PHYTOCHEMICAL INVESTIGATION INTO LEAVES OF *ELSHOLTZIA BLANDA* (BENTH.) BENTH. AND ITS ANTIMICROBIAL ACTIVITY

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Abstract

Elsholtzia blanda (Benth.) Benth.is an aromatic an annual herb, locally known as Ar-pu-ywat which belongs to family Lamiaceae. It's distribution extend to Bangladesh, India, Malaysia, China and Myanmar especially in Rakhaine state. This plant is commonly used as traditional herbal medicine as well as vegetable and spices. After the sample collection in Yepawgyi village, Taunggoke Township, the genus and species were checked by studying the morphological characters with available literatures. The present work was conducted during February 2017 to July 2018 and mainly deals with photochemical analyses, antimicrobial activity of leaf-extracts and elemental analyses of dried powdered leaves. The results of phytochemical analyses indicated the presence of glycosides, reducing sugar, steroid, carbohydrates, phenolic compound, terpenoid, flavonoid, netural compound, alkaloid, saponin, α-amino acid and tannin in the leaves. The cyanogenetic glycoside was not detected. The elemental analysis on the dried leaves of *Elsholtzia blanda* (Benth) Benth revealed that a part from hydrocarbon (91.45%), the most abundant elements were K (4.5%), Ca (2.3) and Si (0.97%). The antimicrobial activity of leaves extracted by acetone, butanol, ethanol, ethyl acetate, methanol, pet-ether and water was studied. The acetone, butanol, ethanol, ethyl acetate and methanol showed distinct inhibitory effects on *Bacillus sublilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican, Escherichia coli and Agrobacterium spp.* The ethyl acetate extract possessed the highest antimicrobial activity on seven test organisms. The pet ether and water extracts did not inhibit the test organisms.

Introduction

German botanist, Johann Heinrich Friedrich Link (1809) firstly described plant parasitic fungi and they were termed as 'microzymas' by French scientist Bechamp. Since 1887 Victor Galipp had discovered bacteria normally living inside the cells and tissues of the plants. Hence, different types of microorganisms inhabit on the plant surfaces and tissues are termed as "Endophytes". According to the concept of "Plant Microbiome", plants are not living alone. But, they are intimately associated with microorganisms present in their close environment (Hardoim *et al*. 2015).

On another point of view, microbes that dwell in plant tissues are endo-symbiotic group of microorganisms. They can be readily isolated from plant parts using suitable culture medium. Through metabolic reaction, endophytes accumulate the useful metabolites which can show novel bioactive potentials. They may be either alkaloids, phenolic acids, quinones, steroids, saponins, tannins, or terpenoids that function as a potential candidate for antimicrobial anti-insect, anticancer and many more properties. In Myanmar, different plant resources are being extensively researched in various aspects such as food, new drug, industrial use, diversity sustainable conservation. Investigators in Botany Department are trying to conduct experimentations aiming to discover new chemical entities for therapeutic purposes.

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In our country, medicinal plants are the most vital sources of traditional as well as modern medicines. Today, pharmaceutical drugs currently use in the world are mostly manufactured from plant resources WHO (1998).

According to Britto *et al.* (2012) the extract of *Elsholtzia blanda* (Benth) Benth had been used in the drug formulation of diuretic, sedative, digestive, anti-parasitic, carminative, appetizer, anti-conversant, and anti-inflammatory. In 2013, Swedan *et al*, pointed out that the aromatic plant members in the Family Lamiaceae, enriched with essential oils, phenolic compounds and terpenoids. The main aim of present works deals with the Botanical study, qualitative investigation on the phytochemical constituent in the leaves of *Elsholizia blanda* (Benth) and extent of antimicrobial activities shown by different extracts using some organic solvents on 7 test organisms.

Materials and Methods

Plant material Collection

The present study mainly emphasize on the leaves of ethno-medicinal plant namely *Elsholtzia blanda* (Benth). Its Myanmar/Rakhine local name is ar-pu-ywat belong to the family Lamiaceae. It is distributed through South Asian as well as ASEAN countries and is also an aromatic shrub. This plant is widely grown in hilly regions of Taung-goke area is Rakhine State. The healthy plant leaves were collected from Ye-paw-gyi village, Taunggoke Township and washed thoroughly in distilled water. The leaves allowed drying in shade place for two weeks. Well dried leaf samples were powdered by conventional methods and stored in air tight containers for phytochemical tests and antimicrobial activities.

