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STUDY ON MORPHOLOGICAL, PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITIES OF *SARACA INDICA L. (THAW-KA)*

Htay Htay Myint[1], Nwe Nwe Aye[2]

Abstract

The medicinal plant *Saraca indica* L. belongs to the family Caesalpiniaceae. This plant is known as Thaw-ka in Myanmar. The specimens were collected from Dagon University, East Dagon Myothit Township, Yangon Region, during the flowering and fruiting period, February to June, 2019. In this research morphological characters, preliminary phytochemical tests and antimicrobial activities tests were studied. In the morphological study, the plant was small tree, dark green leaves and grey to dark brown barks. The leaves contain glycoside, tannin, steroids, carbohydrate, α-amino acid, alkaloid, saponin, phenolic compound and terpenoid. Reducing sugar, and starch were absent. The antimicrobial activities were carried out by agar well diffusion method on six types of test microorganisms. Ethanol extract and acetone extract showed the significant activities on all test microorganisms. Methanol extract showed the moderate against on all test organisms and ethyl acetate, chloroform and pet ether extracts showed the negative results for all types of test microorganisms.

Keywords : Morphological characters, Phytochemical test, Antimicrobial activities

Introduction

Advance in modern science and technology has contributed to an enormous development in the quality of human life. Though, stress in incidence of variety of psychiatric disorders. Drugs currently used in treatment of different neuropsychiatric and neurological disorders like anxiety, depression, schizophrenia, epilepsy, parkinsonism either refractory or have serious side effects or posses unfavorable drug-drug/ drug-food interactions. Plants are as medicine since time immemorial. Drugs from plant sources are being used by about 80 % of the world population. Herbal medicines have stood the test of time for their safely, efficacy, acceptability and lesser side effects.

The plant *Saraca indica* L. (Thaw-ka) belongs to the family Caesalpiniaceae. The common names of the plant are ashoka, asoka, asoka-tree, sorrowless tree of India (English), asok, ashoka, asupala, ashogam (Hindi); vànganh Lánh'o (Vietnamese). The native of the plant is India, Indonesia, Laos, Malaysia, Thiland and Myanmar. The word "Ashoka" means "no grief" in Sanskrit. The flowering season is around February to May. This plant is valued for its attractive foliage and fragrant flowers. It requires full sun or slight shade and soils from slightly acidic to neutral rich of organic substance, well drained and kept humid, adult plants can stand short dry periods. It can be cultivated in pot for the decoration of luminous greenhouses and winter gardens with lowest winter temperatures not under the 14^{o}C.

The plant has been greatly used as traditional medicine for women related problems such as menorrhagia, leucorrhoea, bleeding hemorrhoids, dysfunctional uterine bleeding etc (Mohammad *et al*., 2017). Parts of the tree used in traditional Ayruvedic medicine and homeopathic therapies. Juice obtained from boiling of bark said to be effective against female medicinal disorders like menstrual irregularities. Flowers are eaten against dysentery (website I).

Leaves are useful in stomach pain, help to remove worms from the stomach and thus provide relief from pain and swelling. Bark is used to prepare cosmetics that help to improve

[1] Dr, Lecturer, Department of Botany, Dagon University
[2] Dr, Lecturer, Department of Botany, Dagon University

skin complexion. The bark of the tree also has anti-fungal, anti-bacterial and pain relieving properties. The bark decoction help to treat internal piles. Flowers controls blood loss in stools. Seeds powder helps to control kidney stones. Fruits are used as a masticating kidney stones. Fruits are used as a masticating as a replacement for betel nuts. The wood is used in the buildings, the fruit as fodder and from the flowers get a dye.

The aim and objectives of the present research were to verify the morphological characters of this plant, to know the phytochemical constituents, medicinal uses and to examine the antimicrobial activities of leaves.

Materials and Methods

Botanical Studies

The specimens used in this research were collected from Dagon University, East Dagon Township, Yangon Region. They were collected especially during the flowering and fruiting period from January to June in 2018. The collected fresh specimens of both vegetative and reproductive parts of the plants were identified by using literatures of Lawrences, 1964; Backer, 1965; Hundley and Chit Ko Ko, 1987; Dassanayake, 2000 and Kress *et al.*, 2003.

Chemical Studies

The leaves of *Saraca indica* L. were collected from Dagon University, East Dagon Township, Yangon Region. The leaves samples were washed with water and were cut slices by knife. Then these slices were dried at room temperature for 2-3 weeks. The dried leaves were pulverized by grinding with a blender to get fine powdered and stored in air tight container. For preliminary phytochemical test, the air-dried powdered of the leaves were tested for alkaloids, α-amino acid, glycoside, cyanogenic glycoside, carbohydrates, reducing sugar, starch, saponin, tannin, phenolic compound, steroids and terpenoids were carried out.

Preliminary Phytochemical Test of Leaves of *Saraca indica* Linn.

The preliminary phytochemical tests were carried out according to Vogel, 1956; British Pharmacopoeia 1968, Marini Bettolo *el. al.,* 1981; Robinson 1983 and Central Council for Research in Unani Medicine, 1987.

Test for Alkaloid

One gram of powdered sample was boiled for about 20 minutes with 20ml of 10% HCl and filtered. The filtrate was divided into three portions and tested with Dragendroff's reagent, three Wagener's reagent and Mayer's reagent. The precipitate formed an addition of the reagent indicated the presence of alkaloid (Robinson, 1983).

Test for α-Amino acid

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and then filtered. And then, a few drops of each filtrate was spotted on a filter paper using a capillary tube, allowed it to dry and sprayed with ninhydrin reagent. The filter paper was dried at room temperature and then kept it in over at 110°C for a few minutes after which the purple colour appears due to the presence of α-amino acids (Marini Bettolo *et. al.,* 1981).

Test for Glycoside

One gram of powdered sample was heated in a glass test tube with 10ml of distilled water on the water-bath for 20 minutes. The mixture was filtered and 10% basic lead acetate solution was added drop-wise to the filtrate. Pale yellow precipitate was observed which showed the presence of glycoside (Marini Bettolo *et. al.*,1981).

Test for Carbohydrate

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was introduced into a test tube and a few drops of 10% α-naphthol was added shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of the tube. A red ring was formed between the two layers, showing the presence of carbohydrate (Marini Bettolo *et al.,* 1981).

Test for Reducing Sugar

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was treated with Fehling's solution, then boiled about 20 minutes, it furnished green precipitates, indication the presence of a reducing sugar (Vogel, 1956).

Test for Starch

One gram of dried powdered sample was boiled with 10 ml of distilled water for about 20 minutes. It was then filtered and two drops of iodine solution were added to the filtrate. Blue black colour was formed which indicate the presence of starch (Marini Bettolo *et. al.,* 1981).

Test for Saponin

One gram of powdered sample was boiled with 10 ml of distilled water for about 20 minutes and filtered. The filtered and the filtrate shaken vigorously with distilled water for a few minutes. Market forthing which lasted for about half an hour to take place, indicating the presence of saponin (Marini Bettolo *et al.*, 1981).

Test for Tannin

One gram of powdered sample was boiled with 10ml of distilled water for about 20 minutes and filtered. The filtrate was treated with a few drops of 1% ferric chloride solution. If a bluish black or yellowish brown colour resulted indicating the presence of tannins (Marini Bettolo *et al.,* 1981).

Test for Phenolic compound

One gram of powdered sample was boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was treated with neutral *5%* ferric chloride solution, it gave deep blue colour, indicating the presence of phenol groups (Marini Bettolo *et. al.,* 1981).

Test for Steroids and Terpenoids

One gram of powdered sample was extracted with petroleum ether for 20 minutes and filtered. When the petroleum ether extract was dissolved in 1ml chloroform. The chloroform extract was treated with 3 drops acetic anhydride and concentrated sulphuric acid. The reenish colour was turns to blue green indicate the presence of steroids and deep pink of terpenoid (Central Council for Research in Unani Medicine, 1987).

Antimicrobial Activities of Different Solvent Extracts from Leaves of *Saraca indica* L.

Apparatus Used

Autoclave, clean bench, conical flask, hot air sterilizer, measuring cylinders, micropipettes, steam-drying oven, petridishes, pipettes, water bath and loops.

Extraction of crude drugs

Five grams of powder was soaked with 50ml of different solvent such as ethyl acetate, chloroform, methanol, ethanol, acetone, water and pet-ether for about three days and thoroughly shaked. The mixture was filtered and evaporated.

Aseptic Techniques

Sterilized Pyrex glass- wares used throughout the experiment. Glass- wares were first acid washed and then rinsed in distilled water and were sterilized by using autoclave at 121°C for 15 minutes. Once substances are sterilized, they stay sterile as long as they remain within containers that do not permit living organisms to enter. But in research works, the culture materials are transferred from one container to another. The methods that do not permit the accidental entry of living microorganisms during the transfer process were collectively called as aseptic technique. Inoculation wires, the mouth of the test tubes, flasks and culture bottles are heated during transferring process, and working quickly in a clean area were as aseptic techniques.

Cultivation of Test Organisms

Bacillus subtilis (N.C.T.C-8236), *Staphylococcus aureus* (N.C.P.C-6371), *Pseudomonas aeruginosa* (6749), *Bacillus pumilus*(N.C.I.B-8982), *Candida albicans and Escherichia coli* (N.C.I.B-8134*)* were used for the antimicrobial activities. They were inoculated into the nutrient broth and transferred into nutrient agar media.

Preparation of Culture Media

Nutrient Agar (NA) Medium (Atlas, 1993)

Nutrient Agar	-	28.0 g
Agar	-	16.0 g
Distilled Water	-	1000 ml
pH	-	6.8

After autoclaving, Nystatin 1.5 ml was added to the medium for bacteria and Chloramphenicol was added to the medium for fungi.

Nutrient Broth Medium (Atlas, 1993)

Peptic digest of animal tissue	-	5.0 g
Beef extract	-	1.5 g
Yeast extract	-	1.5 g
Sodium chloride	-	5.0 g
Distilled water	-	1000 ml
pH	-	6.8

After autoclaving, Nystatin 1.5ml was added to the medium for bacteria and Chloramphenicol was added to the medium for fungi.

Preparation of Plates for Antimicrobial Activities Test

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to method described by Cruikshank, 1975. Nutrient agar was boiled and 20-25 ml of the medium was poured into a test- tube and plugged with cotton wool and autoclaves at 121 °C for 15 minutes. Then the tubes were cooled down to 30-35°C and poured into sterilized petridishes and 0.01 ml of spore suspension were also added into the dishes. The agar was allowed to set for 30 minutes after with 5mm plate agar well was made with the help of sterilized cork borer. After that, about 0.1ml of sample was introduced into the agar-well and incubated at 37°C for 24-48 hrs. The inhibition zone appeared around the agar-well indicating the presence of anti-microbial activity. The extent of antimicrobial activity was measured from the zone of inhibition diameter. The results were shown in Table 2, Figures 4 to 5.

Results

Morphological Chracters of *Saraca indica* L.

Scientific name	-	*Saraca indica* L.
Myanmar name	-	Thaw-ka
English name	-	ashoka, asoka, sorrowless tree
Family	-	Caesalpiniaceae
Flowering and fruiting period	-	February to June
Parts used	-	Leaves

Small trees, evergreen about 6 m in high. Stems are cylindrical, woody and greywish-brown. The leaves are alternate, paripinnately compound, petiolate, stipulate; Leaflets are elliptic-lanceolate, 6.5-24-5 cm, rounded at the base, margin entire, acuminate apex, both surfaces shinning and dark green; petioles are cylindrical, 2.0-3.0 cm; 0.4-0.5 × 0.2-0.3 cm; stipules are lanceolate about 0.6 × 0.3 cm, caducous. The inflorescences are axillary, corymb racemose; peduncles are cylindrical, about 1.5-2.5 cm long, reddish green, glabrous, The flowers are yellow to about 2.5 cm in diameter at anthesis, bracteate, bacteolate, pedicellate, complete, bisexual, regular, actinomorphic, tetramerous, hypogynous; bract ovate, about 0.6 × 0.6 cm, pale red; bracteolate ovate, about 0.4 × 0.4 cm, pale red; pedicel are cylindrical, about 0.6 × 0.2 cm and red. The sepals are 4, synsepalous, calyx tube about 2.2 × 0.4 cm, calyx lobe about 1.2 × 1.2 cm, valvate, petaloid (yellow to red) persistent, pubescent, inferior. The petals are absent. The stamens are (6-7), fuse, episepalous, filament about 3.0 cm, filiform, staminal tube very short; the anther is dithecous, about 0.2-0.4 cm, purple, extrorse, dorsifixed, longitudinal dehiscence, inferior. The ovary is superior, oblong, compressed, about 0.2 cm in diameter, slightly curved, borne on the stalk, adnate to the calyx tube, pink, carpel 1, monocarpellary, apocarpous, uniloeular, marginal placentation, one ovule in each locule in T.S; hairy; style is one, terminal, about 2.8 cm long, red, glabrous; stigma is capitate, gynophore present, about 2.5 cm. The fruits are legume, oblong, 1.2 × 4.0 cm and green. The seeds are 5, ovoid and about 3.0 × 2.2 cm.

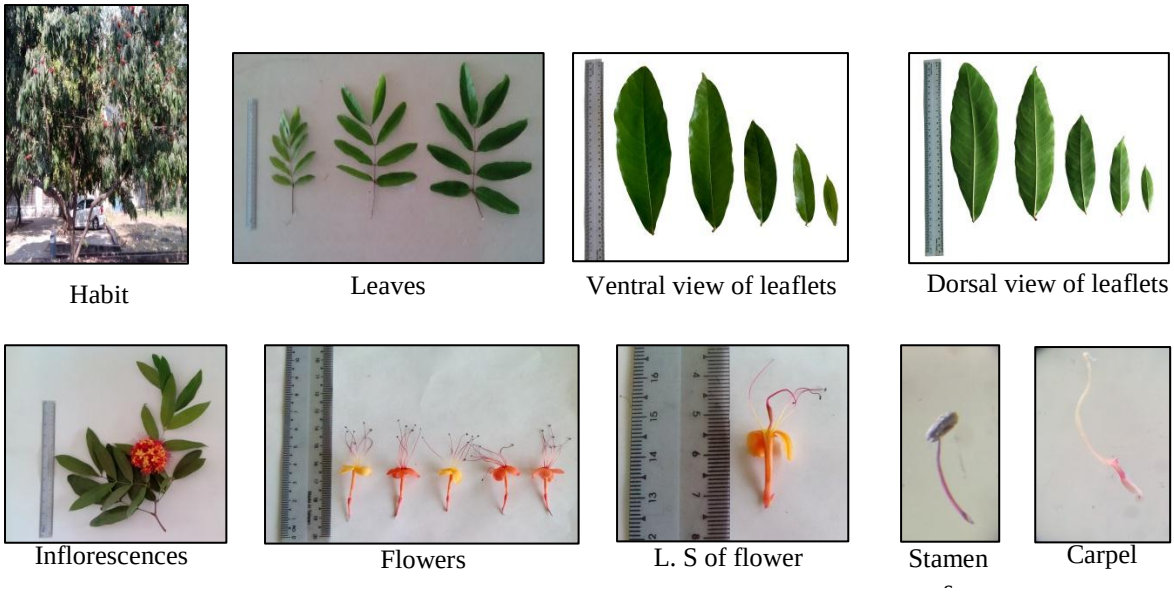


Figure 1 Morphological characters of ***Saraca indica*** **L.**

Preliminary Phytochemical Test of Leaves from *Saraca indica* L.

The results of preliminary phytochemical test of air-dried powdered leaves from *Saraca indica* L. indicated that tannin, steroids, carbohydrate, α-amino acid, alkaloid, saponin, phenolic compound and terpenoid were found to be present and reducing sugar and starch were absent. Among them, the amount of precipitate from glycoside was highest than the other tests.

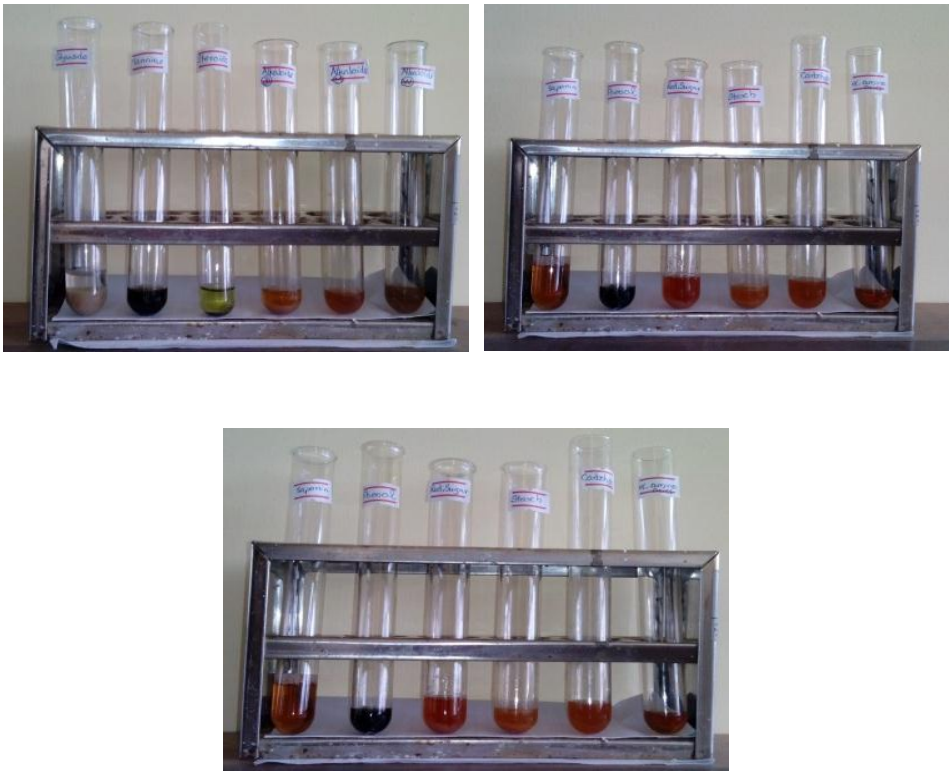


Figure 2 Phytocehmical Test of Leaves from *Saraca indica* L.

Table 1 Preliminary Phytochcmical Test of Leaves from *Saraca indica* L.

No.	Test	Extract	Test reagent	Observation	Results
1.	Alkaloid	10% HCl	Dragendroffs reagent Wagner's reagent Mayer's reagent	Orange ppt Brown ppt White ppt	+ + +
2.	α-amino acid	H_2O	Ninhydrin reagent	Pink spot	+
3.	Glycoside	H_2O	10% lead acetate solution	White ppt	+
4.	Carbohydrates	H_2O	10% α-napthol + H_2S0_4 (Conc:)	Red ring	+
5.	Reducing sugar	H_2O	Fehling's solution	ppt	+
6.	Starch	H_2O	Iodine solution	Brown ppt	+
7.	Saponin	H_2O	Distilled water	Foaming	+
8.	Tannin	H_2O	1% $FeCl_3$ solution	Bluish black	+
9.	Phenolic compound	H_2O	5% $FeCl_3$ solution	Deep blue	+
11.	Steroids	Petroleum ether	Acetic anhydride	Green ppt	+
12.	Terpenoids	Petroleum ether	Acetic anhydride and H_2S0_4 (Conc:)	Black ppt	+

Key to the table

Key to the table (+) = present (-) = absent (ppt.) = precipitate

Antimicrobial Activities of Different Solvent Extracts of Leaves of *Saraca indica* L.

In this study, ethanol and acetone extract showed the significant activities on six test organisms. Methanol extract showed the moderate against on all test organisms. Ethyl acetate, chloroform end petether extract the negative results for all types of test organisms.

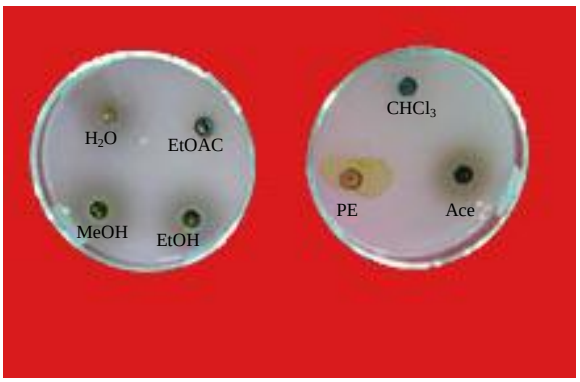
Figure 3 Leaves extracts of ***Saraca indica* L.**

Table 2 Inhibition Zone Exhibited by Different Solvent Extract of Leaves of ***Saraca indica* L.**

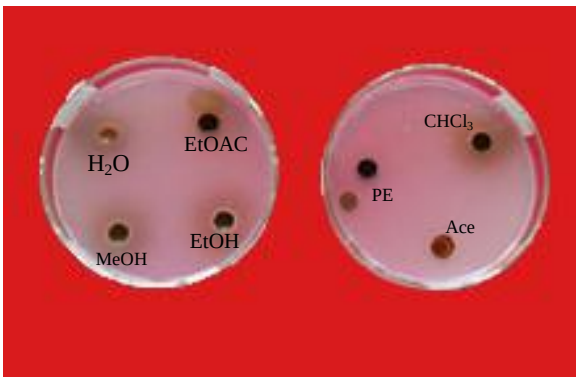
No.	Microorganisms	Inhibitions Zone (mm)						
		H_2O	MeOH	EtOH	EtOAC	$CHC1_3$	P.E	Ace
1.	*Pseudomonas aeruginosa*	-	25 ++	25 ++	-	-	-	25 ++
2.	*Staphylococcus aureus*	25 ++	20 +	25 ++	-	-	-	25 ++
3.	*Candida albicans*	30 ++	25 ++	20 +	-	-	-	25 ++
4.	*Esherichia coli*	-	20 +	30 ++	-	-	-	25 ++
5	*Bacillus subtilis*	15 +	25 ++	25 ++	-	-	-	25 ++
6.	*Bacillus pumilus*	25 ++	25 ++	25 ++	-	-	-	25 ++

Key to the table

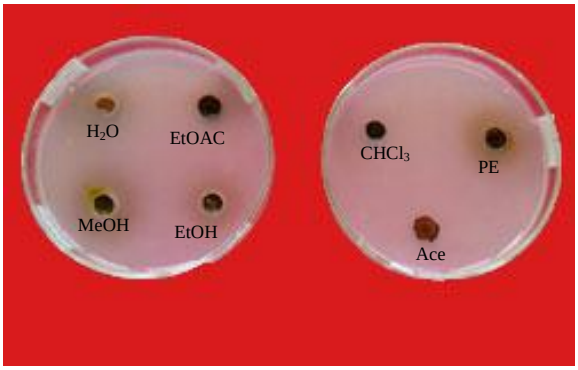
(+) = 10mm-20mm (++) = 21mm-30mm (+++) = 31mm above (10 mm) = Agar well



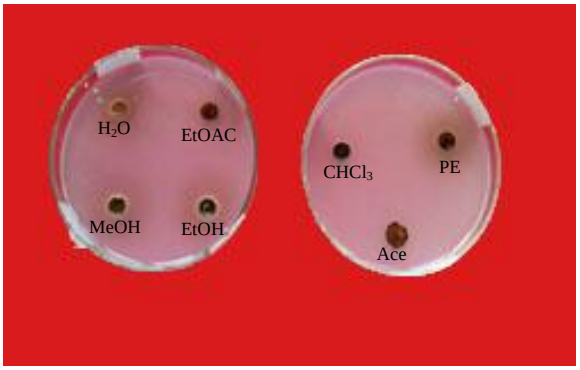
Pseudomonas aeruginosa



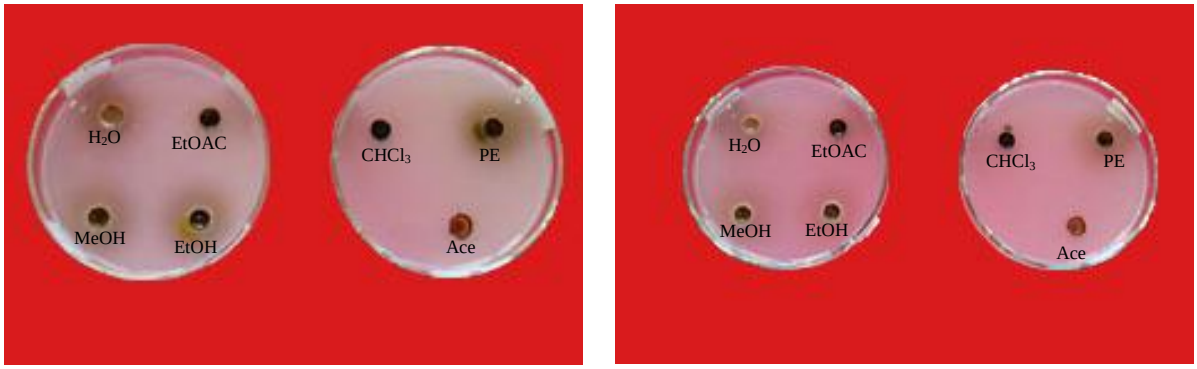
Staphylococcus aureus



Candida albicans



Esherichia coli



Bacillus subtilis *Bacillus pumilus*

H_2O = Aqueous
MeOH = Methanol
EtOH = Etanol
EtOAc = Ethyl acetate
$CHCl_3$ = Chloroform
P.E = Petroleum ether
Ace = Acetone

Figure 5 Antimicrobial activities of leaves of *Saraca indica* L.

