assistant Lecturer, Marine Science Department, Pathein university, Myanmar

Figure 1 A. Hatching of *Artemia* cysts and B-C. Culture chambers.

Results

Scientific classification

Phylum	Arthropoda
Class	Crustacea
Order	Anostreca
Family	Artemiidae
Genus	*Artemia*
Species	*Artemia* sp

Figure 2 Life cycle of *Artemia*.

After about 20 hours the outer membrane of the cysts burst (= "breaking") and the embryo appears, surrounded by the hatching membrane. While the embryo hangs underneath the empty shell (= "umbrella" stage) the development of the nauplius is completed and within a short period of time the hatching membrane is ruptured (= "hatching") and the free swimming nauplius is born.

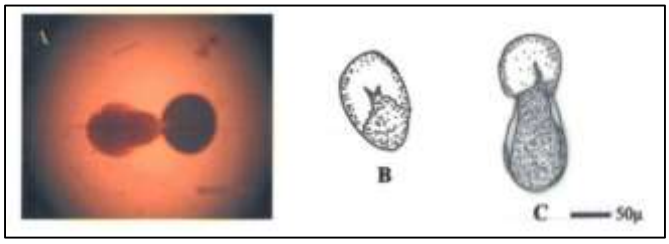

Figure 3 A. Breaking stage (umbrella stage), B- C. Sketch drawing of breaking cyst (umbrella stage).

Day	**Rice Bran**	**Yeast**	***Spirulina* Powder**	**Shrimp Meal**
Day- 1	-Nauplius eye -1st antennae -2nd antennae -Labrum	-Nauplius eye -1st antennae -2nd antennae -Labrum	-Nauplius eye -1st antennae -2nd antennae -Labrum	-Nauplius eye -1st antennae -2nd antennae -Labrum

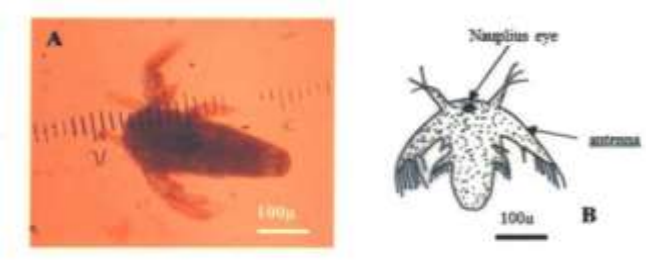

Figure 4 A. The first larval stage (instar I), B. Sketch drawing of the first larval stage (instar I).

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day- 2	-Nauplius eye -Larger & swim faster -Intennae -Intennula -Labrum	-Nauplius eye -Larger & swim faster -Intennae -Intennula -Labrum	-Nauplius eye -Larger & swim faster -Intennae -Intennula -Labrum	-Nauplius eye -Larger & swim faster -Intennae -Intennula -Labrum

Figure 5 A. The 2nd larval stage (instar II), B. Sketch drawing of the 2nd larval stage (instar II).

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day- 3	-Intennae -Nauplius eye -Digestive tract open	-Nauplius eye -Mandible -1st body segment -2nd body segment	-Intennae -Nauplius eye -Digestive tract open	-Nauplius eye -Mandible -1st body segment -2nd body segment

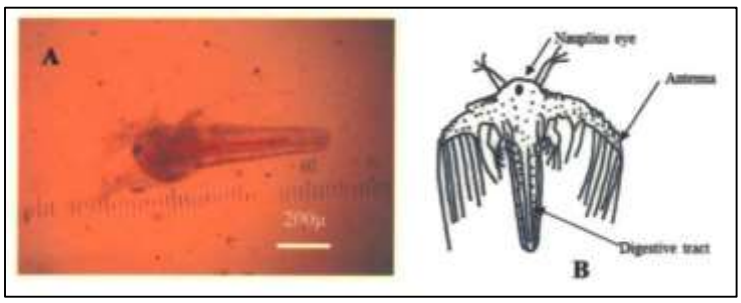

Figure 6 A. The larval stage, B. Sketch drawing of the larval stage.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day- 4	-Intennae -Nauplius eye -Digestive tract open	-Nauplius eye -Mandible -1st body segment -2nd body segment	-Intennae -Nauplius eye -Digestive tract open	-Nauplius eye -Mandible -1st body segment -2nd body segment

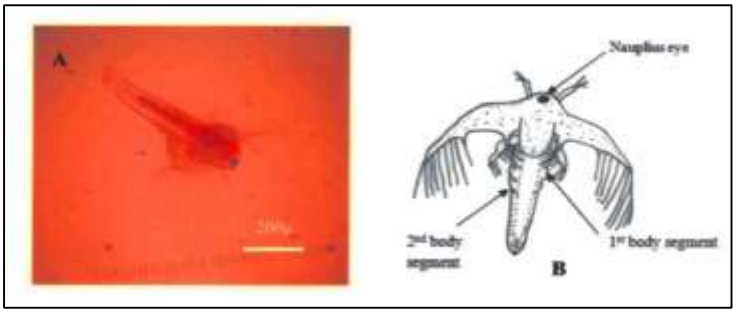

Figure 7 A. The larval stage, B. Sketch drawing of the larval stage.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day-5	-Nauplius eye -Labrum distinct -Pairs of thoracopods (5) buds	-Lateral complex eyes develop -Pairs of thoracopods (7) buds	-Lateral complex eyes develop -Pairs of thoracopods (7) buds	-Lateral complex eyes develop -Pairs of thoracopods (7) buds

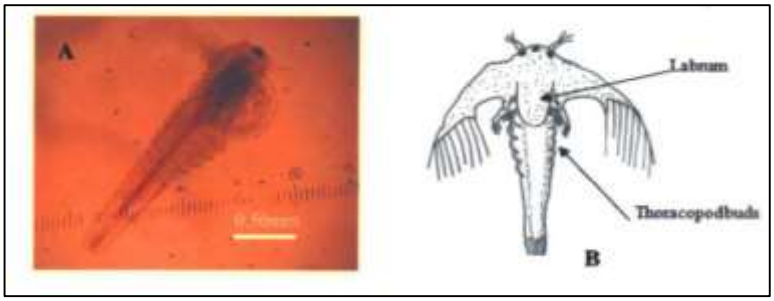

Figure 8 A. The larval stage, B. Sketch drawing of the larval stage.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day-6	-Lateral complex eyes develop -Pairs of Thoracopods (7) buds	-Thoracopods Buds biramous & slightly longer	-Lateral complex eyes develop -Pairs of thoracopods (7) buds	-Thoracopods Buds biramous & slightly longer

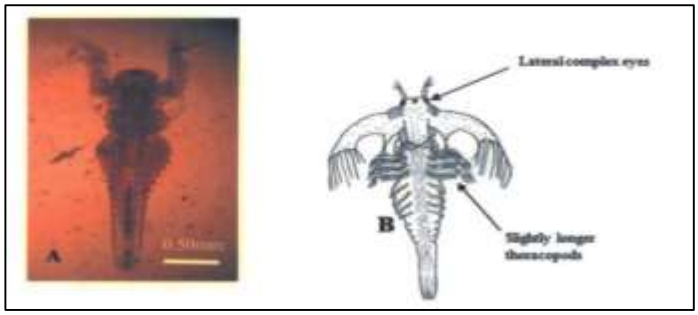

Figure 9 A. The pre-adult stage, B. Sketch drawing of the pre-adult stage.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day-7	-Thoracopods Buds biramous & slightly longer	-Lateral complex eyes develop -Thoracopods buds slightly longer	-Thoracopods buds biramous & slightly longer	-Thoracopods buds slightly longer

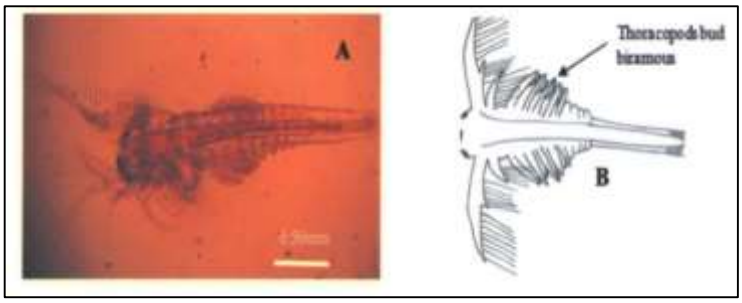

Figure 10 A. The pre-adult stage, B. Sketch drawing of the pre-adult stage.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day- 8	-Thoracopods buds biramous & slightly longer	-Lateral complex eyes develop -Thoracopods buds slightly longer	-Thoracopods buds biramous & slightly longer	-Thoracopods buds slightly longer

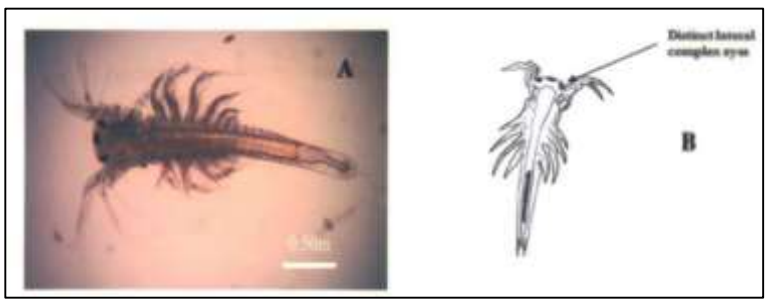

Figure 11 A. The pre-adult stage, B. Sketch drawing of the pre-adult stage.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day- 9	Thoracopods buds biramous & slightly longer	-Lateral Complex eyes develop -Thoracopods buds longer	Thoracopods buds biramous & slightly longer	-Thoracopods buds slightly longer

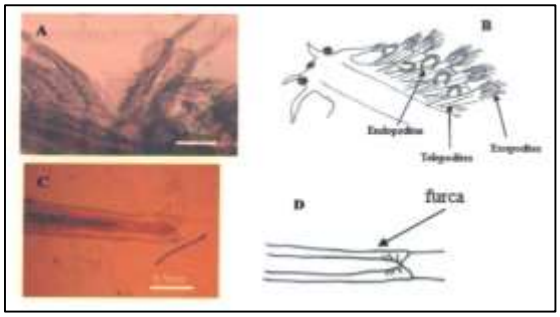

Figure 12 A. Detail structure of thoracopods, B. Sketch drawing of thoracopods, C. Tail of *Artemia*, D. Sketch drawing of tail of *Artemia*.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day-10	-Lateral compound eyes protruding -Furca with setae	-Thoracopods differentiate into 1)exopodites 2)endopodites 3)telepodites -Abdomen longer -Furca appear	-(7) pairs of appendages	-(7) pairs of appendages

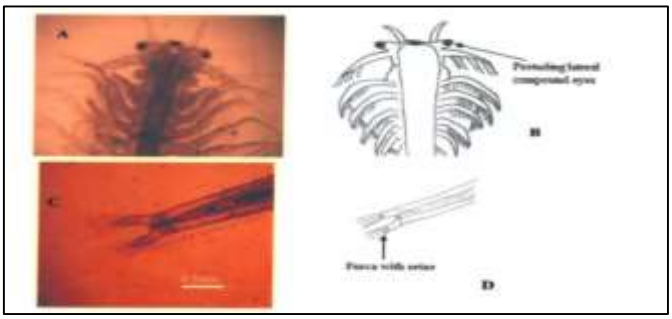

Figure 13 A. Larval development stage, B. Sketch drawing of larval stage, C. Tail of *Artemia*, D. Sketch drawing of tail of *Artemia*.

Day	Rice Bran	Yeast	*Spirulina* Powder	Shrimp Meal
Day- 11	-Stalked complex eyes -Linear digestive tract -Sensorial antennulae -(11) pairs of thoracopods appendages	-Lateral compound eyes protruding -Furca with setae	-Thoracopods differentiate into 1)exopodites 2)endopodites 3)telepodites -Abdomen longer -Furca appear	-Thoracopods differentiate into 1)exopodites 2)endopodites 3)telepodites -Abdomen longer -Furca appear

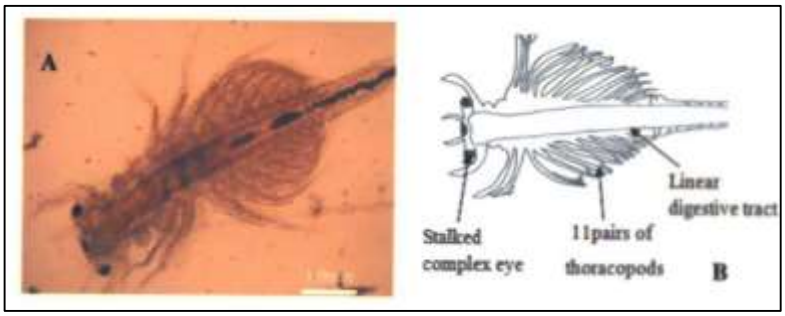

Figure 14 A. The adult stage, B. Sketch drawing of adult stage.

Day	**Rice Bran**	**Yeast**	***Spirulina* Powder**	**Shrimp Meal**
Day -12	-Antennae change for sexual differentiation -In female, sensorial appendages -In male, hooked gasper	-Stalked complex eyes -Linear digestive tract -Sensorial antennulae -(11) pairs of thoracopods appendages	-Lateral compound eyes protruding -Furca with setae	-Lateral compound eyes protruding -Furca with setae

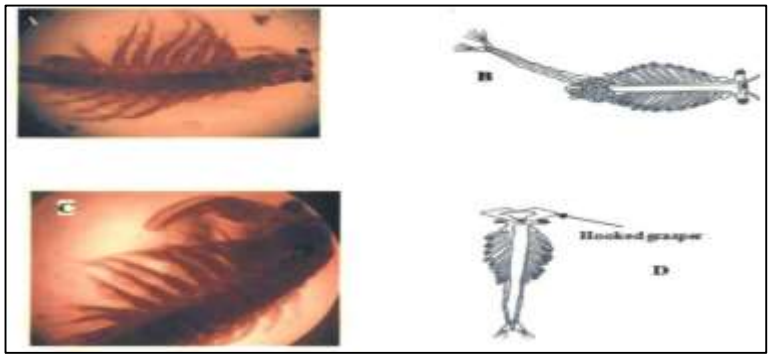

Figure 15 A. Female *Artemia*, B. Sketch drawing of female *Artemia*, C. Male *Artemia*, D. Sketch drawing of male *Artemia*.

Day	**Rice Bran**	**Yeast**	***Spirulina* Powder**	**Shrimp Meal**
Day- 13	-In male, paired penis -In female, brood pouch	-Antennae change for sexual differentiation -In female, sensorial appendages -In male, hooked gasper	-Stalked complex eyes -Linear digestive tract -Sensorial antennulae - (11) pairs of thoracopods appendages	-Stalked complex eyes -Linear digestive tract -Sensorial antennulae -(11) pairs of thoracopods appendages

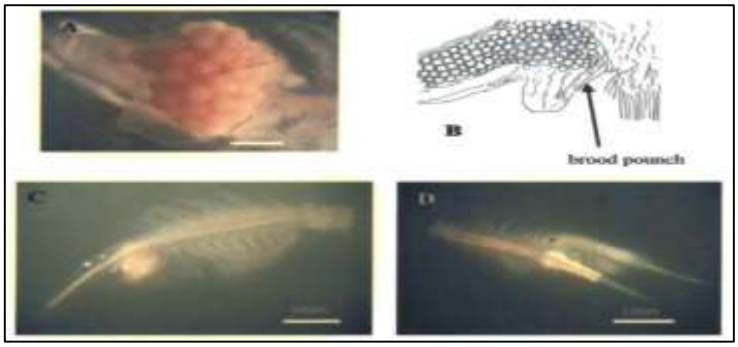

Figure 16 A. Brood pouch of female *Artemia*, B. Sketch drawing of female brood pouch, C. Female *Artemia*, D. Male *Artemia*.

Day	**Rice Bran**	**Yeast**	***Spirulina* Powder**	**Shrimp Meal**
Day- 14	-In male, paired penis -In female, brood pouch	-Antennae change for sexual differentiation -In female, sensorial appendages -In male, hooked gasper	-Stalked complex eyes -Linear digestive tract -Sensorial antennulae -(11) pairs of thoracopods appendages	-Stalked complex eyes -Linear digestive tract -Sensorial antennulae -(11) pairs of thoracopods appendages
Day- 15		-Reproduction -Fertilization take place in uterus	-In male, paired penis -In female, brood pouch	-In male, paired penis -In female, brood pouch
Day- 16			-Reproduction -Fertilization take place in uterus	-Reproduction -Fertilization take place in uterus

Discussion

In this study, the breaking stage started after 18-25 hours. The hatching will be completed within 24-48 hours depending on the quality of the cysts. And if the cysts were not used after opening, it wouldn't be good to use. Broone and et.al (1991) stated that *Artemia*'s ingestion, however, can be interrupted at high particle concentrations: i.e., mandibles and maxillae stop their action and the borlus accumulated behind the labrum is rejected into the medium by the first pair of thoracopods. Furthermore, there are no sizes that can be ingested by the naupliar stages have not been exactly determined, diameters should not exceed 50 to 70µm (Coutteau and Sorgeloos, 1989.In this study, good performances were recorded for the brine shrimp that are fed on baking yeast, obtained good growth rate and survival rate. At the adult stage, the survival rate varied because of their high fraction of water soluble components which cannot be ingested by the brine shrimp and interfere with the water quality of the culture medium. Bacteria and protozoans which

develop easily in *Artemia* cultures are indeed able to biosynthesize essential nutrients as they use the supplied brine shrimp food as a substrate. In this experiment, *Spirulina* powder is a suitable diet for *Artemia* growth in the early culture period. But during the late culture period, these food particles affected the culture medium by their interference at food uptake and propulsion by *Artemia*, or by bacterial growth and consequently, oxygen demand. In this experiment, rice bran is the optimal food for *Artemia* growth. Their main advantages are low cost and availability. Although soluble products in the food material are not taken up by *Artemia* and they will be decomposed by bacteria in the culture medium thereby deteriorating the water quality, the adult *Artemia* was fed the insoluble food material attached to the culture tank and this process provided for quality of culture water. In this study, the formulated diet (shrimp meal) has good result for *Artemia* growth but at later culture stage, the insoluble particles were decomposed in the bottom of the culture tank and polluted the cultured water. The insoluble products attached the appendages of *Artemia* and disturbed their swimming activity. This is the main problem of the diet.

Conclusion

In this study, the best performances of growth rate and survival rate were recorded for the brine shrimp that are fed on baker's yeast. At the adult stage, the survival rate varied because of their high fraction of water soluble components which cannot be ingested by the brine shrimp and interfere with the water quality of the culture medium. Bacteria and protozoans which develop easily in the *Artemia* cultures are indeed able to biosynthesize essential nutrients as they use the supplied brine shrimp.

Spirulina powder is a suitable diet for *Artemia* growth in the early culture period. In later culture period, these food particles affected the culture medium by their interference at food uptake and propulsion by *Artemia*, otherwise bacterial growth and consequently oxygen demand in cultured seawater.

Rice bran is the optimal food for *Artemia* growth. Their main advantages are low cost and their availability. Although soluble products in the food materials are not taken up by *Artemia* and they will be decomposed by bacteria in the culture medium thereby deteriorating the water quality, the adult *Artemia* was fed the insoluble food material attached to the culture tank and this process provided for water quality. The shrimp meal has good result for *Artemia* growth but at later culture stage, the insoluble particles were decomposed in the bottom of the culture tank and polluted the culture tank. The insoluble products attached the appendages of *Artemia* and disturbed the appendages of *Artemia*.

According to the recent study, knowledge of the life cycle of *Artemia* essential to carry out the culture operation successfully. Nowadays it was found that brine shrimp and their cysts could be produced as a by-product of solar salt-works. *Artemia* is the suitable food sources for most of the cultured finfish and shellfish. Furthermore, a better knowledge of the feeding of *Artemia* was the origin of the development of application in hatchery, nursery and brood stock rearing. All these developments resulted in optimized and cost-effective applications of this live food in hatchery production will help to provide in shrimp farming for development of aquaculture in Myanmar.

Acknowledgements

I express my sincere and deep gratitude to my teacher, Dr. Cherry Aung, Head and Professor, Department of Marine Science, for being a patient supervisor and for supporting this work with ideas, criticism, encouragement, etc. I would like to gratefully acknowledge the supervision of Dr. Min Oo, Lecturer, Department of Marine Science, as a valuable guide during this work. I am grateful to Dr. Si Si Hla Bu, Rector, Pathein University, for providing me the necessary facilities to carry out this research work. I would also like to thank all the teachers of my Department who monitored my work and took effort in reading my reports and providing me with valuable comments.

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DISTRIBUTION, ABUNDANCE AND COMPOSITION OF SOME CARANGID FISH IN MON COASTAL WATERS

Thu Thu Min[1],Thant Zin[2],Lwin Mar Aung[3],Soe Thaw Thaw Tun[4]

Abstract

The composition of carangid fish species in the catches from bag net and drift gill net fisheries of three sampling sites, Kyaikkhami, Asin and Zeephyuthaung in Mon coastal waters were recorded during June 2013 and May 2015. A total of seventeen carangid species belonging to ten genera contributed to the catches of Mon coastal waters. Among the seventeen species of Mon coastal waters, the catches of Asin sampling site composed of fifteen species followed by 14 species (Zeephyuthaung) and 9 species (Kyaikkhami). The catch weight and catch effort was found to be highest in Asin with an average of 24829kg and 3.7kg/boat/day followed by Zeephyuthaung (21563kg, 3.1kg/boat/day) and Kyaikkhami (3724kg, 2.6kg/boat/day). The catches were high in November to January at Kyaikkhami, October to December at Asin and September to December at Zeephyuthaung. The highest average percentage of carangid species composition in total catch was observed at Asin (23.825%), followed by Zeephyuthaung (19.82%) and Kyaikkhami (8.8%).

Keywords: catch weight, catch effort, composition, carangid fish.

Introduction

The carangids are pelagic fish widely distributed in Indo-Pacific regions and live in diverse marine habitats. These carangid fish also locally known as Zar-kyan, Zar-byat, Hmee-war and Nga-chin-paung. The Family Carangidae includes ecologically and economically important species such as the jacks, scads, trevallies, pompano, amberjacks and queenfishes.

Carangid fishes are commercially caught in the study areas. Some species are abundant and large in size. They are marketed in fresh locally and dried under the sun. Good quality dried fishes are provided for local markets. Therefore, carangid fishes are valuable food resources for the local people of Mom coastal region. They are mostly taken in bag nets and drift gill net. Most of the study areas in Mon State covered with estuarine regions which are characterized by a variable salinity, a temperature range greater than the sea, and turbid water and muddy bottom. In these areas both of marine and freshwater fishery resources are rich. Thus fish is one of the important protein resources in Mon coastal areas. They can be utilized as food in many forms such as dried, salted, smoked, paste, sauce, and fresh state for local needs and also exported to many other countries to earn foreign currency. Hence they are of great demand by the local people of Myanmar. In the study areas, fish exploitation is almost entirely by traditional boats and gears. Most of the fishing boats were motorized with engines and fishing gears were made of nylon and polyethylene fibers. Most of the villagers of study areas earn their livelihood wholly or partially from marine and estuarine fishing.