Plant extract preparation

The powdered materials (100 g) was put into separate conical flask and successively extract with different solvent (100 ml) of acetone, butanol, ethanol, ethyl acetate, methanol, pet ether and aqueous extracts for one week Then they were filtered through whatmann No.1 paper. The extract were collected and evaporated to dryness using water bath at 100°C so as to obtain a paste.

Phytochemical Screening Test:

Qualitative analysis was done to identify the presence of the following phyto-constituents; glycoside, reducing sugar, carbohydrate, phenolic, cyanogenetic glycoside, steroid, terepenoid, flavonoid, acid/base/neutral compound, α-amino acid, tannin , alkaloids and saponin using standard procedures. Chemical tests were carried out in all the solvent extracts of *Elsholtzia blanda* (Benth.) Benth.using standard producers described by Trease, and Evans (1978) and Harbone (1993).

Elemental Analysis by Using EDXRF

Quantitative Elemental Analysis of this leave samples were done by using Energy Dispersive X-ray Florescence (EDXRF) in Analytical Lab, Department of Chemistry, West Yangon University.

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. 1968. After

autoclaving, 20-25 ml of nutrient agar was poured into petridishes and made plating by using 0.1 to 0.2 ml of seven test organisms (*Bacillus sublilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican, Escherichia .coli and Agrobacterium spp.*). This plate was allowed to set for 2-3 hours. And then, dried and sterilized filter paper discs (8 mm diameter) were then impregnated with known amounts of the test substances (1 mg / 1 ml) using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms and incubated at room temperature for 24 hours. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter and was measured with the help of clipper.

Results

Botanical Studies

The present medicinal plant was observed to be *Elsholtzia blanda* (Benth.) Benth. family Lamiaceae, according to report of Dr. Thet Thet Mar Win (2014) and Kress *et.al* (2003).

Outstanding characters of *Elsholtzia blanda*(Benth.) Benth.

Herbs erect. Stems and branches densely pubescent. Petiole densely pubescent, leaf blade elliptic to elliptic-lanceolate, adaxially pubescent, glandular, abaxially gray-green, glabrous, strigose on veins, base narrowly cunneat, margin serrate, apex acuminate. Spikes terminal or axillary, mostly pubescent; verticillasters flowered, short pediculate; bracts subulate to lanceolate; fruiting calyx slightly dilated at base, ovoid. Corolla white ,strigose outside, subglabrous inside, funnelform; upper lip emarginated; middle lobe of lower lip subcircular, slightly concave; lateral lobes semicircular, margin entire. Anterior stamens exerted, posterior slightly longer. Nutlets yellow-brown, oblong.

Scientific Name - *Elsholtzia blanda* (Benth.) Benth.

Myanmar Name - Ar-pu-ywat

Family - Lamiaceae

Flowering and fruiting period - October

Part used - Aerial shoot

Figure 1 Habit of the *Elsholtzia blanda* (Benth.) Benth.

Figure 2 Botanical study on leaves from *Elsholtzia blanda* (Benth.) Benth.

Chemical Studies

Preliminary phytochemical test of *Elsholtzia blanda* (Benth.) Benth.

The preliminary phytochemical investigation was carried out on the powdered leaves.

Table 1 Preliminary phytochemical test of *Elsholtzia blanda* (Benth.)Benth.