Discussion and Conclusion

Herbal medicine has such as amazing influence that various alternative medicine therapies with herbal remedy, Unani and Ayurveda. The tree has many heath benefits and has long be used in traditional Indian medicine.The medicinal importance of the tree is one of the most important medicinal plant which possess a lot of therapeutic values especially for female disorders.

The plant *Saraca indica* L. belongs to the family Caesalpiniaceae. The plants are small trees. The leaves are alternate, paripinnately compound, petiolate and stipulate. Leaflets are elliptic-lanceolate, rounded at the base, margin entire, acuminate apex, both surfaces shinning, dark green. The stipules are lanceolate and caducous. The inflorescences are axillary, corymb racemose, peduncles are cylindrical, reddish green and glabrous. The flowers are yellow to red, bracteate, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, tetramerous and hypogynous. The sepals are yellowish to red. The petals are lacking. The stamens are fused. The filaments are filiform and distinct. The anthers are dithecous. The ovary is superior and oblong. These datas were agreed with Dassanayake, 1991.

The primary benefit of *Saraca indica* L. is a brain tonic and improve memory and intellect. It can be used to treat epilepsy and headache. It also controls vomiting and help to cure diabetes. The herb of this tree can acts on uterine muscles and endometrium and thus provides relief from abdominal pain and other spasms. It also helps to treat irregular menstrual cycles, amenorrhea, leucorrhea, fibroids, cysts and other related disorders. This tree is widely used to treat gynecological and menstrual problems in women and it helps to remove toxins from our blood and therefore provides excellent benefits for our skin. (website 1).

The flowers were eaten by cooked, aromatic, with a somewhat sour flavour. Eaten as potherb. Fruits are used as masticatons as a replaced for betel nuts. Although the health benefits of this tree are numerous, pregnant women should abstain from consuming products from this tree as it might lead to complications.

In this research, the preliminary phytochemical screening of the extracts of leaves contain glycoside, tannin, steroids, carbohydrate, α-amino acid, alkaloid, saponin, phenolic compound and terpenoid. These results were agreed with Aditya *et al*., 2013 and Mohammad *et al.,* 2017.

In the antimicrobial activities test, six types of test organisms were used. Among them, ethanol and acetone extract of leaves showed the best significant activities on all test organisms. Methanol extract showed the moderate against on all test organisms and ethyl acetate, chloroform and pet-ether extract showed the negative results for all types of test microorganism. These datas were agreed with Aditya, 2013.

Therefore, the present study focused on chemical composition by using preliminary phytochemical test and antimicrobial activities of this plant which could be assumed to be beneficial for human health.

Acknowledgements

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We are greatly indebted to Dr. Khin Latt Latt Mon, Professor, Department of Botany, Dagon University, for her valuable suggestion in this research.

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Website

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

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

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.

[1] Lecturer, Department of Botany, Dagon University
[2] Assistant Lecturer, Department of Botany, Dagon University
[3] Demonstrator, Department of Botany, University of Medicinal "1"
[4] Professor/Head, Department of Botany, Dagon University

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

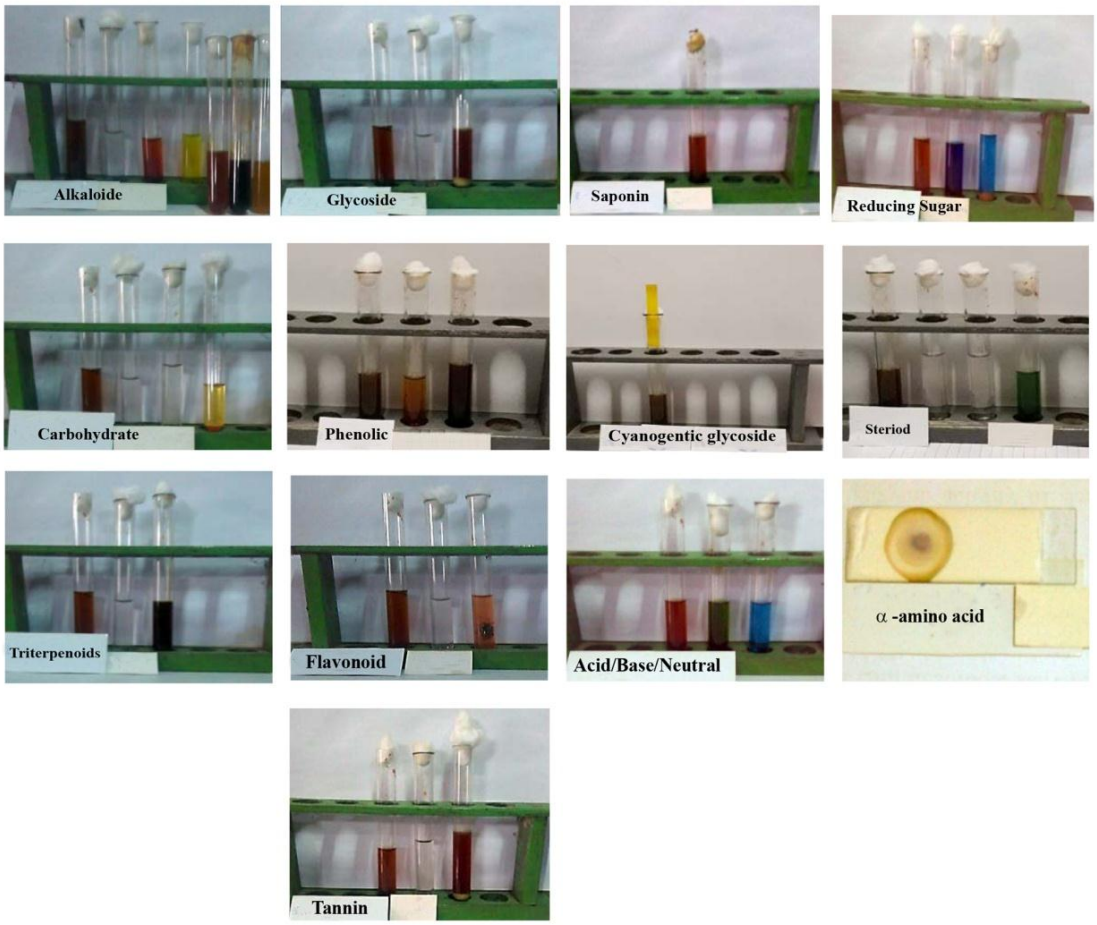

Figure 3 Results of Phytochemical test of dried leaves of *Elsholtzia blanda* (Benth.) Benth.

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.

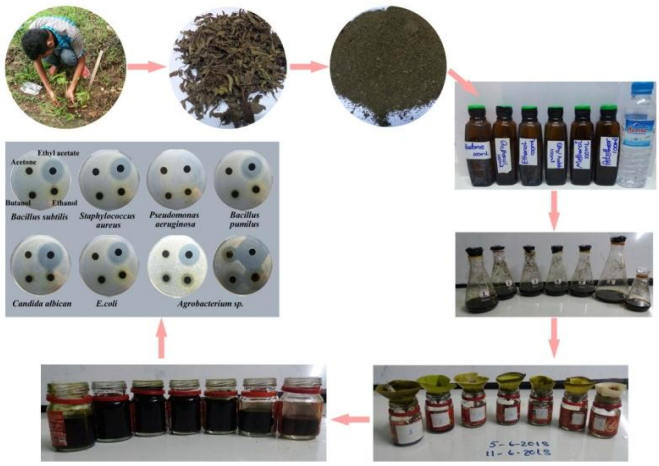

Figure 4 Stages involved in Antimicrobial Activity Tests of *Elsholtzia blanda* (Benth.)Benth.

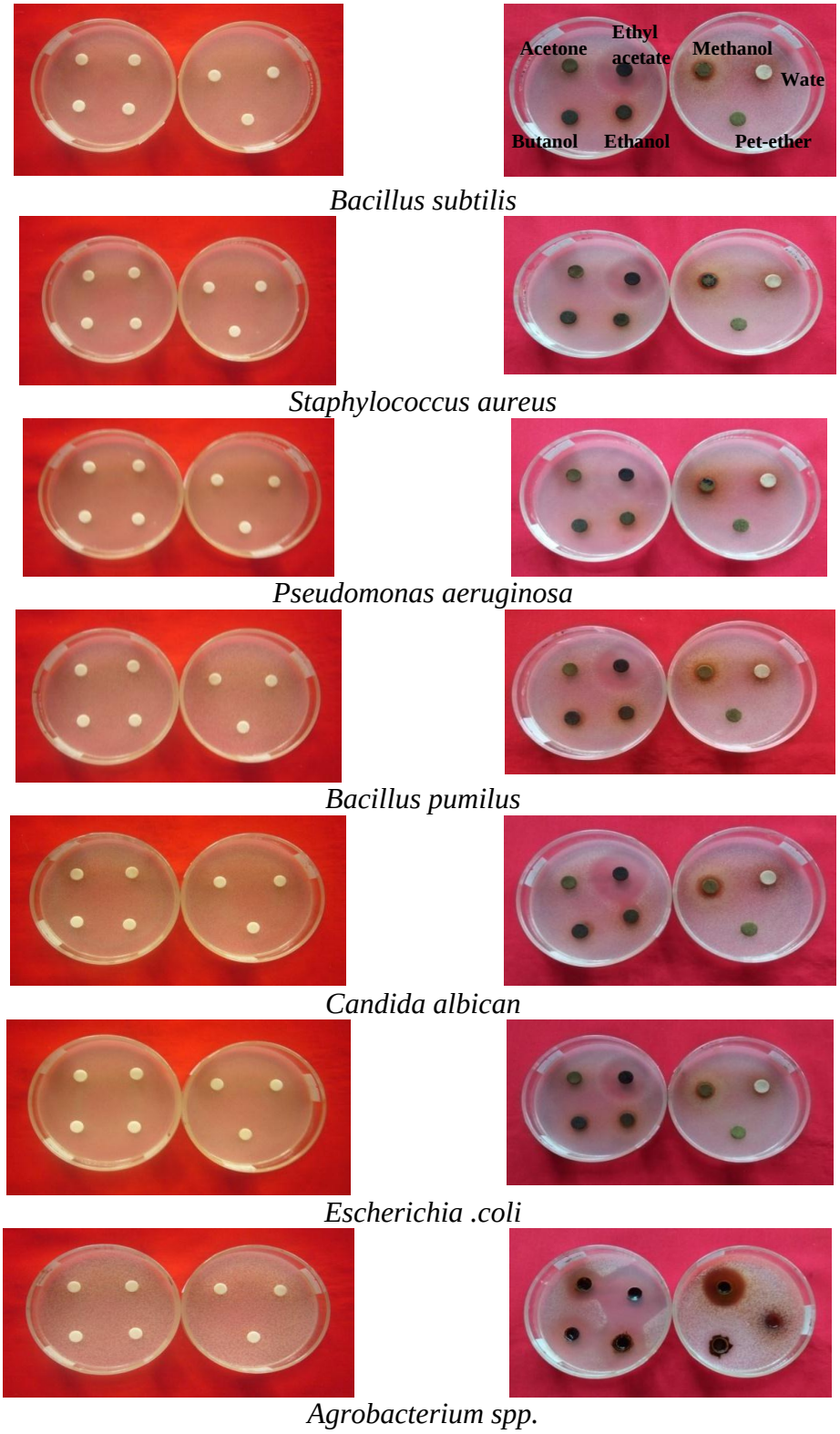

Figure 5 Plate showing zone of inhibition of different extract of *Elsholtzia blanda* (Benth.) Benth. on seven test organism and their control plates

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	-	-	-	-	-	-	-

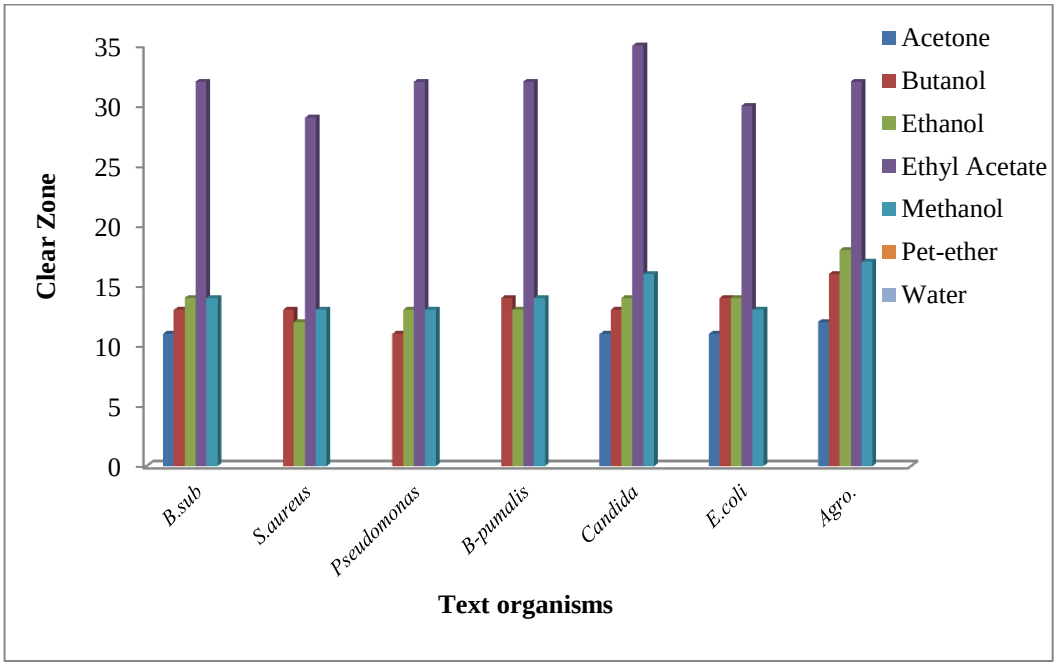

Figure 6 Antimicrobial activity of different solvent extract of dried leaves of *Elsholtzia blanda* (Benth.) Benth.

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.*

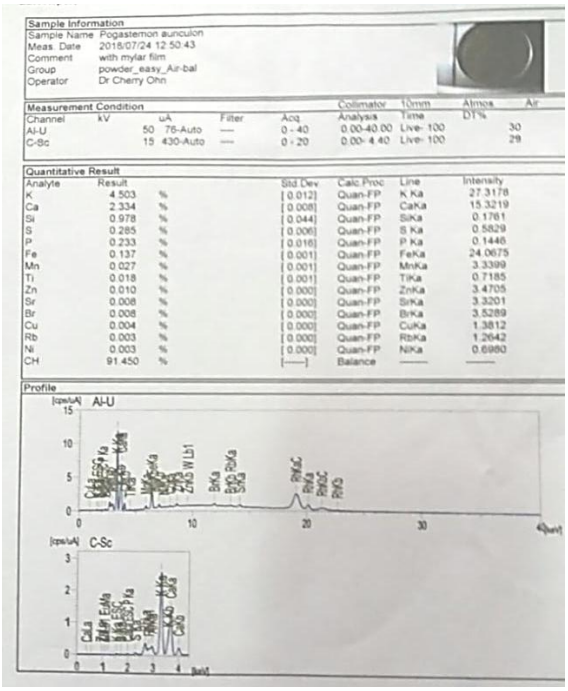

Figure 7 The EDXRF spectrum of *Elsholtzia blanda* (Benth.) Benth.

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%

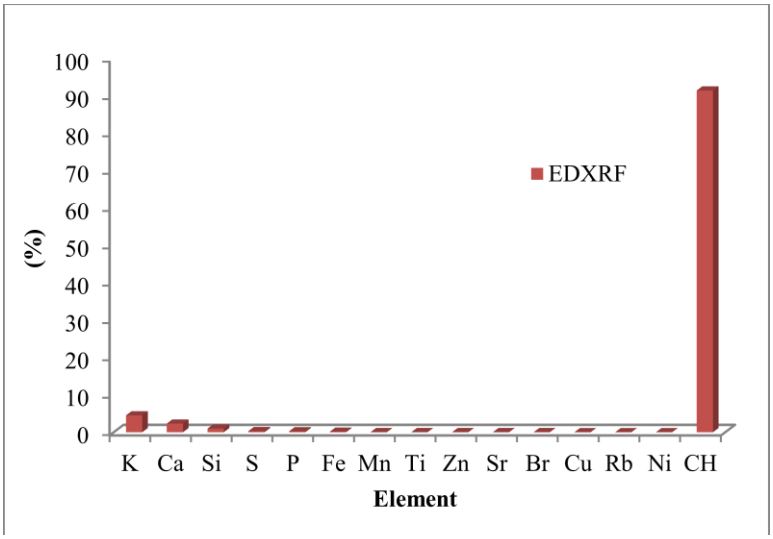

Figure 8 Elemental analysis of *Elsholtzia blanda*(Benth.)

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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EFFECTS OF PLASTIC MULCHING ON GROWTH, YIELD AND COST AND RETURN ANALYSIS OF *CAPSICUM ANNUUM* L. VAR. *ANNUUM* CV. *ACCUMINATUM* FINGERH

Ei Khaing Thwe[1]

Abstract

The field experiment was conducted at Aungmyinttar Quarter, Magway Township from June to November 2017. In the experiment, two different plastic mulches were transparent, black and control (without mulch).Soil temperature and moisture had no significant were observed. Significant differences on plant height, number of leaves, fresh weight of fruit and harvest index of chili pepper were observed. According to the results of different plastic mulches, T_2 black was tallest plant height (22.33cm), maximum number of leaves (30.91), fresh weight of fruit (16.94g) and harvest index (12.46) compared to other T_1 transparent and T_3 control. Production of chili pepper by using plastic mulch was more cost than the fruit production without using the plastic mulching, however, higher yields in the plastic mulched chili pepper fruits than fruits without using it in 2017. The results of 2017, using black plastic mulch gave the highest profit in all treatments and the use of transparent plastic mulch profited five times than without plastic mulch (control). There was a very high difference between black plastic mulch and control. The results clearly suggested that using the black plastic mulch can be performed the best yield components and yields of chili pepper production.

Introduction

Mulching is an agricultural and horticultural technique in which the use of organic is involved. The technique is very useful in protecting the roots of the plants from heat, cold (Bhardwaj, 2013).Mulch can be defined as use of a material that covers soil for a variety of uses. The term was at one time used for the application of organically based plant residue, although the understanding of the term mulch has grown to incorporate use of paper and plastics applied from a roll or polymers applied to the soil (Horst, 2001). Plastic mulch has been used commercially on vegetables since the early 1960s.Currently, it was used on the thousands of acres of vegetables in the United States (Hochmuth, 2001).

Plastic mulches offer many advantages to growers including earlier and higher overall yields, reduced evaporation, fewer weed problems, reduced fertilizer leaching, and elimination of root pruning. The major disadvantages of plastic mulch are initial cost, removal, and disposal (Lamont, 1993). There are various colored plastic such as, clear, black, red, blue, silver, white, yellow polyethylene, etc. Black mulch was used most widely because it suppress weed growth, resulting in less chemical usage (Hochmuth, 2007).

Black plastic mulch applied to planting will warm the soil and promote faster growth in early season, which generally leads to earlier harvest (Bhardwaj, 2013).Even though the use of black plastic mulch was expensive, it has become an accepted practice for commercial growers of many vegetable and fruit crops including chilli, tomato, eggplant, muskmelon, watermelon and strawberry (Lamont, 1999). Transparentplastic mulch was used in some areas due to its increased soil warming characteristics (Bhardwaj, 2013).Transparentplastic transmitted radiation to the soil surface, which was absorbed and converted to sensible heat (Hopen, 1964).Transparentplastic mulch has a repellent effect on pest and vector insects, such as aphids, whiteflies and thrips (Bhardwaj, 2013).

[1] Assistant Lecturer, Department of Botany, University of Magway

Cost and Return Analysis will show weak points of a business that cause of low returned and unnecessary large expenditure (Bautista, 1994).The plan includes the activities to be done, manpower requirement and organization, resource to be used, budget to be utilized, records to be kept, schedule of farm operations, and labor requirement (Bautista, 1994).Based on the plan, and budget is prepared. Budgeting is determining the production requirements, allocating money and non financial resources to different production activities and estimating the financial gain (Bautista, 1994).In both the U.S.A and parts of Europe, cost of production studies became increasingly prevalent from before the First World War up until the 1930s (Mc Cathy, 1975).The major direct cost items were seed, cultivation, herbicides and fertilizer (Mc Cathy, 1975). A goal in sustainable agriculture is more productivity per production costs in crop production systems (Mc Cathy, 1975).The economic analysis is the only tools, which compel the farmers to decide what to grow and what not to grow. Important economical indicators as well, total costs production, gross return, net return, benefit to cost ratio and productivity were calculated to purpose successful management. Finally, minimizing costs production is necessary because in addition to increasing economic benefit as well as increased sustainability of these agroecosystems (Yousefi, 2011).

In facts, the study was carried out to evaluate the plastic mulching on growth and yield of *Capsicum annuum*L. var. annuum cv. *accuminatum* Fingerh (ripen fruits of chilli). To estimate by using plastic mulching techniques the cost and returns associated with these chilli production. To know the gross income and net profit of ripen fruits of chilli production by the application of plastic mulching techniques.

Materials and Methods

Time and Place of the Study

The field experiment was conducted at Aungmyinttar Quarter, Magway Township from June to November 2017 to study the effects of plastic mulching on the growth and yield of *Capsicum annuum*L. var. *annuum* cv. *acuminatum* Fingerh.

Preparation of Materials

The seeds of *Capsicum annuum*L. var. annuum cv. *acuminatum* Fingerh were obtained from Selywar village, Pakokku Township, Magway Region.

Preparation of soil and plots for chilli cultivation

In the study area, the wild grasses were cut and the land was ploughed to transparentthe root stocks and to clean up the land one week before conducting the experimentation.The soil was prepared by thoroughly mixed with cowdung, loamy and compost in the ratio of 2 : 1 : 1 were applied to the soil at final land preparation. Each plot was laied out in 60 cm wide, 450 cm long, 15 cm height and 30 cm apart.

Preparation of plastic mulches and transplanting of seedlings

Plastic mulches were carefully spread over the plots and holes were punched with 4 inches P.V.C pipe and razor blade. After making the holes, seedlings were transplanted to the centre of the bed at a distance of 75 cm between plants. Furrow irrigation once in two weeks about 7 hours after transplanting.

Determination of Meteorological Parameter

The report of meteorological data such as monthly minimum and maximum temperature, rainfall, and relative humidity (RH) of the study area were obtained from Department of Meteorology and Hydrology, Magway Station, Magway Township from May to September in 2017.

Harvest Index

Harvest index was determined by using the following formula (Gomez and Gomez, 1984).

$$\text{Harvest Index (HI)} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Economic yield = seed/fruit yield

Biological yield = total dry matter

Benefit- Cost Ratio (BCR)

Benefit- Cost Ratio (BCR) is the ratio based on the invest (cost) the profit (benefit) was produced. The calculation was followed by the method of Bautista, (1994).

$$\text{Benefit - Cost Ratio}\,(\text{BCR}) = \frac{\text{Gross income}}{\text{Cost of production}}$$

Gross income = sale of produce+ non- cash returns

Cost of production = total investment

Return on Investment (ROI)

Profitability is the rate of Return on Investment (ROI). It was obtained by the following method of Bautista, 1994.

(ROI) = (A) ÷ C x 100

A = net income

C = gross income – cost of production

Measurements of Soil Temperature and Moisture

Soil temperature and moisture under each plastic mulches were done weekly starting from transplanting to final harvesting. Soil temperature was measured at 2 inches soil depth by using a laboratory thermometer. Soil moisture was measured at 2 inches soil depth by using a McGregor's 3 in 1 Soil Tester. Measurements of soil temperature and moisture were done weekly at 9:00 AM.

Data Collection

Three plant samples were randomly taken from each plot at their vegetative stage (35 DAS) (DAS = day after sowing), at reproductive stage (56 DAS) and at harvesting time (98 DAS and 112 DAS). The following data were collected in the study: soil temperature, soil moisture, plant height, number of leaves, , number of flowers, fresh weight of fruits and harvest index (HI).

Statistic Analysis

Two factor factorial design in RCBD with 3 replications was carried out in this study. Factor A was assigned in 2 kinds of plastic mulching (T_1 = transparent, T_2 = black, T_3 = control). Factor B was assigned in 3 kinds of planting times (S_1 = 5th June, S_2 = 10th July, S_3 = 15th August).

The data were analyzed using the IRRISTAT software (Version 7.0). Treatments means were compared by Least Significant Difference (LSD) at 5% level of significance.