Materials and Methods

Study areas and study periods

The study areas were chosen at three stations, namely Asin (Lat. 15°13′ N, Long. 97°47′ E), Zeephyuthaung(Lat. 15° 11′ N, Long. 97° 46′ E) and Kyaikkhami (Lat.16° 03′ N , Long. 97° 35′ E). Samples were collected monthly from June 2013 to May 2015.

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[2] Lecturer, Department of Marine Science, Pathein University.
[3] Assistant Lecturer, Department of Marine Science, Pathein University.
[4] Assistant Lecturer, Department of Marine Science, Pathein University.

Data collection

For the estimation of species composition, data were collected from the commercial catches of drift gill net and bag net. Based on the catch data, catch rate or catch per unit effort (CPUE) was computed by dividing the total weight of carangids by the number of fishing boats and fishing days (kg/boat/day). Catch composition of species was estimated from the total weight (kg) of carangid samples and expressed in percentages. In the field survey, in addition to the fish collection, the fishery status such as fishing areas, fishing gears and fishing boats were recorded at the different coastal areas. Information regarding the fishery of carangid fish has been collected by visiting the fish landing sites in Mon coastal waters. During the visit fish species were collected from the landing sites. The information was gathered through field visit and inquiring directly to the fishermen about the details of the fishing boats, type of fishing implement and gears they used, mechanism of operation and the type of fish caught in the study areas. The fishing boats, fishing gears with accessories were taken photograph. Questionnaires were compiled and evaluated for the status of fishery. The samples were collected from the catches of bag net and drift gill net fisheries in Mon coastal areas for two successive years from 2013 to 2015.Some of the fishery data were recorded by investigating and questionnaires to local fishermen.

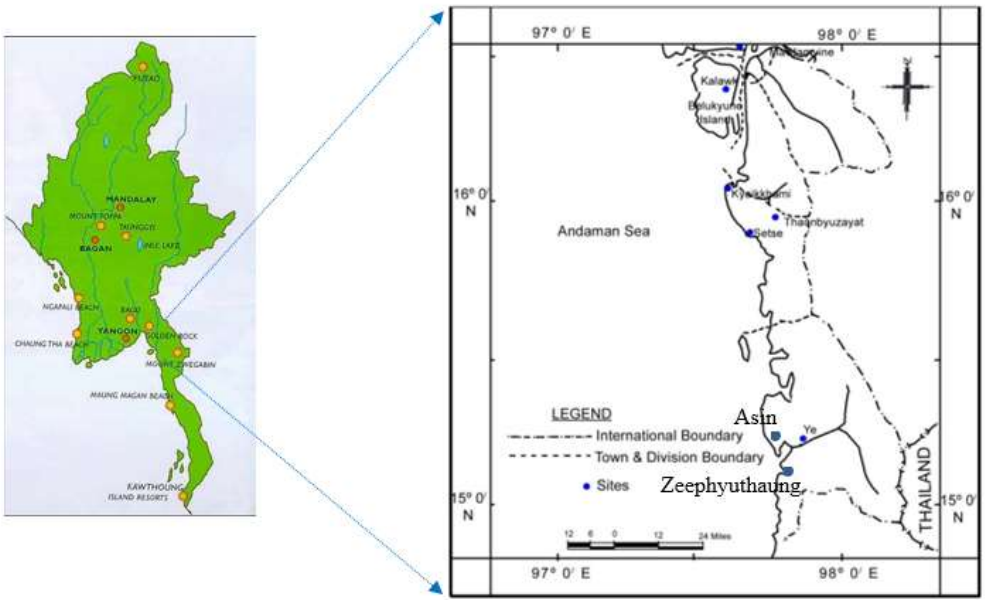

Figure 1 Map showing the sample collection sites in the study areas

Result

Group wise occurrence in the study areas with different gears during the study period

For the group wise occurrence, observation and data collection were made during the study period of June, 2013 to May, 2015. Data used for this study were collected from the drift gill net and bag net fishery. During this study, altogether 23 families including family Carangidae with their catch weight were recorded.

Group wise occurrence in Kyaikkhami areas by drift gill net and bag net

According to this study, altogether nine families with shrimp and trash were caught by drift gill net. Among these families, family Sciaenidae stood first in the catch, estimated 14482 kg (19.3%) of total catch, followed by 12381 kg (16.5%) of Polynemidae, 9605 kg (12.8%) of Lutjanidae and 7879 kg (10.55%) of Engraulidae. Altogether six families with shrimp and trash were caught by bag net. Among these families, family Synodontidae stood first in the catch,

estimated 3756 kg (18.8%) of total catch, followed by 3297 kg (16.5%) of Engraulidae, 2638 kg (13.2%) of shrimp and 2538 kg (12.7%) of Sillaginidae.

Group wise occurrence in Asin areas by drift gill net and bag net

In Asin landing site, the data were collected from drift gill net and bag net. According to data collected by drift gill net, altogether ten families with shrimp and trash were recorded. Among these families, family Polynemidae stood first in the catch, estimated 10402 kg (17.5%) of total catch, followed by 8203 kg (13.8%) of Engraulidae and 7846 kg (13.2%) of Carangidae. Seven families were collected with trash and shrimp by bag net. Among these families, family Carangidae stood first in the catch, estimated 9976 kg (19.5%) of total catch, followed by 8953 kg (17.5%) of Sciaeneidae, 6753 kg (13.2%) of Clupeidae and 5986 kg (11.7%) of Lutjanidae.

Group wise occurrence in Zeephyuthaung areas by drift gill net and bag net

In Zeephyuthaung landing site, the data were collected from drift gill net and bag net. During this study, altogether eleven families with the catch weight percentage were recorded by drift gill net. Among these families, family Sciaeneidae stood first in the catch, estimated 10059 kg (14.8%) of total catch, followed by 7764 kg (11.5%) of Polynemidae and 7223 kg (10.7%) of Carangidae. Altogether twelve families with the catch weight percentage were recorded by bag net. Among these families, family Carangidae stood first in the catch, estimated 8666 kg (12.5%) of total catch, followed by 8112 kg (11.7%) of Polynemidae, 7973 kg (11.5%) of Engraulidae and 7280 kg (10.5%) of Sciaeneidae.

Distribution of carangid fishes along the study areas

A total of 17 species belonging to 10 genera of family Carangidae were recorded from three sampling sites namely Kyaikkhami, Asin and Zeephyuthaung during the study period. The highest species distribution was found at Asin and with 15 species (*Alectis cliaris*, *Alepes djeddaba*, *A. vari*, *Atropus atropos*, *Carangoides chrysophrys*, *Carangoides ferdau*, *Carangoides malabaricus*, *Decapterus macrosoma*, *Decapterus russelli*, *Megalaspis cordyla*, *Parastromateus niger*, *Scomberoides commersonnianus*, *Scomberoides tol*, *Selar crumenophthalmus*, and *Selaroides leptolepis*). The lowest distribution was found at Kyaikkhami with nine species species (Table.1).

Table 1 Stationwise composition of carangid fishes in Mon coastal waters

Sr No.	Species Name	Kyaikkhami	Asin	Zeephyuthaung
1	*Alepes djeddabs*	+	+	+
2	*A. vari*	+	+	+
3	*Alectic ciliaris*	_	+	+
4	*Atropus atropus*	+	+	+
5	*Carangoides chrysophrys*	_	+	+
6	*C. dinema*	_	_	+
7	*C. ferdau*	+	+	+
8	*C. malarbaricus*	+	+	+
9	*Decapterus kurroides*	_	_	+
10	*D. macrosoma*	_	+	_
11	*D. russelli*	_	+	_
12	*Megalaspis cordyla*	+	+	+
13	*Parastromateus niger*	_	+	+
14	*Selar crumenophthalmus*	+	+	+
15	*Selaroides leptolepis*	_	+	_
16	*Scomberoides commersonnianus*	+	+	+
17	*S. tol*	+	+	+

+ = Present; - = Absent

Catch composition of carangid species in Mon coastal waters

Monthly catches of carangids ranged from the minimum of 740 kg (June) to the maximum of 8860 kg (November) in Kyaikkhami fish sampling station with an average catches of 3871 kg. The maximum catch of carangids was found in November (8860 kg) and it decreased slightly to (8270 kg) December and 7880 kg (January) and then sharply decreased to 4480 in February. In 2014-15, the maximum catch was found in December (7960kg) and the amount was slightly lower than 2013-14. The minimum amount (650 kg) was found in June and increased slightly to (675 kg) and then sharply increased to (2050 kg) in August. It was slightly decreased to (7550 kg) in January from the maximum amount and then sharply decreased to (3950 kg) in February.

According to the monthly catch weight of Asin sampling site, it varied from the range of 8800 kg (June) to 51600 kg (December) with an average catches of 25933 kg in 2013-14. The catch weight 8800 kg of June increased to 14400 in July and then slightly decreased to 13500 (August). In September the catch weight reached 30800 kg and then gradually increased to maximum range of 51600 kg in December. After that, it decreased to the 36400 kg (January). In 2014-15, the maximum amount was also found in December (48700 kg) and minimum amount was 6400 kg in June. The catch amounts were lower than the previous year. It was sharply increased to 12500 kg (July) from the minimum of 6400 kg (June). It was slightly decreased to 33600 kg (January) from the maximum of 48700 kg (December) and then increased again to 42300 kg (February). The average catch amount in 2014-15 was 23752 kg which was slightly lower than average catch (25933 kg) in 2013-14. (Figure. 2)

The range of monthly catches of carangids of Zeephyuthaung was from 2900 kg (July) to 48200 kg (December) with an average catches of (22583 kg) in 2013-14. The catches slowly increased from July (2900 kg) to August (4300 kg) and then gradually increased to maximum catch (48200 kg) in December. From the maximum catch, it decreased again to (29000 kg) in January and then slightly increased to (33700 kg) in February. It was found that the maximum catch (45500 kg) in 2014-15 was slightly decreased from the maximum catch of 2013-14. From the maximum catch the amount decreased slowly to 32500 kg (January), 29500 kg (February), 28700 kg (March) and then sharply decreased to 6850 kg (April). The average catch amount was 20541 kg in 2014-15. (Figure. 3)

With regard to monthly catch per unit effort of carangids in Kyaikkhami, it ranged from 1 kg/boat/day to 4.9 kg/boat/day and average effort was 2.7 kg/boat/day in 2013-14. In 2014-15, it ranged from 0.9 kg/boat/day to 3.9 kg/boat/day and average effort was 2.4 kg/boat/day. At Asin, it ranged from 1.6 kg/boat/day to 6.9 kg/boat/day with average effort of 3.9 kg/boat/day in 2013-14. In 2014-15, it ranged from 0.7 kg/boat/day to 6.4 kg/boat/day with average effort of 3.5 kg/boat/day. At Zeephyuthaung, it ranged from 1.5 kg/boat/day to 5.6 kg/boat/day with average effort of 3.1 kg/boat/day in 2013-14. In 2014-15, it ranged from 0.9 kg/boat/day to 6.7 kg/boat/day with average effort of 3.1 kg/boat/day. Average effort was found to be highest at Asin (3.7 kg/boat/day) followed by Zeephyuthaung (3.1 kg/boat/day) and Kyaikkhami (2.5 kg/boat/day).

At Kyaikkhami, the percentage composition of carangids varied from the minimum of 3.9% (June) to the maximum of 14.8% (December) of the total catch with an average composition of 8.8% in 2013-14. In 2014-15, the percentage composition of carangids varied from the minimum of 3.5% (June) to the maximum of 17.4% (December) of the total catch with an average composition of 8.5%. At Asin, the percentage composition of carangids varied from the minimum of from 11.5% (April) to 32.2 % (February) with an average composition of 23.8% in 2013-14. In 2014-15, the minimum percentage was 11% (March) and the maximum percentage was 38.3% (June) with an average of 23.1%. At Zeephyuthaung, the percentage composition of carangids varied from the minimum of from 8.2% (August) to 35% (November) with an average composition

of 19.8% in 2013-14. In 2014-15, the minimum percentage was 5.8% (August) and the maximum percentage was 36.8% (November) with an average composition of 8.1%. (Figure. 2)

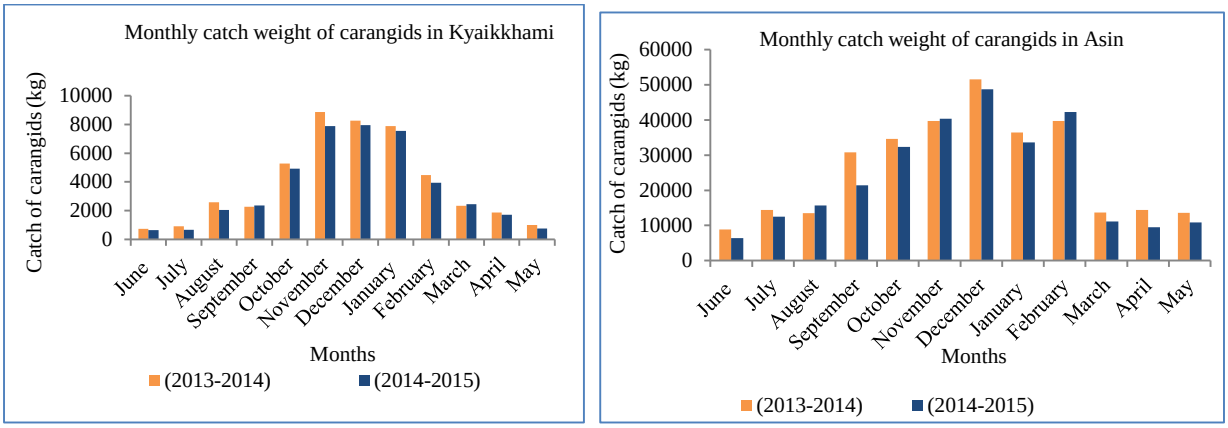

Figure 2 Monthly catch weight of carangids in Kyaikkhami and Asin

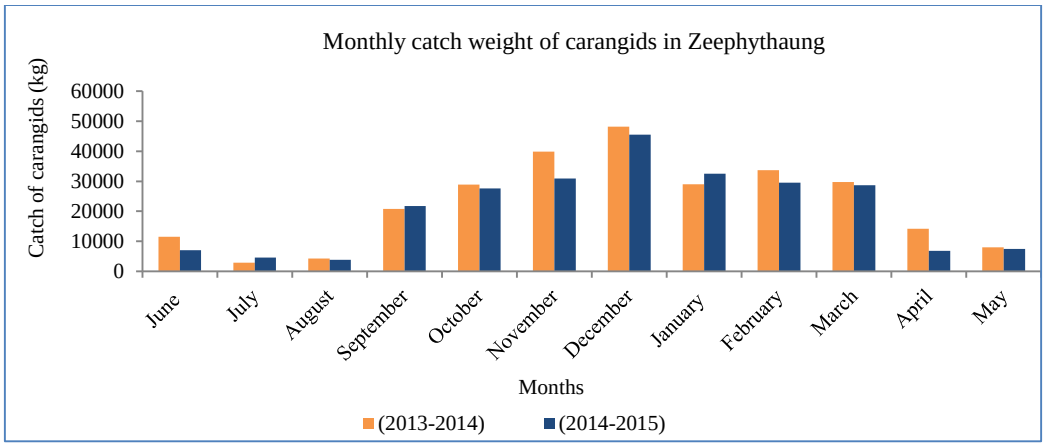

Figure 3 Monthly catch weight of carangids in Zeephyuthaung

Species composition of carangid fishes in Mon coastal waters

The catch composition of species was estimated from the total weight (kg) of carangid samples. *Alepes djdedaba* constituted almost all months except in June, October and May in 2013-14. The highest composition of 13.7% was recorded in January of 2013-14 and 12.5% was highest composition in January of 2014-15. The catch composition of *A. vari* ranged from 4.7% (August) to 19.3% (May) in 2013-14. In 2014-15, the catch composition of *A. vari* ranged from 5.7% (August) to 11.8% (December). The maximum catch composition of *Carangoides ferdau* was 11.4% in January. The catch composition of *Carangoides ferdau* ranged from 7.3% (July) to 13.3% (January). *Megalaspis cordyla* and *Selar crumenophthalmus* occurred in all months. The composition of *M. cordyla* ranged from 17.8% (July) to 34% (September) of 2013-14. In 2014-15, the catch composition of *Megalaspis cordyla* ranged from 19% (July) to 36.3% (May). The distribution of *Scomberoides commersonnianus* to the carangid catches was largest in September (17.2%) of 2013-14. In 2014-15, the catch composition of *Scomberoides commersonnianus* ranged from 9.8% (October, February and April) to 13.7% in March. Fifteen carangid species were contributing to the catches of Asin sampling site. Among them, the catch composition of *Alepes djedaba* ranged from 6.2% (December) to 16.7% (April) in 2013-14. In 2014-15, the catch composition of *Alepedjeddaba* ranged from 6.2% (December) to 20.8% in March. *Aletic ciliaris*

composed the highest percentage of carangid catches was 12.6% (July) in 2013-14. In 2014-15, the catch composition of *Aletic ciliaris* ranged from 3.6% (December) to 13.5% in April. The contribution of *Carangoides chrysophrys* was ranged from 7.2% (December) to 24% (March) in 2013-14. In 2014-15, the catch composition of *Carangoides chrysophrys* ranged from 8.5% (February) to 23.7% (March). The highest percentage of *C. malabaricus* was 13.3% (September) in 2013-14. In 2014-15, the catch composition of *C. malabaricus* ranged from 6.4% (October) to 14.7% (September). *M. cordyla* and *S. crumenophthalmus* were occurred in all months of carangid catches. The maximum composition of *M. cordyla* was found in June (11.4%) in 2013-14. In 2014-15, the catch composition of *M. cordyla* ranged from 2.8% (July) to 14.3% (October). The maximum composition of *S. crumenophthalmus* was 26.4% (May) in 2013-14. In 2014-15, the catch composition of *S. crumenophthalmus* ranged from 9.5% (November, December) to 24% (April). The contribution of *Parastromateus niger* was ranged from 7.2% (November) to 12.1% (May) in 2013-14. In 2014-15, the catch composition of *Parastromateus niger* ranged from 6.7% (November) to 18.4% (April). The maximum catch compositions of *Scomberoides commersonnian*us was 26.4% (February) in 2013-14. In 2014-15, it was ranged from 5.3% (June) to 22.4% (February). Monthly catch weight (kg) and species composition (%) of carangids of Zeephyuthaung was shown in Appendix 8 and 9. The percentage of *Alepes djeddaba* was ranged from 4.7% (August) to 7.5% (October) in 2013-14. In 2014-15, the catch compositions of *Atropus atropus* in 2013-14 was ranged from 5.9% (July) to 7.7% (December, April). In 2014-15, the catch compositions of this species ranged from 5.3% (January)) to 11.4% (August). *Carangoides chrysophrys* were highest in March (9%) and January (18.6%) January in 2013-14. In 2014-15, the catch compositions of this species ranged from 9.2% (March)) to 18.3% (January). The maximum catch composition of *C. ferdau* was found in August (23.3%) and minimum composition was in June (6.2%) in 2013-14. In 2014-15, the catch compositions of this species ranged from 8.6% (June)) to 22.7% (August). The catch compositions of *Megalaspis cordyla* in 2013-14 was ranged from 6.7% (September) to 18.2% (April). In 2014-15, the catch compositions of this species ranged from 7.5% (September) to 18.5% (May). The percentage of *Parastromateus niger* was ranged from 9.6% (November) to 15.8% (September) in 2013-14. In 2014-15, the catch compositions of this species ranged from 9.5% (June) to 15.4% (December). The catch composition of *Selar crumenophthalmus* was ranged from 5% (February) to 14% (August) in 2013-14. In 2014-15, the catch compositions of this species ranged from 5.2% (February) to 13.7% (August).The maximum catch compositions of *Scomberoides commersonnianus* was 19.3% (October) in 2013-14 and 14.6% (June) in 2014-15. The minimum catch compositions of *S. commersonnianus* was 6.9% (July) in 2013-14 and 6.2% (July) in 2014-15. The highest and lowest compositions of *S. tol* were 18.9% (April) and 7.7% (March) in 2013-14. In 2014-15, the highest and lowest compositions of this species were 15.3% (April) and 3.7% (July) respectively.

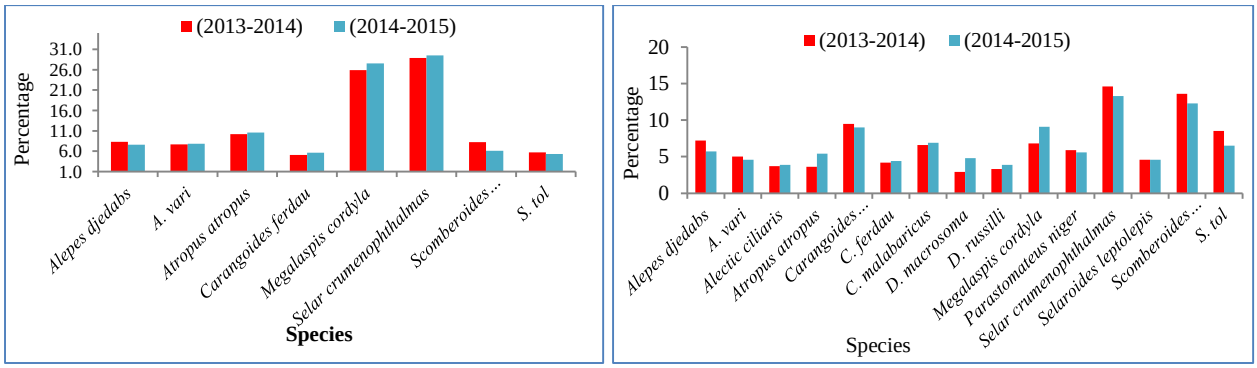

Figure 4 Average percentage species composition of carangids in A) Kyaikkhami and B) Asin

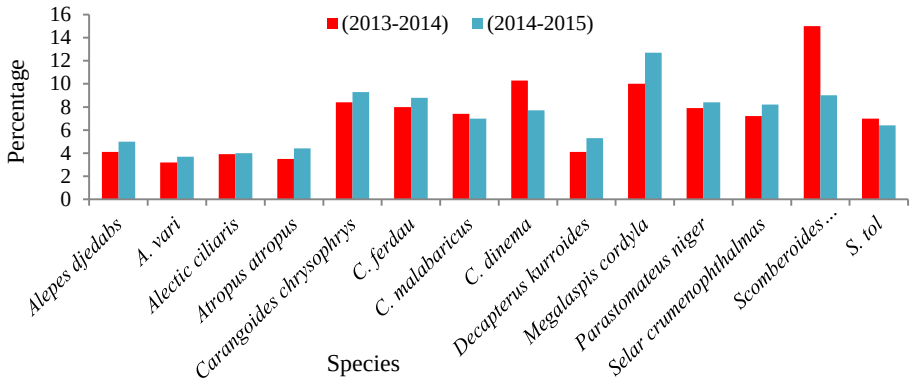

Figure 5 Average percentage species composition of carangids in Zeephyuthaung

Discussion

According to the data based on the fishery, drift gill net and bag net fishing methods were used in the study areas as a major fishing gear although the other fishing apparatus were used. Fishing was operated throughout the year in the sea. The main fishing gears used in this area were bag net, drift gill net and seine net. Among the fishers both genders were involved in the fisheries with the male gender dominating the pre and fish harvesting sectors and the female gender dominating the post-harvest preservation and marketing sectors.