No	Chemical constituents	Extract	Reagent used	Observation	Results
1	Alkaloid	1% HCl	1. Mayer's reagent 2. Dragendroff's reagent 3.Wagner's reagent	white ppts reddish ppts yellow ppts	+ + +
2	Glycoside	H_2O	10% lead acetate solution	White ppts	+
3	Saponin	H_2O	Distilled water	Frothing	+
4	Reducing sugar	H_2O	Fehling solution	Reddish ppts	+
5	Carbohydrate	H_2O	10% α napthol and con H_2S0_4.	Pink ring	+
6	Phenolic	H_2O	$FeCL_3$ solution	Deep brown colour	+
7	Cyanogentic glycoside	H_2O	Sodium picrate paper and con. H_2S0_4.	No change in colour	_
8	Steroid	Pet -ether	Acetic anhydride and con. H_2S0_4	Green colour	+
9	Triterpenoids	EtoH and CH_3CL	con. H_2SO_4acid	Reddish brown colour	+
10	Flavonoid	Methanol	Con HCl acid and Mg turning	Pink colour	+
11	Acid/Base/ Neutral compound	H_2O	Bromocresol green colour	Green colour	Natural compound
12	α-amino acid	H_2O	Ninhydrin	Violet colour	+
13	Tannin	H_2O	1% gelatin solution	White ppts	+

+ = positive - =negative

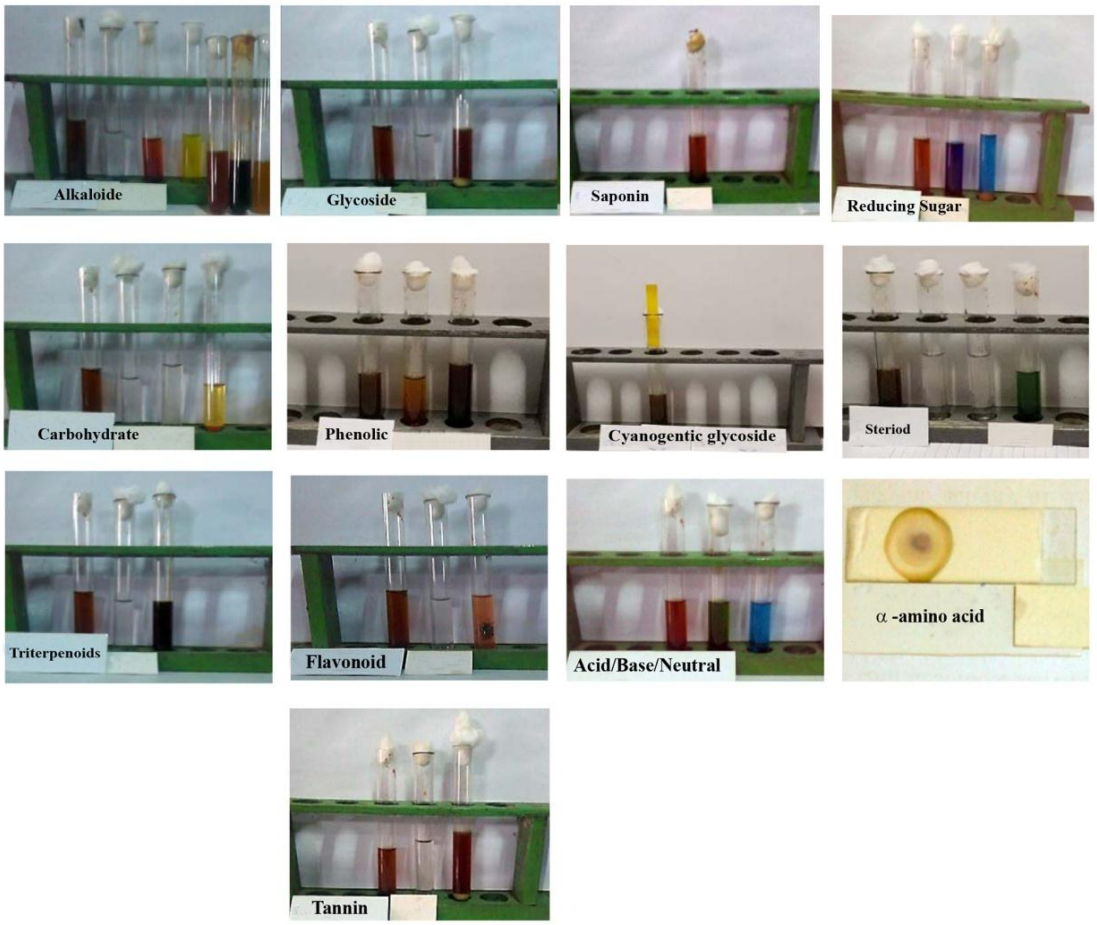

Figure 3 Results of Phytochemical test of dried leaves of *Elsholtzia blanda* (Benth.) Benth.