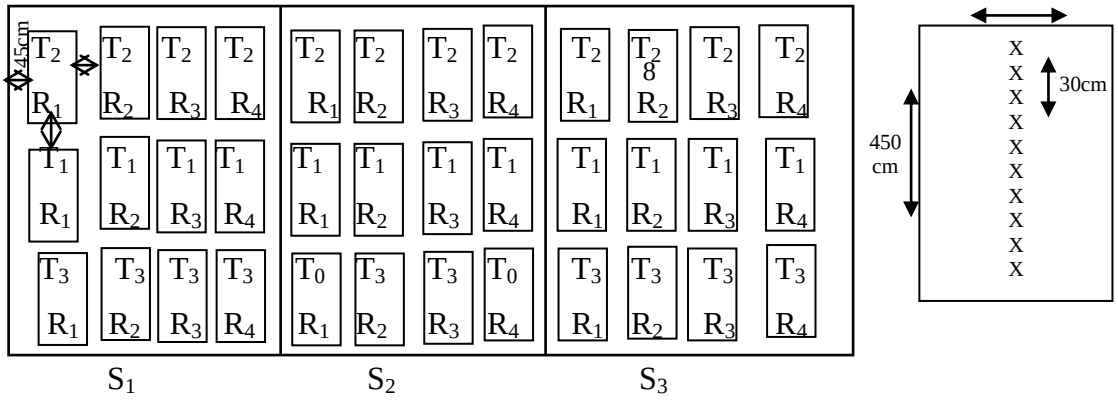

Figure 1 Plot layout of cultivation of chili pepper using plastic mulching techniques

Results

Meteorological Parameters during Growing of *Capsicum annuum* L. var. *annuum* cv. *accuminatum* (2017)

The monthly temperature, rainfall and relative humidity (RH) of the cultivation area were noted from May to September, 2017.

According to the report from Department of Meteorology and Hydrology, Magway Station, maximum temperature (41.7°C) was obtained on May and the minimum temperature (18.5 °C) in September. The maximum rainfall (42.22 inches) was obtained in September and the minimum rainfall (6.06 inches) in July. The maximum RH (83%) was obtained in September and the minimum RH (63%) in May.

Soil Temperature

The result observed that soil temperature had not significantly differenceat 35 DAS(day after sowing), 77 DAS and 112 DAS in different plastic mulching.

Soil Moisture

The result observed that soil temperature had no significant difference at 35DAS, 77DAS and 112 DAS in different plastic mulching.

Plant Height

The result observed that plant height was significantly different at 35 DAS to 105 DAS. When compared to the plastic mulching, T_2 black was tallest plant height (22.33 cm) followed by T_1transparent(22.23 cm) and T_3 control (19.89 cm).

Number of leaves

The result observed that number of leaves was significantly different at 35 DAS to 105 DAS. When compared to the plastic mulching, the maximum number of leaves was found in T_2 black (30.91) followed by T_1transparent (25.99) and T_3 control (23.74).

Fresh weight of fruits

The result observed that fresh weight of fruits was highly significant difference at 98 DAS and 112 DAS in different plastic mulching.When compared to the plastic mulch, T_1transparentwas higher (13.25 g) than T_3 control (10.88 g). The maximum fresh weight of fruits was found in T_2 black (16.94 g).

Harvest Index (HI)

The result observed that harvest index were significantly differences after harvesting at 105 DAS and 119 DAS in different plastic mulching. When compared to the plastic mulching, the harvest index of T_2 (black) was higher than T_1 transparent and T_3 control. The T_2 black was (12.46), T_1transparent(8.49) and T_0 control (9.68).

Estimated Cost and Return Analysis

The total cost estimated included the purchasing fertilizer and chemical pesticide, soil mixes and laborer cost for soil preparation, spraying, watering, weeding and harvesting.The income was based on the yield per acre and current sold price per basket (400 viss).As shown in Table 1, total cost, gross income and net profits were significantly greater in chilli cultivation with black plastic mulches and transparent plastic mulches than control. The total cost estimated was higher using black plastic mulch and without plastic mulch (13,66,000 kyats and 12,00,000 kyats $acre^{-1}$)than transparentplastic mulch (10,34,000 kyats $acre^{-1}$).According to result, in year 2017, gross income was obtained (285,0000 kyats) $acre^{-1}$ with black plastic mulches followed by transparentplastic mulches (205,0000 kyats) and control (175,0000 kyats). In year 2017 showed that the net profits was higher in transparentplastic mulches (1,016,000 kyats) than control (550,000 kyats).In year 2017,the highest net profits was found in black plastic mulches (14,84,000 kyats) $acre^{-1}$ among treatments.

Table 1 Estimated total cost, gross income and net profits per acre of ripen chilli fruits production using the plastic mulching techniques in year 2017

Plastic Mulching		2017		
		Total Cost (Kyat)	Gross income (Kyat)	Net profit (Kyat)
Black plastic		13,66,000	28,50000	14,84,000
Transparentplastic		10,34,000	20,50000	10,16,000
Control		12,00,000	17,50000	550,000

Source of sold price pre basket: Magway and Pakkoku market in September 2016, 2017.

Return On Investment (ROI) and Benefit – Cost Ratio (BCR) results were also presented Table 2. BCR were obtained (2.09) in black plastic mulches and there was slightly difference between transparentplastic mulch(1.45) and control (1.98) in 2017.In 2017, ROI was obtained 108.64% with black plastic and 98.26% with transparentplastic compared to control 45.83%.

Table 2 Benefit Cost Ratio (BCR) and Return on Investment (ROI) percentages of chilli which was black, transparent plastic and control treatments in year 2017

Plastic mulching	BCR	ROI(%)
	2017	2017
Black plastic	2.09	108.64
Transparentplastic	1.98	98.26
Control	1.45	45.83

Table 3 Estimated cost and return analysis of ripen chilli fruits production by using the black plastic mulching in 2017

Items	Unit	Unit amount	Price per unit(Kyat)	Total Price (Kyat hectare^{-1})
Black plastic	Roll	24	35500	852000
Seed	Gram	100	3000	3000
Fertilizer	Bag (13.5kg)	2	45000	90000
Cowdung	Bag (13.5kg)	60	500	30000
Loamy	Bag (13.5kg)	30	2500	75000
Laborer				
Soil preparation	Time	2	50000	100000
Plastic	Laborer x Time	20 x 1	3000	60000
mulchPreparation	Time	2	15000	30000
Spraying insecticide	Time	3	21000	63000
Irrigation	Laborer x Time	7 x 3	3000	63000
Harvesting				
Total cost				1366000
Total income	Basket	675 (50)	4000	2850000
Net profit			(3000)	1484000

Table 4 Estimated cost and return analysis of ripen chilli fruits production by using the transparent plastic mulching in 2017

Items	Unit	Unit amount	Price per unit(Kyat)	Total Price (Kyat hectare^{-1})
Transparentplastic	Roll	10	52000	5200000
Seed	Gram	100	3000	3000
Fertilizer	Bag (13.5kg)	2	45000	90000
Cow dung	Bag (13.5kg)	60	500	30000
loamy	Bag (13.5kg)	30	2500	75000
Laborer				
Soil preparation	Time	2	50000	100000
Plastic mulchPreparation	Laborer x Time	20 x 1	3000	60000
Spraying insecticide	Time	2	15000	30000
Irrigation	Time	3	21000	63000
Harvesting	Laborer x Time	7 x 3	3000	63000
Total cost				1034000
Total income	Basket	475 (50)	4000 (3000)	2050000
Net profit				1016000

Table 5 Estimated cost and return analysis of ripen chilli fruits production by using the without plastic mulching (control) in 2017

Items	Unit	Unit amount	Price per unit(Kyat)	Total Price (Kyat hectare^{-1})
Seed	Gram	100	3000	3000
Fertilizer	Bag (13.5kg)	4	45000	180000
Cowdung	Bag (13.5kg)	60	500	300000
Loamy	Bag (13.5kg)	30	2500	75000
Laborer				
Soil preparation	Time	3	50000	150000
Spraying insecticide	Time	11	15000	165000
Weeding	Laborer x Time	40 x 3	1500	180000
Irrigation	Time	4	21000	84000
Harvesting	Laborer x Time	7 x 3	3000	63000
Total cost				1200000
Total income	Basket	350 (60)	4000 (3000)	1750000
Net profit				550000

Discussion and Conclusion

Two types of plastic mulch materials and control were tested on chilli in order to determine their effects on chilli plant growth and yield in this study. According to plastic mulching, the tallest plant height were found in black plastic mulch followed by transparentplastic mulch. The shortest plant height were found in control.The result was agree with Shinde, (1999) who reported that the increased plant height in mulched plants was possibly due to better availability of soil moisture and optimum soil temperature. According to the plastic

mulching, the maximum number of leaves per plant was found on the plants mulched with black plastic at all growth stages, followed by the transparentplastic mulch. The minimum number of leaves per plant was found in control. Among the plastic mulching treatments, black plastic mulch gave the maximum fruit yields, (3.96) while transparent plastic mulch gave the second highest fruit yields, (3.09) and control (2.29) respectively.Lamont 1993 reported that Plastic mulches offer many advantages to growers including earlier and higher overall yields, reduced evaporation, fewer weed problems, reduced fertilizer leaching, and elimination of root pruning.

This result was agree with Izakovic, (1989) who reported that the microclimate condition improved by the mulches might have provided a suitable condition for producing higher number of leaves in the plants.The estimated cost and return analysis showed that the application of plastic mulching with black and transparentplastic would be more total costs in the cultivation of chilli pepper, however, it can be obtained more profitable high yields than without plastic mulch (control) after studied in 2017. In 2017, using black plastic mulch profited four times than using transparentplastic mulch which was also more profitable five time than control because heavy rainfall onto bare soil leads to the destruction of the soil structure and the texture of the soil.However, mulching prevents the leaching of fertilizer, because it acts as a physical barrier to rainfall.

In year 2017, BCR (Benefit Cost Ratio) showed that more increased in black plastic followed by transparentplastic and then control.According to the results of ROI (return on investment), both the black and transparentplastic mulching indicated more profitability of ROI percentages (about 39– 45%) than control in 2017.Those findings were agreement with Singh *et.al.* (2014), maximum benefit per unit cost of cultivation was observed in summer squash, tomato and capsicum cultivation under black plastic mulch was found to be the best with respect to net returns and benefit cost ratio (BCR).Before computing cost and return analysis there is a indeed to set up the plan to get benchmark information on available resources land use, labour charges, capital including cash and utilized equipment.

According the computation, the gross value can provide relative comparisons between treatments (plastic mulch and control) and can be given an economic indication of the relative potential to increase the producer of chilli net income.According the results of gross income and net profits were very obviously increased in the black and transparentplastic mulches than control in 2017 because plastic mulches conserved soil moisture, maintained a more even soil temperature, suppressed weed growth and increased soil organic matter.However, there will be needed the further studies of relating researches using this plastic mulching techniques on other vegetable and fruit crops to be achievement of more production, greater net profits and maximum gross incomes on the final products.

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ISOLATION AND FERMENTATION CONDITIONS OF SELECTED SOIL FUNGUS TT-27 BETWEEN YENANCHAUNG AND MAGWAY TOWNSHIP, MAGWAY REGION

Thida Than[1], Zar Zar Yin[2]

Abstract

Six soil samples were collected from six different places of Magway and Yenanchaung Township, Magway Region. Twenty-nine fungi were isolated from these soil samples. Isolation of soil fungi were carried out in Biotechnology Development Center (BDC) from Pathein University. Isolations of fungi were undertaken by the serial dilution method, and cultured on Blakeslee's Malt Extract Agar (BMEA Medium), Czapek-Dox Agar (CZA Medium) and Malt Extract Agar (MEA Medium). The isolated fungi were given as TT-1 to TT-29. In the preliminary study of antimicrobial activity, all fungi were evaluated by agar well diffusion method with seven test organisms. Among them, four fungi TT-22, 23, 24 and 27 exhibited the highest antimicrobial activities. Especially, TT-27 showed the highest antimicrobial activity (39.10mm) on *Escherichia coli*. Therefore TT-27 was selected and the fermentation conditions of TT-27 were carried out by the study of fermentation period, proper age, size, different carbon and nitrogen sources, fermentation medium (FM), effects of pH, temperature, static and shaking culture.

Keywords: soil fungi, antimicrobial activity, fermentation

Introduction

Soil fungi play an important role as major decomposers in the soil ecosystem. 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. (Hawksworth *et al*., 1995). Fungi are considered as a good natural source for a production of bioactive secondary metabolites that contain different bioactive agents including antibiotics, antitumor and antioxidants (Elaasser *et al.*, 2011). The secondary metabolite is obtained by fermentation process. During fermentation, the organisms produce the antibiotic material, which can then be isolated for used as a drug (Fenical, 1993).

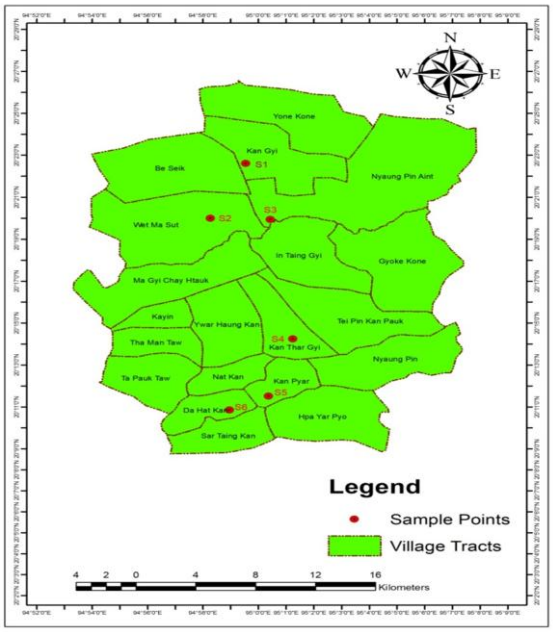
Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. The media used in fermentation process can either be synthetic or complex. Complex media are often used in enzyme and antibiotic production (Chistic, 1999). The composition of the fermentation medium must include the nutrient essential to support the growth of the microbial strains and the formation of the desired products. The fungi have been widely studied for their bioactive metabolites and sources of noval anticancer, antibacterial and anti-viral agents. Therefore, the present study was carried out the isolation and fermentation studies for antibacterial compounds from selected fungus. The aim and objectives of this study were to isolate the soil fungi from six different places, to observe the antimicrobial activities of selected fungi on seven test organisms and to know the optimal fermentation conditions of fungus TT-27.

Materials and Methods

Study Area and Collection of Soil Samples

Soil samples were collected from six different places between Yenanchaung and Magway Township, Magway Region from July, 2017 to August, 2017. These samples were isolated by using three media (MEA, BMEA and CZA).

[1] Lecturer, Department of Botany, Magway University
[2] Associate Professor, Department of Botany, Pathein University



Source from Geography Dept. Pathein University

Figure 1 Location map between Yeanchaung and Magway Township in Magway Region

Table 1 Six different soil samples collected between Yenanchaung and Magway Township

Samples Collected areas	pH	Soil type	Location
Gway gone	6.83	Sandy loam	N20º 22.613′ E 94º 59546′
Pay taw	6.94	Sandy loam	N20º 20.251′ E 94º 59.77′
Nyaung bin aing	6.98	Loamy Sand	N20º 19.944′ E 95º 0.433′
Kanthar gyi	6.84	Sandy loam	N 20º 14.249′ E 95º 1.247′
West of Kanbya	7.09	Sand	N 20º 11.514′ E 95º0.367′
Kan hla	4.6	Sand	N 20º 10.856′ E 54º 58.956′

Isolation of soil fungi by using serial dilution Method

1g of soil sample was introduced into a conical flask containing 99mL of distilled water. The flask was then 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} to10^{-7} dilution in separate test tubes and 0.5 mL each of the above dilution was separately transferred into sterile petridishes under aseptic condition. Chloramphenicol was added to the sterilized medium for preventing bacterial growth before pouring in to petri plate. The sterilized medium in conical flask was cooled down to about 45º and separately poured into each of the petridish containing the respective soil dilutions. The inoculated plates were shaken clock-wise and anticlockwise 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.

Screening for antimicrobial activities (NITE 2005)

The isolated fungi were grown on BMEA medium at room temperature for 5 days. After incubation period, these fungi inoculated into the seed medium (glucose 0.5 g, peptone 0.3 g, yeast extract 0.3 g, $K_2HPO_4$0.01 g, $CaCO_3$0.01 g, DW 100 mL at pH 6.5) for 3 days at room temperature. After three days, the seed medium (2%) was transferred into the fermentation medium (glucose 1.0 g, peptone 0.5 g, yeast extract 0.5 g, $K_2HPO_4$0.01 g, $CaCO_3$0.01 g, DW 100 mL at pH 6.5) and carried out for 3-10days and evaluated the antimicrobial activity by agar well diffusion method.

Screening of antimicrobial activity by agar well method (Collins, 1965)

1 day old culture test broth (0.2 mL) was added to 25 mL warm assay medium (glucose 1.0 g, peptone 0.3 g, KNO_3 0.1 g, DW 100 mL, agar 1.8 g) and thoroughly mixed and poured into plate. After solidification, the agar was left to set. Cork borer was used to make the wells (8 mm in diameter). And then, the fermented broth (20 µL) was carefully added into the well and incubated at room temperature for 24-48 hours. The diameter of the zones of inhibition around each well measured and recorded after 24-48 hours incubation.

Test organisms

Candida albicans NITE 09542, *Bacillus subtilis* IFO 905771, *Bacillus pumilus* IFO 905771, *Escherichia coli* AHU 5436, *Pseudomonas fluorescens* IFO 94307, *Agrobacterium tumefaciens* NITE 09678, *Staphylococcus aureus* AHU 8465, were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan).

Study on the effect of Fermentation Period

The fermentation period of isolated fungi was studied by 3 days to 10 days and 5 mL of seed culture medium was added to 25 mL of fermentation medium. The flasks were incubated at room temperature and the fermentation medium was assayed for antimicrobial activity by using agar well diffusion method.

The effect of ages of inoculum

In this study, incubation time (48, 60, 72, 84, 96, 108, 120, 132, 144 hrs) were used for the production of antimicrobial metabolites 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. Fermentations were worked from 48 to 144 hrs and antibacterial activity was tested by agar well diffusion method.

The effect of sizes of inoculum

The inoculum level (5%, 10%, 15%, 20%, 25%, 30%, 35%) were used for the production of antimicrobial metabolite. In the investigation of size of inoculum, well sporulated selected strain was taken and added into 100 mL of seed culture medium and incubated for 3 days at room temperature. After that 3days old seed cultures were transferred to 100 mL conical flasks containing 25 mL of fermentation medium respectively. The flasks were incubated for 5 days at room temperature and the fermentation medium was assayed for antimicrobial activity by using agar well diffusion method.

Effects of carbon and nitrogen sources for growth

The different carbon sources such as potato, corn, carrot, oat, fructose, tapioca powder, lactose, soluble starch powder, mannitol, maltose, sucrose, glucose, glycerol, molasses and sweet potato were used for growth morphology.And then the different nitrogen sources such as NH_4Cl, NH_4SO_4, NH_4NO_3, $NaNO_3$, KNO_3, soybean, peanut, meat extract, peptone, gelatin, urea, yeast extract, asparagine, casein and fish cake were also used.

The effects of pH

Optimum pH was studied by varying the pH as 4, 5, 6,7, 8, 9 and 10. The different pH of seed medium was adjusted by using HCl and NaOH. The fermentation medium was assayed for antibacterial activity.

The effects of temperature

The selected fungus was inoculated and incubated at five different temperature by using 20°C, 25°C, 30°C, 35°C and 40°C.The fermentation medium was carried out 5 days and antibacterial activity was studied by agar well diffusion method.

Effects of carbon and nitrogen utilization

There were variations in the level of antibacterial activity when the different carbon potato, corn, carrot, oat, fructose, tapioca powder, lactose, soluble starch, mannitol, maltose, sucrose, glucose, glycerol, molasses and sweet potato were used. The different nitrogen sources such as NH_4Cl, NH_4SO_4, NH_4NO_3, $NaNO_3$, KNO_3, soybean, peanut, meat extract, peptone, gelatin, urea, yeast extract, asparagine, casein and fish cake were also used for fermentation.

Effects of fermentation medium

Fermentation media were undertaken with suitable conditions of 25% sizes and 108 hrs ages of inoculum with twenty three different media. Fermentation media was carried out for 5 days and antibacterial activity test was carried out every 24 hrs.

Comparison of static and shaking culture

100 mL conical flask containing 50 mL of the best fermentation medium was incubated on the rotary 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 assay method.

Results

Isolation of soil fungi

In this investigation, 29 fungi were isolated from six different soil samples between Yenanchaung and Magway Township, Magway Region. Three isolated fungi were collected from Gwaygone Village, four from Pawtaw, four from Nyaingbin Aing, thirteen from Kanthargyi, one from West Kanbyar and six fungi from Kanhla Village. These results were shown in Table 2 and Figure 2-3.

Table 2 Isolation of fungi from soil samples

No.	Samples Collected areas	BMEA Medium	MEA Medium	CZA Medium	Total
1	Gway gone	TT-3, TT-10, TT-22	TT-16	-	4
2	Pay taw	TT-1, TT-17, TT-27	TT-12	-	4
3	Nyaung bin aing	TT-8	TT-14, TT-15, TT-26	-	4
4	Kanthargyi	TT-4, TT-5, TT-6, TT-7, TT-9, TT-11, TT-18, TT-19, TT-23, TT-24, TT-28	TT-21, TT-29	-	13
5	West of Kanbya	TT-2,	-		1
6	Kanhla	TT-13, TT-20	-	TT-25	3
Total Isolated fungi		**21**	**7**	**1**	**29**

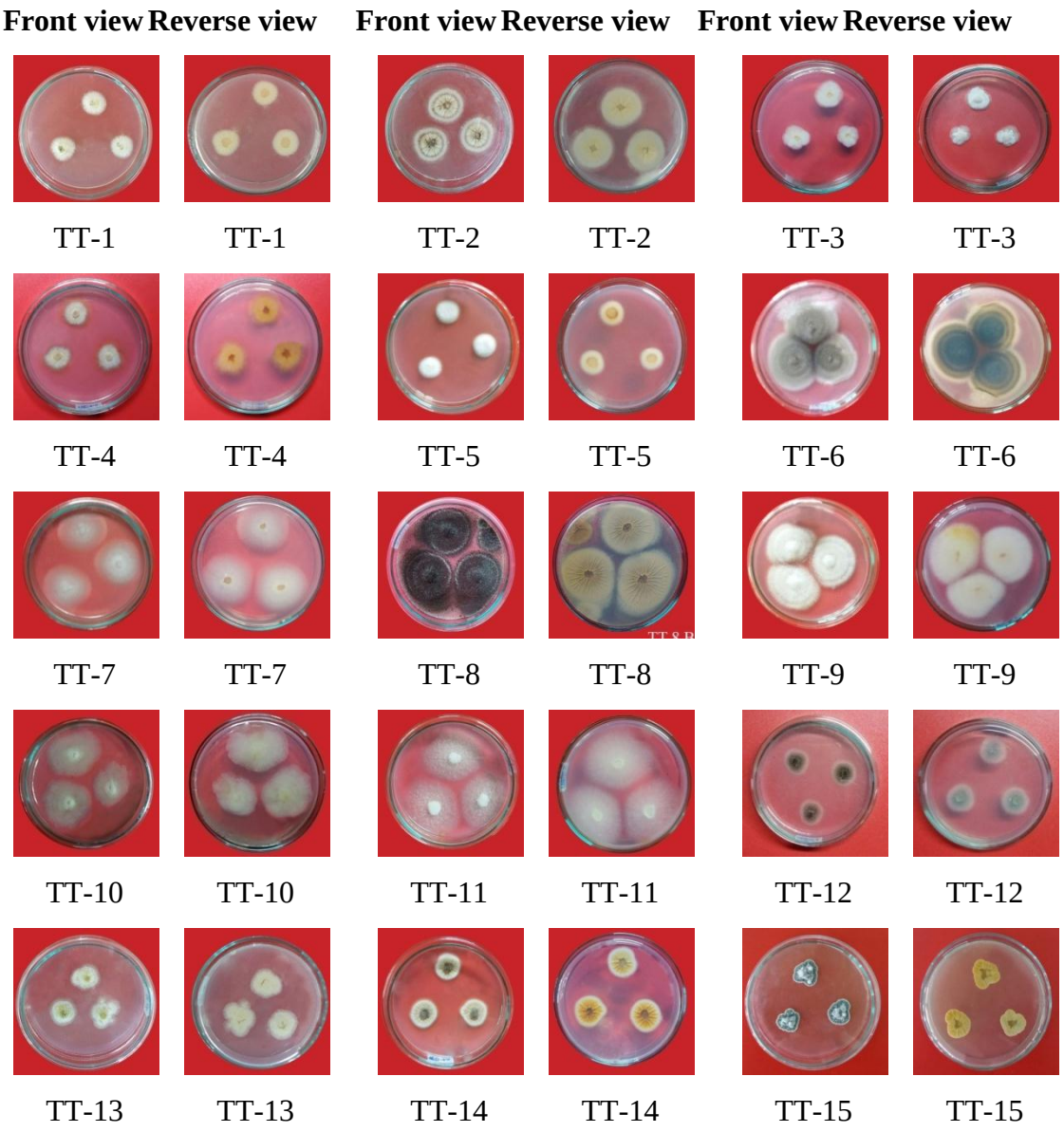
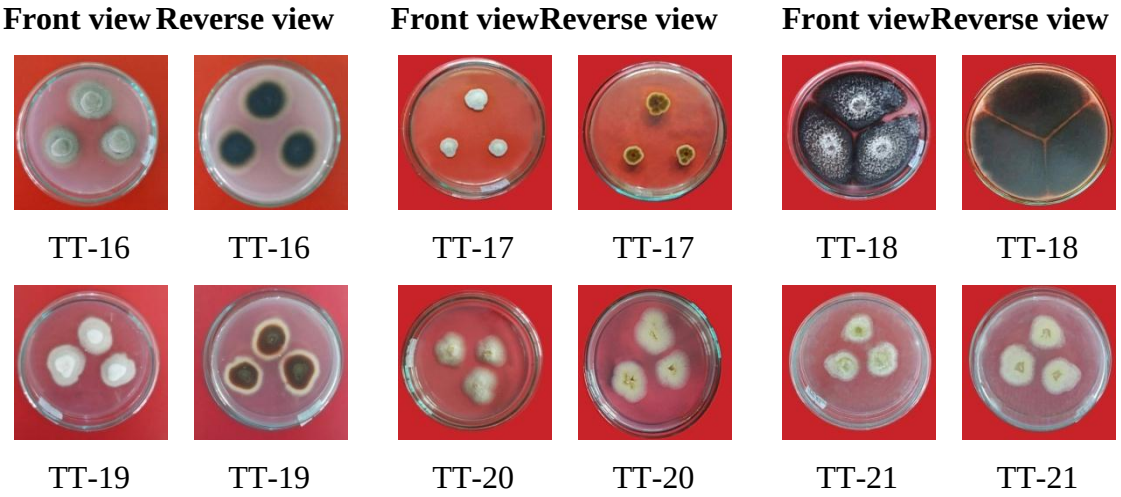