From the information obtained from the field survey, carangid fishes were widely distributed along the study areas of Mon coastal water. In Asin and Zeephyuthaung, the highest species compositions were found with 15 and 14 species respectively. It was found that *M. cordyla* and *Selar crumenophthalmus* observed in almost all sampling stations throughout the study periods. Monthly species distribution of carangids was recorded from three stations of Kyaikkhami, Asin and Zeephyuthaung. Among the seventeen species of carangid occurring in Mon coastal waters, the highest species distribution was found in Asin sampling station with fifteen species, followed by Zeephyuthaung station with fourteen species. *Megalaspis cordyla* and *Selar crumenophthalmus* were observed in all sampling sites throughout the study period. *Decapterus russilli* and *Selaroides leptolepis* were only recorded in the catches of Asin sampling site. There were nine species recorded at Kyaikkhami during the study period. According to the research by Nair *et al* (2000), *Carangoides* was the most diverse group with nine species followed by *Caranx* with seven species in Cochin,Kerala.

According to monthly species distribution in Kyaikkhami, it was found to be highest in July and August with eight species and followed by February with seven species while lowest in May with four species. With regard to the monthly species distribution in Zeephyuthaung, it was found to be highest in June and February with twelve species and lowest in April with seven species. According to monthly catch weight, it was found to be highest in Asin with an average catch of 24829 kg followed by Zeephyuthaung with 21563 kg and Kyaikkhami with 3724 kg. Monthly abundance of carangids in the present study showed that the catches were high during October to January at Kyaikkhami, October to February at Asin and October to December at Zeephyuthaung.

Catch per Unit of effort (CPUE) and catch per unit area (CPUA) are the basic quantity to compute stock density in assessments, and these estimations are utilized to acquire other evaluations such as biomass approximations and abundance indices for essential commercial fish stocks (Hinton and Maunder 2003). With regard to monthly catch per unit effort of carangids, the average CPUE declined from 2.7 to 2.4 kg/boat/day within the study periods in Kyakkhami while

average CPUE decreased from 3.9 to 3.5 kg/boat/day in Asin. In Zeephyuthaung, it was found that average CPUE was 3.1 kg/boat/day in both years. In the results by Hosseini *et al* (2018), CPUE for *Caranx ignobilis* and *S.Crumenophthalmus* were 0.159 and 0.078kg/hr, while the results of CPUA were 2.44 and 1.91 kg/km^2 respectively in the Motaf fishing grounds, Bushehr Province, Persian Gulf, Iran. A decline in CPUE reflects a state of over-exploitation, with a fishing pressure exceeding the carrying capacity of the ecosystem (Kantoussan *et al* 2014). So, declining CPUE in Kyaikkhami and Asin showed that the fish population cannot support the level of harvesting. Unchanging CPUE in Zeephyuthaung indicates the sustainable harvesting.

Conclusion

In the present study, a total of 17 species of 10 genera belonging to family Carangidae were collected from the Mon coastal waters. Among these species, some species were dominant in the catches in some areas while others were collected in moderate quantity in the catch and some were found rarely in the catches. Carangid fishes are not exported to foreign country and they are less commercially important. But these fishes were locally marketed and they offer the important source of fish meal and food. Finally, findings of this study gave basic information on the distribution, abundance and composition of carangid fish in the Mon Coastal Waters.

Acknowledgement

The author would like to express deep gratitude to Rector, Pro-Rector of Pathein University and Head of Marine Science Department, Pathein University for their valuable advice and permission to conduct this research.

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REPRODUCTIVE BIOLOGY OF BOMBAY DUCK, (*HARPADON NEHEREUS*) IN MON COASTAL WATER

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Abstract

The reproductive biology of *Harpadon nehereus* from Mon coastal water was investigated during May (2019) to April (2020). *H. nehereus* have a prolonged spawning season with two peaks during May to June and December to January. GSI showed peak values in May (4.16), June (5.16), December (4.83) and January (5.69) in female. The Kn values ranged from 0.989 (August) to 1.004 (October) in male and 0.998 (July) to 1.027 (December) in female. The overall sex ratio (1:0.66) showed significant difference at 5% ($p<0.05$) level and males outnumbered females. The length at first maturity of females was estimated as an average of 230 mm. The fecundity varied between 23,625 and 117,498 with an average fecundity of 55054 ova. The fecundity indicated higher relationship with ovary weight (r = 0.934) rather than total length (r = 0.724) and body weight (r = 0.713). The mature ovaries (from stage IV to VI) of *H. nehereus* contained mature ova which are distinctly separated from the immature stock.

Keywords: *Harpadon nehereus*, reproductive biology, spawning, fecundity.

Introduction

Harpadon nehereus (Hamiltom, 1822) popularly known as Bombay duck, forms one of the most important commercial fish along the Mon coastal areas. These species was mainly caught by bag-net locally in shallow water of estuaries within the depth of 10 to 40 meter. The fishery sector of Sepalar, Setse and Zeephyuthaung coastal areas are important to the local populace where artisanal fishery is developed including the production of dried fish, dried shrimp, fish paste and fish sauce as their business. Bombay duck is a very soft fish and due to its highly perishable body composition, a large amount of the catch particularly during the peak fishing season is sundried and a small portion is sold fresh in the markets.

Reproductive parameters such as size at first maturity, sex composition, spawning frequency and spawning season, fecundity and ova diameter studies can help in the fisheries forecast (Bal and Rao, 1984). The knowledge on length at first maturity and spawning season detects when and at which length the fish should be protected and therefore it is important for the proper management and conservation of fish stocks (Hunter *et al.* 1992).

Several biological information such as length-frequency distribution, length-weight relationship and food and feeding habit *H. nehereus* along the coast of Mon state have been studied by Tint Swe (2011) but there was no record, so far, on the reproductive biology of this species. Thus, the objective of this study is to provide the first information on reproductive biology of *H. nehereus* including maturity stages, spawning season, gonado-somatic index (GSI), relative condition factor (Kn), length at first maturity, sex ratio, fecundity and ova diameter measurement.

Materials and Methods

Study areas

The present study on the reproductive biology of *H. nehereus* was conducted at three stations in Mon coastal area, namely Sepalar (Lat. 16° 14′N, Long. 97° 32′E) in Chaungzone

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Township, Setse (Lat. 15° 56′N, Long. 97° 35′E) in Thanbyuzayat township and Zeephyuthaung (Lat. 15° 19′ N, Long. 97° 51′ E) in Ye Township.

Sample collection and study period

A total of 843 fish samples (508 males and 335 females) ranging in size from 180-305 (mm) total length and 19.55 - 206 (g) body weight were collected monthly from Sepalar, Setse and Zeephyuthaung fish landing sites during May (2019) to April (2020). The samples were preserved in ice box until it reaches to the laboratory.

Maturity stages and spawning season

After recording the total length and body weight by using digital clipper (0.01 mm) and digital balance (0.01 g), ovaries were separated from fresh specimen. The maturity stages of female were identified based on macroscopic examination like appearance of ovary (shape, size, color, condition, extend of yolk formation) and microscopic structure such ova diameter measurement (Clark, 1934). Maturity stages were classified into seven stages such as immature (stage I), maturing (Stage II), developing (stage III), mature (stage IV), pre-spawning (stage V), ripe (stage VI) and spent (stage VII) followed by Mackie and Lewis, (2001). Samples of ovary were weighted to nearest 0.01g with digital balance and preserved in 5% formalin for further study. Spawning season was ascertained by the proportion of the maturity of stage IV, stage V and stage VI (mature, pre-spawning and ripe) females, and gonad-somatic index values during the different months of the study period.

Gonado-somatic index (GSI)

Gonado-somatic index provides information about the spawning period and GSI was calculated for monthly by using the following formula, (Nikolsky, 1963).

$$\text{Gonado-somatic index (GSI)} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

Relative condition factor (Kn)

The relative condition factor was calculated by using the formula:

$$Kn = W/W' \text{ (Le Cren, 1951)}$$

Where, Kn = Relative condition factor, W = Observed weight, and W' = Calculated weight.

Sex ratio

To know the homogeneity of the distribution of males and females, Chi-square test (Snedecor and Cochran, 1976) was applied.

$$\text{Chi-square } (x^2) = \frac{\sum /O - E/^2}{E}$$

Where, **'**O**'** is observed frequency and **'**E**'** is expected frequency of males or females per month or length groups.

Length at first maturity

Length at first maturity was estimated by plotting the percentage of cumulative frequency against the length groups. The length at which 50% of fish attained sexual maturity was considered as length at first maturity (Lm_{50}). (Beverton and Holt, 1957).

Fecundity

For estimation of fecundity, twenty ovaries in the V (mature) and VI (ripe) stages of maturity were selected. An amount of 0.05g of ovary from the anterior, middle and posterior regions of each ovaries was taken by using digital electronic balance. The sub-samples were placed on the counting slide with aid of few drop of water. The numbers of mature ova were counted and average number of ova of the three portions determined. Fecundity was calculated by using the following formula, Fecundity = Total weight of gonad/ sub-sample weight of gonad* No. of ova in the sub-sample. The relationship between fecundity (F) and total length (TL); body weight (BW); ovary weight (OW) and their respective correlation coefficients (r) were expressed by least square method:

$$\text{Log } F = a + b \text{ Log } X$$

Where: F = fecundity, a = constant, X = variable (Total length; body weight; and ovary weight) and b = regression coefficient (The exponent).

Ova diameter

Twelve ovaries were selected for the ova diameter study, where two each belong to stage I to VI. Random samples of ova from three portions of each ovary were taken and the diameters of nearly 200 ova were measured in straight line under the microscope using calibrated ocular micrometer. Ova diameter measurements were grouped into 80 µm class interval size groups and expressed in percentage of the total number of ova for each ovary (Clark, 1934).

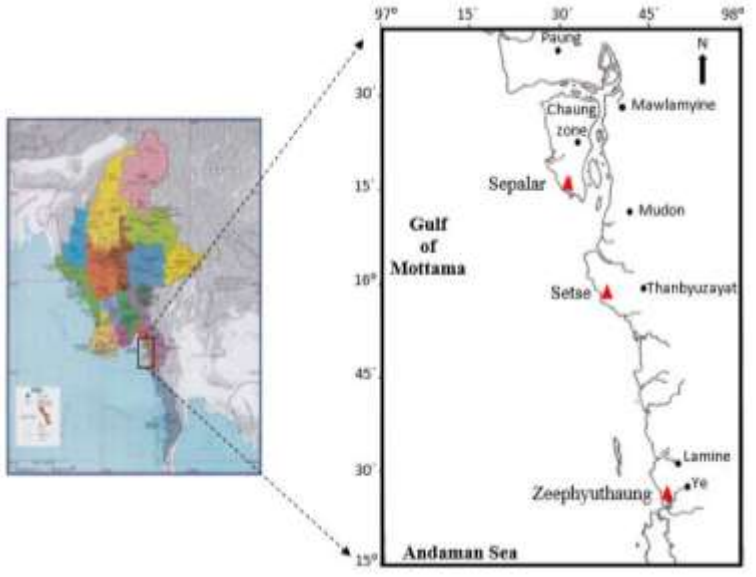


Figure 1 Map showing the study sites of *H. nehereus* in Mon coastal water

Results

Maturity stages and spawning

Based on a total of 335 females ranging from 180 to 305 mm total length and 22.33 to 206 g body weight, the maturity stages and spawning season were examined. From the result of monthly percentage distribution of maturity stages, the maturity stages I–III ovaries were observed throughout the study period and the maximum percentage for stage I was recorded in April, stage II in October and stage III in February, respectively. Stage IV ovaries were also observed all the month except in April and the peaks values were observed in December and January. The percentage occurrence of stage V ovary showed the lowest value in September and the highest in

June followed by December and January. Stage VI ovaries occurred from May to August, November to January and March showing two peaks in June and December. Samples of stage VII ovary were recorded in June, August to November and February to March showing three similar peaks in August, September and February. The lowest percentage were observed in March and November (Table 1.). The proportion of maturity (%) for stage IV, stage V and stage VI indicated the peaks in May (44.82%), June (50%), December (66.66%) and January (58.06%) and the lowest values were observed in September (7.14%), October (8.33%), and November (7.41%). There was no record in April (Fig. 2).

Table 1 Monthly percentage distribution of maturity stages in female *H.nehereus*

Months	No. of fish	Maturity stages (%)						
		I	II	III	IV	V	VI	VII
May	29	6.90	20.69	27.59	13.79	17.24	13.79	
June	36	5.56	13.89	19.44	11.11	22.22	16.67	11.11
July	48	4.17	22.92	37.50	14.58	16.67	4.17	
August	28	10.71	17.86	25.00	17.86		14.29	14.29
September	28	28.57	28.57	21.43	3.57	3.57		14.29
October	24	16.67	41.67	20.83	8.33			12.50
November	27	29.63	29.63	25.93	3.70		3.70	7.41
December	33	3.03	9.09	21.21	30.30	21.21	15.15	
January	31	3.23	9.68	29.03	29.03	19.35	9.68	
February	21	23.81	14.29	38.10	9.52			14.29
March	25	28	12	20	24	4	8	4
April	5	40	40	20				

Gonado-somatic index (GSI)

Monthly gonado-somatic index of female *H. nehereus* varied between 0.94 to 5.69 and the peak values were observed in May (4.16), June (5.16), December (4.83) and January (5.69). The values were found to be lower in September (1.24), October (0.94), November (1.1) and April (1.46) as expressed in (Fig. 2).

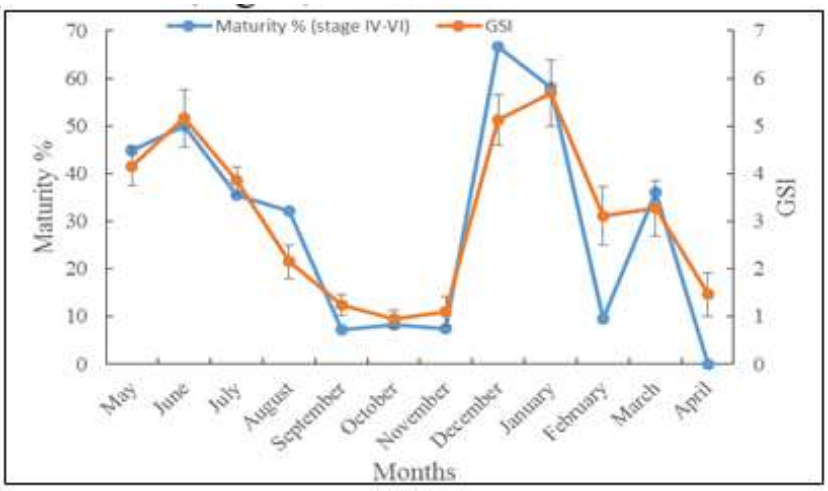


Figure 2 The percentage occurrence of maturity (Stage IV-VI) and GSI of female *H. nehereus* in different months

Relative condition factor (Kn)

The Kn values in male showed slightly fluctuation from the minimum 0.989 in August to the maximum 1.004 in October. In female, the peak values were recorded in May (1.021), June (1.025), December (1.027) and January (1.026). The low Kn values were observed in July (0.998) and February (0.999). Average Kn values for male and female were 0.999 and 1.01.

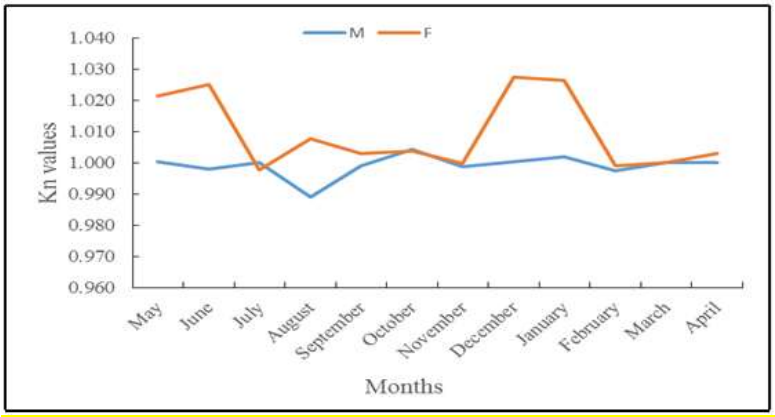


Figure 3 Monthly variation of relative condition factor in male and female

Sex ratio

Since *H. nehereus* have no external differentiating characters between the two sexes, the abdomen of the specimens were cut open for distinguish the sex. The sex ratio was tested by 1:1 method of Chi-square (x^2). The overall sex ratio of population (1: 0.66) showed significant difference at 5 % level ($n = 843$, $p < 0.05$). Monthly sex ratio was significant during September to October and January to April, indicating the predominance of males on females.

Table 2 Monthly variation in sex ratio of *H. nehereus*

Months	No. of fish	Males	Females	Expected (E)	Sex ratio (M:F)	Chi-square
May	55	26	29	27.5	1 : 1.12	0.16
June	86	50	36	43	1 : 0.72	2.28
July	86	38	48	43	1 : 1.26	1.16
August	68	40	28	34	1 : 0.70	2.12
September	77	49	28	38.5	1 :0.57	5.73*
October	72	48	24	36	1 : 0.50	8*
November	69	42	27	34.5	1 : 0.64	3.26
December	73	40	33	36.5	1 : 0.83	0.68
January	80	49	31	40	1 : 0.63	4.06*
February	79	58	21	39.5	1 : 0.36	17.32*
March	72	47	25	36	1 : 0.53	6.72*
April	26	21	5	13	1 : 0.24	9.84*
Total	843	508	335	421.5	1 : 0.66	35.5*

* Significant at 5% level ($P < 0.05$) with 1 df

Length at first maturity

Length at first maturity of *H. nehereus* was determined based on 135 females ranged in size from 180 to 301 mm total length. Females in maturity stages IV and above were considered as matured fish and they were grouped into 10 mm length groups and analyzed into different maturity stages. The estimated length at which 50% of females *H. nehereus* reached sexual maturity was 230 mm.

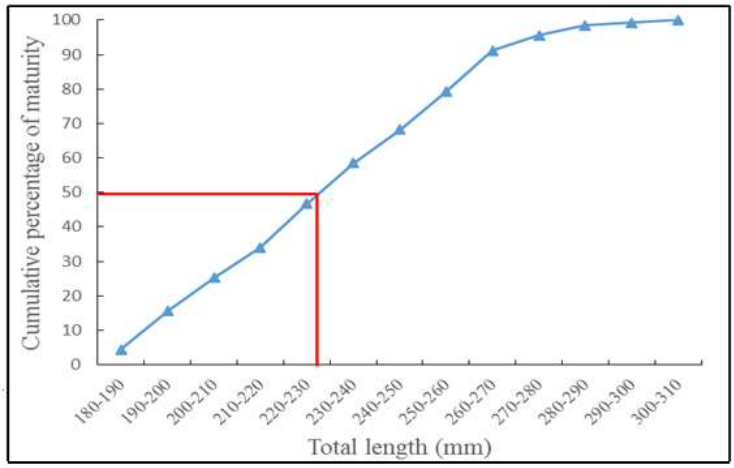


Figure 4 Length at first maturity of female *H. nehereus*

Fecundity

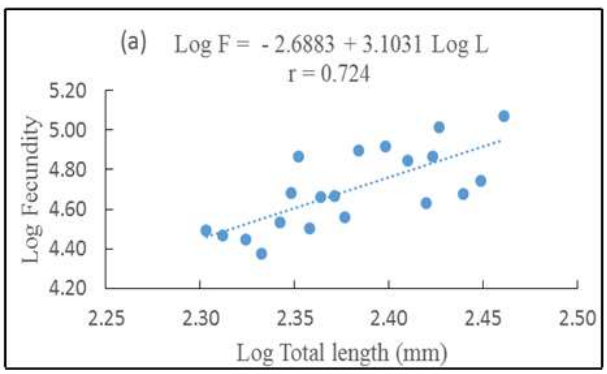
Fecundity was determined based on 20 specimens which ranged from 201 mm to 289 mm (TL) and 42.28 to 153.44 g (BW). The estimated fecundity varied between 23,625 and 117, 498 ova with an average fecundity of 55054. The minimum fecundity was observed in fish with 215 mm (TL), 50.9 g (BW) and 3.36 g (OW). The maximum fecundity was recorded in fish with 289 mm (TL), 153.44 g (BW) and 16.78 g (OW). Regression equations of fecundity in relation to total length, body weight and ovary weight of fish were given as:

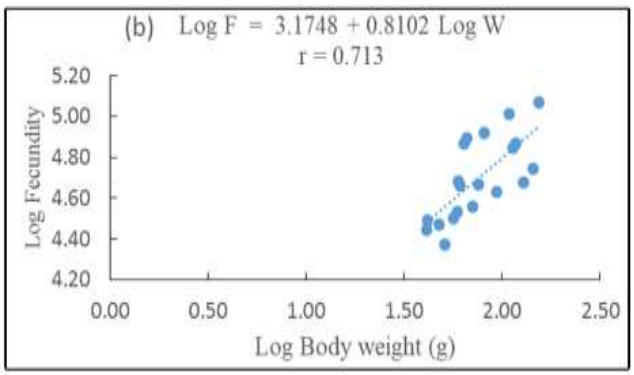
$$\text{Log } F = -2.6883 + 3.1031 \text{ Log TL}, \quad r = 0.724$$

$$\text{Log } F = 3.1748 + 0.8102 \text{ Log BW}, \quad r = 0.713$$

$$\text{Log } F = 4.0849 + 0.8371 \text{ Log OW}, \quad r = 0.934$$

According to the correlation coefficient (r) values from the above regression equations, there were highly positive relationship between fecundity against total length, body weight and ovary weight. This means that the fecundity increased according to total length, body weight and ovary weight.





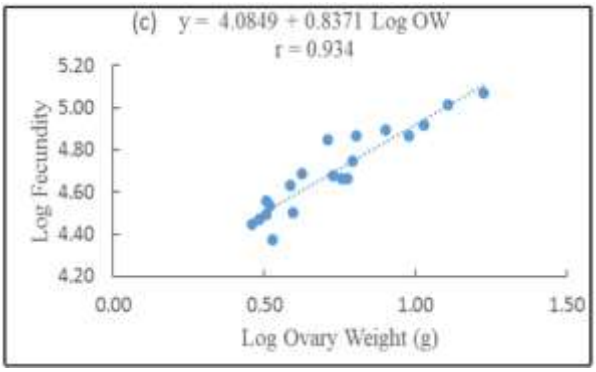


Figure 5 Relationship between fecundity and (a) total length, (b) body weight and (c) ovary weight of *H. nehereus*

Ova diameter

In stage I, most of the ova ranged from 57 to 300 µm with a mode at 80-160 µm (65.4%) and stage II showed ova diameter range from 160 to 440 µm with a mode at 240-320 µm (38.9%) size group for the immature stock. In stage III, most of the ova ranged from 240 to 600 µm and two modes, one 'a' at 240-320 µm (13.5%) and 'b' at 400-480 µm (45.9%) were observed. In stage IV, most of the ova varied between 320 and 600 µm and two modes were at 'a' 320-400 µm (14.4%) and 'b' at 560-640 µm (43.2%). All the ova belong to mature stock. Stage V showed two modes 'a' and 'b' at 400-480 µm (11.9%) and 640-720 µm (47.8%) size groups ranging the ova diameter from 400 to 680 µm. In stage VI, two modes 'a' and 'b' were observed at 480-560 µm (11%) and 640-720 µm (37.8%) size groups. Ova in this stage is the largest varied between 560 to 720 µm.