In the present investigation, qualitative phytochemical screening test were analyzed in powdered leaf extract of *Elsholtzia blanda* (Benth.) Benth. The result were showed in table (1) which indicated the present or absence of compounds in *Elsholtzia blanda* (Benth.) Benth. leaf extract. Results indicated that, alkaloids, glycoside, saponin, reduction sugar, carbohydrate, phenolic, steroid, tri-terpenoids, flavonoid, ∞-amino acid and tannin. Cyanogenetic glycosides were not present in the leaf plant extract.

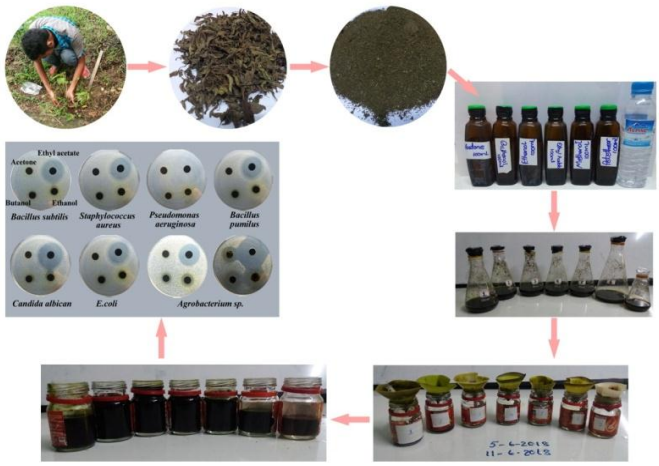

Figure 4 Stages involved in Antimicrobial Activity Tests of *Elsholtzia blanda* (Benth.)Benth.