Figure 2 Mophology of isolated fungi on BMEA medium (TT-1 to TT-15)



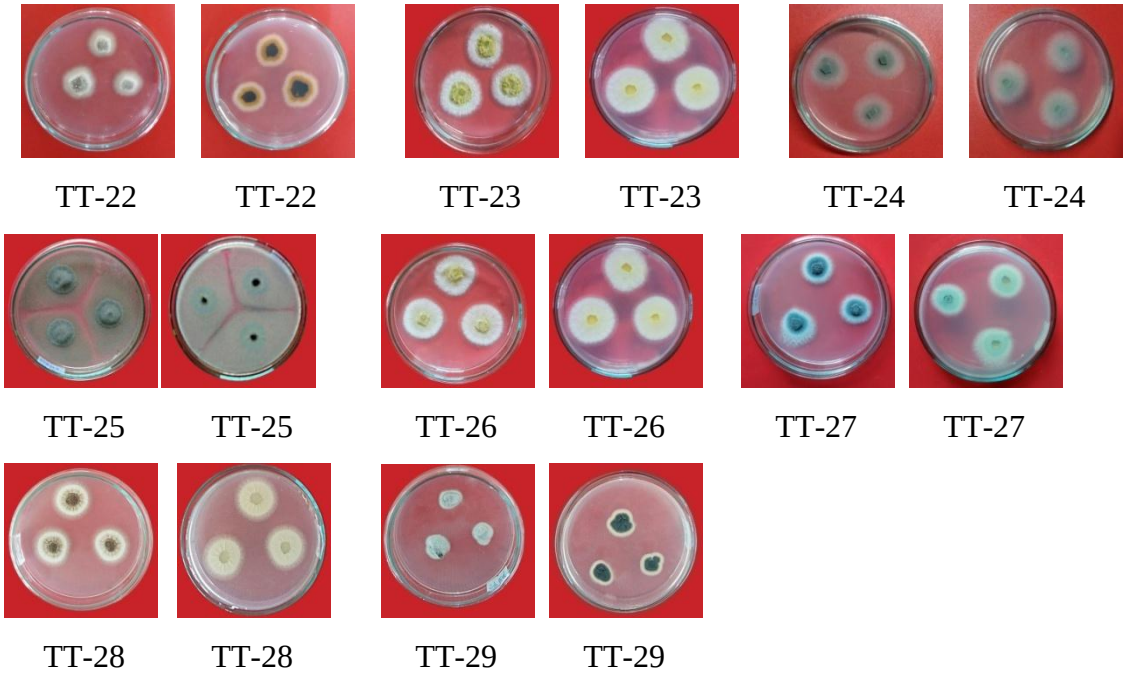


Figure 3 Mophology of isolated fungi on BMEA medium (TT-16 to TT-29)

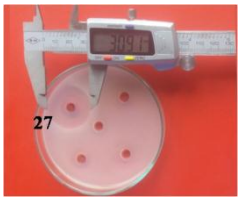
Antimicrobial activities of isolated fungal strains

Four isolated stains (TT-22, 23, 24 and TT-27) were tested for antimicrobial activity with seven test organisms. Agar well diffusion methods were employed for assay performance. Among them, TT-27 showed the maximum antimicrobial activities on all test organisms. TT-27 showed the highest antimicrobial activity (36.20 mm, 36.37 mm, 30.91 mm and 21.86 mm) at 5 days fermentation periods on *C. albicans*, *B. pumilus*, *B. subtilis* and *S. aureus* respectively. Moreover the highest antibacterial activities (39.10 mm, 34.92 mm and 30.95 mm) were found at 6 days fermentation periods on *E. coli*, *A. tumefaciens* and *P. fluorescens.* These results were shown in Table 3 and Figure 4.

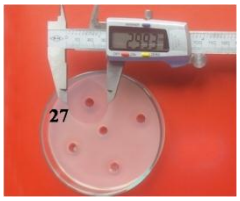
Table 3 Antimicrobial activity of isolated fungus TT-27 on the fermentation period

Fermentation period (days)	Test organisms and antimicrobial activity (mm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
3	14.47	14.32	13.00	16.74	15.94	15.68	14.47
4	16.36	15.36	22.31	19.43	20.18	19.74	16.19
5	36.20	28.25	36.37	28.64	30.91	21.02	21.86
6	29.93	34.92	35.33	39.10	29.13	30.95	18.39
7	21.58	19.55	23.96	20.31	20.33	20.99	15.34
8	18.17	13.97	15.68	12.17	14.17	16.68	12.10

1-Candida albicans 4-*Escherichia coli* *7- Staphylococcus aureus*
2-*Agrobacterium tumefaciens* *5- Bacillus subtilis*
3-*Bacillus pumilus* 6-*Pseudomonas fluorescens*



Bacillus subtilis



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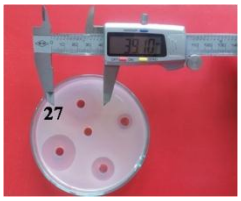
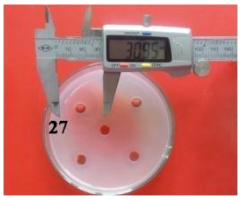
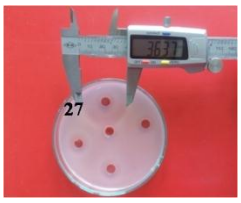


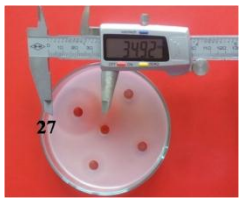
Escherichia coli



Pseudomonas fluorescens



Bacillus pumilus



Agrobacterium tumefaciens

Staphylococcus aureus

Figure 4 The Antimicrobial activity of isolated fungus TT-27 against seven test organisms

The effect of ages of inoculum of TT-27 on *Escherichia.coli*

In the effect of age of inoculum, TT-27 was investigated by using 48, 60, 72, 84, 96, 108, 120, 132 and 144 hrs old culture age of inoculums. The results showed that 108 hrs age of inoculum gave the highest activities (20.21 mm) followed by (19.81 mm) at 96 hrs and (19.53 mm) at 84 hrs age of inoculum.

Table 4 The effect of ages of inoculum

Sr. No	Age of Inoculum (hrs)	Antibacterial Activity (mm)
1	48	14.27 mm
2	60	16.68 mm
3	72	17.63 mm
4	84	19.53 mm
5	96	19.81 mm
6	108	20.21 mm
7	120	15.31 mm
8	132	14.90 mm
9	144	12.64 mm

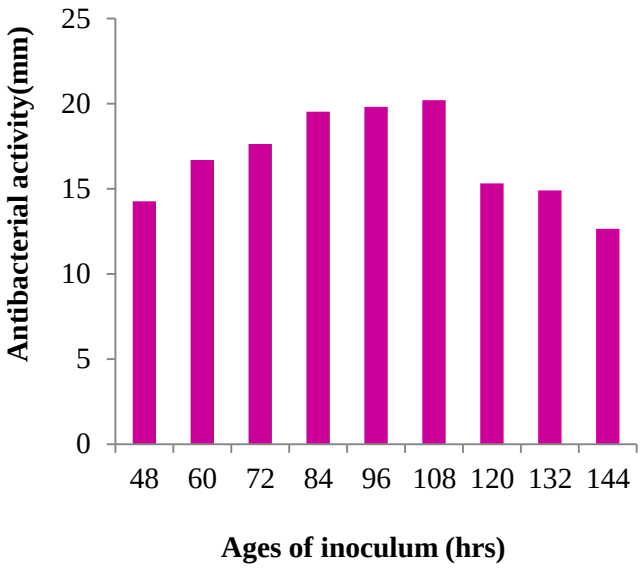


Figure 5 The effect of ages of inoculum

The effects of sizes of inoculums of TT-27 on *Escherichia. coli*

In this research work, the effect of size of inoculums was studied by using 5%, 10%, 15%, 20%, 25% and 30% inoculums (Table 5). Using 25% inoculums showed significantly higher (23.09 mm) than others, followed by 10% and 15% (20.05 mm and 21.09 mm) respectively in Figure 6.

Table 5 The effect of sizes of inoculum

Size of inoculums (%)	Antibacterial Activity (mm)
5	19.51mm
10	20.05mm
15	21.09mm
20	16.14mm
25	23.09mm
30	18.05mm

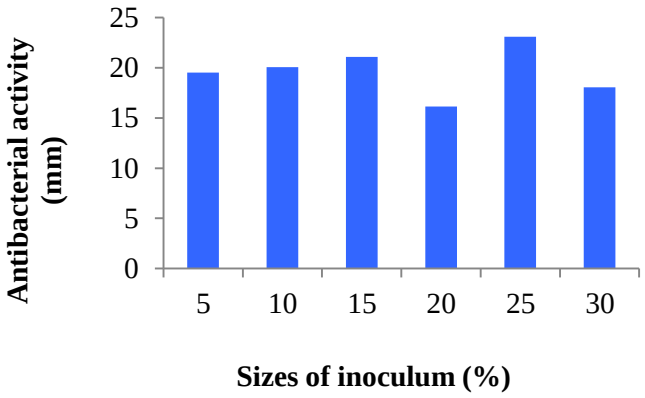


Figure 6 The effect of sizes of inoculum

Effects of Carbon and Nitrogen sources on TT-27

The different carbon sources such as potato, corn, carrot, oat, fructose, tapioca powder, lactose, soluble starch, mannitol, maltose, sucrose, glucose, glycerol, molasses and sweet potato were used for growth morphology of selected fungus TT-27. The selected fungus TT-27 showed that molasses, sweet potato and glucose were excellent growth and good growth on potato, carrot, fructose, mannitol, maltose, sucrose and glycerol but the other five carbon sources showed poor results. The nitrogen sources such as casein, $NaNO_3$, gelatin and asparagine showed the excellent growth and good growth on KNO_3 and urea but the remaining nine nitrogen sources were showed poor growth on TT-27.

Table 6 Growth of TT-27 on carbon and nitrogen sources

Carbon Sources	Growth Size (cm)	Nitrogen Sources	Growth Size (cm)
Potato	2.30 cm	NH_4Cl	1.40 cm
Corn	1.80 cm	NH_4SO_4	1.70 cm
Carrot	2.50 cm	NH_4NO_3	1.50 cm
Oat	1.90 cm	$NaNO_3$	3.70 cm
Fructose	2.70 cm	KNO_3	2.90 cm
Tapioca	2.10 cm	Soybean	2.20 cm
Lactose	1.90 cm	Peanut	2.20 cm
Soluble starch	2.00 cm	Meat	2.40 cm
Mannitol	2.60 cm	Peptone	2.40 cm
Maltose	2.90 cm	Gelatin	3.30 cm
Sucrose	2.80 cm	Urea	2.80 cm
Glucose	3.00 cm	Yeast Extract	2.40 cm

Carbon Sources	Growth Size (cm)	Nitrogen Sources	Growth Size (cm)
Glycerol	2.30 cm	Asparagine	3.10 cm
Molasses	3.40 cm	Casein	4.10 cm
Sweet Potato	3.00 cm	Fish cake	2.10 cm

3.00cm -3.40cm = Excellent
2.30cm -2.90 cm = Good
1.80cm -2.00 cm = poor

3.10cm -4.1cm = Excellent
2.60cm -3.00 cm = Good
1.40cm -2.50 cm = poor

Antibacterial activity of TT-27 on carbon and nitrogen utilization

There were variations in the level of antibacterial activity when the different carbon such as potato, corn, carrot, oat, fructose, tapioca powder, lactose, soluble starch, mannitol, maltose, sucrose, glucose, glycerol, molasses and sweet potato were used. The highest antibacterial activity was obtained by the addition of maltose and carrot (each 24.91mm), followed by sucrose (22.75mm), mannitol (22.75mm), corn (22.08mm), tapioca powder (21.68mm), glucose (21.36mm) and glycerol (20.79mm), oat (18.50mm), lactose (18.30mm), potato (18.27mm), molasses (17.87mm), sweet potato (17.40mm) and fructose (14.69mm) and soluble starch (14.24mm) respectively.

Similarly, when the various nitrogen sources were added, the significant inhibition zones (26.05 mm, 24.23 mm, 23.18 mm, 22.98 mm, 22.37 mm and 20.83 mm) were obtained in soybean, peptone, KNO_3, asparagine, meat and $NaNO_3$. TT-27 showed the moderate inhibition zones in peanut (19.95 mm), yeast (19.37 mm), NH_4NO_3 (19.31 mm), gelatin (19.04 mm), urea (18.81 mm), casein (17.80 mm), NH_4Cl (16.95 mm), fish cake (16.91 mm) and NH_4SO_4 (15.53mm) were regarded as poor inhibition zone. These results were shown in Figure (7) and (8).

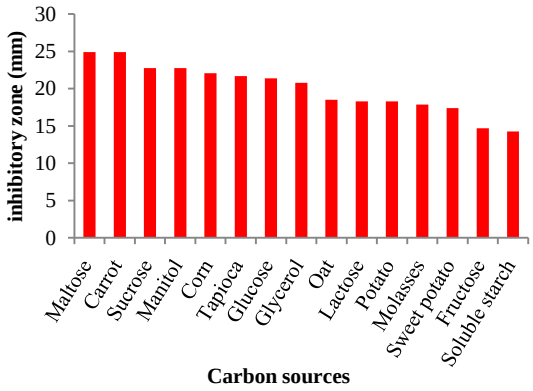


Figure 7 The effect of carbon utilization on fermentation

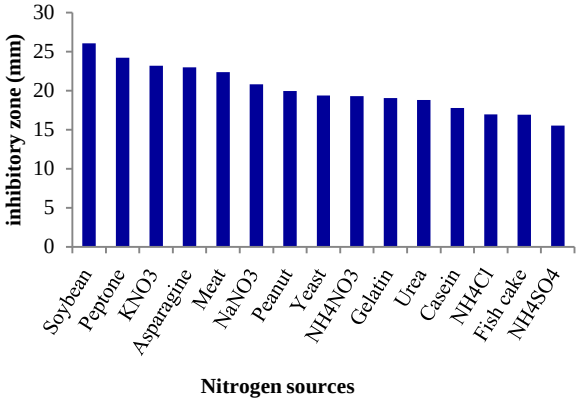


Figure 8 The effect of nitrogen utilization on fermentation

Antibacterial Activity medium of TT-27 on various fermentation media

In the fermentation medium (FM), the best antibacterial activity was obtained by using FM-1, (glucose and peanut, 29.17 mm) followed by FM-2, (maltose and meat, 26.79 mm) and FM-3, (corn and meat, 26.70 mm) respectively.

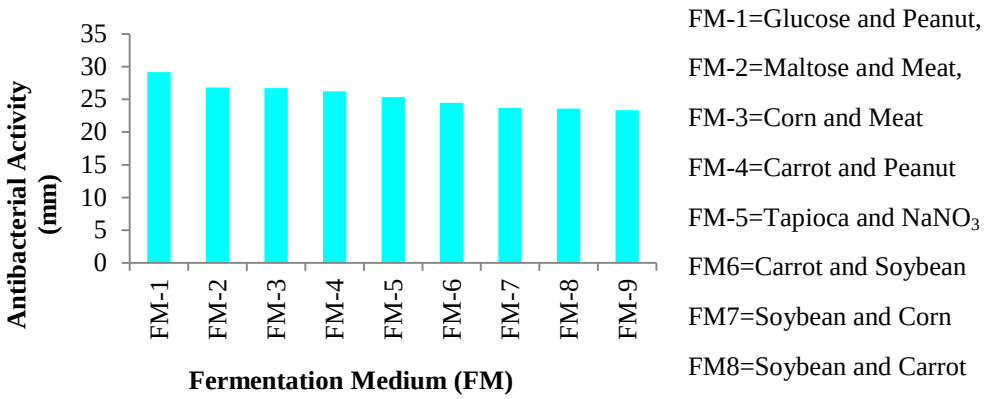


Figure 9 Antibacterial activity of TT-27 on various fermentation media

The effects of pH and temperature

Effect of pH was studied by varying pH 4, 5, 6, 7, 8, 9 and 10. The best antibacterial activity was found at pH-5 (22.10 mm).

In the temperature effect, TT-27 was incubated at changing temperature 20°C, 25°C, 30°C, 35°C and 40°C. The increased activity of antibacterial metabolite was recorded at temperature 25°C (18.44 mm).

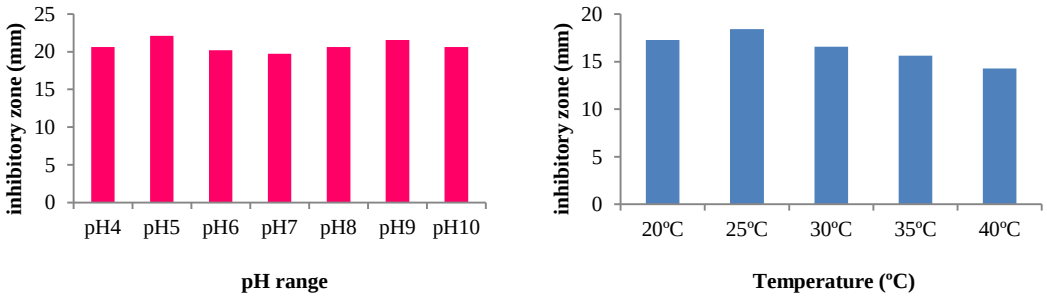


Figure 10 The effect of different pH and temperature

Comparison of static and shaking culture

In the comparison of shaking and static culture, the antibacterial activity of shaking culture (23.40 mm) was more than that of static culture (20.50 mm).

Static culture Shaking culture

Figure 11 Comparison of static and shaking culture

Discussion and Conclusion

The present research was focused by isolation of soil fungi between Yenanchaung and Magway township and preliminary antimicrobial activities with seven test organisms. In all

fungal strains, twenty strains showed different antimicrobial activities. Among them, fourteen selected fungi TT-1, 3, 4, 8, 15, 17, 18, 19, 22, 23, 24, 26, 27 and 29 exhibited moderate antimicrobial activities. TT-27 showed the best antibacterial activity (22.76mm) at five days on *E. coli.* Thus, TT-27 and *E. coli* was selected for the optimum fermentation conditions. TT-27 reached the highest activities (34.92mm) in 6 days fermentation period. To study the optimization of inoculums age, incubation time (48 ,60 ,72 ,84, 96, 108, 120, 132 and 144 hrs) were used and the highest antibacterial activity was found at 108 hrs. In the proper size of inoculums, 25% was the most suitable and the maximum activities (23.09mm) in TT-27 followed by 15% (21.09 mm) and 10% (20.05mm) respectively. In the carbon sources, the colony of TT-27 was the excellent growth on molasses, sweet potato and glucose.

The antibacterial substance production of TT-27 was influenced by addition of maltose and carrot reached the highest activity (24.91mm) followed by sucrose, mannitol and corn. Nitrogen is required by all organisms as an essential nutrient. The nature of the nitrogen source has notable effect on the production of antibacterial metabolites in TT-27. Especially, TT-27 showed the moderate growth on nearly all nitrogen sources. Colony morphology of TT-27 was excellent growth on casein, $NaNO_3$, gelatin and asparagine. Maximum antibacterial metabolite of TT-27 was found on soybean (26.05mm) followed by peptone, KNO_3, asparagines ad meat respectively as nitrogen sources. This result was in agreement with the description of EL-Gammal, 1986. He found that peptone has been reported by the suitability of nitrogen sources for the production of metabolites from microorganisms. 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 the fermentation (El-Tayeb *et al.,* 2004).

In the fermentation medium (FM), FM-1 gave the highest antibacterial activity (29.17 mm) by using glucose and peanut. Effects of pH was studied by varying pH-4, 5, 6, 7, 8, 9 andpH-10. The best antibacterial activity was found as pH- 5 (22.10mm). Effects of temperature was studied by varying from 20°C, 25°C, 30°C, 35°C and 40°C. The best antibacterial activity for temperature was found at 25°C (18.44mm), followed by 20°C(17.28mm), 30°C(16.59mm), 35°C(15.63mm) and 40°C(14.29mm) respectively. Thus the results of optimum fermentation conditions indicated that antibacterial metabolites of TT-27 were obtained by optimally 6 days fermentation period, in the presence of maltose and soybean, 108 hrs age of inoculums, 25% inoculums size, FM-1, pH-5, temperature 25°C and shaking culture. It was concluded that the present study revealed to observe the fermentation period of four isolated fungi and to investigate the optimization parameters of fermentation conditions on TT-27 against *E coli*.

Acknowledgements

I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, University of Pathein and Professor Dr Wah Wah Lwin, Department of Botany, University of Pathein 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, University of Pathein, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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ISOLATION AND FERMENTATION CONDITIONS OF SOIL FUNGUS PP-15 FROM GAWECHAUNG FORT

Phyu Phyu Aung[1], Zar Zar Yin[2]

Abstract

Soil samples were collected from six different places of Gawechaung fort, Magway Township, Magway Region. Thirty fungi were isolated from these six different soil samples. Isolations of fungi were undertaken by the serial dilution method and cultured by using Blakeslee's Malt Extract Agar (BMEA Medium), Czapek-Dox Agar (CZA Medium) and Malt Extract Agar (MEA Medium). Isolated fungi were given as PP-1 to PP-30. Antimicrobial activities of these fungi were evaluated by agar well diffusion assay with seven test organisms. Among them, ten fungal strains showed the antimicrobial activity.Especially, PP-15 gave the best antibacterial activity on *Agrobacterium tumefaciens* NTTE 09678.Therefore, different fermentation parameters of PP-15 were studied by the fermentation period, proper age and size of inoculums, effect of various carbon and nitrogen sources, pH, temperature, fermentation medium, shaker and static on *Agrobacterium tumefacines*.

Key words: Soil fungi, Antimicrobial activity, Fermentation

Introduction

Life on earth would have been impossible without microorganism in nature. There are numerous varieties which are living on earth and are deeply involved with human life. Microorganisms have significant function in ecosystems and are found in all kinds of habitats. Soil microbiology is the study of organisms in soil, their function and how they affect soil properties. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Soil sample is the most effective and popular materials for especially isolating a number of microorganisms such as fungi (Harayama, *et al.,* 2002).

Soil fungi play an important role as major decomposer in the soil ecosystem. There are about 75,000 species of soil fungi in the world (Finlay, 2007). Antibiotic is a drug used to treat infection caused by bacteria that can cause illness to humans and animals. Antibiotic functions to inhibit or destroy the bacterial cells that cause certain disease (Duerden,1993).The fermentation process is basically dependent on the transformation of carbohydrate, proteins and lipids to acidic, alcoholic and organic metabolites. Production of antibiotic metabolite has been known to be influenced by media components and cultural conditions, such as aeration, agitation, pH, temperature and glycerol concentration, which vary from organism to organism (Iwai *et al*.,1982).