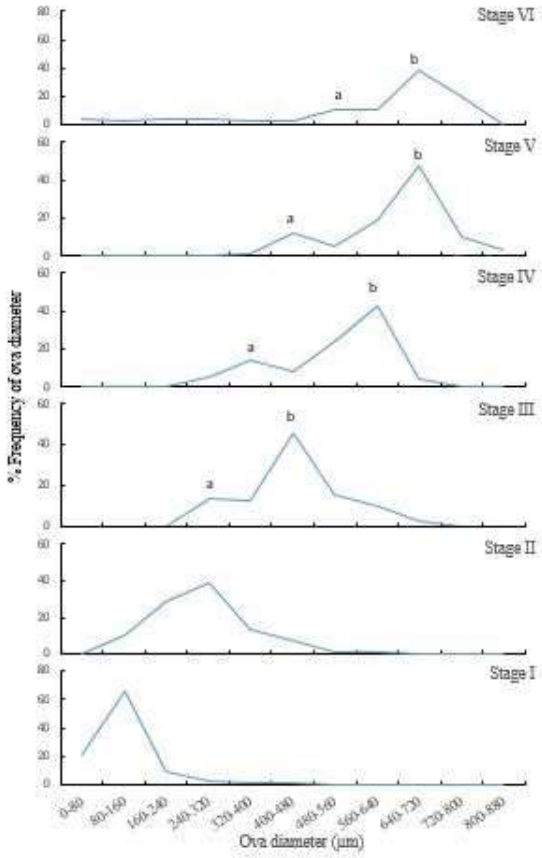


Figure 6 Ova diameter frequencies for different maturity stages in *H. nehereus*

Discussion

In the present study, all stages of maturity of *H. nehereus* were observed throughout the study period. The proportion of maturity (%) for stage IV, stage V and stage VI indicated the peaks in May, June, December and January and the low values were observed in September to November and April (Table. 1 and Fig. 2). It can be suggested that *H. nehereus* probably have a prolonged breeding season with two peaks during May-June and December- January. This result is in an agreement with the finding of Kham, *et al.,* (1992) who reported that peak spawning activity of *H. nehereus* was during December-January and June along the Saurashtra coast. Ghosh (2014) revealed that *H. nehereus* breed throughout out the year with peak in the summer period (Arpil-July) along the Saurashtra coast. However, Johnson (2006) reported the peak spawning season was found to be in November and March in Mumbai water.

The study of gonado-somatic index (GSI) determined the reproductive conditions and spawning season of fishes. GSI of *H. nehereus* varied from 0.94 to 5.69 in female. The high GSI values were observed during May, June, December and January, and this found to be coincide with the peak spawning period. The low values were recorded in September to November when there was the predominance of immature (stage I) and maturing females (stage II) in the catch (Fig. 2). Kumar, *et al.* (2016) stated that GSI values of *H. nehereus* fluctuated between 2.158 and 8.173 and peak value was observed in February along Sunderband region. David (1963) observed the GSI peak which indicates active spawning.

The high Kn values were recorded in May to June and December to January in female and this observation confirmed that the spawning period of *H. nehereus* was the highest during May to June and December to January (Fig. 3). Nair *et al.* (1983) reported that 'Kn' cycle of the female closely follows the seasonal pattern of GSI, the periods of heavy breeding activity showing comparatively high 'Kn' value and vice-versa in *A. commersoni*. Monthly variation in sex ratio showed that there was significant difference at 5% ($p<0.05$) level in September, October and January to April between males and females. The overall sex ratio of population indicated the dominance of males on females in the catch (Table. 2). Similarly, Gosh *et al*. (2009) found the sex ratio of *H. nehereus* as 1:0.99 along Saurashtra coast. Bapat (1970) suggested that the presence of large number of male in July-August was due to the fact that the spent females move out of the fishing grounds after the main spawning season in November-March.

The length at first sexual maturity is an important parameter in fisheries research to determine the optimum mesh size and minimum legal size that may be needed to maintain the suitable spawning stock and to ensure at least one spawning for the mature individuals. (Amin *et al.,* 2016). In this study, length at first maturity of *H. nehereus* was estimated as an average of 230 mm (Fig. 4). This result is consistent with the finding of Kurian and Kurup (1992) and Kurian (2000). The result of the present study on the length at first maturity of *H. nenereus* was smaller than that of Johnson (2006) estimated the length at first maturity of *H. nehereus* as 255 mm. The variations in the size at first maturity from different coast and from same area can be attributed to the temporal and spatial changes in ecological conditions as well as possible error in sampling (Jaiswar and Chakaborty, 2016).

Fecundity is the total number of eggs produced per fish. The fecundity of *H. nehereus* ranged from the minimum (23625) in 215 mm to the maximum (117498) in 289 mm total length. The result of the present study is in an agreement with Kumar, *et al.* (2016) reported that the minimum fecundity (18156) was recorded in the length group of 210–220 mm and that of maximum (92012) in length of 280–290 mm from Sunderban. The estimated fecundity in the present study was higher than that of Khan, *et al.* (1992) found the fecundity of *H. nehereus* varied between 17075 and 79 631 along the Saurashtra coast. According to the result of Johnson, (2006), the fecundity of *H. nehereus* ranged from 21,182 to 116,067 in 246-356 mm in Mumbai water.

Ghosh (2014) estimated that the number of ova varied from 8467 to 102,079. Bromage *et al.*, (1992) reported that fecundity varied with the seasons, climatic conditions and environmental factors, nutritional status and genetic potential.

The correlation coefficient values (r) from the linear regression equations showed that the fecundity indicated higher relationship with ovary weight rather than that with total length and with total weight (Fig. 5). Thus, the present study on fecundity can be suggested that the weight of ovary is more suitable indices for estimating the fecundity than length and weight of fish. However, (Ghosh, 2014) stated that fecundity was more closely related with length of fish although the relationship between length, weight and gonads weight of fish was observed to be linear.

As a result of ova diameter measurement, two modes of ova representing the immature and mature were observed in maturity stage III to VI ovaries of *H. nehereus*. The mature ovaries (from stage IV to VI) contain mature ova which are distinctly separated from the immature stock. This observations probably indicated that *H. nehereus* spawns once a year with a prolonged spawning season. Similarly Bapat (1970) and Johnson *et al.* (2006) reported that the ovaries of *H. nehereus* have ova with two modes representing the immature and mature crop where individuals spawn once a year and the species breed throughout the year. This observation was not in an agreement with Ghosh (2014), suggested that the presence of one batch of mature ova, one batch of maturing ova and one batch of immature ova in a mature ovary showed the individual fish spawns continuously in a year. Maturation and spawning period varies in different fish species and even the same species from different waters. It depends upon several ecological and physiological factors mainly temperature and intensity as well as duration of light that control maturation of gonads (Khanna and Singh, 2003).

Conclusion

In conclusion, *Harpadon nehereus* have a prolonged spawning season with two peaks during May to June and December to January and males were dominant in the catch throughout the year. The fecundity indicated higher relationship with ovary weight than total length and body weight, therefore the weight of ovary is more suitable indices for estimating the fecundity. The length at first maturity of female *H. nehereus* was estimated as 230 mm. The ova diameter measurement revealed that the fish spawns once a year.

Acknowledgement

I am thankful to Rector Dr. Si Si Hla Buu, Pathein University for her permission accepting the research. I am greatly indebted to Dr. Cherry Aung, Professor and Head of Marine Science Department, Pathein University. I am also greatly obligated to Dr. Tint Swe, Professor (Retired) Mawlamyine University, for his invaluable guidance and suggestions.

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LABORATORY TO OUTDOOR CULTIVATION OF *ARTHROSPIRA PLATENSIS* IN NATURAL SEAWATER

Sit Thu Aung[1], Sein Than Lin [2] and Min Oo [3]

Abstract

In the experiment of laboratory, the growth rates of *Arthrospira platensis* were tested at five different media (modified F-2 medium with $CaCO_3$, modified F-2 medium with $NaHCO_3$, modified Zarrouk's medium with $CaCO_3$, modified Zarrouk's medium with $NaHCO_3$, and urea and T-super with $NaHCO_3$) at pH 8.5 to 10.5 and salinity 30‰ and 35‰. Then they were also tested with urea and T-super with $NaHCO_3$ medium at pH 10 and salinity 30‰ for outdoor culture. The optimum growth of *A. platensis* (OD 0.56) was observed in modified Zarrouk's medium with $NaHCO_3$ at pH 10.5 in both salinities and at pH 10 in salinity 30‰ and also observed in urea and T-super with sodium bicarbonate at pH 10.5 of salinity 30‰ in the laboratory cultivation. For outdoor culture the optimum growth was OD 0.47. The minimum growth rate was occurred in modified F-2 medium with $CaCO_3$ at pH 8.5 of salinity 30‰.

Keywords – *Arthrospira platensis*, medium, seawater, pH, laboratory culture, outdoor culture

Introduction

Spirulina are multicellular and filamentous blue-green microalgae belonging to two separate genera *Spirulina* and *Arthrospira*, consisting of about 15 species (Habib *et.al* 2008). It is a primitive organism originating some 3.5 billion years ago that has established the ability to utilize carbon dioxide dissolved in seawater as a nutrient source for their reproduction (Hill 1980). *Arthrospira* is a photosynthesizing cyanophyte (blue-green algae) that grows vigorously in strong sunshine under high temperatures and highly alkaline conditions. It is found in soil, marshes, freshwater, brackish water, seawater and thermal springs. Alkaline, saline water (>30 g/l) with high pH (8.5–11.0) favour good production of *Arthrospira* (Habib *et.al* 2008).

It grows naturally in tropical regions inhabiting alkaline lakes containing sodium carbonate or sodium bicarbonate, especially the place where there is a high level of solar radiation at altitude in the tropics. These lakes are found near volcanoes. The natural lakes of the world are found in Chad, China, Ethiopia, Kenya, Mexico, Myanmar and Peru. In Myanmar, there are four natural lakes namely Twin Taung, Twin Ma, Taung Pyauk and Yae Kharr which are located at Sagaing Region in upper part of Myanmar. Spirulina is motile but do not have heterocyst. The new cells are reproduced by separating into a number of shorter segments.

Arthrospira platensis contains high levels of protein (50-70%), lipids (7-16%) and vitamins, especially vitamin A and B complexes which make it suitable for animal feeding (Cohen 1997). In many countries of Africa, it is used as human food as an important source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in many countries of Asia it is used as protein supplement and as human health food. *Arthrospira* has been used as a complementary dietary ingredient of feed for poultry and increasingly as a protein and vitamin supplement to aqua feeds (Habib *et.al* 2008).

Cultivations of *A. platensis* are influenced by physical and chemical variable such as temperature, light intensity, nitrogen and carbon sources and pH. In particular, the success of *A. platensis* growth depends on maintenance of both cultivation temperature near the optimum and light intensity below a photoinhibition threshold (Vonshak and Richmond 1988)

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The conventional nitrogen sources for the production of *Arthrospira* are sodium or potassium nitrates. High pH and temperature are the key factors for large scale *A. platensis* culture indoors. The optimum temperature for *A. platensis* culture is in the range of (28-33°C). In addition, *A. platensis* requires relatively high pH values which may effectively inhibit the contamination of most algae in the culture (Henrikson 2010). The ability to flourish in extreme pH is a strategy of cyanobacterial species to avoid contamination by other micro-organisms (Touloupakis *et.al* 2016).

The mass cultivation of *Arthrospira* depends on a number of factors, including the availability of nutrients, temperature, and light. *Arthrospira* also requires a relatively high pH, which inhibits the growth of other algae in the system. In order to maintain high pH and avoid fluctuations, high amounts of sodium bicarbonate must always be there in the culture medium (Soni *et.al* 2017). Moreover, the light and temperature are the main factors for growth in nutrient-operated outdoor pond (N, P, CO2, etc.) and well mixed conditions because the specific growth rate of the selected strain must be determined based on these two variables (Huesemann *et.al* 2016)

The first successful culture of *Arthrospira* using untreated seawater in laboratory condition was reported in Italy in 1984 by Materassi *et.al* (1984). The culture technique developed in the laboratory has been successfully applied to outdoor mass culture of *Arthrospira*. The climatic condition is very suitable for *Arthrospira* culture especially in south of Italy throughout the year (Tredici *et.al* 1986).

There are about 1.4 trillion cubic meters of water on Earth. Among them, about 97% is in the oceans and seas. The remaining, about 3% is fresh water. In fresh water, nearly 70% is frozen in icecaps of Antarctica and Greenland. Most of the remainder is present in soil. Only about 1% of fresh water is used for 7 billion people and animals. In 2025 at least 2.5 billion people won't have enough water to drink (Gershwin & Belay 2007). So, we will have to use seawater for *Spirulina* cultivation. The two advantages of *Spirulina* cultivation in seawater are: 1) lower fertilizer cost and 2) saving farm land by using waste sea beach. The aim of this present study is to observe the suitable media, pH and salinity for *A. platensis* culture and to know whether or not cultivation on coastal regions with natural seawater.

Materials and Methods

For laboratory condition, the growth rates of *Arthrospira platensis* were tested with mainly five media which are two modified F-2 media: medium (1) F-2 with $CaCO_3$ and medium (2) F-2 with $NaHCO_3$, two of modified Zarrouk's medium: medium (3) Z-1 with $CaCO_3$, medium (4) Z-1 with $NaHCO_3$, and medium (5) urea and T-super, and medium at five different pH values ranged from 8.5 to 10.5 and two salinity values were 30‰ and 35‰. For outdoor culture condition, the growth rate was only tested with urea and Triple super phosphate medium, 10 of pH value and 30‰ of salinity.

The initial cell density of all experiments was OD 0.2. The cultivations were carried out for a period of 7 days. The materials and apparatus used in this experiment were (8′×3.5′×8″) pond, cultural shelf, 40-watt fluorescent lamps, aeration pump, digital photocolorimeter (Model – 312), microscope, heater, Whatman No. 540 filter paper, refractometer, pH meter, four-digit digital balance and laboratory apparatus (pipettes, conical flasks, beakers). The culture designs for our researches were shown in figures 1 & 2 as flow chats.

Table 1 List of nutrients used in the experimental culture.

Nutrients	Medium				
	1	**2**	**3**	**4**	**5**
$CaCO_3$	+	-	+	-	-
$NaHCO_3$	-	+	-	+	+
$NaNO_3$	+	+	+	+	-
$NaH_2PO_4.2H_2O$	+	+	+	+	-
$Na_2SiO_3.9H_2O$	+	+	-	-	-
Thiamine HCl (B_1)	+	+	-	-	-
Biotin (B_6)	+	+	-	-	-
Vitamin (B_{12})	+	+	-	-	-
K_2SO_4	-	-	+	+	-
KOH	-	-	+	+	-
Urea	-	-	-	-	+
Triple super phosphate	-	-	-	-	+
Na_2EDTA	-	-	+	+	-

\+ present
\- absent

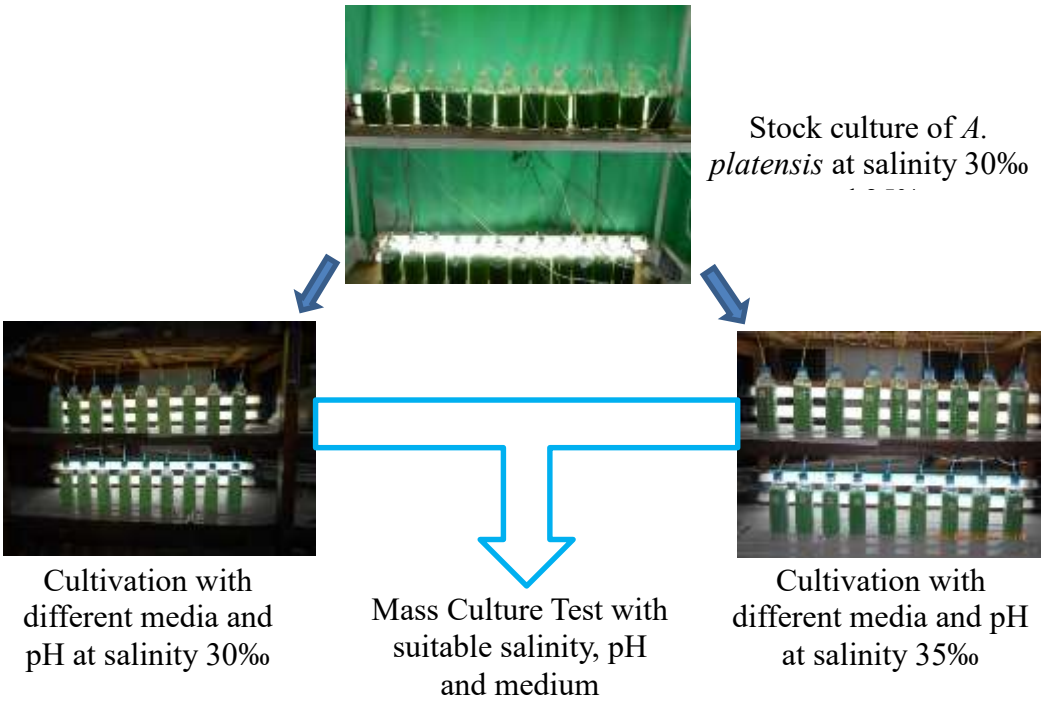

Figure 1 Flow Chat of *A. platensis* cultivation for Laboratory.

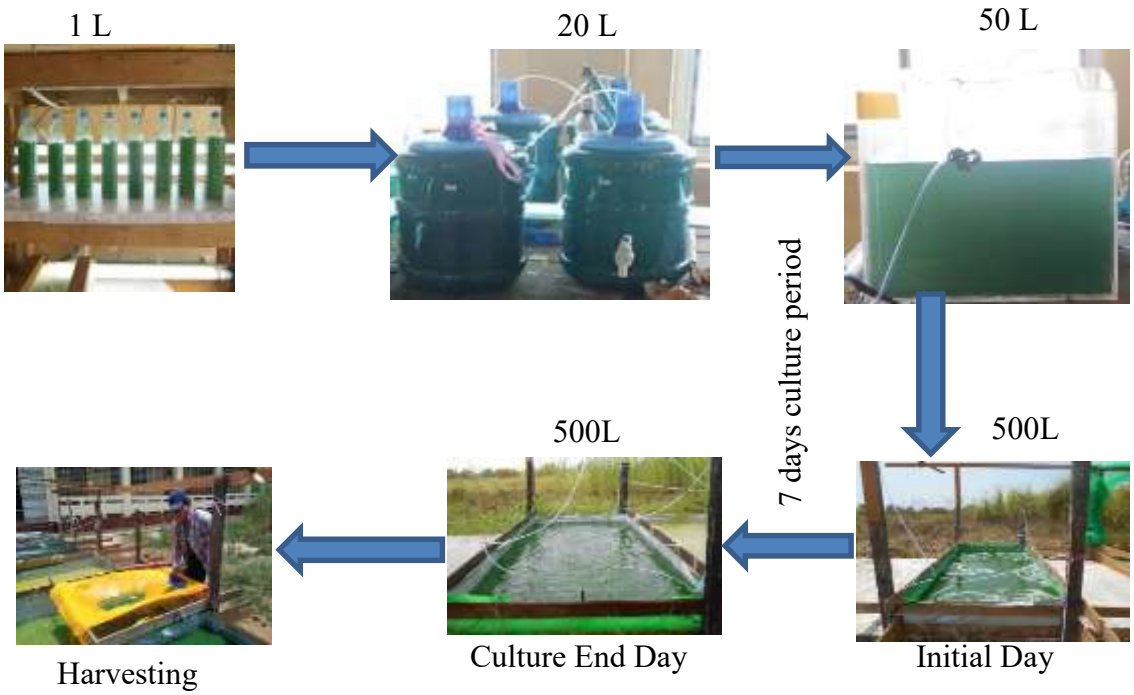

Figure 2 Flow Chat of *A. platensis* cultivation for outdoor.

Results

Laboratory Experiments on the Growth Rate of *Arthrospira platensis* in modified F-2 medium

Arthrospira platensis was cultivated in five different pH values (8.5, 9.0, 9.5, 10.0 and 10.5) which were adjusted by using calcium carbonate or sodium bicarbonate at salinities of 30‰ and 35‰ with modified F-2 medium (Table- 2). Total 20 of 1 litter plastic bottle capacity containing 800 ml were cultured with nearly same amount of aeration. All bottles were kept at room temperature.

Among the 20 bottles, ten bottles were prepared at salinity 30‰ and others were at salinity 35‰. Each three bottles of salinities 30‰ and 35‰ were adjusted to reach the require pH value with calcium carbonate ($CaCO_3$) or sodium bicarbonate ($NaHCO_3$). Growth rates of *A. platensis* were illustrated in Figures (3-6).

Table 2 Chemical composition of modified F-2 medium.

Nutrient Elements	**g/L of seawater**
$NaNO_3$	0.075
$NaH_2PO_4.2H_2O$	0.005
$Na_2SiO_3.9H_2O$	0.030
Thiamine HCl (B_1)	0.0001
Biotin (B_6)	0.0000005
Vitamin (B_{12})	0.0000005

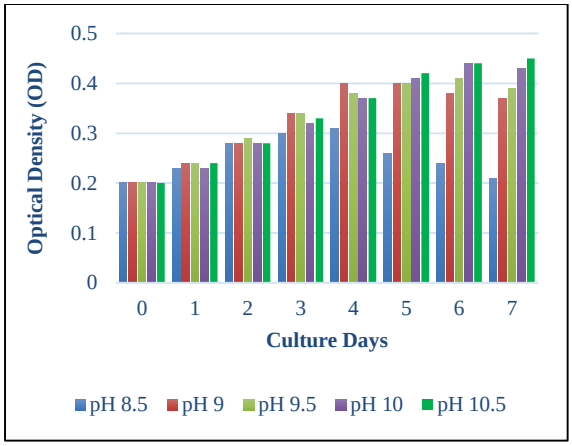

Figure 3 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with F-2 medium at salinity 30‰.

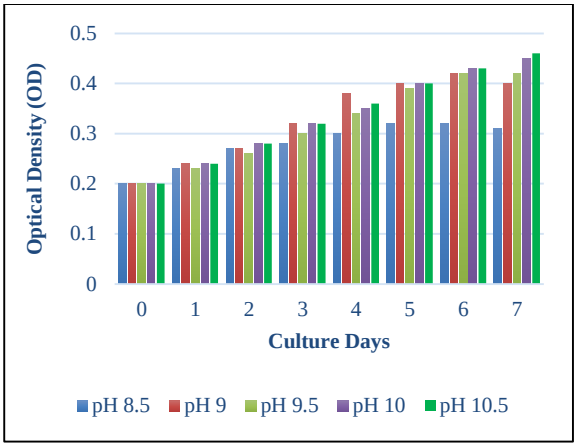

Figure 4 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with F-2 medium at salinity 35‰.