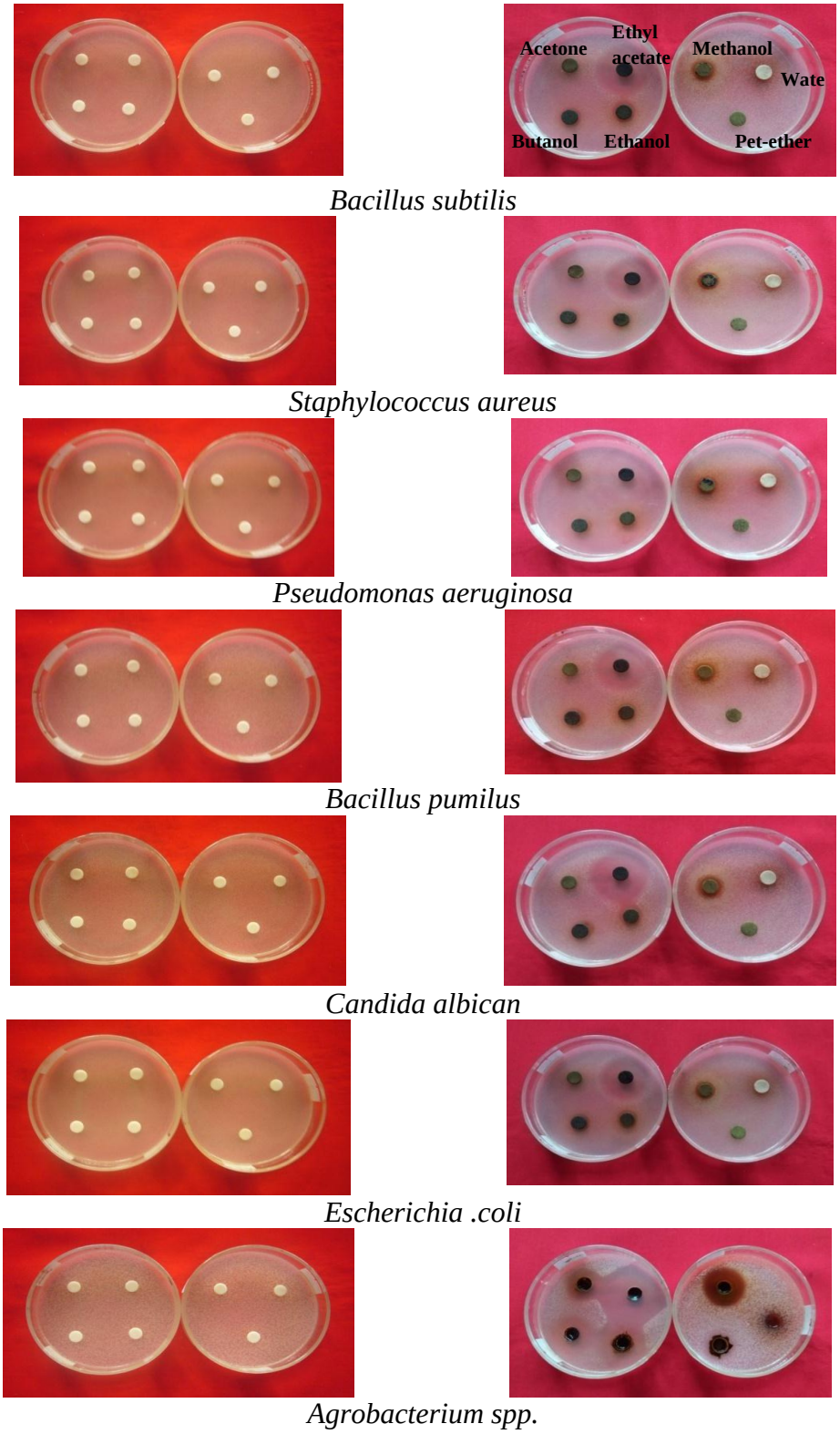

Figure 5 Plate showing zone of inhibition of different extract of *Elsholtzia blanda* (Benth.) Benth. on seven test organism and their control plates

Table 2 Antimicrobial activity of different solvent extract of dried leaves of *Elsholtzia blanda* (Benth.) Benth.

Extracted Solvent	*B.sub*	*S.aureus*	*Pseudomonas*	*B-pumalis*	*Candida*	*E.coli*	*Agro.*
Acetone	11 mm	-	-	-	11 mm	11 mm	12 mm
Butanol	13 mm	13 mm	11 mm	14 mm	13 mm	14 mm	16 mm
Ethanol	14 mm	12 mm	13 mm	13 mm	14 mm	14 mm	18 mm
Ethyl Acetate	32 mm	29 mm	32 mm	32 mm	35 mm	30 mm	32 mm
Methanol	14 mm	13 mm	13 mm	14 mm	16 mm	13 mm	17 mm
Pet-ether	-	-	-	-	-	-	-
Water	-	-	-	-	-	-	-