This research paper aims to investigate the isolation of soil microorganisms and to study the different soil microorganisms from various soil samples and to investigate the effect of fermentation, pH, temperature, static and, shaker culture of selected fungus on *Agrobacteriumtunefaciens.*

Materials and Methods

Collection of Soil Samples

Soil samples were collected from Gawechaung Fort in Magway Township, Magway Region. Soil samples of six different places were collected during July 2017 to August 2017.

[1] Assistant Lecturer, Department of Botany, Magway University
[2] Associate Professor, Department of Botany, Pathein University

Table 1 Six Different Soil Samples Collected from Gawechaung fort

Soil samples	Sample collected areas	pH	Soil Type	Location
S-I	South of Fort	10.15	Loamy Sand	N 19˚ 57.863' E 95˚ 3.93'
S-II	Front of fort landmark	8.66	Sandy Loam	N 19˚ 57.942' E 95˚ 3.951'
S-III	Entry Street of fort	7.59	Loamly Sand	N 19˚ 57.509' E 95˚ 4.76'
S-IV	West of fort	7.79	Loamly Sand	N 19˚ 57. 905' E 95˚ 3.906'
S-V	North East of fort	7.63	Sandy loam	N19˚ 57. 905' E 95˚ 3.906'
S-VI	Short landmark of Gawechaung	7.77	Sandy Loam	N19˚ 57.873 ' E 95˚ 4.519'

Isolation of Fungi From the Soil Samples

The soil fungi were enumerated by serial dilution method (Dubey, 2002) and media such as Blakeslee's Malt Extract Agar (BMEA Medium), Czapek-Dox Agar (CZA Medium) and Malt Extract Agar (MEA Medium).

Serial dilution Method

1g of soil sample was introduce into a conical flask containing 99 mL of distilled water. The flask was then 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} to10^{-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. Chloramphenicol was added to the sterilized medium for preventing bacterial growth before pouring into petri plate. The sterilized medium in conical flask was cooled down to about 45ºC and separately poured into each of the petri dish containing the respective soil 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 27ºC-30ºC for 3-6 days(Dubey , 2002).

Preliminary study for antimicrobial activity

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

Screening of Antimicrobial Activity by Agar Well Diffusion Method

1 day old culture test broth (0.01mL) was added to 25mL 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 (20µL) 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 hoursincubation (Collins, 1965).

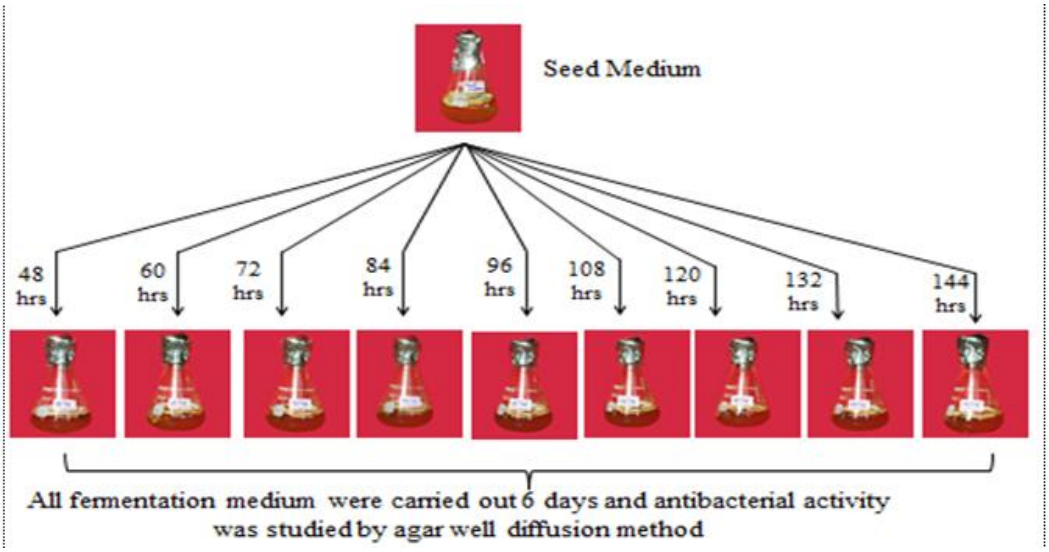


Figure 1 Study on the effects of ages of inoculums of PP-15

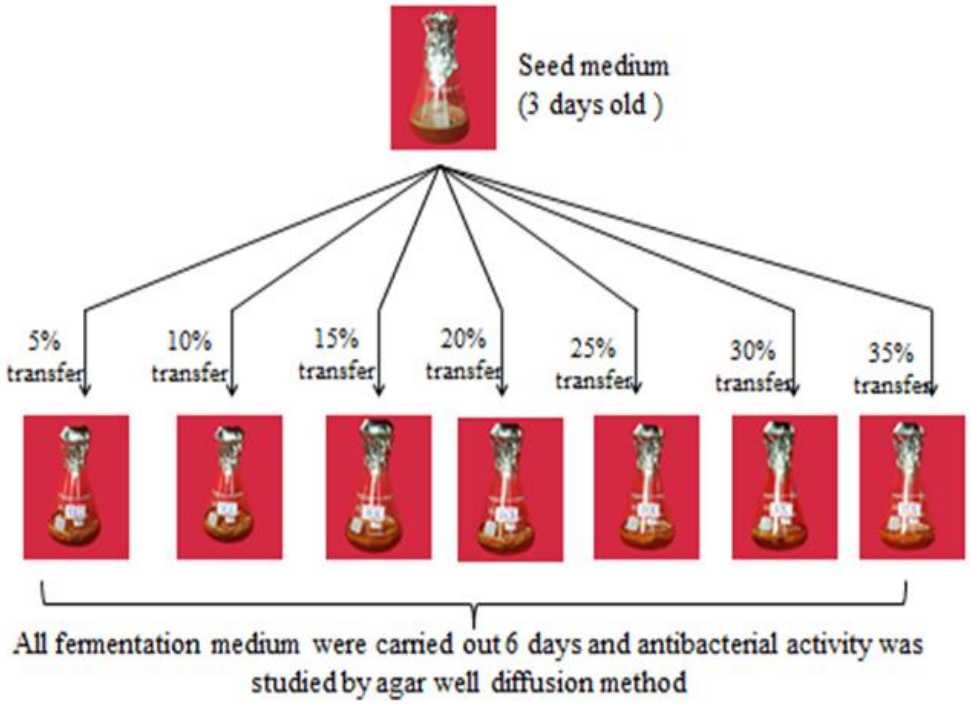


Figure 2 Study on the effects of sizes of inoculums of PP-15

Effect on the Carbon and Nitrogen Utilization of selected fungus(PP-15)

Optimal fermentations are 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 carrot, corn powder, dextrose, fructose, glycerol, glucose, lactose, maltose, mannitol, molasses, oat, potato, rice powder, soluble starch, sucrose, xylose and tapioca powder were used. Nitrogen sources such as asparagine, casein, fish cake, gelatin, KNO_3 malt extract, meat extract, $NaNO_3$, NH_4N_3, $(NH_4)_2SO_4$, peanut cake, NH_4CL, peptone, polypeptone, rice bran, soybean, urea and yeast extract were also used.

The effect of pH on fermentation

Effects of different pH were used for antibacterial activity of pH 4, 5, 6, 7, 8 and 9.These different pH were adjusted by NaOH and HCL.

The effect of temperature on fermentation

The selected fungus PP-15 was inoculated and incubated at five different temperature by using 20ºC, 25ºC, 30ºC, 35ºC and 40ºC.

Study on the fermentation media of PP-15

Fermentation was undertaken with suitable conditions of 10% sizes and 72 hrs ages of inoculum with fourteen different media. Fermentation was carried out for 6 days and antibacterial activity test was carried out every 24 hrs.

Comparison of static culture and shaking culture

100 mL conical flask containing 50 mL of the best fermentation medium was incubated on the rotary shaker (100 rpm) 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.

Results

Isolation of fungi from soil samples

In this investigation, 30 fungi were collected from the six different soil samples of Gawechaung Fort, Magway Township, Magway Region. Isolated fungi PP-1 to PP-5 were collected from south of Fort , PP-6 to PP-10 from front of Fort landmark, PP-11 to PP-15 from entry street of Fort, PP-16 to PP-20 from west of Fort, PP-21 to PP-25 from north east of Fort and PP-26 to PP-30 from short landmark of Gawechaung Fort.

Table 2 Isolated Fungi from Soil Samples

Soil Samples	Isolated Fungi
S –I	PP- 1 to PP-5
S -II	PP- 6 to PP-10
S -III	PP- 11 to PP-15
S -IV	PP- 16 to PP-20
S –V	PP-21 to PP-25
S-VI	PP-26 to PP-30

Front view Reverse view Front view Reverse view Front view Reverse view

(PP-1) (PP-2) (PP-3)

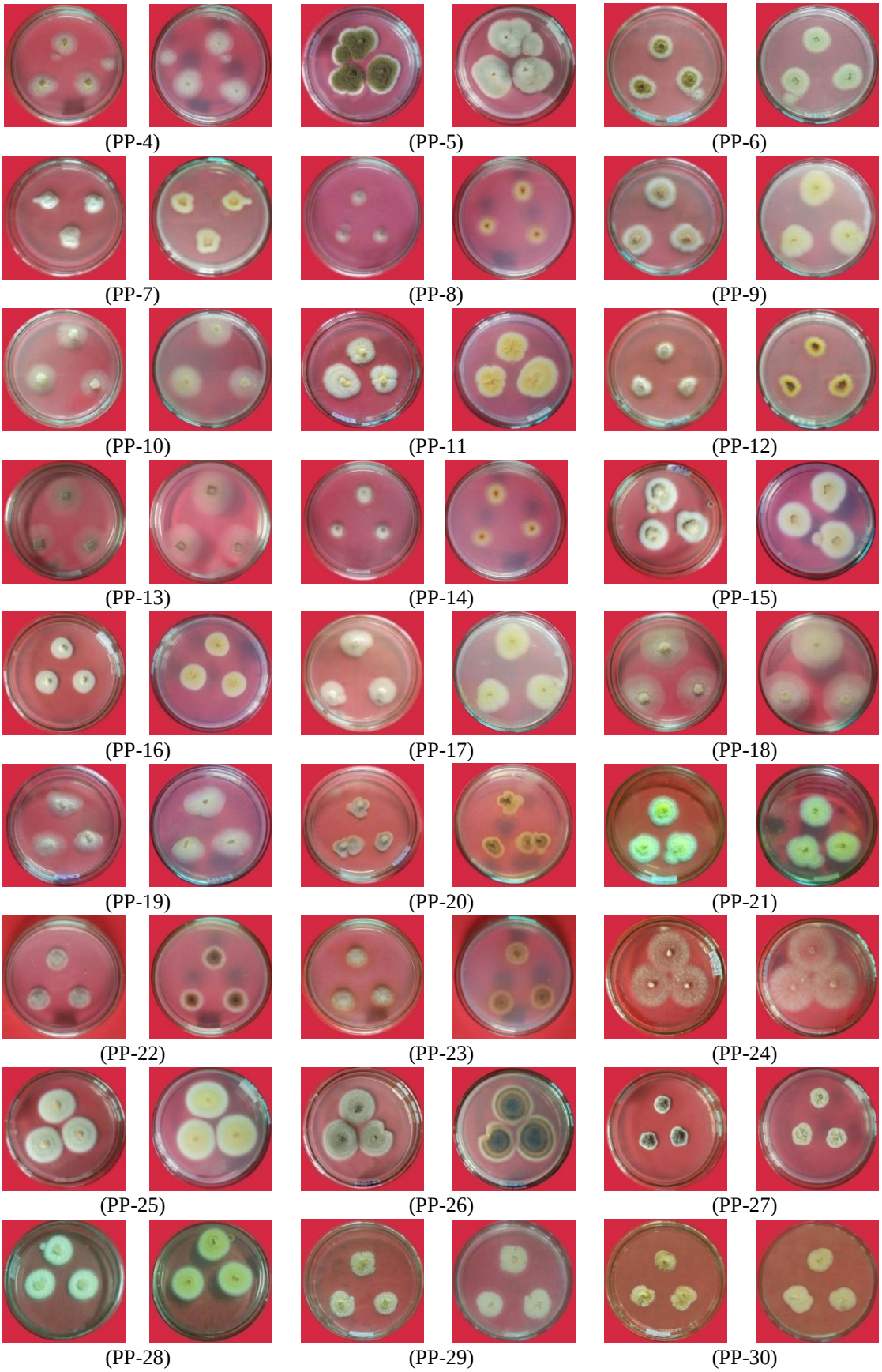


Figure 3 Morphological Character of Isolated Fungi PP-1 to PP-30

Isolated Fungi and their Antibacterial Activity

In this study, ten fungi strains were tested with *Agrobaterium tumefaciens* by agar well diffusion method. PP-15 gave the best activity on *Agrobaterium tumefaciens*.

Table 3 Isolated Fungi and their Antimicrobial Activity

No	Isolated fungi	Fermentation period of (days) and inhibition zone (mm)				
		3 days	4 days	5 days	6days	7days
1	PP-2	-	-	-	-	-
2	PP-3	-	-	-15.51	21.37	14.72
3	PP-8	17.34	12.79	16.68	20.02	-
4	PP-12	-	-	-	-	-
5	PP-14	15.22	17.97	19.09	15.32	-
6	PP-15	14.56	18.96	25.48	27.02	24.18
7	PP-21	14.29	12.34	16.16	-	-
8	PP-23	12.41	21.42	14.`11	24.50	17.25
9	PP-27	-	13.73	16.36	18.28	15.84
10	PP-28	14.30	15.13	21.97	23.30	23.29

Figure 4 Antibacterial Activity of Ten Selected Fungi Against *Agrobaterium tumefaciens*

The effects of ages of inoculum on the fermentation

In the effect of age of inoculum, PP-15 was investigated by using 48, 60, 72, 84, 96, 108, 120, 132 and 144 hrs old culture age of inoculums. The results showed that 72hrs age of inoculum gave the highest activity (25.35 mm) followed by (23.01 mm) at 96 hrs and (22.66 mm) at 84 hrs age of inoculum.

Table 4 The Effects of Ages of inoculums on the Fermentation for PP-15

Sr. No	Fermentation Period (hrs)	Antibacterial activity (mm)
1	48	20.21
2	60	21.78
3	72	25.53
4	84	22.66
5	96	23.01
6	108	22.35
7	120	17.46
8	132	16.32
9	144	16.04

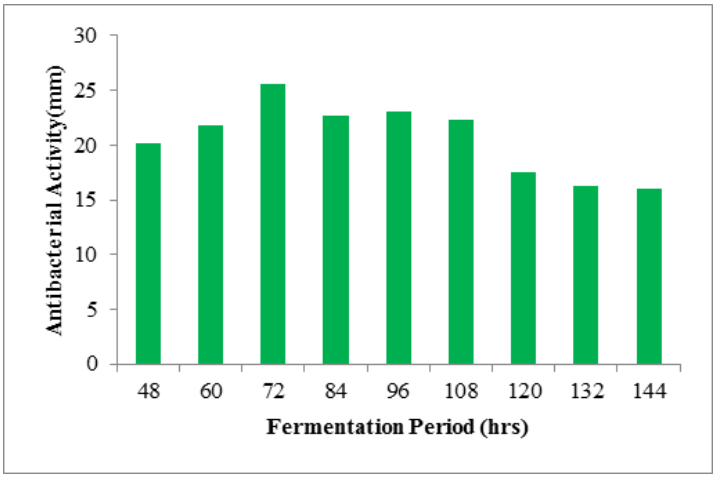


Figure 5 The Effects of Ages of inoculums on the Fermentation for PP-15

The effects of sizes of inoculums on the fermentation for PP-15

In this research work, the effect of size of inoculums was studied by using 5%, 10%, 15%, 20%, 25%, 30% and 35% inoculum. Using 10% inoculums showed significantly higher (27.95 mm) than others, followed by 30% and 20% (23.52 mm and 22.05 mm) respectively.

Table 5 The Effects of Size of inoculums on the Fermentation for PP-15

Sr. No	Size of inoculums (%)	Antibacterial activity (mm)
1	5	21.10
2	10	27.95
3	15	19.22
4	20	22.05
5	25	20.24
6	30	23.52
7	35	18.47

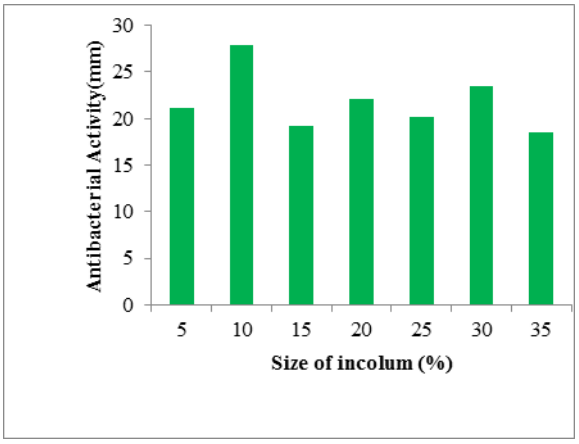


Figure 6 The Effects of Size of inoculums on the Ferment

The effects of carbon and nitrogen sources utilization for growth of PP-15

The selected fungus PP-15showed that the carbon sources such as glucose, and tapioca powder were excellent growth, good growth on starch, poor growth on corn powder, while other nine carbon sources showed moderate growth .The excellent growth were found on gelatin and yeast extract, good growth on Asparagine, moderate growth on malt extract, peptone, NH4CL, KNO_3, $(NH_4)_2SO_4$ and left four nitrogen sources were poor growth.

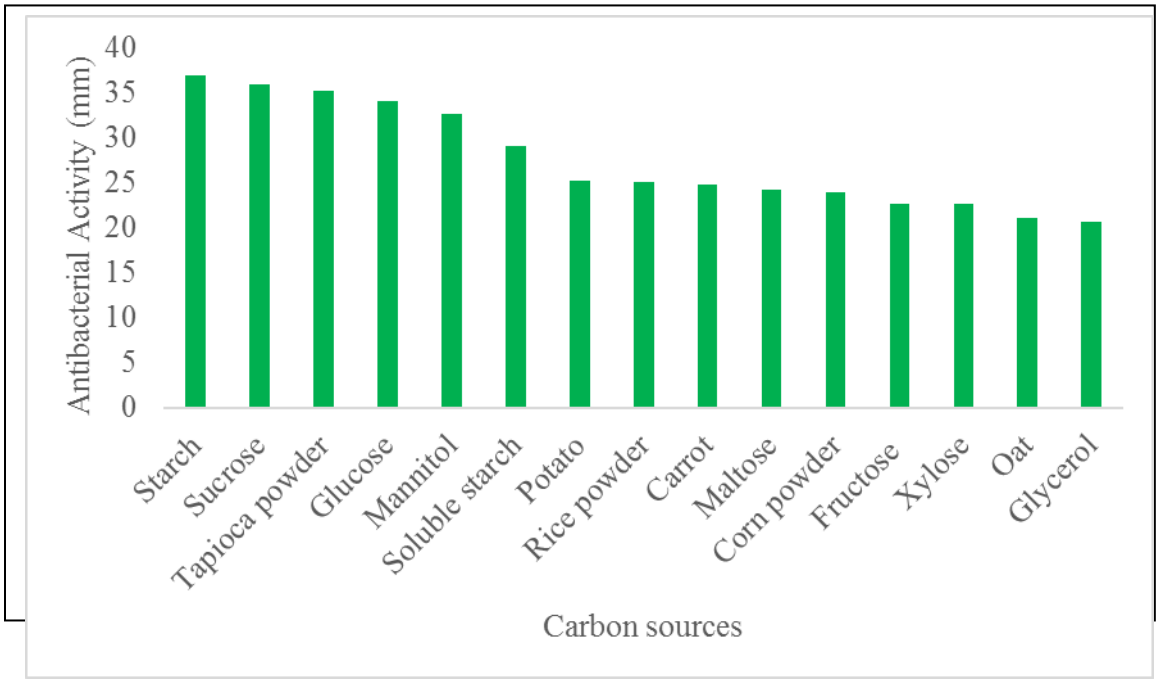
Table 6 Growth of PP-15 on carbon and nitrogen sources

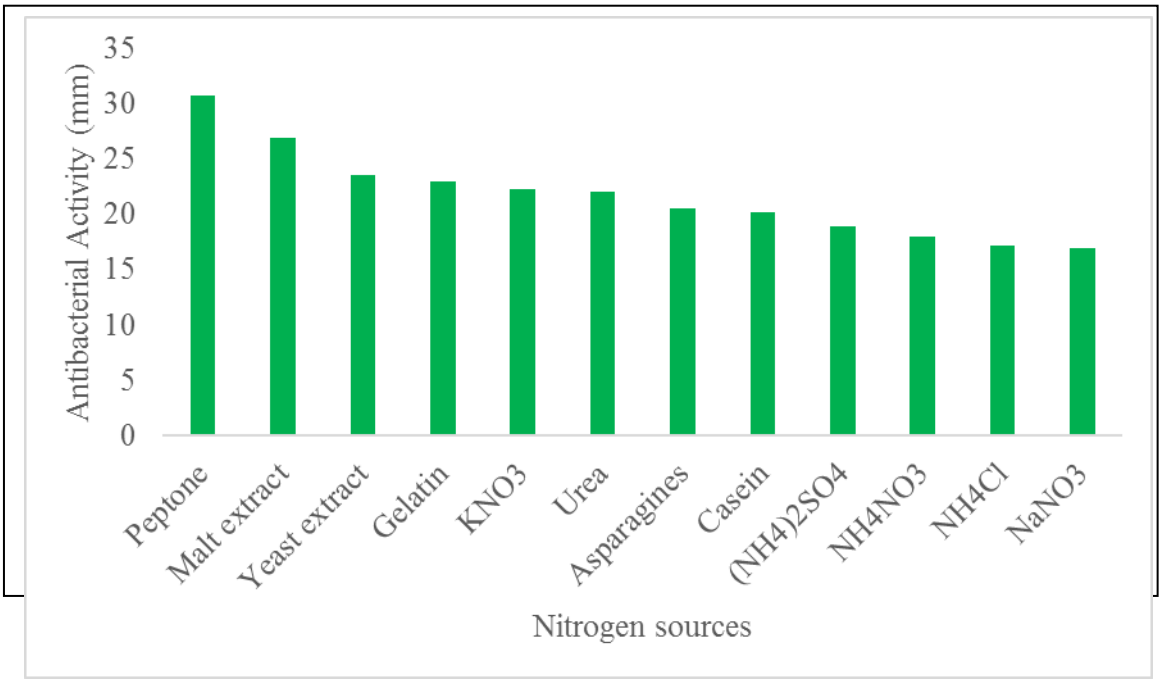
Sr. No	Carbon sources	Growth	Nitrogen Source	Growth
1	Glucose	5.5cm(Excellent)	Gelatin	4.5cm(Excellent)
2	Tapioca powder	4.5cm(Excellent)	Yeast Extract	4.5cm(Excellent)
3	Starch	4cm(Good)	Asparagine	3.9cm(Good)
4	Sucrose	3.5cm(Moderate)	Malt extract	3cm(Moderatet)
5	Soluble starch	3cm(Moderate)	Peptone	3cm(Moderate)
6	Potato	2.8cm(Moderate	NH_4Cl	2.7cm(Moderate)
7	Rice powder	3cm(Moderate)	KNO_3	3cm(Moderate)
8	Glycerol	2.5cm(Moderate)	$NH_4(SO_4)_2$	2.1cm(Moderate)
9	Carrot	2.7cm(Moderate)	Casein	1.7cm(Poor)
10	Fructose	3cm(Moderate)	NH_4NO_3	1.5cm(Poor)
11	Xylose	2.5cm(Moderate)	Rice bran	1.5cm(Poor)
12	Corn powder	2cm(Poor)	Urea	1cm(Poor)

1cm to 2cm = Poor, 2.1cm to 3cm = Moderate,
3.1cm to 4cm = Good 4.1cm to above = Excellent

Effect of carbon and nitrogen utilization on fermentation of PP-15

The significant inhibition zone (36.85mm, 35.84mm, 35.24mm, 34.03mm and 32.58mm) were obtained in starch, sucrose, tapioca powder, glucose and mannitol. Soluble starch (29.01mm), potato (25.21mm), rice powder (25.12mm), carrot (24.72mm), maltose (24.15mm), corn power (23.97mm), fructose (22.67mm), xylose (22.61mm), oat (21.06mm) and glycerol (20.66mm) showed moderate inhibition zone. Similarly, the addition of peptone exhibited the greatest activity (30.79mm) followed by malt extract (26.97mm), yeast extract (23.55mm), gelatin (22.95mm), KNO_3 (22.27mm), urea (22.05mm), asparagines (20.52mm), casein (20.21mm). The poor inhibition zone (18.97mm, 18.04mm, 17.23mm and 16.97mm) were obtained $(NH4)_2SO_4$, NH_4Cl, $NaNO_3$.