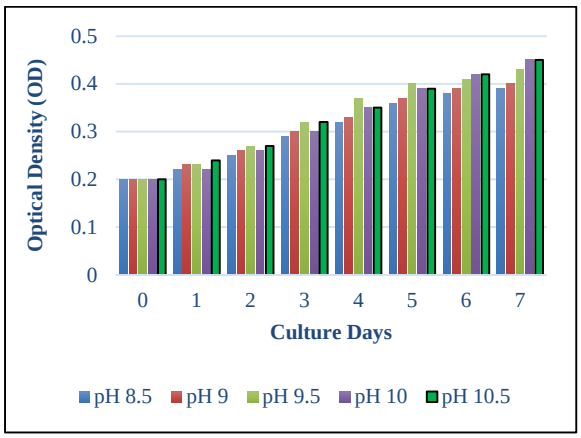

Figure 5 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with F-2 medium at salinity 30‰.

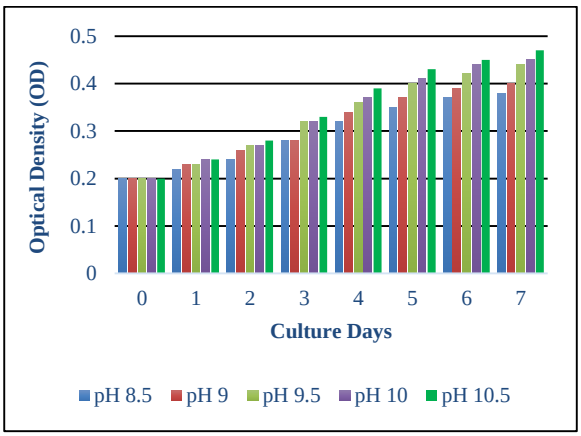

Figure 6 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with F-2 medium at salinity 35‰.

Laboratory Experiments on the Growth Rate of *Arthrospira platensis* in modified Zarrouk's medium

Arthrospira platensis was cultivated in five different pH values (8.5, 9.0, 9.5, 10.0 and 10.5) which were adjusted by using calcium carbonate or sodium bicarbonate at salinities of 30‰ and 35‰ with modified Zarrouk's medium (Table- 3). Total 20 plastic bottles of one litter capacity containing about 800 ml of the medium were cultured with nearly same amount of aeration. All bottles were kept at room temperature. Among the 20 plastic bottles, ten were prepared at salinity 30‰ and others were at salinity 35‰. Growth rates of *A. platensis* were shown in Figures (7-10).

Table 3 Chemical composition of modified Zarrouk's medium.

Nutrient Elements	g/L of seawater
K_2SO_4	1.148
$NaNO_3$	2.500
NaH_2PO_4	0.344
KOH	0.227
Na_2EDTA	0.080

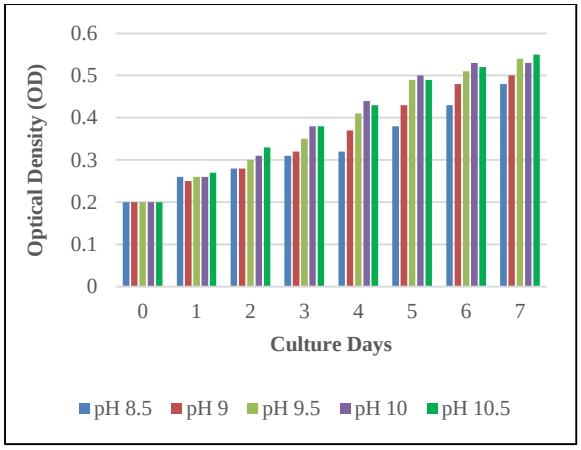

Figure 7 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with modified Zarrouk's medium at salinity 30‰.

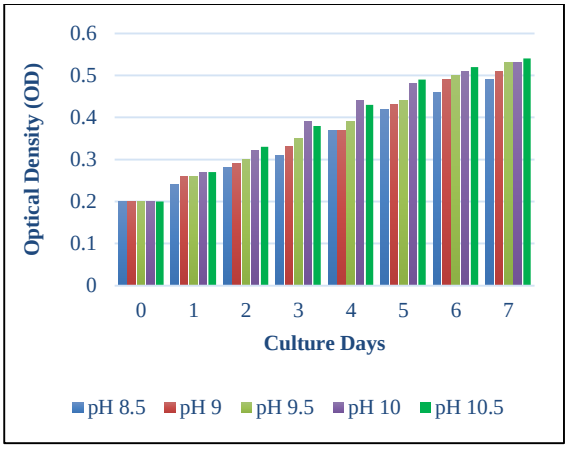

Figure 8 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with modified Zarrouk's medium at salinity 35‰.

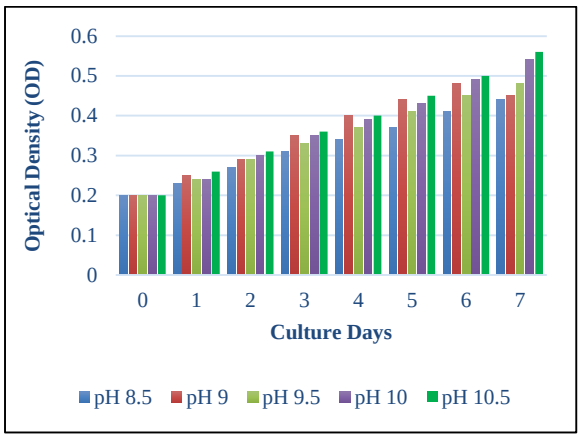

Figure 9 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with modified Zarrouk's medium at salinity 30‰.

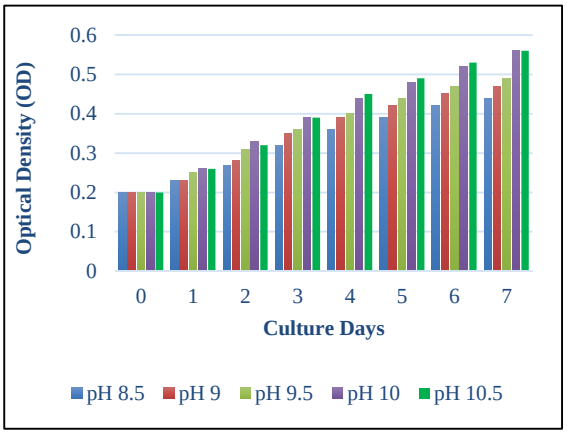

Figure 10 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with modified Zarrouk's medium at salinity 35‰.

Laboratory Experiments on the Growth Rate of *Arthrospira platensis* in Urea and Triple Super Phosphate medium

Arthrospira platensis was cultivated in five different pH values (8.5, 9.0, 9.5, 10.0 and 10.5) which were adjusted by using only sodium bicarbonate at salinities of 30‰ and 35‰ with urea and T-super as the ratio 5:1. The nutrient medium was prepared that 10 g of urea and 2 g of T-super were separately soluted with each one liter of distil water. Total 10 plastic bottles of one litter capacity containing about 800 ml of the medium were cultured with nearly same amount of aeration. All flasks were kept at room temperature.

Among them, five bottles were prepared at salinity 30‰ and others were at salinity 35‰. Each five bottles of salinity 30‰ and 35‰ were adjusted to reach the require pH value with sodium bicarbonate. In this test, 10 ml of each nutrient solution was daily feed into the culture bottles. The growth rates of the cells were described in figures11 and 12.

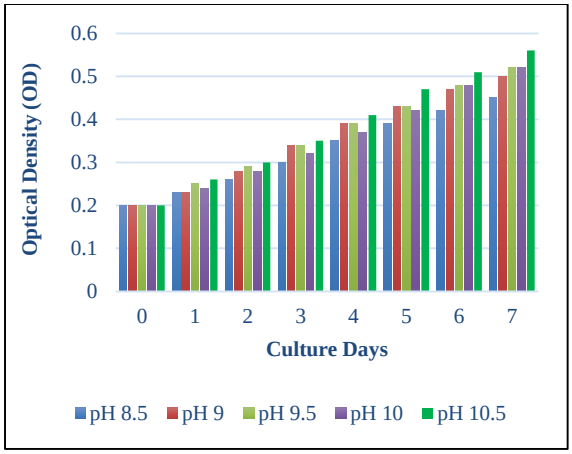

Figure 11 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with urea and T-super medium at salinity 30‰.

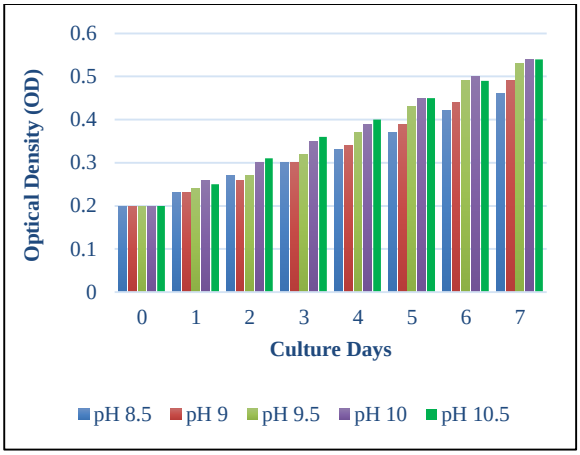

Figure 12 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with urea and T-super medium at salinity 35‰.

Outdoor Experiments on the Growth Rate of *Arthrospira platensis*

Arthrospira platensis was cultivated in seawater by using sodium bicarbonate ($NaHCO_3$) to control the pH value. The salinity of this experiment is 30‰. The nutrient medium was urea and T-super as the ratio 5:1. The cultivated water volume was 0.5 ton. The growths of *A. platensis* in seawater for outdoor culture were shown in figure 14. In this culture period, the doubling time for the growth was observed at second day. The maximum OD 0.47 was occurred at the end of the culture.

Figure 13 Small scale mass culture of *A. plantensis* in seawater.

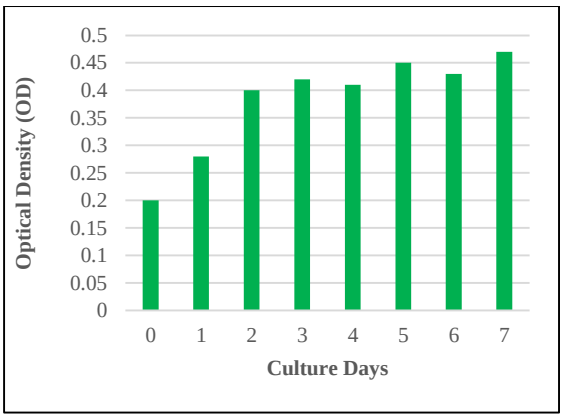

Figure 14 Comparison of the growth of *A. platensis* in outdoor culture by using $NaHCO_3$ with urea and T-super medium at salinity 30‰.

Production of *Arthrospira platensis*

Arthrospira platensis was harvested by using (300μm) screen and then were dried into the oven (70°C) for 24 hours. The yield was 4 kg (wet weight) and 0.3 kg (dry weight). The process for production was shown in figure 15.

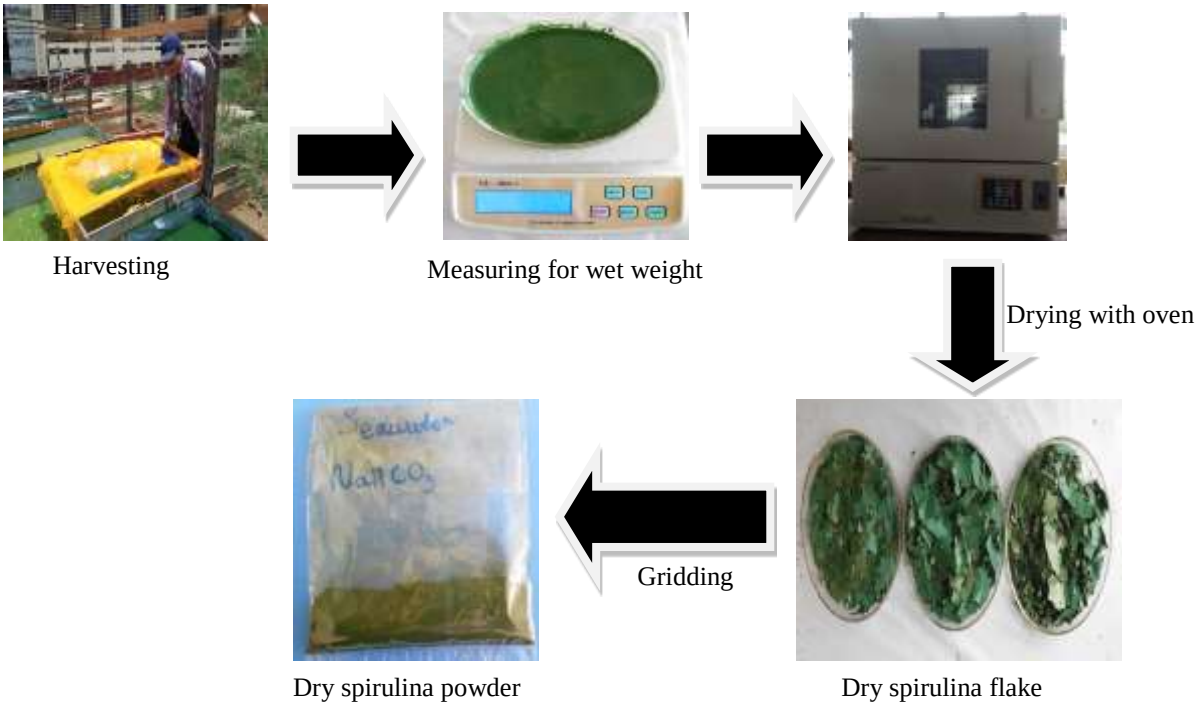

Figure15 Flow chart of the processing of *A. platensis* in sea water.

Discussion and Conclusion

Ciferri (1983) said that eight major environmental factors such as luminosity, temperature, inoculation size, stirring speed, nutrient, salinity, pH and water quality influence the productivity of spirulina.

Arthrospira shows an optimum growth between 35 and 37 °C under laboratory conditions. Outdoors, it seems that an increase in temperature up to 39 °C for a few hours does not harm the blue-green alga, or its photosynthetic ability. Thermophilic or thermotolerant strains of spirulina can be cultivated at temperatures between 35 and 40 °C. Such a property has the advantage of eliminating microbial mesophilic contaminants. The minimum temperature which growth of *Arthrospira* takes place is around 15 °C during the day. At night, *Arthrospira* can tolerate relatively low temperatures (Richmond 1988). In this study *A. platensis* was cultivated at room temperature in the laboratory and the water temperature ranges for outdoor condition were between 32℃ and 35℃.

Khin Mar Soe (2009) studied the growth of *A. platensis* on seawater-based Provasoli (PES) medium, seawater-urea medium, seawater-based medium I and seawater-based medium II and seawater-based medium III in the laboratory. The optimal growth of *A. platensis* was found in seawater-based medium III which contained a low concentration of phosphate, a small amount of bicarbonate, nitrate and Fe-EDTA. She reported that the maximum OD 0.79 with initial OD 0.2 was obtained at 15th day of the experimental period.

In the present laboratory study, the growth of *A. platensis* in seawater were studied in different five pH values (8.5, 9.0, 9.5, 10.0 and 10.5) at salinity 30‰ and 35‰ with various media. Among this experiment, the highest optimum density (OD 0.56) was observed in modified Zarrouk's medium and urea & T-super nutrient medium with $NaHCO_3$ over pH 10.0 at the end of culture. The lowest was in modified F-2 medium with $CaCO_3$ at pH 8.5. Therefore *A. platensis* possesses a high tolerance of alkaline pH for cultivation.

Faintuch *et.al* (1991) also studied the influence of the nutritional sources on the growth rate of cyanobacteria. They reported that there is very significant influence of mixtures of defined proportions of KNO_3, urea and ammonia on the growth of *Arthrospira*. The most favourable growth rates of *A. platensis* occurred in the presence of 2.57 g/litre KNO_3 with growth rate of 0.3-0.4/day. Chang *et.al* (1999) studied the possibility of using nitrifying bacteria for the fulfilment of nitrogen fertilizer in *Arthrospira* mass culture. They first adapted the nitrifying bacteria with pH 8-10, 0.6-2.2 percent salt and 6-12 mg/litre of $NaHCO_3$ in the culture solution. They found that the concentration of NO_3 reached over 20 mg/litre after the nitrifying bacteria was inoculated in the *Arthrospira* culture solution and then incubated for 6 days at 25–35 °C. In the present study we mainly used nitrogen sources as $NaNO_3$ or urea. The growth rates of *A. platensis* were observed OD 0.56 at pH 10.5 in salinity 30‰ when using $NaNO_3$ or urea for laboratory cultivation and OD 0.46 was occurred in outdoor cultivation by using urea.

Growth performance of *A. platensis* was studied in three different concentrations of banana leaf ash added with 0.4 g/litre jackfruit seed powder and 0.2 g/litre with urea in the laboratory (Toyub *et.al* 2005). Rice husk ash (RHA) and $NaHCO_3$ were used as a source of carbon in *Arthrospira* culture (Akhter *et.al* 1996). They reported that the addition of 2.0 g $NaHCO_3$/litre every two days supported better growth of *Arthrospira* than 1.0 g RHA/litre every day, although this might not be supported on economic grounds.

May Yu Khaing (2007) reported that the optimum pH for the growth of *A. platensis* biomass was 8.5 to 9.5. In the present study salinity ranges (30 g/l and 35 g/l) with pH (8.5-10.5) for laboratory condition and 30 g/l of seawater with pH 10 for outdoor culture were used.

In modified F-2 and Zarrouk's media the growth rate of *A. platensis* was found that content of sodium bicarbonate medium was higher than containing calcium carbonate media in seawater. From this study, using sodium bicarbonate into culture media may be one of the important factors for *A. platensis* growth. Calcium carbonate rapidly raised the pH values but the responding growth of *A. platensis* was not achieved to a suitable rate in seawater media. Sodium bicarbonate was suitable to use in seawater because it slowly increased the pH values and nearly steadied at the required pH.

Lagoon water can be used with some nutrient supplementation to grow *A. platensis* (Costa *et.al* 2004). They used Mangueira lagoon water rich with carbonates and a high pH, and in addition of 1.125 or 2.250 mg/litre of urea and 21 or 42 mg/litre of $NaHCO_3$ during fed-batch culture of *A. platensis*, respectively using a 32-factorial design. They found that lagoon water in addition of 1.125 mg/litre of urea resulted in a 2.67-fold increase in the final biomass of spirulina.

The first successful culture of *Arthrospira* using untreated seawater in laboratory condition was reported in Italy in 1984 by Materassi *et.al* (1984). The culture technique developed in the laboratory has been successfully applied to outdoor mass culture of spirulina. The climatic condition is very suitable for *Arthrospira* culture especially in south of Italy throughout the year (Tredici *et.al* 1986). The mean annual yield of biomass on sea-water plus urea was 7.35 g (dry weight)/m2/day, which was slightly lower value than that obtained on the standard sodium bicarbonate medium with sea-water (8.14 g/m2/day) under controlled pH, ranged from 8.0 to 8.3.

Dineshkumar *et.al* (2016) and Bharat *et.al* (2011) said that *Arthrospira* was survived in seawater but growth was not flourished, achieving maximum dry weight of 1.86 dw/L on 30^{th} day and 0.28 dw/L on 18^{th} day of cultivation. Natural seawater fortified with different amount of $NaHCO_3$ and $NaNO_3$ did not shown significant impact on *Arthrospira* growth. In this study, *A. platensis* was also survived in seawater and growth contained $NaHCO_3$ in media was flourished during cultural period.

Florian *et.al* (2017) used modified Zarrouk's medium in order to reduce the production cost. The modified Zarrouk's medium was diluted up to five times without impacting the growth rates in 28-days batch cultivation. Higher dry weights (1.21 g/L and 0.84 g/L) were observed after 21 days of batch cultivation. In the present study, maximum growth rates in modified Zarrouk's medium were observed at culture end day in pH 10.5.

From this study, the growth rate of *A. platensis* was found that containing sodium bicarbonate media were higher than containing calcium carbonate media in seawater. So, using sodium bicarbonate into culture media may be one of the important factors for *A. platensis* growth.

Modified Zarrouk's medium was suitable for culturing in seawater but the treatments of *A. platensis* are expensive. Other, modified F-2, are not suitable to use in seawater because the growth rates of *A. platensis* were poor and the costs are expensive. Therefore, urea and T-super medium was the most suitable medium in seawater. These results will be expected to provide good ideas for *A. platensis* production in seawater and to cultivate in coastal region.

Acknowledgements

The authors greatly appreciate Dr. Cherry Aung, Professor and Head of Marine Science Department, Pathein University for her kind permission to do this research work and supporting. We would like to express our gratitude to Dr. Myat Kyaw Wai, Associated Professor and Head of Department of Marine Science in Sittway University for her permission and advices to do this research work. We also greatly thank to Special thanks go to Dr. Htay Aung, Professor (Retired), Marine Science Department of Pathein University for his suggestions and necessary helps.

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STUDY ON SHELL MORPHOLOGY AND INTERNAL SOFT TISSUE ANATOMY OF GOLD-LIPPED PEARL OYSTER *PINCTADA MAXIMA* (JAMESON, 1901) IN MYEIK ARCHIPELAGIC WATERS

Zaw Myo Hein[1], Myat Thu[2]

Abstract

Pearl oyster of *Pinctada maxima* is the largest species in genus *Pinctada* and commonly found in Myeik Archipelagic waters. Morphology of *Pinctada maxima* mainly based on shell features such as external scale sculpture, internal features of shell. Its internal organs, especially anal papilla had different shape from other *Pinctada* taxa. Mantle was enclosing all soft tissues and organs. The adductor muscle was strongest and controls the closing of valves.The fundamental function of mantle is to secrete shell valves and ensure their growth. Gills performed the sieving and sorting of food particles. Labial palps accepted the food particles that filtered from gills or released the unnecessary particles as pseudofeces. The adductor muscle is strongest and controls the closing of valves.

Keywords: Nacre boundary, morphology, anal papilla, labial palps, anatomical, pseudofeces.

Introduction

Pearl oysters are members of the Phylum Mollusca and belong to the class Bivalvia. Having two shells (two valves), a soft body with a small foot, a byssal gland and paired gills are distinguished of bivalve mollusks. There are many different species of mollusk that can produce pearls but the pearls which are not made of true pearly nacre have small value except as curiosities (Mar Lar Myo Sein, 1982). Pearl producing molluscs can be divided in two group; those producing nacreous and non-nacreous pearls (as cited in Mamangkey, 2009). Pearl oysters are not closely related to true oysters or edible oysters (Family Oysteridae), being members of a distinct family, the feathered oysters (Family Pteriidae) in the order Pterioida. In family Pteriidae; two genera are cultivated for pearl production, they are *Pinctada* and *Pteria*. In these two genera, the most important is the genus *Pinctada* (Marques & Barbier, 2015). Of the taxa within the genus *Pinctada*, *Pinctada maxima* is the most important in terms of commercial cultured pearl production. The species of *Pinctada maxima* was recorded for the first time in northern Australian waters (as cited in Southgate & Lucas, 2008). It is the largest species in the genus *Pinctada* and it produces the largest and finest pearls. *Pinctada maxima* is differentiated from other species of *Pinctada* by the absence of denticles on the hinge (Humphrey and Norton, 2005).