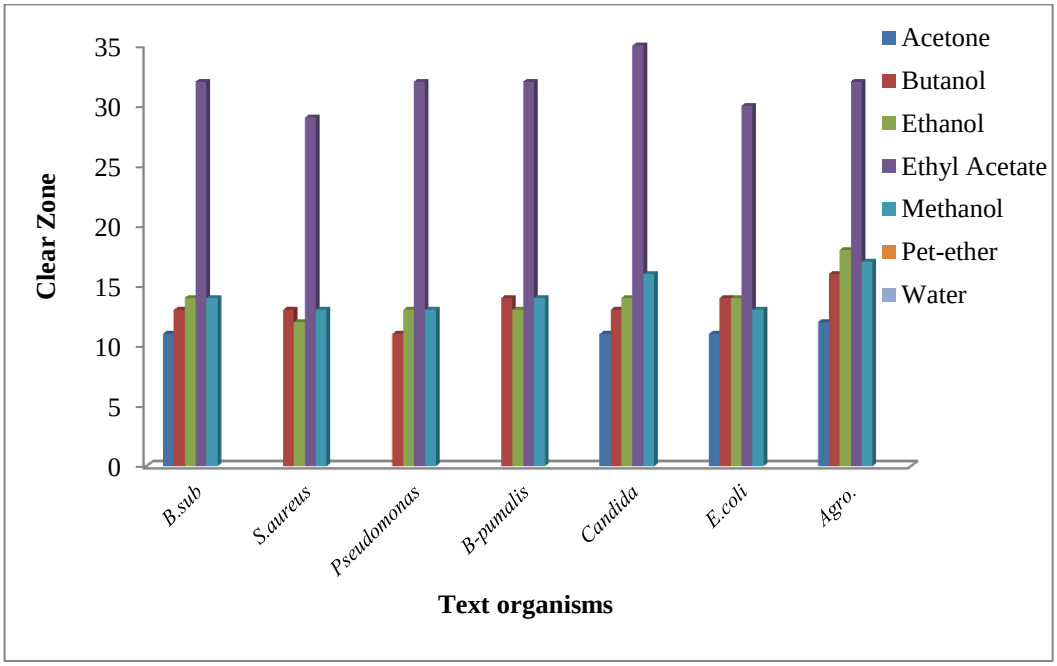

Figure 6 Antimicrobial activity of different solvent extract of dried leaves of *Elsholtzia blanda* (Benth.) Benth.

In the present study, the presence of antimicrobial potential in different solvent extracts of *Elsholtzia blanda* (Benth.) Benth. leaves using seven test organisms was investigated and shown in table 2. It was found that maximum antimicrobial activity (29-35 mm clear zone) was recorded in the extracts of ethyl-acetate by applying disc method (size of disc, 8 mm) whereas acetone extract provided minimum activity (11 mm clear zone). Moderate activity of 12 to 18 mm clear zone was measured in the extracts of ethanol and methanol. The water and petroleum ether extracts showed no activities on seven test organisms with disc method. On the other hand, when used by well method, the ethyl-acetate extract showed high activity (42 mm),

moderate activities each (30 mm) with ethanol and methanol, low activity (11mm) with water extract against *Agrobacterium.*

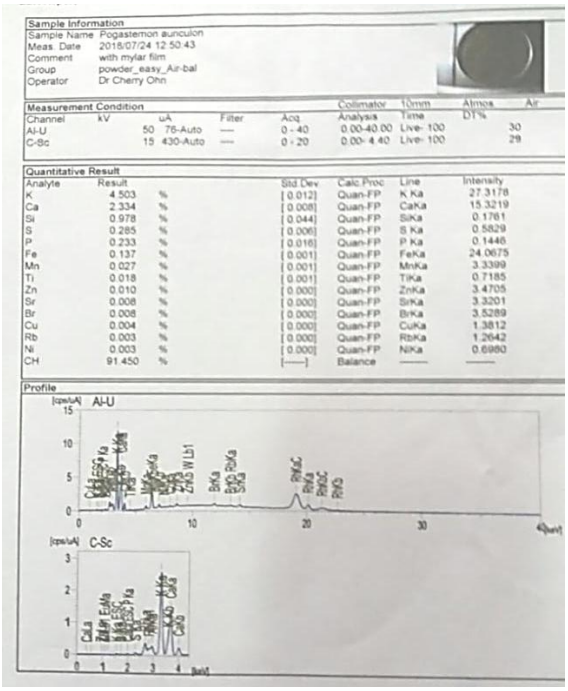

Figure 7 The EDXRF spectrum of *Elsholtzia blanda* (Benth.) Benth.

Table 3 Elemental analysis of *Elsholtzia blanda* (Benth.) Benth.

No	Element	EDXRF
1	K	4.50%
2	Ca	2.30%
3	Si	0.97%
4	S	0.28%
5	P	0.23%
6	Fe	0.13%
7	Mn	0.02%
8	Ti	0.02%
9	Zn	0.01%
10	Sr	0.01%
11	Br	0.01%
12	Cu	0.004%
13	Rb	0.003%
14	Ni	0.003%
15	CH	91.45%