The effect of pH on the fermentation conditions of PP-15

In this study, the highest antibacterial activity was obtained at pH 6 (27.78 mm) against *Agrobacterium tumefaciens*.

Table7 The Effects of pH on the Fermentation Conditions of PP-15

Sr. No	pH	Antibacterial activity (clear zone , mm)
1	4	24.41
2	5	22.28
3	6	27.78
4	7	25.86
5	8	21.75
6	9	21.01

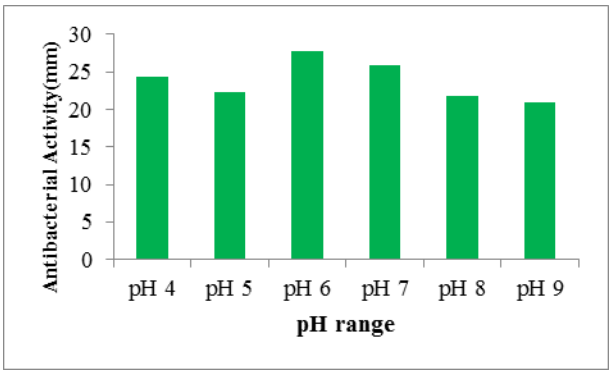


Figure 9 The Effects of pH on the Fermentation Conditions of PP-15

The effect of temperature on the fermentation condition

In this investigation, temperature 25˚C showed the highest antibacterial activity (28.83 mm) against on *Agrobacterium tumefaciens*.

Table 8 The Effects of Temperature on the Fermentation Conditions of PP-15

Sr. No	Temperature (˚C)	Antibacterial activity (clear zone, mm)
1	20	20.98
2	25	28.83
3	30	25.57
4	35	20.57
5	40	19.65

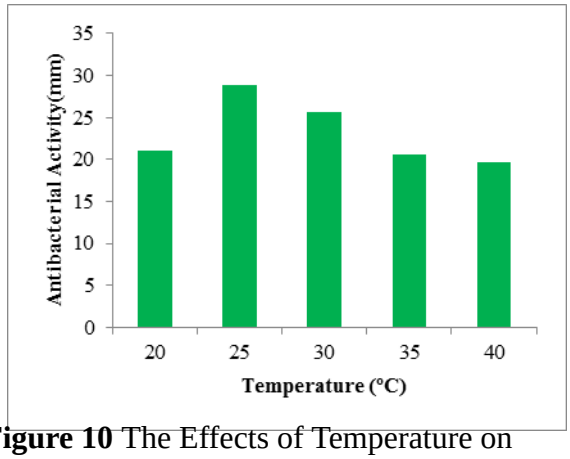


Figure 10 The Effects of Temperature on the Fermentation Conditions of PP-15

Antibacterial activity of PP-15 on Fermentation media

In the fermentation medium (FM), the best antifungal activity was obtained by using starch and peptone in FM-1 (30.89 mm) followed by 29.71 mm, FM-2 (starch and malt extract), 29.04 mm, FM-10 (glucose and peptone) and 28.14 mm, FM-3 (starch and gelatin) respectively.

Table 9 Antibacterial activity of PP-15 on various fermentation medium

Fermentation medium (FM)	Antibacterial activity (mm)
FM-1	30.89
FM-2	29.71
FM-3	28.14
FM-4	27.29
FM-5	28.10
FM-6	27.21
FM-7	25.82
FM-8	26.19
FM-9	24.29
FM-10	29.04
FM-11	27.72
FM-12	26.43
FM-13	25.76
FM-14	26.52

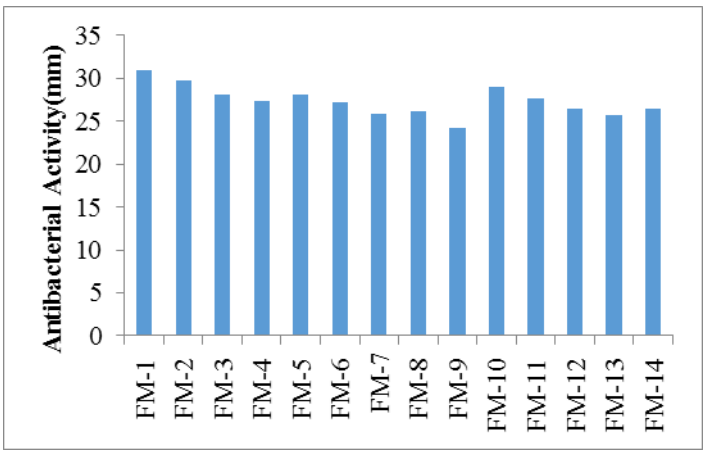


Figure 11 Antibacterial activity of PP-15 on various fermentation medium

Comparison of static culture and shaking culture

When comparing the static culture and shaking culture on fermentation medium of PP-15 antibacterial activity from shaking culture is better than (30.92 mm) than that of static culture (20.01 mm).

Table 12 TheAntibacterial Effects on Static and Shaking Culture of PP-15

Sr. No	Fermentation condition	Antibacterial activity (clear zone , mm)
1	Static	20.01
2	Shaker	30.92

Static Shaker

Figure 12 The Antibacterial Effects on Static and Shaking Culture of PP-15

Discussion and Conclusion

The soil serve as a reservoir for many microbial communities of plants and herbs which can be producing CO_2 and nitrogen cycle. The microorganisms plays major role in soil ecosystem. Microbial composition and functioning changes the soil quality through decomposition of organic matter, recycling of nutrients and biological control (Stefanis, *et al.*, 2013). Soil samples were collected from six inches depth after removing the surface soil for the isolation of fungi. The color of soil samples were red, brown and pale brown. In general the majority of microbial population is found in the upper six to twelve inches of soil and the number decreases with depth (Cattle, *et al*., 2002). Ten isolated fungi (PP-2, 3, 8, 12, 14, 15, 21, 23, 27

and 28) were tested with one test organism by agar well diffusion method. Among them, the selected fungus PP-15 showed potent antibacterial activity against *Agrobacterium tumefaciens*.

Therefore, PP-15 was selected for the study of the optimum fermentation condition. In the fermentation period, PP-15 reached the moderate activity (23.23mm) in 6 days fermentation period. To study the optimization of inoculum age, inoculation time (48, 60, 72, 84, 96, 108, 120, 132 and 144 hrs) were used and the highest antibacterial activity was found at 72 hrs (25.53 mm). In the proper size of inoculum, 10% was the most suitable and the maximum activities of PP-15 reached up (27.95 mm) followed by 30% and 20% respectively. According to Tomita (1988) in the fermentation studies, 72 hrs age and 10% size of inoculum were optimized for the production of antibacterial metabolite. The highest biomass and antibiotic activity was observed at an incubation time of 72 hrs by some other investigators (Srinivasulu *et al*., 2002).In addition, effects of variation of carbon and nitrogen sources were observed for the growth of colony morphology and maximum antimicrobial metabolite production. In the carbon source, the colony of PP-15 was the excellent growth on glucose, starch and tapioca powder. There was a high degree of variation in the level of antimicrobial activity in the present study when the different carbon sources were tested in the fermentation medium. Moderate growth and the antimicrobial substance production of PP-15 were influenced by addition of starch reaching the highest activity 36.85mm, followed by sucrose (35.84mm) and tapioca powder (35.84 mm).Katoke*et al* 1992 studied that different carbon sources like sucrose, glycerol, starch, dextrose, lactose and fructose have been reported to be suitable for production of secondary metabolite in different organisms. Fungi have 40-55% carbon use efficiency so they store and recycle more (C) compared to bacteria (James *et al*., 2011).

The nature of the nitrogen source has notable effect on the production of antibacterial metabolite in PP-15. Especially PP-15 showed the moderate growth on almost all nitrogen sources. Maximum production of antibacterial metabolite of PP-15 was observed on peptone (30.79mm) followed by malt extract, yeast extract and gelatin respectively as nitrogen sources. El-Gammal AA, 1986 described that peptone has been reported by the suitability of nitrogen sources for the production of metabolites from microorganisms. Effect of pH was studied by varying from pH 4, 5, 6, 7, 8 and 9. The best antibacterial activity was found at pH-6 (27.78 mm). The change of pH is also important for the enzyme activity of microorganisms, for the intermediate products, their dissociation and solubility (Rizk*et al*.,2007).

The maximum production of antibacterial metabolite (28.83 mm) was obtained at 25ºC. Physical factors incubation such as temperature, can exert different effect on the growth and production phases of secondary metabolism (Rizk*et al*.,2007).

Fermentation media (FM) were studied and FM-1 gave the highest activity (30.89mm). The choice of the good fermentation medium is virtually as important to the success of an industrial fermentation as is the selection of an organism to carry out the fermentation (E1-Tayeb*et al*., 2004).In the comparison between shaking and static culture, the antibacterial activity of shaking culture PP-15 more than the static culture. Stevens *et al*., 1975 indicated that adequate agitation was found to increase antibiotic metabolite production. Thus, the results of the optimum fermentation tests indicated that antimicrobial metabolites obtained from PP-15 may be produced optimally in the presence of 6^{th}days fermentation period,72 hrs age of inoculums and 10% inoculums size, glucose and starch in the carbon source, gelatin and peptone in the nitrogen source, pH-6, temperature 25ºC, FM-1 and shaking culture. It was concluded that the present

study revealed to observe the fermentation period of isolated fungi and to investigate the optimization parameters of fermentation condition on PP-15 against *Agrobacterium tumefaciens.*

Acknowledgements

I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, University of Pathein and Professor Dr Wah Wah Lwin, Department of Botany, University of Pathein 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, University of Pathein, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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TAXONOMIC STUDY ON TWELVE SPECIES OF FAMILY FABACEAE FROM NAT YAY GAN MOUNTAIN AREA IN NGAPHE TOWNSHIP

Thida Cho[1], Dr Nwe Nwe Yi[2]

Abstract

The present research work deals with the taxonomic study on some species of family Febaceae from Nat Yay Gan mountain area in Ngaphe Township which is located in Middle part of Myanmar. This area lies between 20º10'N Latitude and 94º30'E Longitude. The flowering plants from the floristic area were collected, preserved and identified from 2017 to 2018. Altogether 12 species which are belonging to 8 genera were included. The characteristics of the family, the morphological characters of the genera and species were presented with the colour photographs. The names of the genera were arranged in alphabetically. All the plants were recorded and described.

Keywords: Taxonomy Field.

Introduction

The present research work deals with the floristic study on flowering plants (Angiosperms) growing in Nat Yay Gan mountain area in Nagphe Township. The present research work deals with the taxonomic study on some species of family Febaceae from Nat Yay Gan mountain area in Ngaphe Township which is located in Middle part of Myanmar.Altogether 12 species which are belonging to 8 genera were included.

The floristic study of Nat Yay Gan mountain area is located between Nagphe township and Rakhaine Yoma. It has an area of 132 .8 square kilomater. It has 15 villages. Nat Yay Gan Mountain has near the Gokkyi village. Nat Yay Gan Mountain is apart 7 miles from Gokkyi village. It lies between 19° 52' N latitude and 94° 24' E longitude. The elevation of Nat Yay Gan Mountain is 1744 meter high.

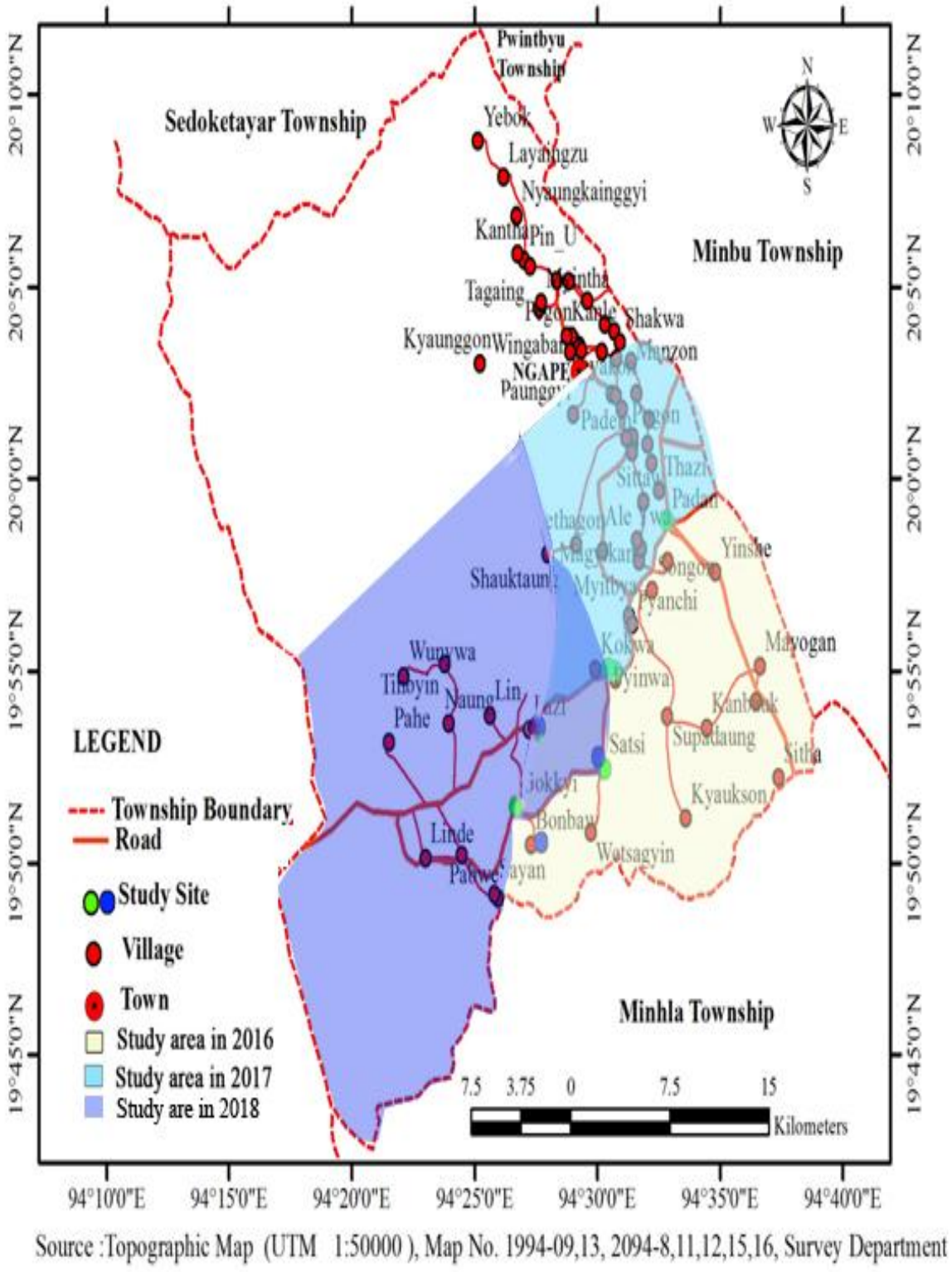
The aims and objectives of the present work were to classify and identify the plants of Angiospermae on Nat Yay Gan mountain area in Nagphe Township, to record the taxonomical characters of the information of floristic natural plant resources and to understand the information of wild species and its vegetation from the study area.

Materials and Methods

The flowering plants specimens were collected from Nat Yay Gan mountain area in Nagphe Township from the year during 2017 to 2018. Field notes were made on the natural habit and plant location by using the GPS (Global Positioning System). The inflorescence and fruit portions were recorded in the form of the photographic records. The fresh specimens were used for the characterization of the plants.

The plants collected specimens of flowering plants have to contain flowers, fruits and seeds. Keys are constructed mainly on the basis of these characters. Identification of an unknown specimen is carried out by utilizing the floras or manuals and checklist of the particular region. The taxonomic identification of collection plants were carried out by referring to Hooker (1879), Hutchinson (1959), Backer (1963-1968) and Dassanayake (1980- 2001). Myanmar names were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003). The families of the arrangements were followed by the classification system of APG IV (2016).

[1] Asistant Lecturer, Department of Botany, Magway University
[2] Associate Professor, Department of Botany, Pathein University



Source :Topographic Map (UTM 1:50000), Map No. 1994-09,13, 2094-8,11,12,15,16, Survey Department

Figure 1 Location Map of Study Area in Ngape Township

Results

Altogether 12 species which are belonging to 8 genera were included.

Bauhinia vahlii Wight & Arn., Prod. 297. 1834. (Figure 1(A))

Phanera vahlii Benth., Pl. Jungh. 263. 1852.

Myanmar name : Swe daw; Bwe gyin

English name : Unknown

Flowering period : February to April

Perennial, trees, up to about 5.0 m high; stems and branches terete, pubescences dense grey or ferruginous; internodes 1.5-2.0 cm long. Leaves simple alternate; stipules linear, caducous; petioles 3.0-4.5 cm long, densely pilose; blades suborbicular, 13.0-15.0 cm by 12.5-13.0 cm, rounded to cordate at the base, entire along the margin, rounded to acute at the apex, green above, pale green beneath, glabrous on both surfaces. Inflorescences terminal, racemes, dense, corymbose; peduncles terete, 10.0-12.5 cm long, densely, ferruginous tomentose. Flowers bisexual, hypogynous, zygomorphic, pentamerous, 5.0-6.0 cm in diameter, creamy-white; bracts triangular, very small, early caducous; bracteoles linear, persistent, inserted below the middle of pedicel; pedicels 4.0-5.0 mm long. Calyx 5- lobed, linear-lanceolate, splitting spathaceous into five petals, pilose on both surfaces. Petals broadly obovate, subequal, creamy-white, bright yellow tanged in the medium petal; claws 1.0 cm long, densely pilose on both surfaces. Stamens 10, free, fertile 3; exserted; filaments 3.0-4.5 cm long, pale green; anthers oblong, dithecous, dorsifixed, dehiscing by longitudinal slit, black or grey. Carpel 1; ovary superior, oblongoid, about 5.0-8.0 mm in diameter, unilocular with many ovules per locule on the marginal placenta; styles simple, slightly oblique; stigmas peltate. Pods 4.0-5.0 cm long, pods linear, glabrous. Seeds flattened, yellowish-brown, endospermic.

Distribution: This species had recorded in the checklist of Myanmar (Kress *et al*. 2003). In the study area this species distributed as a wild plant.

Specimen examined: Nat Yay Gan, Nagphe Township, Nat Yay Gan forest, N 19° 52' 33" and E 94° 24' 59", 303 m; 18 June, 2016; Thida Cho; collection no. 128.

Bauhinia variegata L., Sp. Pl. 1: 375.1753. (Figure 1(B))

Myanmar name : Swe daw

English name : Orchid tree

Flowering period : January to March

Perennial, tree, up to 15.0 m high; stems and branches terete, glabrescent, Leaves simple, alternate; stipules about 0.2 cm long, caducous; petioles 2.0-3.0 cm long, glabrous; blades orbicular or broadly ovate, 4.0-7.0 cm by 3.4-7.0 cm, cordate at the base, entire along the margin, rounded at the apex, glabrous above, pubescent beneath. Inflorescences axillary, racemose, fewflowered; peduncles 1.0-2.0 cm long, pubescent. Flowers bisexual, zygomorphic, pentamerous, hypogynous, pinkish-white, or violet, 5.0-8.0 cm in diameter, showy, fragrant; bracts triangular, about 0.2 cm long, early caducous; bracteoles 0.7-0.8 cm long, pubescent. Calyx splitting spathaceous; tube 2.0-2.5 cm long, glabrous. Petals 5, free, slightly unequal,

obovate-oblong, 4.0-6.0 cm long, pale purple, the medium one larger and deep red in colour in the centre, with prominent veins, clawed. Fertile stamens 5, free, exserted; filaments unequal; the longer 3, 3.5-4.0 cm long, pink, the shorter 2, 2.0-3.0 cm long, white, turning pinkish-white; anthers dithecous, basifixed, oblong; staminodes 5. Carpel 1; ovary superior, linear, 1.0-1.5 cm long, unilocular, many ovules in the locule on the marginal placentae; style 0.7-0.8 cm long, pink, glabrous; stigma simple. Pods strap-shaped, dehiscent, 12.0-22.0 cm long, beaked. Seeds orbicular, flattened, brown.

Distribution: Kress *et al.* (2003) mentioned that this species was distributed in Shan State of Myanmar. It was found in the forest area of the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Khonzu village, N 19° 54' and E 94° 29', 768 m; 2 March, 2018; Thida Cho; collection no. 181.

Butea monosperma (Lam.) Taub., In Pflanzenfam. 3(3) 365. 1894. (Figure 1(C))

Erythrina monosperma Lam., Encyc. 2: 391. 1784.

Myanmar name	:	Pauk
English names	:	Flame of the forest; Parrot tree
Flowering period	:	February to April

Perennial, deciduous large trees, up to 3.0 m high; stems and branches terete, pubescent. Leaves pinnately trifoliolate-compound, alternate; stipules linearlanceolate, caducous; petioles 7.0-24.0 cm long; leaflets broadly obovate in terminal one and obliquely ovate in lateral ones, 9.0-19.0 cm by 7.0-15.0 cm, rounded or subcuneate at the base, entire along the margin, obtuse or emarginated at the apex, glabrous above and densely silky- pubescent beneath. Inflorescences axillary orterminal, fasciculate-racemose; peduncles 5.0-35.0 cm long, with many tubercles, tomentose. Flowers bisexual, zygomorphic, hypogynous, pentamerous ,2- to 5-nate on each tubercle, rather large, orange, 4.0-5.0 cm in diamete; bracteoles subulate, brownvelvety, fugacious. Calyx campanuate, 5-lobed; tube about 1.0cm long, tomentose;lobes short, the 2 upper ones completely united. Corolla papilionaceous, much exserted, standard ovate, 3.5-5.0 cm by 2.5-5.0 cm by 2.5-5.0 cm, recurved, clawed, basally inflexed biauriculate, tomentoses, scarlet-orange; wings oblong, 4.5-5.0 cm by 1.5-2.0 cm; keels falcate, 4.5-5.0 cm by 1.5-2.0 cm, beaked. Stamens 10, diadelphous; free filaments 6.0-8.0 cm long; anthers uniform, dithecous, basifixed, longitudinally dehiscent. Ovary linear-oblong, 8.0-10.0 mm long, long-stipitate, densely tomentose, superior, unilocular, with 2 to5 ovules on the marginal placentae; style filiform, curved, glabrous; stigma small, capitates. Pods flat, oblong, strap-shaped, indehiscents, 10.0 -15.0 cm by 4.0-5.0 cm, stipitate, light brown. Seeds suborbicular, flat, pale brown, endospermic.

Distribution: This species had recorded in the checklist of Myanmar (Kress *et al*. 2003). In the study area this species distributed as a wild plant.

Specimen examined: Nat Yay Gan, Nagphe Township, Pyinwa village area, N 19° 53' 47" and E 94° 30' 21", 216 m; 19 June, 2016; Thida Cho; collection no. 157.

Dalbergia cultrata Grah. ex Benth., in Miquel, Pl. Jungh. 254. 1852. (Figure 1(D))

Myanmar names	:	Yin daik; Zaungi
English name	:	Unknown
Flowering period	:	February to March

Perennial tree, up to 10.0 m high; stems and branches slender, finely appressed pubescent. Leaves unipinnately compound, imparipinnate, alternate; stipules linear, about 2.0 mm long, caducous; petioles slender, 2.5-5.0 cm long, glabrous; racheae 10-15 cm long; leaflets 7- to 10-paired per rachis , alternate, obovate-oblong, 2.0-4.0 cm by 1.0-2.0 cm, cuneate at the base, entire along the margin, round or emarginate at the apex, glabrous on both surfaces. Inflorescences terminal or axillary panicles with slender ascending branches, many-flowered; primary peduncles 4.0-5.0 cm long, glabrous; secondary peduncles 2.0-3.0 cm long pubescent. Flowers bisexual, zygomorphic, hypogynous, pentamerous, white, about 5 mm in diameter; pedicels about 2.0 mm long, slender, pubescent; bracts and bracteoles linear, minute. Calyx campanulate, 5-toothed; teeth traingular, about 1.0 mm long. Corolla papilionaceous; standard broadly obovate, about 4.0 mm long, rounded at the apex, clawed; wings elliptic, about 3.0 mm long; keels obutse, about 3.0 mm long. Stamens 10, monadelphous; staminal tube about 4.0 mm long; anthers dithecous, basifixed, dehiscent by apical pore. Carpel 1; ovary superior, oblong, about 2.0 mm long, unilocular with few ovules in the locule on the marginal placentae; styles curved, about 1.0 mm long; stigmas simple. Pods oblong to strap-shaped, 6.0-9.5 cm by 0.9 -1.3 cm, lathery, 1-2 seeded, both end obtuse. Seeds reniform, compressed.

Distribution: Widely distributed in Myanmar (Kress *et al.* 2003). It was found in the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Maylat village, N 19° 52' and E 94° 29', 417 m; 2 March, 2017; Thida Cho; collection no. 199.