Pearl oysters are filter feeders; feed on small algae found in the water column. The gills in bivalves are large, and tiny hair-like cilia on the gills are used to trap plankton and particles in the mucus of gill, and from there are transported to the mouth, where they are eaten, digested, and expelled as feces or pseudofeces. Both adults and larvae feed on algae and other small organisms. Clear tropical waters contain limited amounts of algae. Therefore, a large amount of water must be filtered daily in order for the pearl oyster to obtain sufficient food (Maria Haws, 2002). An oyster can filter up to 5 L (1.3 US gal) of water per hour. The commercial harvesting and farming of pearl oysters of the genus *Pinctada* comprises an expanding industry in tropical marine environments worldwide.

Earlier methods of harvesting oysters for their shell and for natural pearls have generally been superseded by farming of world harvested oysters, by the hatchery production of seed stocks and by the cultivation of pearls (Humphrey, Norton, *etal*. 1998). In Myanmar, the natural pearls

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had been produced since the later part of the 19thcentury and the mother of pearls was gathered from natural stock. In 1957, the first cultured pearl were successfully produced from the pioneer pearl culture farm; Myanmar Pearl Enterprise (MPE) at Pale Kyun, Myeik Archipelago, Taninthayi Region. (Nyo Nyo Tun and Htay Aung, 2009). In Myanmar, pearl culture initiated as Japanese-Myanmar joint-venture farm in 1954. The pearl oysters were collected from wild and seeding was implemented by Japanese technicians. In 1987, hatchery trials on *Pinctada maxima* have been lead (Tint Tun, 1998). Today, there are 12 pearl oyster farms extended in Myeik Archipelago. The selective farm for the present study is the Myanmar Oriental Pearl Farm, located on Narr Kho Island in the southern part of Myeik Archipelago. As an introduction to this study, the taxonomy of *Pinctada maxima* (Jameson), the external and internal structures of shell and the anatomy of internal tissues and organs, those are the objectives of this study.

Materials and Methods

Study area

This study was carried out in the pearl farm of Myanmar Oriental Pearl Company located in Narr Kho Island (Lat 10° 42' N and Long 97° 95' E). Narr Kho Island is situated about 80km far from Kawthaung and is located in the southernmost part of Myanmar (Figure 1).

Figure 1 Map showing the study pearl farm area.

Methods for shell morphology and anatomical study

The specimens of different ages and sizes were collected from the long lines. And the external shell morphology of juvenile and adult oysters was studied. To study the internal shell feature, valves of oysters was opened by using openers and the adductor muscle that attached to valves was detached. After removing the adductor muscle, the internal features of shell such as prismatic layer, nacre boundary, nacre layer, adductor muscle scar, pallial muscle scar, retractor muscle scar and ligament were clearly seen. For anatomical study of internal organs, only the left valve of oyster shell was carefully removed not to damage internal organs by detaching the attachment of adductor muscle in the left valve. And then the left lobe of mantle was carefully removed by using dissecting set. Then the internal organs of pearl oyster were dissected and photographed. The filaments of gills and anal papilla were examined under compound microscope.

Results

Classification of *Pinctada maxima*

Phylum – Mollusca

Class – Bivalvia

Order – Pterioida

Family – Pteriidae

Genus – *Pinctada*

Species – *Pinctada maxima* (Jameson 1901)

Description

Pinctada maxima has the large shell up to 30 cm in dorsal ventral measurement, subcircular in outline and typically has pale fawn in colour. The juveniles display the following colours: green, purple-black, yellow, cream, grey, brown and zigzag patterns of purple maroon. Hinge teeth were absent but anodyne (a series of transverse ligament grooves) were strong. Hinge line is long, straight and continues with subtriangular shaped anterior auricle but sometime nearly obsolete in large individuals. The posterior auricle is short, broadly rounded sinuated and it is well developed in young individuals. The internal nacreous layers were highly lustrous, silvery with a variably extended golden border. The anal funnel is flat, relatively long and paddle-shaped: a few narrow at the distal part and end with a blunt tip.

Shell morphology of *Pinctada maxima*

Pinctada maxima possess a pair of valves. Both valves are attached with a ligament in the dorsal hinge region. Each shell valve is composed of three layers: (1) the outer layer is the periostracum or conchiolin layer; (2) the middle layer is the ostracum or prismatic layer; and (3) the inner layer is the hypostracum or nacre (mother of pearl) layer. These structures are embedded within an organic matrix framework composed mainly of protein.

External features of shell

The shell is proscline, typically rounded in outline, with length typically equaling height (Figure 2). The right valve is usually flat to weakly convex and the left valve is more convex. The convexity decreases with age. The exterior colour of the shell is usually pale fawn, sometimes with valves partially green or ochre distally broadening radial rays. However, the juveniles display the following colours: green, purple-black, yellow, cream, grey, brown and zigzag patterns of purple maroon. By the time the oysters are about 120 mm dorsoventral measurement (DVM), the majority of them have brown coloured shells. The exterior shell margins and scales have commarginal (transverse for scales) alternating dark and light bands. The anterior auricles are very small. The byssal notch is small, narrow, slit-like, vertically elongated, and nearly obsolete in the large individuals. The posterior auricles are indistinct from the valves; they are rarely very inconspicuously and broadly sinuated. The sculpture of the exterior surface of the shell consists of commarginal prismatic scales which are most prominent in young individuals and typically abraded in large individuals

4.1.5.3. Internal features of shell

The internal surface of the shell is in direct contact with the external surface of the mantle and is generally covered by nacre. The interior nacreous surface is thick, lustrous, iridescent, silvery, rarely showing any dark tints with a rich golden rim that develops on the periphery of the

nacreous layer (Figure 2). At the margins of the shell, the nacreous layer merges sharply with a zone of dark, semi-soft proteinaceous conchiolin forming the periostracum. Finger-like projections of this material extend beyond the general periphery of the shell. The inner prismatic margin is of the same ground colour as the exterior. The adductor muscle scar is seen as a comma-shaped depression in the center of the shell. The posterior adductor muscle scar is large, wide, very slightly curving, with the dorsal edge narrower than the ventral edge, adjacent to the smaller, narrow, oval shaped, and anteriorly positioned posterior pedo-byssal retractor muscles scar. The pallial muscles are also seen as a discontinuous curve in the anterior side, stretching from the anteroventral extremity of the adductor muscles scar to the dorsoanterior region.

General structure of internal tissues and organs

After removal of one valve, the mantle lobes are covered and enclosed all other tissues and organs. The mouth, foot and byssus are situated in the anterior region. Mouth is surrounded by the labial palps. A visceral mass is located in the very dorsal region of internal tissue. A large part of digestive organs and gonad are enclosed within visceral mass. The large adductor muscle is obvious in a posterior-ventral position. It is separate from the visceral mass, except dorsal part where a portion of free rectum binds it to the digestive mass.The heart, the byssal/pedal retractor muscle and the brown excretory system (running along the anterior part of the branchial axis) are positioned in the central part between the adductor muscle and the foot. The “pearl sac” or nucleus insertion place is located in the posterior end of the foot. The large pigmented gills are connected anteirorly between the labial palps and they run ventrally and end posterior-ventrally at the pallial fold, in front of the anal papilla. Anal papilla is situated adjacent to the posterior-ventrally part of adductor muscle. The diagrammatic and photo views of internal tissues and organs structure of *Pinctada maxima* were shown in figure 3 and figure 4.

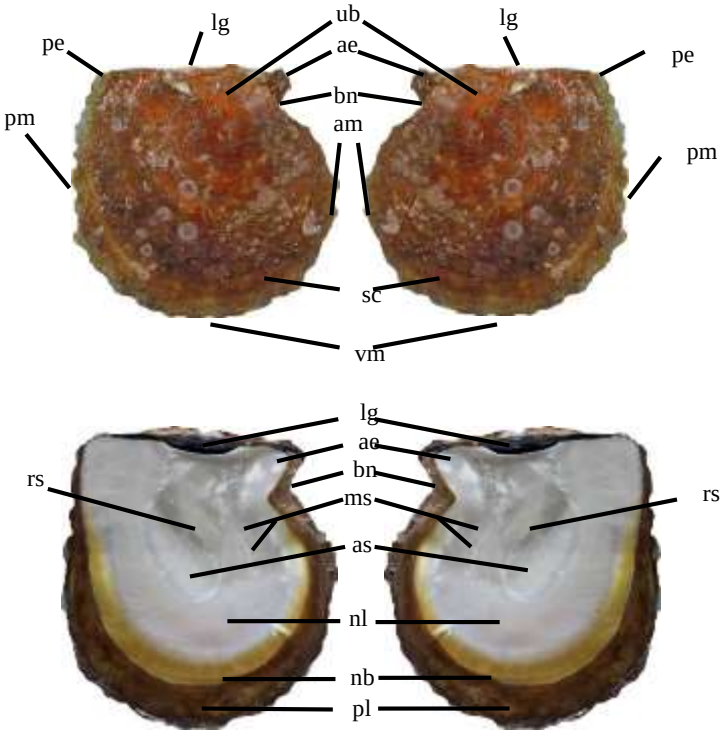

Figure 2 Shell morphology of the exterior and interior surfaces of *Pinctada maxima*. Abbreviations: ae: anterior ear (auricle); am: anterior margin; as: adductor muscle scar; bn: byssal notch; lg: ligament; ms: pallial muscle scar; nb: nacre boundary; nl: nacre layer; pe; posterior ear (auricle); pl: prismatic layer; pm: posterior margin; rs: retractor muscle scar; sc: scales; ub: umbo; vm; ventral margin.

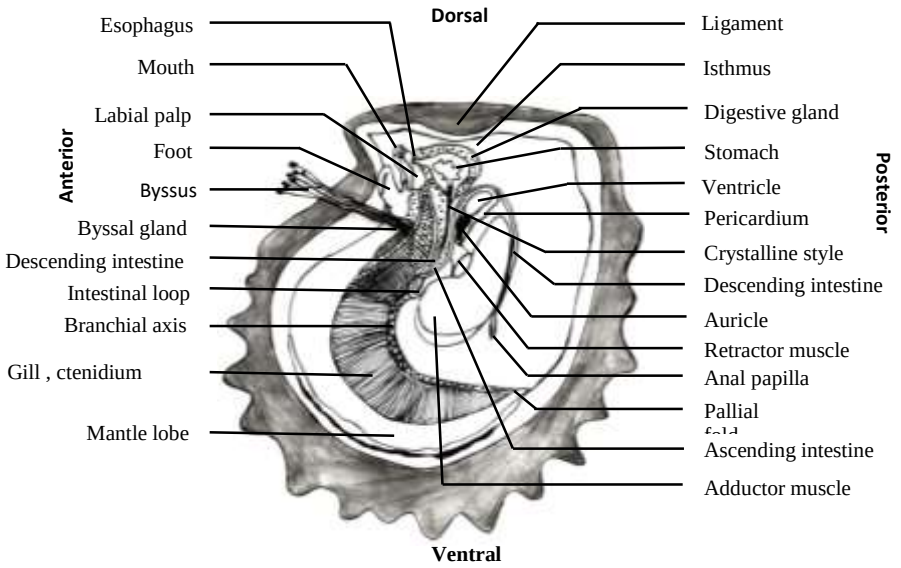

Figure 3 Diagram of internal anatomy of *Pinctada maxima*.

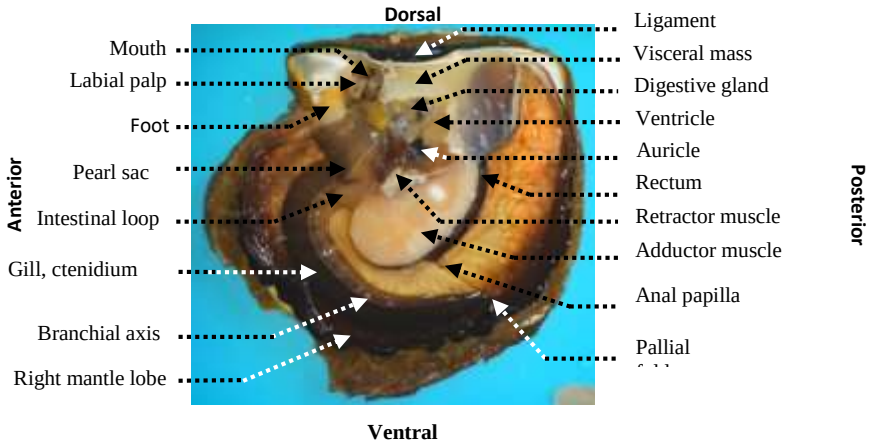

Figure 4 Lateral view of *Pinctada maxima* after removal of left valve, left mantle lobe, left gill.

The mantle

The mantle is a thin, most external and flattened expansive organ, lining the internal surfaces of the shell valves and enclosing all other soft tissues and organs. The fundamental function of the mantel layer is to secrete the shell valves and ensure their growth. The mantle consists of two lobes, with each lining on the inner surface of the shell valve. These two lobes are separated anteriorly, ventrally and posteriorly. But they are fused to the visceral mass and the adductor muscle; they join together at the dorsal portion along the hinge line, forming the isthmus. Each mantle lobe can be divided into four zones: (1) the marginal zone that is thick and pigmented and fringed with branched tentacles, (2) the distal or pallial zones that is attached to the shell, composed primarily of muscular threads used in mantle retraction that are visible to the naked eye (3) the central zone, adhering and covers the soft tissue of visceral mass and adductor muscle, and (4) the isthmus zone of fusing lobes along the hinge dorsally (Figure 5). Along the extremities of the outer margin of the mantle, a distinct pigmented thickening is visible. The outer marginal zone splits into three folds: the outer, middle and inner folds, each with specific roles. The mantle cavity is closed by each side of inner folds, when oyster opens its valves, forms two apertures of inhalant and exhalant to the pallial zone. Tissue from the pallial zone is used as graft tissue in the operation for seeding.

The gills

There are two symmetrical gills that lie between the mantle lobes and enclosed within the mantle cavity, just above the adductor muscle. They are flat, crescent-shaped and filamentous gills, characteristic of bivalves with lamellibranches gills. Each gill is composed of four elongate lamellae and is W-shaped in transverse section. Each gill is fused anteriorly with the visceral mass by its brachial axis and then it is fused with the ventral zone of adductor muscle. The fused gills end at the pallial fold facing the anal papilla, in a posterior-ventral position. The elongate W-shaped lamellae are comprised of ordinary filaments and principle filaments (Figure 6). The ciliated discs connect the ordinary filaments and also join to the principle filaments by long thick and inter-locking cilia. The functions of the gills are respiration and filter feeding as sieving and sorting of food particles. The gills are normally suspended in the water within the shell cavity but collapse on opening of the shell.

The adductor muscle

The adductor muscle is the largest and most important muscle in the body of oyster. It is a conspicuous crescent-shaped mass lies generally central to the shell valve; apposed the gills and the mantle (Figure 3 and 4). The adductor muscle is firmly attached to the inner surface of each shell valve. It is a massive wedge-shaped bundle of muscle fibres and control the closing of the shell valve.

The byssal/pedal retractor muscle

A pair of pedo-byssal rectractor muscle is projecting laterally from the visceral mass. This muscle is V-shaped and originates from the byssal gland and running to posterior and form two large white oval masses adhering to the adductor muscle (Figure 7).

The foot

The foot arises as a protrusive, tongue-like organ that is oval in transverse section. It is located in the anterior part of the body between the mouth and the byssus, extending towards the byssal notch (Figure 3). A pedalgroove runs along the ventral portion of the foot and it extends into the byssal organ. The foot is turgescent muscle and its function as locomotion performs during the early stages before settles as spat.

The byssal gland

The byssal gland is located in the central part of the retractor muscles. Byssal threads are secreted by the byssal gland and pass down the pedal groove which is formed into a tube. Byssal threads are extending toward and through the byssal notch in the shell. The distal end of byssal threads flatten as a disc, which help to anchor with the substrate (Figure 8).

The nucleus insertion place (pearl sac)

The “pearl sac” is located in the posterior end of foot and under the retractor muscle. It is a transparent turgescent organ where the nucleus and mantle tissue are inserted for pearl formation (Figure 8). The intestinal loop comprises in the posterior region of the “pearl sac”. The “pearl sac” is usually recognized as gonad because the gonad invades into it when the animal is sexually matures.

The gonad

The sexes of pearl oysters are indistinguishable from the external appearance of the gonad. The gonads are paired but asymmetrical. They generally occupy as a layer between the connecting tissue and digestive gland and also protruding to antero-ventral part of visceral mass and intestinal loop.

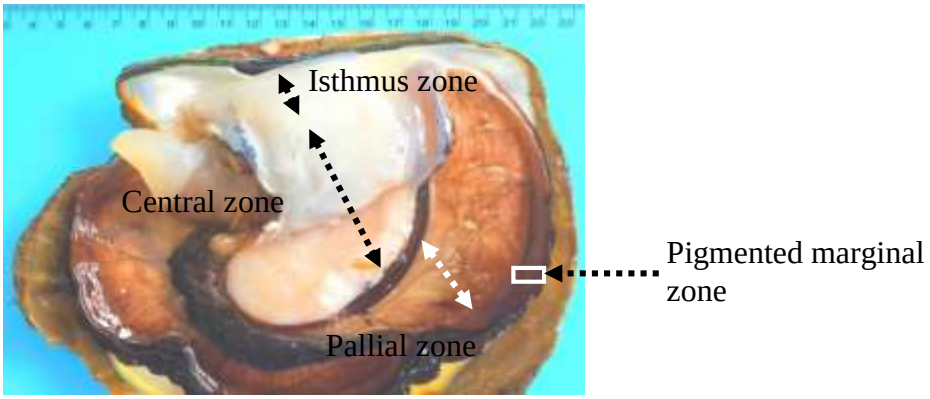

Figure 5 The four zones of mantle lobe of *Pinctada maxima*: (1) marginal zone; (2) pallial zone; (3) central zone; (4) isthmus zone.

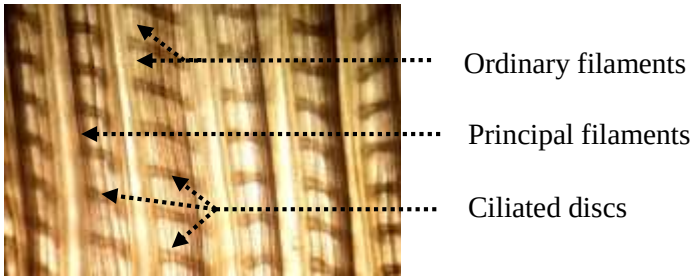

Figure 6 Microscopic view of branchial lamellae of *Pinctada maxima*.

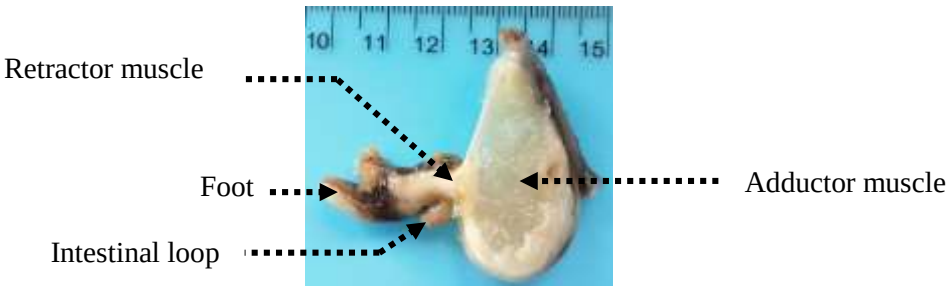

Figure 7 Attachment of retractor muscle, adductor muscle and foot of *Pinctada maxima.*

At the sexual maturity stage, the gonad invades the “pearl sac”. Both sex of the gonad are whitish in the initial stage. In the matured state, gonad of the male is pale creamy in coloured and that of female is yellowish creamy.

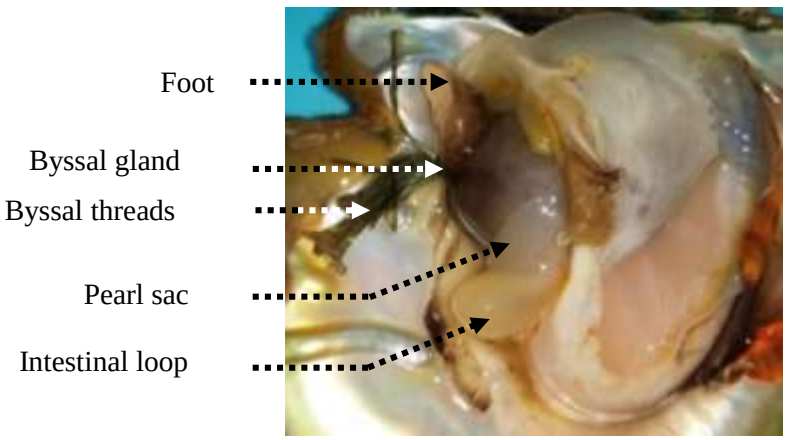

Figure 8 The foot, byssal gland, byssal threads, pearl sac and intestinal loop of *Pinctada maxima*.

The heart

After one of the shells is removed, the heart is seen as a dark mass within a pericardial membrane (Figure 3 and 4). It is located between visceral mass and the adductor muscle. It is limited dorsally by the small portion of free rectum and limited ventrally by the retractor muscles. The heart consists of a single ventricle and a pair of contractile thin walled auricles, one on each side (Figure 9).

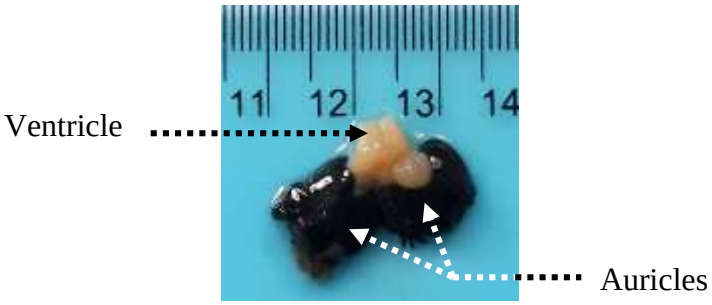

Figure 9 The heart of *Pinctada maxima*: single ventricle and a pair of auricles.

The digestive tract

The visceral mass covers mostly parts of digestive tract of the pearl oyster. The mouth is concealed with two labial palps (Figure 3). These labial palps accept the organic and inorganic particles that are filtered by gills. And also labial palps released unnecessary materials and particles of low nutritional value as pseudofeces. The mouth is connected to short esophagus which opens into the stomach. The stomach has many chambers which are covered by the digestive gland (Figure 10). In the sexually immature stage of pearl oyster, the stomach can be seen through transparent visceral mass tissue. A long semi-transparent crystalline style rod protrudes across the stomach cavity and bears the stomach wall and then into the initial descending intestine (Figure 3 and 10). This crystalline style rod produced gastric fluid.