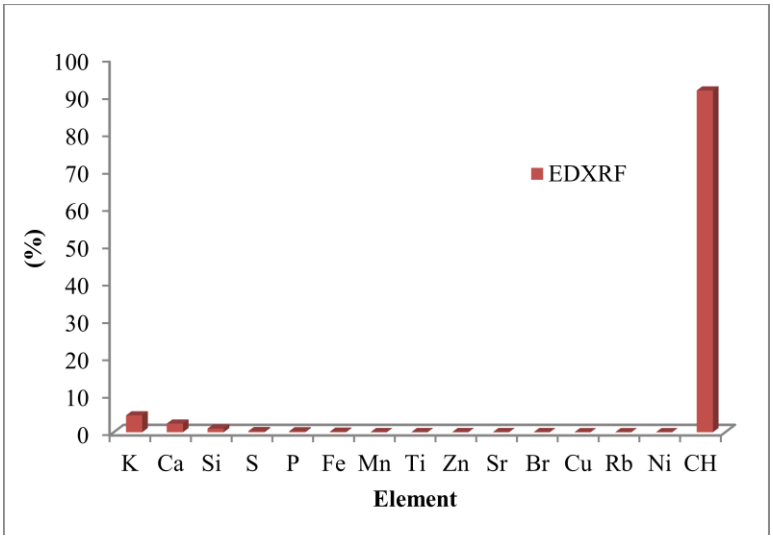

Figure 8 Elemental analysis of *Elsholtzia blanda*(Benth.)

The quantitative estimation of mineral contents in the dried leaves of *Elsholtzia blanda* (Benth.) Benth. was performed by using EDXRF in analytical laboratory of Chemistry Department in West Yangon University. The results showing intensities of containing elemental were showed in Table (3).Hydrocarbon was detected to be 91.45%.Apart from this the most abundant elements were K (4.50%), Ca (2.30%) and Si (0.97%) as shown in Table (3).

Discussion

Angiosperms are treasure houses of potential drugs and in the recent years there has been an increasing awareness on plant derived drugs investigation studies (Cathrine and Prabavathi, 2011). Drugs from the plants are easily obtained, less expensive, safe and efficient and without side effects as compare with synthetic drugs. The importance of medicinal plants in Myanmar and the management of human ailments cannot be overemphasized.

In Myanmar, most people are interested in using plant derived medicine rather than chemically derived medicine because they feel that those medicines can provide various side–effects, more expensive and some may be imitated by dangerous substances. Traditional herbal medicine become more and more popular and are getting significant attention in Myanmar health debates. In one of the neighboring countries, China, people are successfully applying traditional herbal medicines for instance, in the treatment of disease like Severe Acute Respiratory Syndrome (SARS). Also in Africa, 80% of African people use some form of traditional herbal medicine. In the world annual markets for these plant-derived products approach US$ 60 billion. Many scientists postulated that traditional herbal medicine research will play a critical role in global health. China, India, Nigeria, the United States of America and WHO have all made substantial research investments in traditional herbal medicines. Industry has also invested millions of US dollars looking for promising medicinal herbs and novel chemical compounds. This is still a relatively modest investment compared to the overall pharmaceutical industry; but it raises interesting ethical questions, why this type of fundamental investigation on a medicinal plant named *Elsholtzia blanda* (Benth.) Benth. is an essential study as a graduation term paper.

Elsholtzia blanda (Benth.) Benth. is a genus *Elsholtzia* in the family Lamiaceae, containing at least 33 species. It is native to warmer parts of Asia (including Myanmar), Africa, and North America. They are widely distributed in hilly grassland, waste areas, forests, thickets or valleys in warm area. *Elsholtzia blanda* (Benth.) Benth. is an industrially valued aromatic medicinal plant currently having a huge demand for its essential oil. It is widely used in flavors and fragrance industries as well as in pharmaceuticals. They are used as domestic folk medicine, herbal tea, food, spices, beverages, perfumeries, cosmetics, aromatherapies and the source of honey manufacture. (Zhiqin Guo *et.al*., 2012)

The morphological characters of this species are Herbs erect. Stems and branches densely pubescent. Petiole densely pubescent, leaf blade elliptic to elliptic-lanceolate, adaxially pubescent, glandular, abaxially gray-green, glabrous, strigose on veins, base narrowly cunneat, margin serrate, apex acuminate. Spikes terminal or axillary, mostly pubescent; verticillasters flowered, short pediculate; bracts subulate to lanceolate; fruiting calyx slightly dilated at base, ovoid. Corolla white ,strigose outside, subglabrous inside, unnelform,; upper lip emarginated; middle lobe of lower lip subcircular, slightly concave; lateral lobes semicircular, margin entire. Anterior stamens exserted, posterior slightly longer. Nutlets yellow-brown, oblong.