Dalbergia oliveri Gamble ex Prain, J. Asiat. Soc. Bengal 2, Nat. (Figure 1(E))

Hist.66:451. 1897.

Myanmar names	:	Tabauk; Tamalan
English name	:	Burmese rose wood
Flowering period	:	March to April

Perennial, deciduous trees, up to 15.0 m high; stems and branches terete; brown silky pubescent; bark dark gray, thick. Leaves unipinnately compound, imparipinnate, alternate; stipules linear, 3.0-5.0 mm long, caducous; petioles slender, 4.5-6.0 cm long, brown silky pubescent; racheae 10.5-22.0 cm long; leaflets 7 to 13 paired per rachis, alternate; elliptic-oblong, 2.5-4.0 cm by 0.9-1.5 cm, rounded at the base, entire along the margin, acute at the apex, young leaves pale pink with silky hairs; mature leaves dark green, glabrous. Inflorescences terminal or axillary panicles,many-flowered; primary peduncles 10.0-15.5 cm long; secondary peduncles 5.0-6.0 cm long. Flowers bisexual, zygomorphic, hypogynous, pentamerous, pale purple, 0.8-1.0 cm in diameter; pedicels 4.0-5.0 mm long, glabrous; bracts and bracteoles caducous. Calyx campanulate, 5-toothed; tube cup-shaped, about 3 mm long; teeth linear, unequal, about 2 mm long, brownish purple. Corolla papilionaceous; standard broadly ovate, 7.0-9.0 mm long, rounded at the apex, purple with white center; clawed; wings oblong, 5.0-6.0 mm long; keel obtuse, 4.0-5.0 mm long, white. Stamens 10, diadelphous, (9)+1; staminal tube about 5.0 mm long; anthers dithecous, dorsifixed, dehiscent by apical pore. Carpel 1; ovary superior, oblongoid, about 2.0 mm long, unilocular with few-ovules in the locule on the marginal placentae; styles short, about 2 mm long; stigmas simple. Pods oblongoid, flat, pointed at both ends, pale brown, 2-3 seeded. Seeds reniform.

Distribution: According to Kress *et al.* (2003) stated that this species was distributed in Bago and Mandalay Regions. It occurs as a wild plant in the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Satsi village, N 19° 52' and E 94° 29', 411 m; 12 April, 2018; Thida Cho; collection no. 144.

Dalbergia rimosa Roxb., Fl. Ind., (ed. 1832), 3:233. 1832. (Figure 1(F))

Myanmar name : Unknown

English name : Unknown

Flowering period : June to August

Perennial small trees, 4.0-6.0 m high; stems and branches slender, glabrous. Leaves unipinnately compound, imparipinnate, alternate; stipules subulate, about 3 mm long; petioles terete, 2.5-5.0 cm long, pubescent; racheae 15.0-20.0 cm long, pubescent; leaflets 5 to 9 paired per rachis, alternate, ovate-oblong, 3.0-5.0 cm by 2.0-2.5 cm, rounded at the base, entire along the margin, acute at the apex, firm papery, bright green and glabrous above, grey and adpressed hairy beneath. Inflorescences terminal and axillary panicles with corymbose branches; many-flowered; primary peduncles 15.0-20.0 cm long; secondary peduncles 5.0-6.5 cm long. Flowers bisexual, zygmorphic, hypogynous, pentamerous, greenish-white, about 3.0 mm long; pedicels subsessile; bracts and bracteoles lanceolate, about 1.0 mm long, persistent. Calyx campanulate, 5-toothed; teeth unequal, obtuse, about 2.0 mm long. Corolla papilionaceous; standard obovate, emarginate at the apex, about 2.0 mm long, short clawed; wings elliptic, about 2.0 mm long; keels falcate, about 2.0 mm long. Stamens 10, monadelphous; staminal tubes about 4.0 mm long; anthers dithecous, basifixed, dehiscent by apical pore. Carpel 1; ovary superior, oblong, about 2.0 mm long, unilocular with few ovules in the locule on the marginal placentae; styles short; stigmas simple. Pods oblong, 5.0-7.0 cm by 2.0-3.0 cm, leathery, 1-2 seeded, glabrous. Seeds reniform, compressed, brown shiny.

Distribution: Kress *et al*. (2003) stated that it was found in Kachin State and Sagaing Region of Myanmar. It was commonly found in study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Maylat village, N 19° 52' and E 94° 29', 417 m; 21 July, 2018; Thida Cho; collection no. 198.

Erythrina stricta Roxb., Fl. Ind. 3: 251-252.1832. (Figure 2(A))

Myanmar name : Ka thit

English name : Unknown

Flowering period : March to April

Perennial, deciduous trees, 7 to 12- m high; stems and braches with short whitish prickles; barks pale creamy, soft. Leaves pinnately trifoliate compound, alternate; stipules lanceolate, caducous; petioles 12.0-15.0 cm long, glabrous, pulvinate, glabrous; stiples 2.0-3.0 mm long; leaflets rhomboid, unequal, terminal larger than lateral ones, 7.0-19.0 cm by 7.0-20.0 cm, rounded or truncate at the base, entire along the margin, mucronate at the apex, lateral veins 5-to 8-paired, glabrous on both surfaces; petiolues terminal 2.0-2.5 cm long; lateral ones about 5.0 mm long. Inflorescences terminal racemes, many-flowered; peduncles about 15.0 cm long, glabrous. Flowers bisexual, zygomorphic, pentamerous, hypogynous, red, 2.0-3.0 cm in

diameter; pedicels 4.0-5.0 mm long; bracts and bracteoles caducous. Calyx spathaceous, truncate at the apex, 1.3-1.6 cm long, membranous, dark purple without, glabrous. Corolla papilionaceous; standards elliptic-lanceolate, 3.5-4.0 cm long, glabrous; wings obovate, 2.5-3.0 cm long; keels broadly ovate, 2.5-3.5 cm long, white, membranous, glabrous. Stamens 10, diadelphous, (9)+1; filaments stout, 1.5-1.8 cm long, red; anthers dithecous, basifixed, dehiscing longitudinally. Carpel 1; ovary superior, oblong, unilocular with many ovules in the locule on the marginal placentae, stipitate, hairy; styles curved, about 5.0 mm long, glabrous; stigma simple. Pods linear-oblong, 8.5-10.0 cm long, flat, 1-to 3-seeded, glabrous. Seeds reniform, pale brown.

Distribution: Widely distributed in Myanmar (Kress *et al.* 2003). It was widely distributed in the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Lintel village, N 19° 51' and E 94° 22', 1109 m; 12 April, 2018; Thida Cho; collection no. 129.

Indigofera tinctoria L., Sp.Pl. 2: 751. 1753. (Figure 2(B))

Myanmar name : Me nai; Me nat

English name : Indigo; Indian indigo

Flowering period : January to May

Perennial, suffrutescent, erect shrubs, up to 2.0 m high; stems and branches angular-ribbed, appressed pubescent. Leaves unipinnate-compound, imparipinnate, alternate; stipules subulate, 0.2-0.3 cm long, pubescent; petioles 1.0 cm long; racheae 5.0-15.0 cm long, pubescent; leaflets 5-10, opposite, elliptic to obovate, 1.0-2.5 cm by 0.8 -1.0cm, cuneate at the base, entire along the margin, rounded at the apex, glabrous above, appressed-pubescent beneath. Inflorescences axillary, spicateracemose, many-flowered; peduncles 6.0-10.0 cm long, pubescent. Flowers bisexual, zygomorphic, cyclic, pentamerous, hypogynous, pink, 0.3-0.8 cm in diameter; bracts linear, 0.1 cm long, pubescent, caducous. Calyx broadly campanulate, 5- lobed; tube 0.1-0.2 cm long, pubescent; lobes deltoid, about 0.2 cm long, pubescent. Corolla papilonaceous, exserted; standard orbicular-obovate, about 0.8 cm by 0.5 cm, inner side greenish yellow with radiating purple streaks, pubescent without; wings oblong, about 0.5 cm by 0.3 cm, pinkish, pubescent; keels about 0.5 cm long, with an obliquely backward pointing spur on both sides, creamy-white, pubescent without. Stamens 10, diadelphous; free filaments about 0.4 cm long, glabrous; anthers uniform, gland tipped. Carpel 1; ovary superior, linear-oblongoid, 0.5 cm long, unilocular, 6-10 ovules in the locule on the marginal placentae; style 0.2 cm long, curved, glabrous; stigma capitate. Pods linear-oblongoid, 3.0-4.0 cm by 0.5-1.0 cm, 8- to 12- seeded, appressed -pubescent to glabrous. Seeds cylindric, glabrous.

Distribution: This species was widespread in the Old World tropoics (Rudd as cited in Dassanayake 1991). Kress *et al*. (2003) recorded that this species was found in Myanmar. This species is found in the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Maylat village, N 19° 52' and E 94° 29', 417 m; 13 April, 2018; Thida Cho; collection no. 192.

Millettia brandisiana Kurz, J. Asiat. Soc., Bengal, Pt.2, Nat. Hist. 42(2): 69.1873. (Figure 2(C))

Myanmar name : Thit pagan

English name : Unknown

Flowering period : February to March

Perennial, deciduous trees, 5.0-10.0 m high; stems and branches terete, glabrous; barks dark grey, rough. Leaves unipinnately compound, imparipinnate, alternate; stipules lanceolate, caducous; petioles 3.5-4.0 cm long; racheae cylindrical, 15.0-25.0 cm long; leaflets 7- to 12-paired, opposite, ovate, 2.0-4.0 cm by 1.5-2.0 cm, obtuse at the base, entire along the margin, acute at the apex, glabrous above and pubescent beneath. Inflorescences axillary paniculate racemes, many-flowered; peduncles 4.0-5.0 cm long, glabrous. Flowers bisexual, zygomorphic, pentamerous, hypogynous, pinkish-purple, about 1.0 cm in diameter; pedicels 3.0-4.0 mm long, reddish, pilose. Calyx campanulate, 5-toothed; tube about 5.0 mm long, eddishpurple; teeths linear, minute. Corolla papilionaceous; standards orbicular, 7.0-8.0 mm long, lawed; wing oblong, about 3.0 mm long; keels curved, 5.0-6.0 mm long, beaked. Stamens 10, monadelphous; free filaments about 3.0 mm long; anthers dithecous, dorsifixed, dehiscing longitudinally. Carpel 1; ovary superior, unilocular with many ovules in each locule on the marginal placentae, hairy; styles curved, about 3.0 mm long; stigma simple. Pods ovate-oblongoid, flat, 5.5-7.5 cm long, smooth, woody, 1-to 3-seeded.

Distribution: According to Kress *et al.* (2003), this species was widely distributed in Myanmar. It was commonly found in the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Zynbun village, N 19° 54' and E 94° 29', 349 m; 2 March, 2018; Thida Cho; collection no. 212.

Millettia cinerea Benth. in Miq., Pl. Jungh. 2:249.1852. (Figure 2(D))

Myanmar name : Win U

English name : Unknown

Flowering period : March to May

Perennial, woody climbers; stems and branches terete, covered with grey pubescent. Leaves unipinnate compound, imparipinnate, alternate; stipules linear, 0.3-0.4 cm long, pubescent; petioles 5.0-7.0 cm longs, pubescent; pulvinous at the base; petiolules 0.5-1.0 cm long, pubescent; leaflets 5 to 7, obovate-oblong, 7.0-20.0 cm by 7.0-10.0 cm, oblique and rounded at the base, entire along the margin, obtuse at the apex, pubescent on both surfaces. Inflorescences axillary, paniculate racemes, many-flowered; peduncles 4.0-8.0 cm long, pubescent. Flowers bisexual, zygomorphic, cyclic, pentamerous, hypogynous, pale yellow, 0.1-1.5 cm in diameter; bracts ovate, 1.0-0.2 cm long, pubescent. Calyx tubular, 5-lobed; tube 0.2-0.3 cm long, glabrous; lobes slightly dentate, about 0.1 cm long, pinkish-red, glabrous. Corolla papilionaceous; standard orbicular, 1.0-1.5 cm long, clawed; wings oblong, 0.7-1.0 cm long, clawed; keel obtuse, 0.5-0.6 cm long, clawed, pubescent. Stamens 10, diadelphous; included; free filaments linear, 0.5-0.7 cm long; anthers dithecous, dorsifixed, oblong, about 0.1 cm long, yellow, dehiscing longitudinally. Carpel 1; ovary superior, oblong, 0.3-0.5 cm long, unilocular, 4-5 ovules in the locule on marginal placentae; style terminal, 0.3-0.4 cm long, slightly curved; stigma capitate. Pods, flattened, turgid 14.0-20.0 cm long, 3- to 5- seeded, glabrous. Seeds globoid, yellowish brown, glabrous.

Distribution : This species was distributed in Mandalay Region (Kress *et al*. 2003). This species is found of the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Khonzu village, N 19° 54' and E 94° 29', 768 m; 13 April, 2018; Thida Cho; collection no. 180.

Mucuna pruriens (L.) DC., Prod.2: 405.1825. (Figure 2(E))

Myanmar name : Khweleya

English name : Purple flowered velvet bean

Flowering period : October to December

Annual, twining herbs; stems terete, densely pubescent, glabrous atmaturity. Leaves pinnately trifoliolat compound, alternate; stipules linear, about 4.0 mm long; stiples setaceous, 2.0-3.0 mm long, persistent; petioles 4.5-8.0 cm long, pubescent; leaflets obovate, 8.0-12.0 cm by 3.0-5.0 cm, rounded and oblique at the base, entire along the margin, obtuse at the apex, pubescent on both surfaces; petiolules about 4.0 mm long. Inflorescences axillary, pendulous racemes, many flowered, 2 or 3 flowered at each node; peduncles 13.0-15.0 cm long, tuberculate. Flowers bisexuat, zygomorphic, pentamerous, hypogynous, dark violet, about 8.0 mm in diameter; pedicels 2.0-4.0 mm long,tomentose. Calyx campanulate, 4- lobed; tubes 4.0-5.0 mm long; lobes acute, about 3.0 mm long. Corolla papilionaceous; standard broadly ovate, 1.3-1.5 cm long, clawed; wings oblong, 1.0-1.2 cm long; keels beaked, 0.9-1.1 cm long. Stamens 10, diadelphous, (9)+1; staminal tube 7.0-8.0 mm long; free filaments about 4.0 mm long; anthers dithecous, basifiexed, dehiscing longitudinally. Carpel 1; ovary superior, oblong, about 4.0 mm long, unilocular with many ovules in the locule on the marginal placentae; styles 1.0-1.5 cm long; stigmas capitate. Pods linear-oblong, swollen around the seeds, 4.0-6.0 cm long, slightly compressed, covered with densely orange-brown pubescent, 2- to- 8 seeded, dehiscent.

Distribution : According to Kress *et al.* (2003), this species was distributed in Chin, Kayin, Shan States and Bago, Mandalay, Sagaing and Yangon

Regions of Myanmar.

Specimens examined: Nat Yay Gan, Nagphe Township, Sonteat village, N 19° 51' and E 94° 22', 1001 m; 18 December, 2017; Thida Cho; collection no.134.

Xylia xylocarpa (Roxb.) Taub., Bot. Centralbl. 67: 395. 1891. (Figure 1(F))

Mimosa xylocarpa Roxb., Pl. Corom. 1 : 68. t. 100. 1798.

Myanmar name : Pyinkado

English name : Burmese iron wood

Flowering period : March to April

Perennial, deciduous large tree, up to 25.0 m high; stems and branches terete, bark grey to red, glabrous. Leaves bipinnately compound, paripinnate, alternate; stipules filiform, 3.0-5.0 mm long; petioles terete, 3.0-5.0 cm long, gland at the junction of the pinnae; racheae 25.0-30.0 cm long; pinnae 1-paired; leaflets 8-10 paired per pinnae, opposite, ovate to broadly elliptic, 3.5-10.0 cm by 2.5-6.0 cm, rounded to cuneate at the base, entire along the margin, acuminate at the apex, glabrous above and velutinous below. Inflorescences axillary pedunculate globose head, many-flowered; peduncles 4.0-6.0 cm long, tomentose. Flowers bisexual, actinomorphic, pentameorus, hypogynous, pale yellow, about 4.0 mm in diameter; sessile; bracts spathulate, about 2.0 mm long. Calyx tubular, 5-toothed; tube about 3.0 mm long; teeths triangular, about 2.0 mm long. Petals 5, free, slightly connate at the base, linear, about 4.0 mm long, glabrous. Stamens 10, free, exserted; filament 0.5-1.0 cm long; anthers dithecous, versatile, oblong,

dehiscing longitudinally. Carpel 1; ovary superior, ovoid, unilocular with many ovules in the locule on the marginal placentae; styles terminal, filiform; stigmas simple, minute. Pods oblong-obovate, compressed, 12.0-15.0 cm by 3.0-6.0 cm, woody, pale brown. Seeds ellipsoid, flat, shining.

Distribution: Widely distributed in Myanmar (Kress *et al.* 2003). It occurs as a wild plant in the study area.

Specimens examined: Nat Yay Gan, Nagphe Township, Khonzu village, N 19° 54' and E 94° 29', 768 m; 3 March, 2018; Thida Cho; collection no. 187.

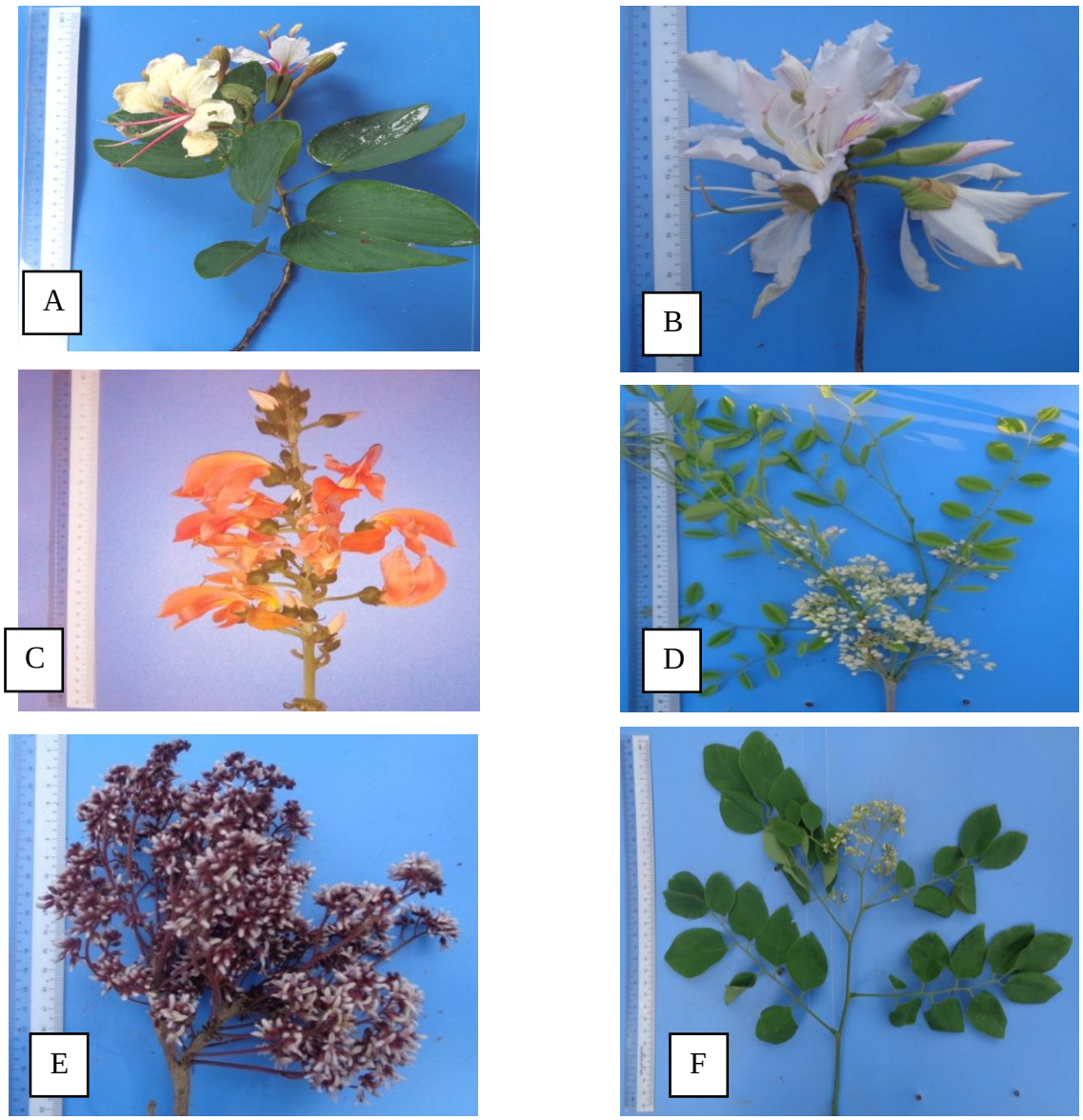


Figure 1 (A) *Bauhinia vahlii* Wight & Arn., (B) *Bauhinia variegata* L., (C)*Butea monosperma* (Lam.) (D) *Dalbergia cultrata* Grah. ex Benth., (E) *Dalbergia oliveri* Gamble ex Prain (F) *Dalbergia rimosa* Roxb., Fl. Ind.,

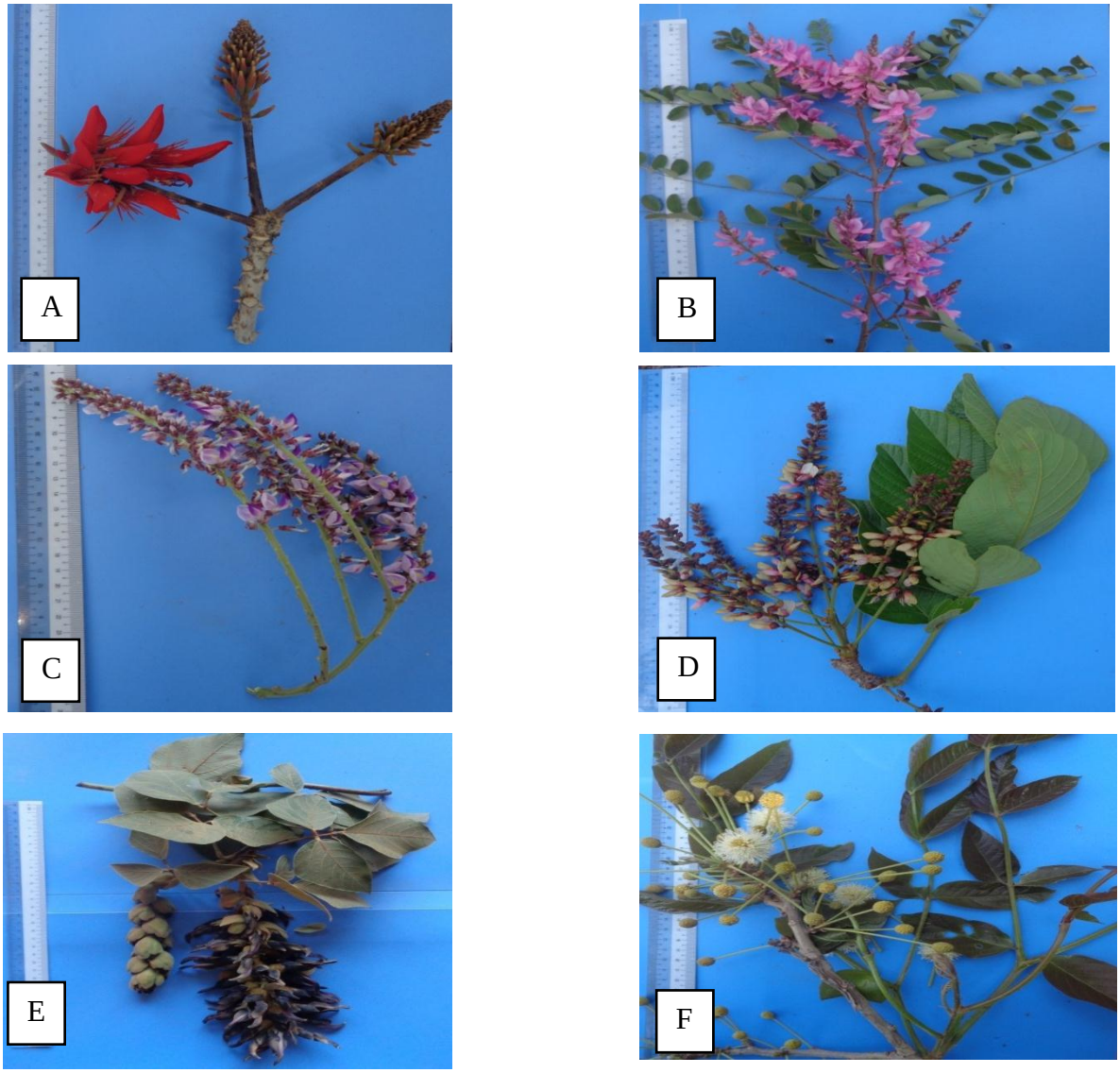


Figure 2 (A) *Erythrina stricta* Roxb., (B) *Indigofera tinctoria* L., *(C) Millettia brandisiana* Kurz, (D) *Millettia cinerea* Benth. (E) *Mucuna pruriens* (L.) DC., (F) *Xylia xylocarpa* (Roxb.) Taub.,

Discussion and Conclusion

The present research work deals with the floristic study on flowering plants (Angiosperms) growing in Nat Yay Gan mountain area in Nagphe Township. The species of family Fabaceae were widely distributed in temperate, tropical and subtropical of the world. About 727 to 732 genera and 19,000 to 19,700 species were recorded in the world (Heywood 2007). Simpson (2006) stated that about 643 genera and 18,000 species were worldwide in distribution. Qi-ming and De-lin (2009) recorded that about 56 genera, 2800 species were distributed in tropical and subtropical regions, especially in south America, a few species extend into temperate area; about 17 genera and 66 species in China; 9 genera and 17 species in Hong Kong. However, Kress *et al.* (2003) recorded that 84 genera and 510 species of Fabaceae were mentioend in Myanmar.