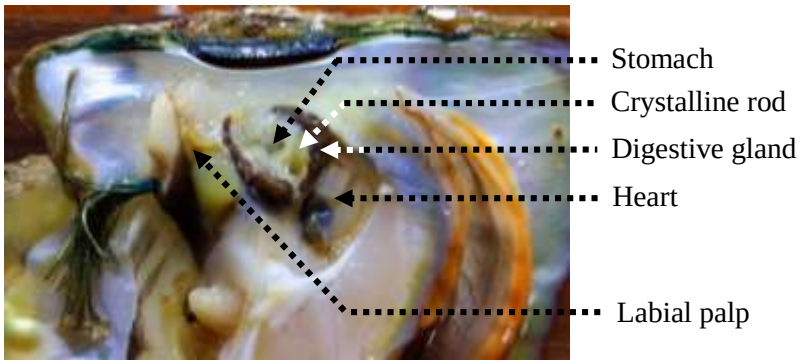

Figure 10 The sagittal section of the digestive gland and stomach in the visceral mass of *Pinctada maxima*.

The intestine of the pearl oyster can be divided into three significant zones: (1) the anterior descending intestine, (2) the ascending intestine and (3) the posterior descending intestine or rectum (Figure 3). The anterior descending intestine emerges from the stomach and runs ventrally between the retractor muscles and extends to the posterior region of the "pearl sac" where the junction between the anterior descending intestine and the ascending intestine is formed as an intestinal loop. The ascending intestine passes around the anterior descending intestine to the left and then suddenly rises and parallel to anterior descending intestine and enter the dorso-posterior part of visceral mass. And then, it emerges from the visceral mass above the heart and curved down

ventrally and then passes around the convex side of the adductor muscle in the median line as the posterior descending intestine or rectum. The rectum terminates as anus and bonds to a paddle-shaped anal papilla which is positioned back to back of the posterior-ventral region of the adductor muscle (Figure 11).

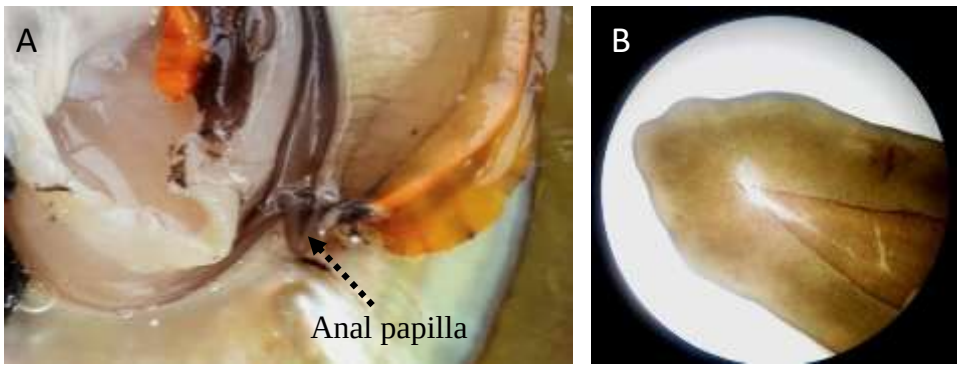

Figure 11 A) The paddle-shaped anal papilla located posterior-ventral part of adductor muscle in *Pinctada maxima*; B) The microscopic view of anal papilla.

Discussion

The pearl oyster *Pinctada maxima* was the largest pearl oyster in the genus *Pinctada*, up to 30 cm in dorsal ventral measurement. According to their nacre boundary, the *Pinctada maxima* can be separated as gold-lipped and silver-lipped. In the present study, oysters with gold-lipped are mostly found and silver-lipped oysters are rarely observed in the pearl farm of Myanmar. They can produce the largest gold and silver south sea pearl. The silver-lipped or gold-lipped pearl oyster, *P. maxima* has no teeth or denticles on the hinge; this character is the difference from other genus. In the study site, another species of genus *Pinctada* such as, *P. margartifera* and *P. fucata* were found. The size of *P. margartifera* reached 20-25 cm in dorsal ventral measurement that is smaller than *P. maxima*. *P. fucata* is the smallest size compared to the previous two because the largest size of *P. fucata* is about 10 cm in dorsal ventral measurement. In general, the colour of external shell of *P. maxima* is pale fawn and has no trace of radial marking. In some cases, the umbonal region is coloured green, dark brown or purple (as cited in Gervis, 1992). The shells of juvenile are green, purple-black, yellow, dark-red, brown and grey colours in the present study. But the external shell colour of *P. margartifera* is dark grayish brown and has lighter striations that are radiating from the umbo. The nacre boundary of *P. margartifera* is black colour and it produced black colour pearl. The external shell colour of *P. fucata* was varying from the cream, brown to bronzes and black. And the colour of internal shell nacre boundary is cream to golden with a hard metallic luster.

The shell valves of *P. maxima* are less convex and hinge lines are longer than *P. margartifera* (Gervis, 1992). In the juvenile stages, the growth processes are slightly convoluted and the distal part is wider than proximal. Their ends are blunt not tapered like *Pinctada fucata*. The dorsal lobe of adductor muscle scar of *P. maxima* is significantly larger and broader compared to *Pinctada margaritifera*. The spat and juvenile stages of *Pinctada maxima* can able to detach their byssal threads and can reattached other suitable place. The byssal attachments are retained up to about three years of age and then byssus are lost they retained on the bottom and kept in position by their shell weights. But the *Pinctada margaritifera* usually attache to the substrate by byssus throughout their life and they can secrete a new byssal threads within a week when they are severed (Gervis, 1992). The anal funnel shapes are also unusual according to different species of pearl oysters. In the *Pinctada maxiam*, the anal funnel is flat, little long and paddle-shaped: a few narrow at the distal part and end with a blunt tip. And the shape of anal funnel of *Pinctada fucata* is thin

and elongated. The distal part is narrow and wider in the middle and end with the elongated tip. But the anal funnel of *Pinctada margaritifera* is wider than others and look like shaped of banyan leaf (Gervis, 1992).

It is very limited scientific researches of pearl oysters *Pinctada maxima* in Myanmar. The present study was mainly emphasized on morphology and anatomy of pearl oyster *Pinctada maxima*. Further study should be carried out for detail process for their histological of gonad and reproduction for the purpose of hatchery, culture and final intension of pearl production.

Conclusion

Pinctada maxima is largest species of oysters and commonly distributed in Myeik Archipelagic waters. It produces the largest and finest gold or silver colour pearls, is also called south sea pearl. It is economical important species because of high price in global market. Water temperature, salinity and nutrition have directly impact on their growth and survivals of pearl oyster. The present study was not complete to express the biological process of *Pinctada maxima*. But it is believed that this study will contribute to promote the fundamental knowledge of morphology and anatomy of pearl oyster *Pinctada maxima*. This could be partly supported for further study of pearl culture.

Acknowledgements

I am thankful to Rector Dr. Si Si Hla Buu, Pathein University for her permission accepting the research. I am greatly indebted to Dr Nyo Nyo Tun, Professor and Head of Marine Science Department, Myeik University and Dr Cherry Aung, Professor and Head of Marine Science Department, Pathein University for their supporting for this research work. I am also greatly obligated to U Myint Lwin (Myanmar Oriental Pearl Company) for his kindly help to be convenient during field study.

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FERMENTATION CONDITIONS FOR THE PRODUCTION OF ANTIBACTERIAL METABOLITE FROM THE ENDOPHYTIC FUNGUS, *ASPERGILLUS TURCOSUS*

Tint Lwin[1], Khin Maung Naing[2], Zaw Lin Aung[3]

Abstract

Seventeen different kinds of selected mangrove leaves collected at Ma Gyi coastal area, Shwe Taung Yan Township, Ayeyarwady Region during monsoon period, 2016. The selected endophytic fungus, *Aspergillus turcosus* was isolated from *Bruguiera cylindrica* (L.) Blume (Byu-Kyet-Tet). In the investigation of antimicrobial activities, this endophytic fungus exhibited highly antibacterial activity on *Agrobacterium tumefaciens* IFO5431. Based on the growth kinetics of this fungus, it was found that 54 hr of ages and 10% of sizes of inoculum were suitable for the fermentation. The good production of antibacterial metabolite from the endophytic fungus was occurred in glycerol and corn powder as carbon sources and in peptone and beef extract as nitrogen sources against *Agrobacterium tumefaciens*. Five fermentation medium (FM-1, FM-2, FM-3, FM-4 and FM-5) were prepared for the fermentation study, according to the results of the effects of carbon and nitrogen sources utilization on the fermentation. It was observed that FM-1 medium was the most suitable for the production of antibacterial metabolite.

Keywords: antibacterial activity, *Agrobacterium tumefaciens*, *Aspergillus turcosus*, *Bruguiera cylindrica*, endophytic fungus, Ma Gyi coastal area, mangrove leaves.

Introduction

In developing countries, numerous communities have been using local plants in different ways to treat various diseases including gastroenteritis. Previous study on secondary metabolites of endophytic fungi from mangrove revealed that the fungi produced antibiotic, including griseofulvin, which commonly found in *Penicillium griseofulvum* (Strobel, 2002).

A clear understanding of microbial growth is necessary if the large-scale may be properly managed. Suitable ages and sizes of inoculum were crucial for the production of primary and secondary metabolite (Omura, 1985 and Crueger, 1989).

Many of the endophytic fungal strains have attracted special attention because they have the capability of producing different colour pigments with high chemical stability (Tan and Zou, 2001).

The physical and chemical parameters like pH, temperature, incubation period, carbon and nitrogen sources and amino acid plays a major role on production of bioactive compounds and antimicrobial agents (Gunasekaran and Poorniammal, 2008).

The objectives of this research are to investigate the fermentation conditions for the production of antibacterial metabolite against *Agrobacterium tumefaciens*, and to optimize fermentation to get the highest yields of antibacterial compound. Therefore, the fermentation optimization was investigated for the production of antibacterial metabolite from the endophytic fungus, *Aspergillus turcosus*.

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Materials and Methods

Sample collection and isolation of endophytic fungus

The selected endophytic fungus, *Aspergillus turcosus* was isolated from *Bruguiera cylindrica* (L.) collected at Ma Gyi coastal area, Shwe Taung Yan Township, Ayeyarwady Region during monsoon period, 2016.

Use of Test Organisms on the Antibacterial Activity

In the investigation of antibacterial activities of endophytic fungus *Aspergillus turcosus*, the test organisms *Agrobacterium tumefaciens* IFO5431 was used.

Studies on Microbial Growth Kinetics of *Aspergillus turcosus*

Microbial Growth Kinetics of *Aspergillus turcosus* was carried out by the methods of (Omura, 1985, Crueger and Crueger, 1989). The fungus *Aspergillus turcosus* was inoculated into 100 mL of GYN medium and incubated for 132 hrs. The culture sample (5 mL) was checked in 12 hrs intervals for the growth. The sample (5 mL) was centrifuged at 2000 rpm for 30 minutes and Packed Cell Volume (PCV) was calculated.

GYN MEDIUM

Medium Composition (g/L)

Glucose	10 g
Yeast Extract	2 g
NZ amine type A	3 g
pH	6.5

Effects of Ages of Inoculum on the Fermentation

Based on the results of microbial growth kinetics of the fungus, seed cultures of (24, 30, 36, 42, 48, 54, 60, 66, 72, 78 hrs) were utilized for the fermentation. Fermentation was carried out 8 days and antibacterial activity was tested by paper disc diffusion assay.

Effects of Sizes of Inoculum on the Fermentation

For the suitable fermentation conditions, the sizes of inoculum were also investigated. According to the results of the ages of inoculum of fungus, (5 %, 10 %, 15 %, 20 %, 25 % and 30 %) of 54 hrs seed cultures of this fungus were utilized for the fermentation. Fermentation was carried out 8 days and antibacterial activity was tested by paper disc diffusion assay.

Effects of Different Carbon Sources Utilization on the Fermentation

In this study, 54 hrs of ages and 10 % of sizes of inoculum were utilized for the fermentation with basal medium. Various carbon sources such as glucose, wheat, sucrose, soluble starch, glycerol, corn powder, rice powder and potato powder were used to study the different carbon sources utilization on the fermentation of fungus. Fermentation was carried out 8 days and 100 mL of fermentation media were prepared for carbon sources test. Each carbon source (2.0 g) was added to 100 mL basal fermentation medium.

Figure 1 Effects of ages of inoculum of fungus on the fermentation

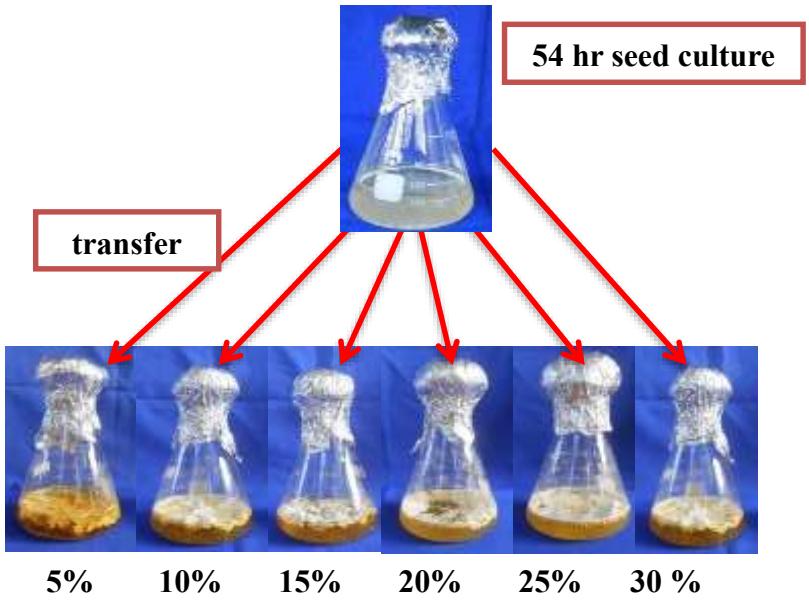

Figure 2 Effects of sizes of inoculum of fungus on the fermentation

Basal fermentation medium used in carbon sources test

Basal Medium (For Carbon sources)	
Medium Composition (g/ L)	
Yeast Extract	**10 g**
KNO_3	**2 g**
$MgSO_4$	**0.01 g**
K_2HPO_4	**0.01g**
pH	**6.5**

Effects of Different Nitrogen Sources Utilization on the Fermentation

Nitrogen sources like yeast extract, peptone, potassium nitrate, fish, peanut and beef extract were employed to study the different nitrogen sources utilization on the fermentation of fungus. Fermentation was carried out 8 days and 100 mL of fermentation media were prepared for nitrogen sources test. Each nitrogen source (0.5 g) was added to 100 mL basal fermentation medium.

Basal fermentation medium used in nitrogen sources test

Basal Medium (For Nitrogen sources)	
Medium Composition (g/ L)	
Glucose	**10 g**
Glycerol	**10 mL**
$MgSO_4$	**0.01 g**
K_2HPO_4	**0.01g**
pH	**6.5**

Establishment and Selection of Media for the Fermentation

Five fermentation medium (FM-1, FM-2, FM-3, FM-4 and FM-5) were prepared for the fermentation study, according to the results of the effects of carbon and nitrogen sources utilization on the fermentation of fungus. In the preparation of five fermentation medium, suitable carbon sources (Glycerol and Corn powder) and suitable nitrogen sources (Peptone and Beef extract) were utilized as suitable ratios and compositions. Fermentations were carried out for 8 days in the Rotary shaker and the activities were checked by paper disc diffusion assay method with 8 mm diameter paper discs.

Five fermentation medium prepared for the fermentation study Medium Composition (g/L)

FM-1	
Glycerol	10 mL
Peptone	8 g
K_2HPO_4	0.1 g
$MgSO_4$-$7H_2O$	0.1 g
pH	6.5

FM-2	
Glycerol	10 mL
Beef extract	8 g
K_2HPO_4	0.1 g
$MgSO_4$-$7H_2O$	0.1 g
pH	6.5

FM-3	
Corn powder	10 g
Peptone	8 g
K_2HPO_4	0.1 g
$MgSO_4$-$7H_2O$	0.1 g
pH	6.5

FM-4	
Corn powder	10 g
Beef extract	8 g
K_2HPO_4	0.1 g
$MgSO_4$-$7H_2O$	0.1 g
pH	6.5

FM-5	
Glycerol	5 mL
Peptone	5 g
Corn powder	5 g
Beef extract	5 g
K_2HPO_4	0.1 g
$MgSO_4$-$7H_2O$	0.1 g
pH	6.5

Results

Studies on Microbial Growth Kinetics of fungus *Aspergillus turcosus*

In the microbial growth kinetics study, as shown in Figure 3 and Table 1, it was found that the lag phase was between 24 hrs and 36 hrs. Growth phase was between 36 hrs and 72 hrs. It was observed that the growth of fungus declined after 84 hrs. According to Crueger and Crueger (1989), it was considered that ages of inoculum (42 hrs, 48 hrs, 54 hrs, 60 hrs, 66 hrs and 72 hrs) were used to optimize the fermentation.

Table 1 Microbial growth kinetics of fungus *Aspergillus turcosus*

Culture Time (hr)	PCV of 5 mL	PCV %
24	0.20	4
36	0.32	6.4
48	0.54	10.8
60	0.78	15.6
72	1.15	23
84	1.32	26.4
96	1.29	25.6
108	1.20	24
120	1.15	23

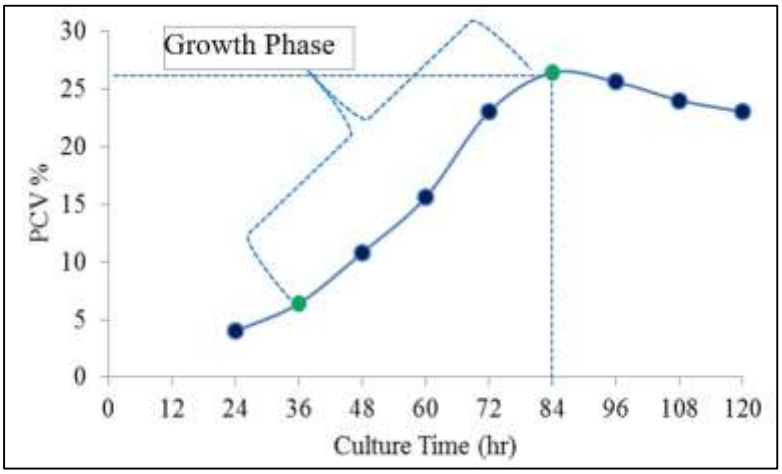

Figure 3 Microbial growth kinetics of fungus *Aspergillus turcosus*

Effects of Ages of Inoculum on the Fermentation

In the investigation of the age of inoculum, ten different hours of 24 hrs,30 hrs, 36 hrs, 42 hrs, 48 hrs, 54 hrs, 60 hrs, 66 hrs, 72 hrs and 78 hrs were used and the results showed the inhibitory zone of 14 mm, 15 mm, 15 mm, 17 mm, 18 mm, 22 mm, 20 mm, 18 mm, 15 mm and 15 mm, respectively against *Agrobacterium tumefaciens* (Table 2).

Table 2 Effect of ages of inoculums on the fermentation

Culture time (hrs)	Activity (Clear zone, mm)
24	14
30	15
36	15
42	17
48	18
54	**22**
60	20
66	18
72	15
78	15

Effects of Sizes of Inoculum on the Fermentation

In the investigation of the sizes of inoculum, 5%, 10%, 15%, 20%, 25% and 30% as six different percentages were used and the results showed the inhibitory zone of 20.06 mm, 22.53 mm, 21.96 mm, 21.06 mm, 21.72 mm and 21.06 mm, respectively against *Agrobacterium tumefaciens* (Table 3).

Table 3 Effect of sizes of inoculums on the fermentation

Sizes of Culture %	Activity (Clear zone, mm)
5	20.06
10	**22.53**
15	21.96
20	21.06
25	21.72
30	21.06

Effects of Different Carbon Sources Utilization on the Fermentation

In the investigation of different carbon sources utilization on the fermentation of fungus, various carbon sources such as glucose, wheat, sucrose, soluble starch, glycerol, corn powder, rice powder and potato powder were used and the results showed the inhibitory zone of 12.25 mm, 11.17 mm, 13.61 mm, 12.80 mm, 18.42 mm, 15.48 mm, 13.74 mm and 12.08 mm respectively against *Agrobacterium tumefaciens.* The activities were tested by paper disc diffusion assay method with 8 mm diameter paper size as shown in Table 4.

Effects of Different Nitrogen Sources Utilization on the Fermentation

In the investigation of different nitrogen sources utilization on the fermentation of fungus, yeast extract, peptone, fish extract, peanut cake and beef extract were employed and the results showed the inhibitory zone of 13.20 mm, 20.90 mm, 12.70 mm, 14.57 mm, 11.90 mm, respectively

against *Agrobacterium tumefaciens.* Only one source, Potassium nitrate gave no activity on *Agrobacterium tumefaciens*. The activities were tested by paper disc diffusion assay method with 8 mm diameter paper size as shown in Table 5.

Table 4 Effects of different carbon sources utilization on the fermentation

Carbon sources	Activity (Clear zone, mm)
Glucose	12.25
Wheat	11.17
Sucrose	13.61
Soluble Starch	12.80
Glycerol	**18.42**
Corn powder	15.48
Rice powder	13.74
Potato powder	12.08

Table 5 Effects of different nitrogen sources utilization on the fermentation

Nitrogen sources	Activity (Clear zone, mm)
Yeast extract	13.20
Peptone	**20.90**
Potassium nitrate	No Activity
Fish extract	12.70
Beef extract	14.57
Peanut cake	11.90

Establishment and Selection of Media for the Fermentation

In the study of media selection for the fermentation, five kinds of fermentation media were used. According to the results of antibacterial activity, fermentation medium FM-1 showed the highest inhibitory zone of 23.56 mm against *Agrobacterium tumefaciens* than that of other media (Table 6 and Figure 4.). Therefore, FM-1 was selected for the production of antibacterial metabolite.