In the present investigation, qualitative phytochemical screening tests were analyzed in powdered leaf extract of *Elsholtzia blanda* (Benth.) Benth. The result was showed in table (1) which indicated the present or absence of compounds of *Elsholtzia blanda* (Benth.) Benth. leaf extract. Results showed that, alkaloids, glycoside, saponin, reduction sugar, carbohydrate, phenolic, steroid, tri-terpenoids, flavonoid, ∞amino acid and tannin. Cyanogenetic glycosides were not present in the leaf plant extract. These compounds also can be correlated with the medicinal potential of the plant.

In 2012, Zhiqin Guo *et al.* reported that similar results that revealed triterpenoids are major constituents in this genus. Lou *et. al.*, (2007) also experiment the presence of flavone and its effect on xiongbi symptom.In 2005, Ling Haiyun *et al.* discovered the protective effect of total flavones from *Elsholtzia blanda* (Benth.) Benth. on cardiac disease in dogs.

Nature and plant parts have been a source of medicinal agents for thousands of years and a striking number of modern drugs have been isolated from plant source which were based on their use in traditional medicines or phytomedicines. Over the years, World Health Organization (WHO) advocated traditional medicines as safe remedies for aliments of both microbial and non-microbial (plant) origins. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. Some antibiotics have become almost old-fashion because of drug resistant and consequently new drugs must be sought for which herbal treatment is one possible way to treat diseases caused by multi drug resistant pathogenic bacteria. In the present study, the presence of antimicrobial potential in different solvent extracts of *Elsholtzia blanda* (Benth.) Benth. leaves using seven test organisms was investigated and shown in Table 2. It was found that maximum antimicrobial activity (29-35 mm clear zone) was recorded in the extracts of ethyl-acetate by applying disc method (size of disc, 8 mm) whereas acetone extract provided minimum activity (11 mm clear zone). Moderate activity of 12 to 18 mm clear zone was measured in the extracts of ethanol and methanol. The water and petroleum ether extracts showed no activities on seven test organisms with disc method. On the other hand, when used by well method, the ethyl-acetate extract showed high activity (42 mm), moderate activities each (30 mm) with ethanol and methanol, low activity (11mm) with water extract against *Agrobacterium.* The results were in agreement with Ai-Lin Liu *et. al.*,(2007) who discovered that the leaf extracts of *Elsholtzia blanda* (Benth.) Benth. showed antimicrobial activity against *S. aureus, B. typhimurium, Diplococcus intracellularis, E. coli* and also on many other *Bacillus* species. Over the past few years, the screening of new antibiotic producing resources has become more common due to the increased rate of development of antibiotic resistant organism. The inhibition of bacteria growth in- vitro by the extracts of dried leaves of *Elsholtzia blanda* (Benth.) Benth. could be due to the presence of some active compounds in the extracts. These active compounds may act alone or in combination to inhibit bacterial growth. The crude extracts containing multiple organic components including flavonoids, tannins, alkaloids, triterpenoids, all of which are known to have antibacterial affects.

The quantitative estimation of mineral contents in the dried leaves of *Elsholtzia blanda* (Benth.) Benth. was performed by using EDXRF in analytical laboratory of Chemistry Department in West Yangon University. The results showing intensities of containing elements such as higher concentrations K, Ca and Si were showed in Table (3).

Conclusion

The bioactive substances in plant such as flavonoids, tannins, alkaloids, triterpenoids in the present work are produced as secondary metabolites, which may not only be developmental stage specific but also organ and tissue specific. While plant leaf, stem and root extracts have been widely evaluated for bioactive compounds. Screening of plant flower has not been extensive. Secondary metabolites belonging to polyketide and non ribosomal peptide families constitute a major class of natural products with diverse biological functions and they have a variety of pharmaceutically important properties. In the present work only the investigation is limited to the leaves of *Elsholtzia blanda* (Benth.) Benth. due to the time allotment. It is necessary to continue to find out in same research trend on the other parts so as to discover how this plant *Elsholtzia blanda* (Benth.) Benth.is of value to Rakhaine State.,

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