Table 6 Effect of media in fermentation study

Fermentation medium	Inhibitory zone (mm)
FM-1	**23.56**
FM-2	11.57
FM-3	19.58
FM-4	No Activity
FM-5	11.30

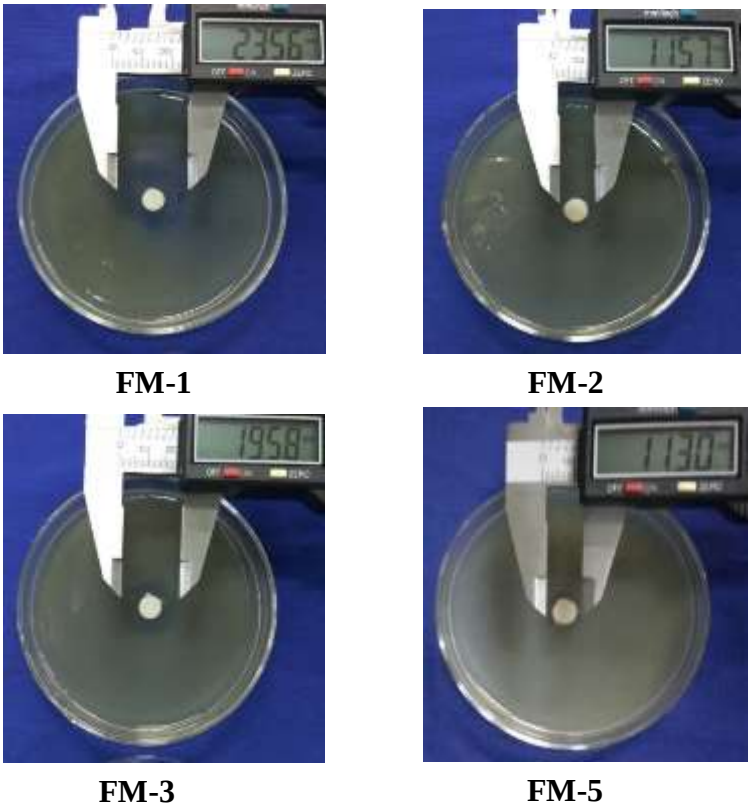

Figure 4 Antibacterial activities of the endophytic fungus *Aspergillus turcosus* on the fermentation media FM-1, FM-2, FM-3 and FM-5

Discussion and Conclusion

In the investigation of microbial growth kinetics of *Aspergillus turcosus*, it was observed that growth phase (trophophase) was between 36 hr and 72 hr. This result is in accordance with the statement of Crueger & Crueger, 1989.

Based on the growth kinetics of this fungus, it was determined that 54 hr of age of inoculum and 10 % of size of inoculum were suitable for the production of metabolite. In the study of carbon and nitrogen sources utilization on the fermentation, the results showed that organic nitrogen sources such as peptone support growth and good production of antibacterial metabolite. Inorganic salts (such as potassium nitrate) can be used as nitrogen sources for biocontrol agents, which are able to assimilate ammonium and to reduce nitrate. These inorganic nitrogen sources probably contain only the nutrients that satisfy no more than minimal requirement for growth and production of antibacterial metabolite (Gibbins 1978). However, the growth obtained for endophytic fungus *Aspergillus turcosus* was lower in potassium nitrate and no activity was shown in the fermentation.

The type of carbon source promotes growth of endophytic fungus influenced by the capacity of the microorganism to use the available nitrogen source (Gibbins 1978). In general, glycerol and corn powder were good carbon sources and peptone was good nitrogen source in fermentation media.

Medium formulation is necessary for each fermentation process. It is necessary to optimize each and every component of fermentation media by varying the concentration of media constituents in order to achieve maximum antibiotic production. The purpose of media optimization is to support efficient growth of microorganisms. Different combinations of medium constituents and sequences of optimized fermentation conditions need to be investigated to

determine growth conditions that produce biomass that is physiologically best suited for antibiotic production (Antal *et al*., 2012).

Based on the result of carbon and nitrogen sources utilization on the fermentation, 5 different kinds of fermentation media were established and utilized in fermentation. The result obtained in the study of 5 fermentation media showed that fermentation medium FM-1 was the most suitable for the production of antibacterial metabolite. Natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development. The large proportion of natural products in drug discovery has stemmed from the diverse structures and the intricate carbon skeletons of natural products (Chen *et al*., 2003).

The present study was carried out on the antibacterial activity of marine microbes from mangrove plants in the coastal region of Myanmar. But there is still a need for the extensive study of marine microbes and their relationships to their environment.

Acknowledgements

I am sincerely to thank Dr. Nyunt Phay, Director General, Department of Monitoring and Evaluation (Education), Ministry of Education, Nay Pyi Taw, for his valuable instructions, advices and care guidance. I am very grateful to Dr. Cherry Aung, Professor and Head, Department of Marine Science, Pathein University, for her helping hand in this study.I wish to special thanks to Dr. Zaw Lin Aung, Lecturer, Department of Botany, Pakokku University, for his supervision, encouragement and care guidance.

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ASSESSMENT ON SEAGRASS COVER AND DENSITY AT MAGYI (SHWETHAUNGYAN) COASTAL AREA, AYEYARWADY REGION

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Abstract

In the present study, analysis of seagrass cover and density have been carried out at MaGyi (Lat. 17° 04' 25" N, Long. 094° 27' 55" E), October, 2019. Three 100m-transect are laid perpendicular to the shoreline and each transect is at a distance of 50m from each other. A total of three transects is generally enough in a given area, thus seagrass cover and density are recorded from a total of 33 quadrats and 12 quadrats respectively. 20.06 % of seagrass are covered in this study area. Average shoot density in a site was 107 and 1280 total shoot density in a site.

Keywords: seagrass cover and density

Introduction

Seagrasses are flowering plants that are fully adapted to live submerged in the marine environment (Short 2001). Seagrasses are commonly found in shallow waters, tidal areas, and estuaries in the tropical and subtropical region (Hemminga and Duarte 2000). Unlike seaweeds, seagrasses have stems, leaves and rhizomes and produce flowers, fruits and seeds for their propagation (Duarte et al., 1996). Seagrass distributes all along the three coastal Regions of Myanmar, namely the Rakhine Coastal Region, the Ayeyarwady Delta and the Gulf of Mottama (Martaban) Coastal Region and the Thanintharyi Coastal Region. Their ability to accumulate and store organic carbon enables seagrass habitat to contribute to15% of the total carbon deposited in the ocean. This is due to high production, particularly in large species, high capacity to store organic matter in the sediment, and the ability for long-term accumulation and storage (IPCC. 2017). With their tremendous ability to absorb and store carbon, seagrass habitats can significantly contribute to climate change mitigation (Laffoley and Grimsditch 2009).

Seagrass often grow in dense meadows, with relatively uniform patches over the seafloor, although patchy mosaics of seagrass, sand and other habitats is also common. The seagrass canopy forms a structurally complex system, providing habitat for other organisms and facilitating complex ecological interactions among seagrass species. The habitat provision and ecological interactions within the meadows are unique features of the seagrass ecosystem and distinguish them from neighboring habitats such as bare sand, coral reefs, and macroalgae stands.

To determine seagrass community structure, we perform several measurements and extrapolate the results into a single value for the entire seagrass beds; therefore, the sampling techniques must be adequate for community level. For the site selection, minimum requirement for seagrass coverage to take the data is 10% coverage from the overall or total area. Some information is essential to establish an appropriate sampling area such as the homogenous seagrass area, seagrass bed extent, and safety and accessibility. Line transects and quadrats were used to determine community structure of seagrass in the intertidal area.

After the sampling site is selected, permanent transect can be established and its Global Navigation Satellite System (GNSS) position should be recorded using a standard Grid system (e.g. WGS84) and a standard unit format (e.g. decimal degrees).

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Materials and Methods

Site Selection and Sampling Considerations

Analysis of seagrass cover and density have been carried out at MaGyi (Lat. 17° 04' 25" N, Long. 094° 27' 55" E), October, 2019 (Fig.1). The assessment is focused on intertidal area of seagrass ecosystems, because: 1) seagrass meadows generally occur in the intertidal area, and 2) sampling in intertidal area is simpler and requires less equipment and logistic support compared to one in subtidal area or in deeper water. If intertidal seagrass ecosystem is not present, then subtidal seagrasses could be considered, but boat and diving equipment would be necessary. Larger species, like *Enhalus acoroides* may best sampled in subtidal area, as they are fully submerged allowing the full extension of their canopy.

We employ line transect and quadrat to determine community structure of seagrass in the intertidal area. If the seagrass meadows are large or extend seaward, three 100m-transect are laid perpendicular to the shoreline and each transect is at a distance of 50m from each other (Fig.2). Narrow meadows might also justify the transects to not be fully extended to 100m, as the meadows already reach its edge before 100m.

A total of three transects is generally enough in a given area, thus seagrass cover and density are recorded from a total of 33 quadrats and 12 quadrats respectively.

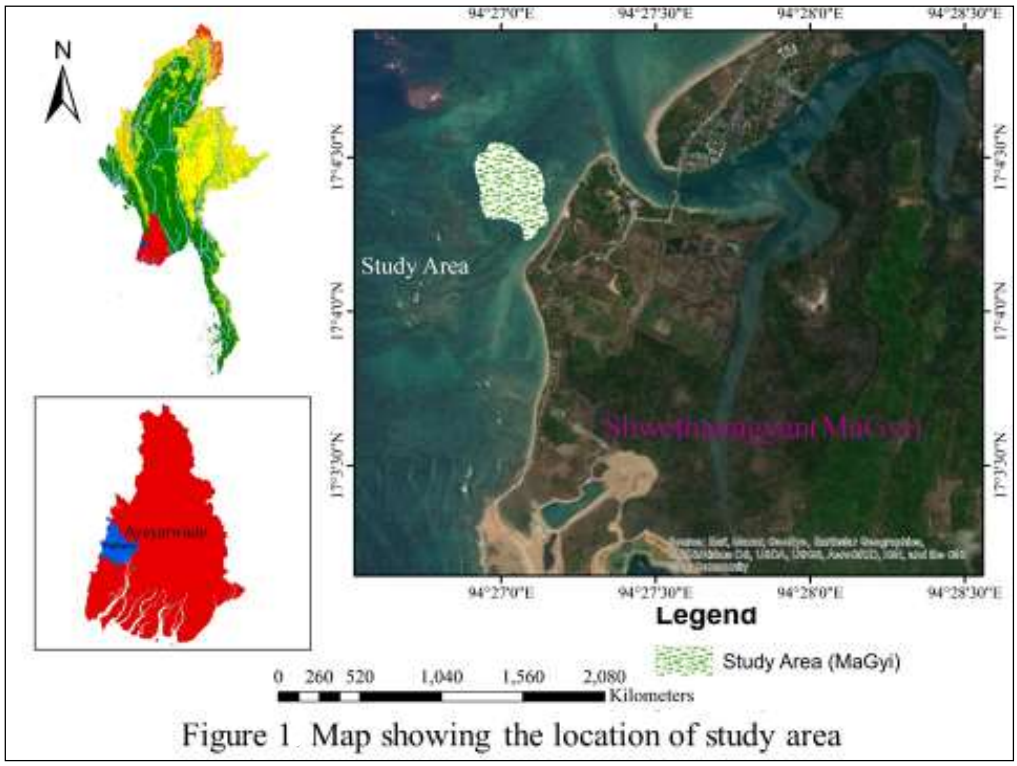

Figure 1 Map showing the location of study area

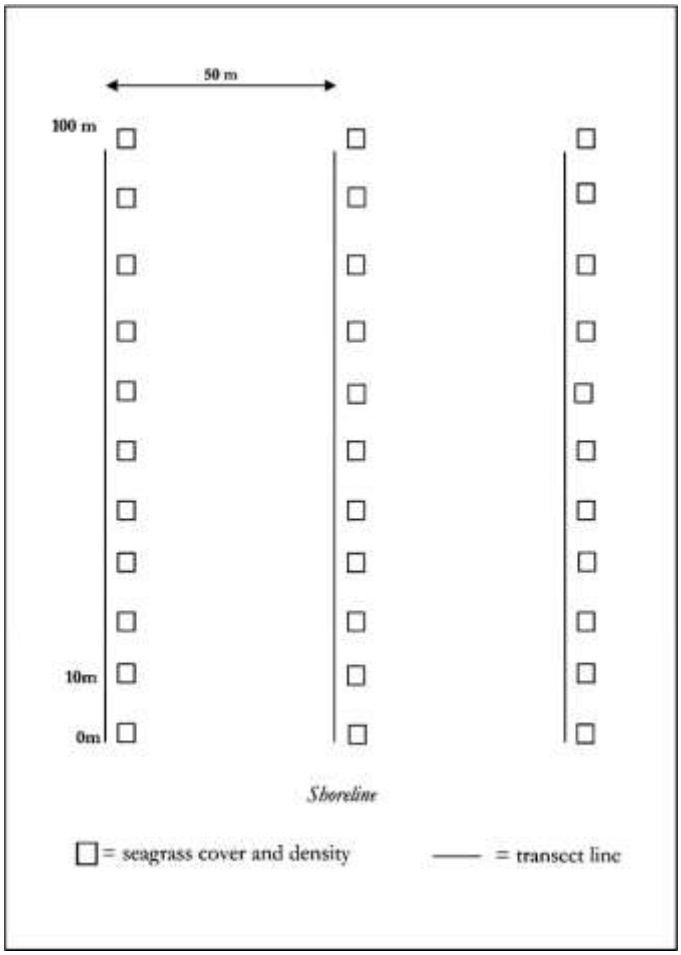

Figure 2 Basic sampling design to collect seagrass communities' structure The transect is modified from Seagrass-Watch (McKenzie and Campbell, S. J., 2002).

Site Description and Additional Data

Before samples are collected, it is recommended to take a general overview (e.g. by snorkeling) about the meadows and its surrounding. The following background information should be recorded:

1. sediment characteristic (mud, sand, etc)
2. presence of potential disturbances (e.g. pollution, fishing activities, human settlement, etc.)
3. presence of epiphyte or algae
4. general description about the sampling site (extent of meadows, etc.)

When resources are available, environmental data should be collected, for example temperature, pH, DO, salinity, turbidity, suspended particulate matter, conductivity, and nutrient level. These data would be of significance when more detailed analysis is needed.

Seagrass Cover

Seagrass cover is defined as the proportion of area covered by perpendicular projection of seagrasses' canopy within a given quadrat (Brower et al. 1998). This will clearly vary if the meadow is exposed at low tide when the leaves lie flat on the ground, or when the meadow is submerged and the leaves float more vertically in the water column. Therefore, if sampling is

planned to be repeated over time, and the site is intertidal, it should be carried out at low tide to ensure comparable measures over time.

In any case record whether the cover was estimated at low tide or sub tidally, when the meadow was submerged.

At each point along the transect indicated in Fig. 2 the cover of seagrass is estimated. A photo reference is provided in Appendix A as a standard to estimate seagrass cover (% cover). Estimate cover to the nearest 5%, with the exception of low cover areas where smaller increments are recommended.

Sampling Procedures

1. Materials needed:
    a. Measuring tapes (100m Roll meter)
    b. Quadrat 50cm x 50cm (divided into four grids)
    c. Data sheet (waterproof paper), slate and pencils
    d. Photo guide for cover estimates
2. Place the quadrat at the first plot (0m on the line transect).
3. Record coordinate position with a GNSS receiver.
4. Identify species present at each plot.
5. Estimate seagrass cover based on photo standard
6. Record all the data (total seagrass percent cover and cover by species) on the data sheet
7. Move to the next sampling plot (10m forward along the transect).
8. Repeat step 3 - 6.
9. Repeat the measurement for the rest of plots in a site. The number of plots in a site may vary depending on the extent of seagrass. In extensive meadows, the maximum number of plots is 33, because there are 3 transect lines and each line contains 11 plots.
10. To determine total seagrass cover in a site, calculate the average of all plots in a transect (n=11 quadrats or possibly less), then the average per site (n=3transects), see Formula 1.

Seagrass cover

$$Seagrass\ cover\ (\%) = \frac{\%\ cover\ plot\ \ (1 + 2 + \cdots + N)}{N}$$

N = number of plots

Seagrass Density

Seagrass density is represented by the number of shoots per unit area. Shoots are the above-ground, predominantly leafy parts of the plant (Dennison 1990). In this guideline, shoot density is determined by manually counting the shoots without uprooting the plants. Most seagrass species have shoots which are long, linear and strap- like that are encased by a sheath at the bottom of the

shoot (e.g. *Thalassia*) and each shoot can have multiple leaves. Some seagrass species have vertical stems with clusters of leaves (e.g. *Halophila beccari*) and others have leaves arranged in pairs that are attached directly to the rhizome by petioles (e.g. *Halophila ovalis*). For species with stems, we recommend that the leaf clusters are counted. For species with leaf pairs, we recommend that the total number of leaves are counted. Clearly, the appearance of a shoot or what needs to be counted, can vary depending on the species. Therefore, to ensure that shoots, not leaves are counted, and the shoot is identified correctly, refer to Fig. 5. In all cases, clearly record the part of the plant that you count for the shoot density component. For smaller seagrass species it may be too time consuming to count all the leaves in the recommended quadrat size, if this is the case, a smaller quadrat such as 10 x 10 cm for species like *Halophila* can be used, but ensure that the size of the quadrat you use is recorded.

Seagrass density can also be used to calculate Leaf Area Index (LAI), which is a useful indicator of standing stock. Leaf area index (LAI) defined as the single-sided leaf area per unit ground area is one of the most important factors for characterising plant canopy structure and process.

Sampling Procedures

1. Materials needed:
   a. Small quadrat (25cm x 25cm, can be a division in the larger 50 x 50 cm quadrat, or smaller quadrat e.g. 10 x 10 cm if you are only targeting small species like *Halophila* and it is too time consuming to use a 25cm x 25cm).
   b. Data sheet (waterproof paper), slate and pencils.
2. Place the small quadrat in the first plot (the 0m in the transect line)
3. Count the number of shoots of each species in a small quadrat (25cm x 25cm).
4. Total shoot density in a quadrat is the sum of all species present.
5. Repeat this at four quadrats, at 0m, 30m, 60m and 90m along each of the three transects (Total of 12 shoot density measures).

6. To estimate average total seagrass density at a site, average the total seagrass density across all quadrats and to estimate average density per species at a site, repeat this but for each species separately. Follow the Formula 2 below.

Seagrass Density

$$Dsx\ (shoot\ m^{-2}) = number\ of\ shoots\ of\ Sp\ X\ per\ small\ quadrat * 16$$

Note: 16 is a factor to convert to per square meter if the quadrat is 25 x 25 cm. A different multiplication factor is required if a different sized quadrat is used.

Average shoot density in a site:

$$Average\ Dsx\ (shoot\ m^{-2}) = \frac{Dsx\ (plot\ 1 + plot\ 2 + \cdots + plot\ N)}{N}$$

Total shoot density in a site:

$$Ds\ (shoot\ m^{-2}) = Average\ Ds\ (Sp\ 1 + Sp\ 2 + \cdots + Sp\ X)$$

Note:

Dsx = Shoot density per species
Ds = Total shoot density
N = Number of plots
X = Number of species

Results

Table 1. Seagrass Cover and Density of Transect A

Country: Observer: _ FIELD WORK DATA SHEET:
Province/ District: Date/ Time: -
Site_ID / Site_Name: Transect No. : _____ (A)

Plot		Coordinate		Cover (%)	Density (25x25 cm^2)						
		Latitude	Longitude		*Cymodocea rotundata*	*Cymodocea serrulata*	*Thalassia hemprichii*	*Halodule uninervis*	*Halodule pinifolia*	*Halophila major*	*Syringodium isoetifolium*
1	0 m	17° 04' 08"N	094° 26' 55"E	5	3						
2	10 m			10	1	4					
3	20 m			10		5		2			
4	30 m			70		13					12
5	40 m			50		10			1		6
6	50 m			10	2	5					
7	60 m			50		5					
8	70 m			20		7				2	
9	80 m			5		2					
10	90 m			-	-	-	-	-	-	-	-
11	100 m			-	-	-	-	-	-	-	-

Table 2 Seagrass Cover and Density of Transect B

Country: Observer: _ FIELD WORK DATA SHEET:
Province/ District: Date/ Time: _
Site_ID / Site_Name: Transect No. : _____ (B)

Plot		Coordinate		Cover (%)	Density (25x25 cm^2)						
		Latitude	Longitude		*Cymodocea rotundata*	*Cymodocea serrulata*	*Thalassia hemprichii*	*Halodule uninervis*	*Halodule pinifolia*	*Halophila major*	*Syringodium isoetifolium*
1	0 m	17° 04' 09"N	094° 26' 59"E	2		2					
2	10 m			10		5					
3	20 m			20		3					
4	30 m			50		7			8		5
5	40 m			20	2	10					
6	50 m			-	-	-	-	-	-	-	-
7	60 m			5		2					
8	70 m			20	3	5		2		1	
9	80 m			5		2					
10	90 m			5					2		4
11	100 m			-	-	-	-	-	-	-	-

Table 3 Seagrass Cover and Density of Transect C

Country:		Observer: _			FIELD WORK DATA SHEET:						
Province/ District:		Date/ Time: _									
Site_ID / Site_Name:		Transect No. : _____ (C)									
Plot		Coordinate		Cover (%)	Density (25x25 cm^2)						
		Latitude	Longitude		*Cymodocea rotundata*	*Cymodocea serrulata*	*Thalassia hemprichi*	*Halodule uninervis*	*Halodule pinifolia*	*Halophila major*	*Syringodium isoetifolium*
1	0 m	17° 04' 10"N	094° 27' 00"E	5		5					
2	10 m			80	2	15		1			
3	20 m			70	1	10				3	
4	30 m			50		1					6
5	40 m			25		4		3		2	
6	50 m			20		3	2		1		
7	60 m			20		5					
8	70 m			20							
9	80 m			-	-	-	-	-	-	-	-
10	90 m			-	-	-	-	-	-	-	-
11	100 m			5			5				

According to the result, the study area was approximately covered with 20.06 % of seagrass. Among them *Cymodocea serrulata* is dominant species and *Thalassia hemprichii* is the least. In shoot density, *Cymodocea serrulata* is also larger than other species and second is *Syringodiun isoetifolium.*

Discussion and Conclusion

This study revealed that the total seven species belonging to five genera of two families of seagrasses in MaGyi namely, *Cymodocea rotundata, Cymodocea serrulata, Thalassia hemprichii, Halodule uninervis, Halodule pinifolia, Halophila major, Syringodium isoetifolium. Cymodocea serrulata* was dominant species along the MaGyi because *Cymodocea serrulata* is a runner and possess more root density and also drop-off all it leaves during the seasonal changes (especially during monsoon wind wave action) (Minikandan et al. 2011). *Syringodium isoetifolium* was the second most dominant and *Thalassia hemprichii* was the least abundant in study area.

The cover of seagrass was large in transect line between 30 m and 70 m. This range was nearly submerged zone. Above and below this range, the seagrass cover was low in percentage. Seagrass density is also related with seagrass cover.

From the data analysis, we can also predict the cover and density of seagrass at study area. We are mainly intended how to calculate the cover and density of seagrass using with ASEAN standard methods. For further study, we will be also carried out biomass and organic carbon content in seagrass.

Acknowledgements

We would like to express special thank is extended to Dr. Si Si Hla Bu (Rector, Pathein University, Myanmar) for giving the chance of this work. We would also like to thank Dr. Cherry Aung, Professor, and head, Department of Marine Sciences, Pathein University, for her advice and encouragement during this work. Special thanks are due to Dr. Htay Aung Professor (Retired), Department of Marine Science, Pathein University, for valuable guidance.

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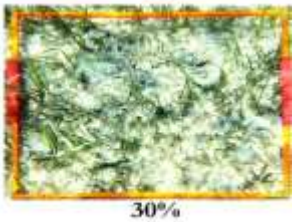
APPENDIX

Percent cover standards

5%

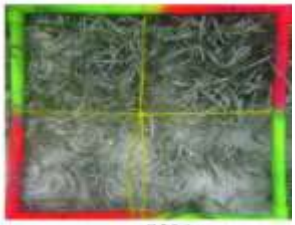
10%

20%



30%

40%



50%

60%

70%

80%

90%

100%