Zinbyun village is situated 19° 53' N latitude and 94° 25' E longitude. It lies 349 m above sea level. The trees found in this area was *Millettia brandisiana* Kurz.

Maylat village is located 19° 52' N latitude and 94° 29' E longitude, 417 m above sea level. The trees found in this area were *Dalbergia cultrate* Grah. ex Benth., *Dalbergia rimosa* Roxb., The shrubs were *Indigofera tinctoria* L., *Dalbergia volubilis were* also found in this area.

Lintel village is situated 19° 51' N latitude and 94° 22' E longitude. It lies 1109 m above sea level. The trees found in this area were *Erythrina stricta* Roxb.,

In the study area, the species *Dalbergia oliveri* Gamble, was less commonly found throughout the study area. Ng and Wee (1994) mentioned that these plants were recorded as globally threatened and endangered species in the Red Data Book of Singapore. The species *Dalbergia oliveri* Gamble ex Prain was mentioned as velnerable species and endangered species by IUCN (Appendix IV). These plants were sparsely found in the study area.

Therefore, these threatened and endangered species are needed to conserve. It is hoped that the resulting of the floristic information and knowledge of natural plant resources are beneficial to the further studies and this research intends not only to provide the information of natural vegetation but also to partially fulfill the compilation the Flora of Nat Yay Gan mountain area in Nagphe Township in Myanmar.

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ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF FUNGI FROM BEIKTHANO ANCIENT CITY AND THEIR ANTIMICROBIAL ACTIVITY

Khin Nilar Oo[1], Zar Zar Yin[2]

Abstract

Soil samples were collected from four different places of Beikthano Ancient city, Taungdwingyi Township, Magway Region. Twenty fungi were isolated from these four different soil samples. Isolation of fungi were undertaken by the chemical treatment method. The soil fungi were isolated by using Low Carbon Agar medium and Malt Extract Agar medium for first culture and Potato Glucose Agar medium for pure culture. The isolated fungi were given as NLF-1 to NLF-20. The surface color of isolated fungi NLF-1 to NLF-20 were white, green, orange white, greenish yellow, yellow, pale brown, black, gray and their reverse color were pale yellow, white, cream, greenish yellow, brown, gray and pink. Antimicrobial activity of isolated fungal strains was evaluated by the agar well diffusion assay with four test organisms. These isolated fugal strains were tested by using 3 days to 10 days old culture. Nine strains showed the antimicrobial activity against *Agrobacterium tumefaciens*, *Aspergillus paraciticus*, *Micrococcus luteus* and *Pseudomonas fluorescens.* Among them, NLF-14 showed the highest antibacterial activity (32.53 mm) on *A. tumefaciens* followed by NLF-7 (29.40 mm) and NLF-16 (29.96 mm) respectively. And then, NLF-14 also showed the highest antifungal activity (32.43 mm) on *A. paraciticus* followed by NLF-9 (31.09 mm) and NLF-6 (30.73 mm). Among all the selected fungi, NLF-12 showed the best inhibition zone (34.85 mm) against *M. luteus.*NLF-6 showed the highest antibacterial activity (30.26 mm) on *P. fluorescens.*

Keywords: Isolation, Soil Fungi, Antimicrobial activity

Introduction

Soils are highly complex system, with many components playing diverse function mainly due to the activity of soil organisms (Goddeyya. G., 2012). Microorganisms live in every part of the biosphere including soil hot spring, seven miles deep in the ocean 40 miles high in the atmosphere and inside rocks for down within the Earth's crust. Soil is the largest source of microorganisms. There are billions to hundreds of billions of soil microorganisms in a mere handful of a typical garden soil.

That single handful might well contain thousands of different species of bacteria, hundreds of different species of fungi and protozoa. Almost all of these countless soil organisms are not only beneficial, but essential to the life giving properties of soil. Microorganisms constitute an important source of biodiversity in soils and are an integral part of terrestrial ecosystems. They contribute to major biological functions such as nutrient and gas cycling, biogeochemical processes and decomposition and transformation of organic matter (Kiran *et al.,* 1999).

The spectrum of antimicrobial activity of the active substance is very important for the competition in nature. In the soil, where most antimicrobial producing microorganisms are found, life is competitive (Lancini *et al.,* 1982).The aim and objectives of this study were to isolate the soil fungi , to study the morphology of isolated fungi and to observe the preliminary study of antimicrobial activities by using four test organisms.

[1] Lecturer, Department of Botany, Magway University
[2] Associate Professor, Department of Botany, Pathein University

Materials and Methods

Collection of Soil Samples

Soil samples were collected from four different places of Beikthano Ancient city, Taungdwingyi Township, Magway Region. Soil samples of four different places were collected during July 2016 to August 2016.

Isolation of fungi from the soil samples

The soil fungi were enumerated by chemical treatment dilution method or media such as (LCA) Low Carbon Agar medium, (MEA) Malt Extract Agar medium and (PGA) Potato Glucose Agar medium.

Table 1 Four different soil samples collected from Beikthano Ancient City

Soil samples No.	Samples collected areas	pH	Soil types	Location
S.I	Gokkone	9.54	Loam	N 19º 59.421' E 95º 24.432'
S.II	Shwe-yaung-taw	6.87	Sandy Loam	N 20º 0.874' E 95º 23.371'
S.III	Nan-twin-taw ya Monastery	9.04	Sandy Loam	N 20º 0.852' E 95º 23.277'
S.IV	Beikthano station	6.80	Sandy Loam	N 19º 59.646' E 95º 23.353'

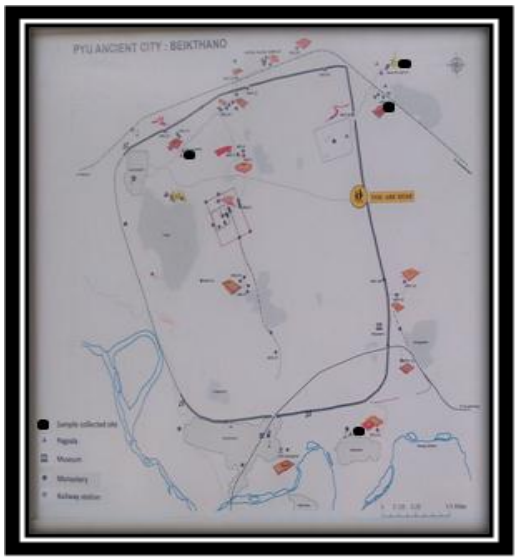

Figure 1 Map of Beikthano Ancient City (Source Beikthano Museum)

Soil isolation method

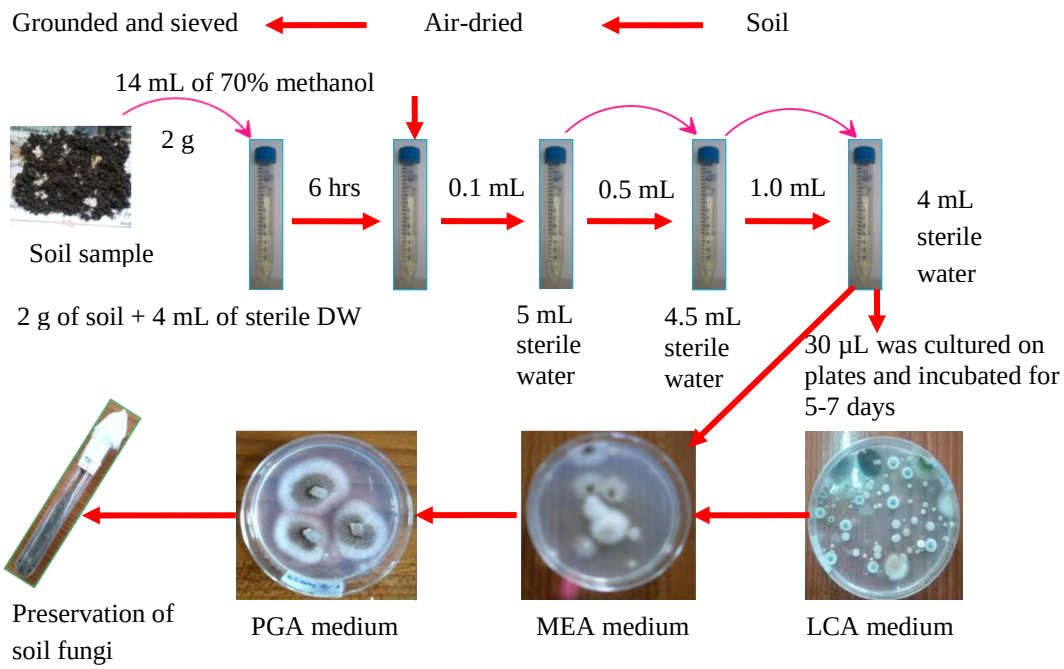

Figure 2 Isolation procedure for fungi from soil samples

Chemical treatment dilution method (Phay & Yamamura, 2005)

- Soil was air-dried at room temperature
- Grounded and sieved
- 2 g of the sieved soil is put into test tube
- 4 ml of sterilized distilled water is also put into the tube containing soil
- 14 ml of 70% of Methanol solution is then added into the tube containing soil suspension and shaken for 1 minute and dilution with sterile water
- Culture on LCA medium and MEA medium

Media Used for the Isolation of Fungi (Ando, 2004)

Low Carbon Agar (LCA) medium

Components per liter (for first culture)

Glucose	2 g
Sucrose	2 g
K_2HPO4	1 g
$MgSO_4.7H_2O$	0.5 g
KNO_3	1 g
KCL	0.5 g
Agar	18 g
pH	6.5

After autoclaving chloramphenicol was added into the medium.

Potato Glucose Agar (PGA) medium

Components per liter

(for transfer culture)

Potato	20 g
Agar	18 g
Glucose	2 g
pH	6.5
DW	1000 ml

After autoclaving chloramphenicol was added into the medium.

Malt Extract Agar (MEA) medium
Components per liter (for first culture)

Malt Extract	20 g
Agar	20 g
Glucose	20 g
Peptone	1.0 g
pH	6.5
DW	1000 ml

After autoclaving chloramphenicol was added into the medium.

Medium used Antimicrobial activity test (Ando, 2004)
(Seed Medium)

Glucose	15 g
Yeast extract	3 g
Polypeptone	2 g
K_2HPO_4	0.01 g
DW	1000 ml

Medium used Antimicrobial activity test (Ando, 2004)
(Fermentation Medium)

Glucose	10 g
Yeast extract	3 g
Polypeptone	2 g
K_2HPO_4	0.01 g
DW	1000 ml

(Assay Medium)

Glucose	5 g
Polypeptone	2 g
Agar	16 g
DW	1000 ml

Isolated strains were subjected with antimicrobial activities by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3-10 days old culture fermented broth (20 µl) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones has observed as potent activity shown by respective strain. Clear zones surrounding the test wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively (Collins, 1965).

Table 2 Test organisms used in antimicrobial activities (NITE, 2004)

Sr No	Test organisms	Infections
1	*Agrobacterium tumefaciens* NITE 09678	Plant disease
2	*Aspergillus paraciticus* IFO 5123	Fruit disease
3	*Micrococcus luteus* NITE 83297	Skin disease
4	*Pseudomonas fluorescens* IFO 94307	Rice disease

Results

Isolation of fungi from soil samples

In the investigation, 20 fungi were isolated from the four different soil samples of BeikthanoAcient city, Taungdwingyi Township, Magway Region. Isolated fungi NLF-1 to NLF-5 were collected from Gokkone village, NLF-6 to NLF-15 from Shwe-yaung-taw, NLF-11 to

NLF-15 from Nan-twin-taw-ya Monastery and NLF-16 to NL-20 were collected from Beikthano station. These results were shown in Table 3 and Fig. 3-4.

The isolated fungi were designated as NLF-1 to NLF-20. The surface color of isolated fungi NLF-1, 3, 17 and 20 were white and their reverse colors were white, pink, pale yellow and yellow. The surface color of isolated fungi NLF-2 is greenish yellow and reverse color was cream. The surface color of isolated fungi NLF-4, 5, 8, 10 and 18 were green and their reverse color were white, pale orange, pale yellow, cream and black green. The surface color of isolated fungi NLF-6 and 9 were pale orange and their reverse color were white and greenish yellow. The surface color of isolated fungi NLF-7 was black and its reverse color was gray.

The surface color of isolated fungi NLF-11 and 13 were pale brown and their reverse color were brown and cream. The surface color of isolated fungi NLF-12 was gray and its reverse color was gray. The isolated fungi NLF-14 was greenish white and its reverse color was cream. The surface color of isolated fungi NLF-15 was yellowish brown and its reverse color was brownish white. The surface color of isolated fungi NLF-16 and 19 were brownish yellow and their reverse color were brownish orange.

Table 3 Isolated fungi from soil samples

Soil samples No.	LCA Medium	MEA Medium
S.I	NLF-1, NLF-2	NLF-3, NLF-4, NLF-5
S.II	NLF-6	NLF-7, NLF-8, NLF-9, NLF-10
S.III	NLF-11, NLF-12	NLF-13, NLF-14, NLF-15
S.IV	NLF-16, NLF-17	NLF-18, NLF-19, NLF-20

Table 4 Morphological character of isolated fungi

No.	Isolated Fungi	Character	
		Surface color	Reverse color
1	NLF-1	White	White
2	NLF-2	Greenish yellow	Cream
3	NLF-3	White	Pink
4	NLF-4	Green	White
5	NLF-5	Green	Pale orange
6	NLF-6	Pale Orange	White
7	NLF-7	Black	Gray
8	NLF-8	Green	Pale yellow
9	NLF-9	Pale Orange	Greenish yellow
10	NLF-10	Green	Cream
11	NLF-11	Pale brown	Brown
12	NLF-12	Gray	Gray

No.	Isolated Fungi	Character	
		Surface color	Reverse color
13	NLF-13	Pale brown	Cream
14	NLF-14	Greenish white	Cream
15	NLF-15	Yellowish brown	Brownish white
16	NLF-16	Brownish yellow	Yellow
17	NLF-17	White	Pale yellow
18	NLF-18	Green	Black green
19	NLF-19	Brownish yellow	Brownish orange
20	NLF-20	White	Yellow

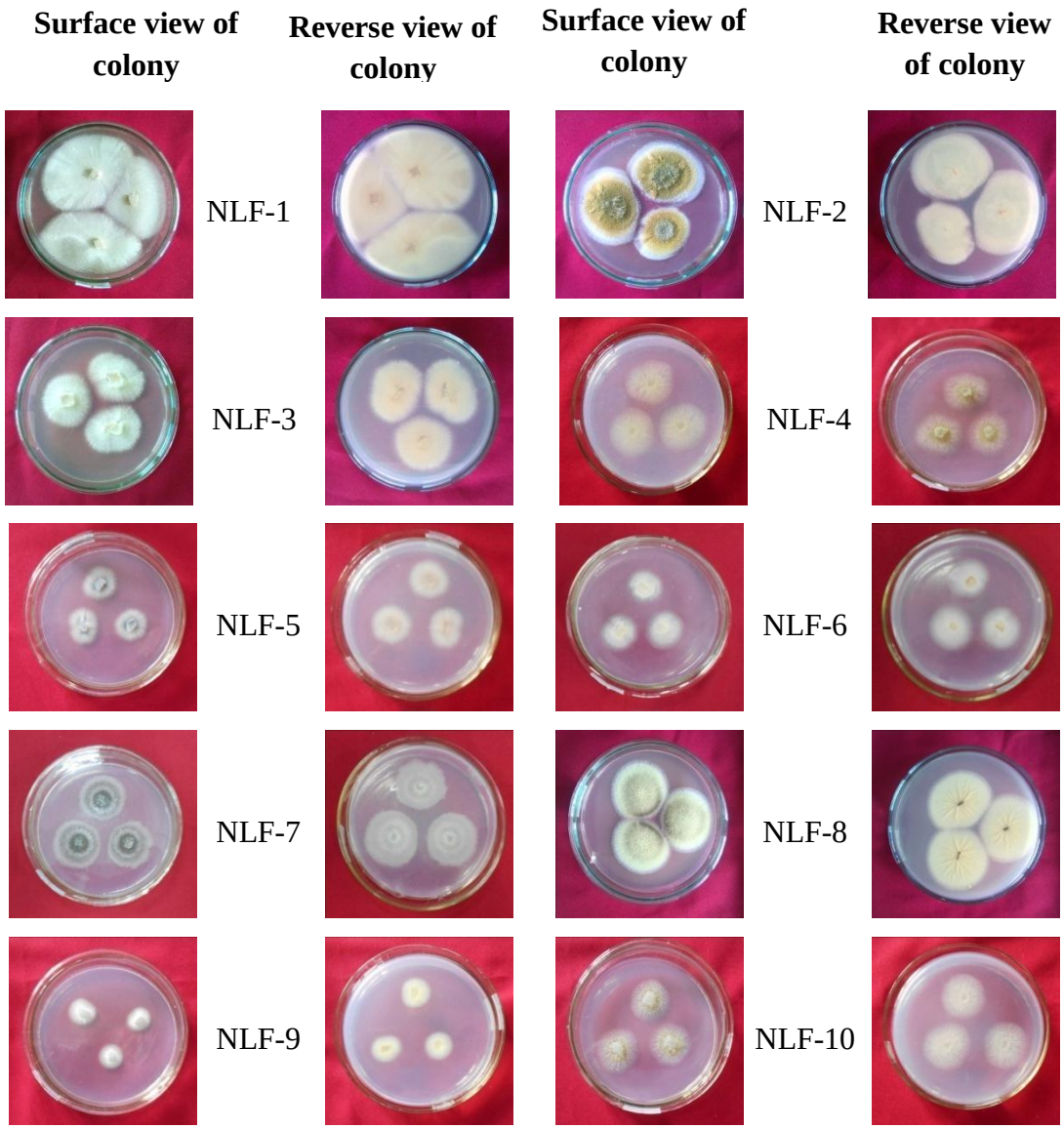

Figure 3 Morphological characters of isolated fungi NLF-1 to NLF-10 (5-days)

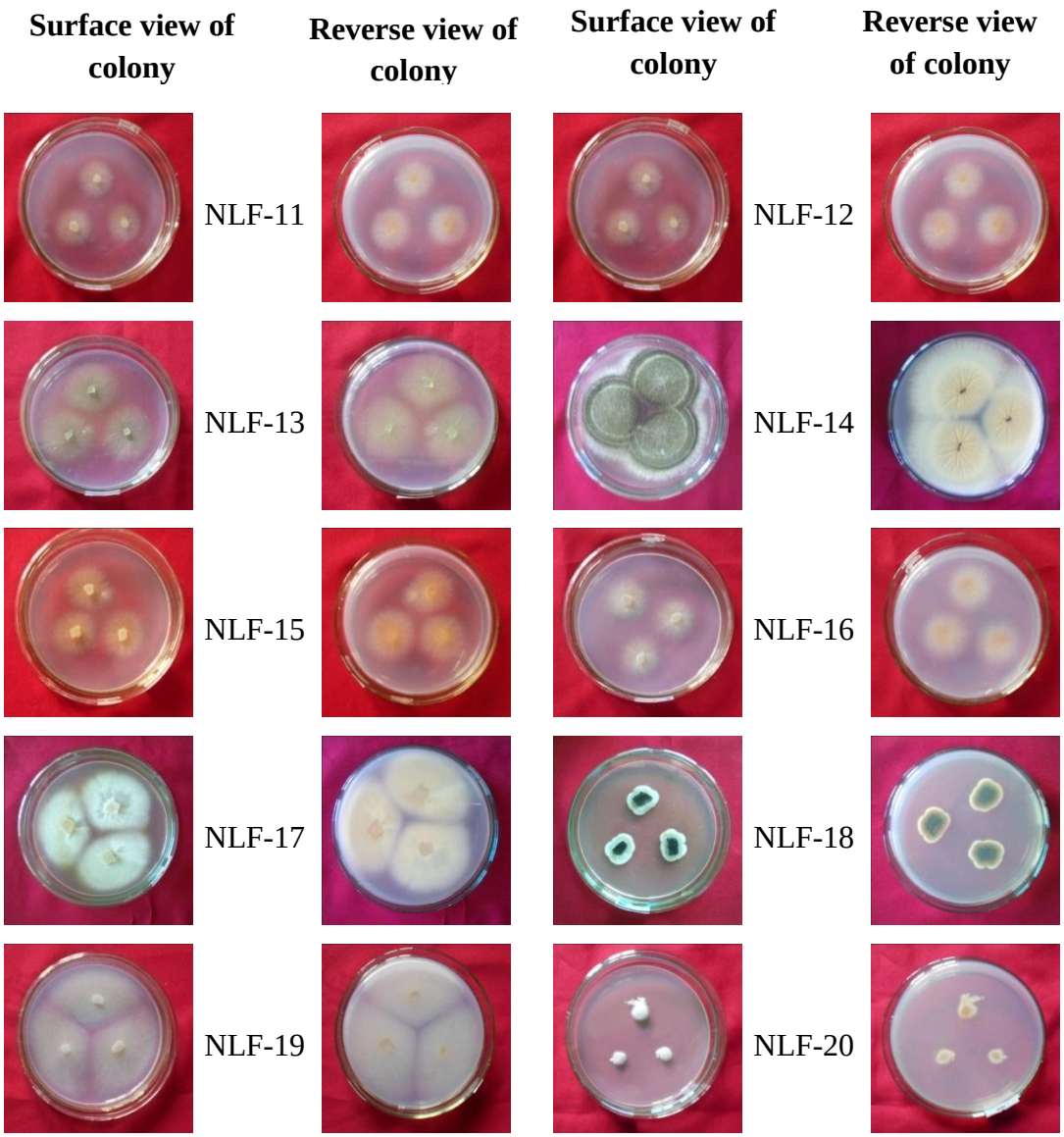

Figure 4 Morphological characters of isolated fungi NLF-11 to NLF-20 (5-days)

Figure 5 Pure culture of isolated fungi from soil sample 4 (NLF 1-20)

Antimicrobial activities of isolated fungal strains

Nine isolated fungi (NLF-6, 7, 9, 10, 12, 14, 15, 16 and 18) had antimicrobial activity and remaining eleven isolates (NLF-1, 2, 3, 4, 5, 8, 11, 13, 17, 19 and 20) could not produce antimicrobial metabolites. NLF-14 showed the highest antimicrobial activity (32.53mm and

32.43mm) against *Agrobacterium tumefaciens* and *Aspergillus paraciticus* in 6 days fermentation period respectively. NLF-12 showed the best inhibition zone (34.85mm) on *Micrococcus luteus* and NLF-6 showed the antibacterial activity (30.26mm) against *Pseudomonas fluorescence* in 5 days fermentation period. These results were displayed in figure (6, 7, 8). Among them, antibacterial activity of isolated fungus NLF-12 showed the maximum inhibitory zone against *Micrococcus luteus*.

Table 5 Antibacterial Activity of Nine Selected Fungi against *Agrobacterium tumefaciens*

Sr No.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	17.8	19.56	21.84	28.49	26.50	24.32	23.66	20.71
2	NLF-7	19.3	25.99	29.40	28.70	25.43	19.80	17.23	15.63
3	NLF-9	18.4	20.56	23.73	18.43	15.66	14.73	13.56	12.00
4	NLF-10	13.6	16.54	17.06	23.43	18.95	15.12	14.00	13.21
5	NLF-12	16.3	16.66	18.95	24.38	25.41	22.80	21.32	17.00
6	NLF-14	23.1	25.04	29.00	32.53	30.05	28.74	25.00	20.00
7	NLF-15	14.5	18.00	21.52	24.00	27.00	25.12	23.00	19.00
8	NLF-16	12.5	13.73	17.60	29.56	18.74	14.20	13.18	-
9	NLF-18	19.7	20.93	25.11	27.43	22.00	23.48	21.73	18.56

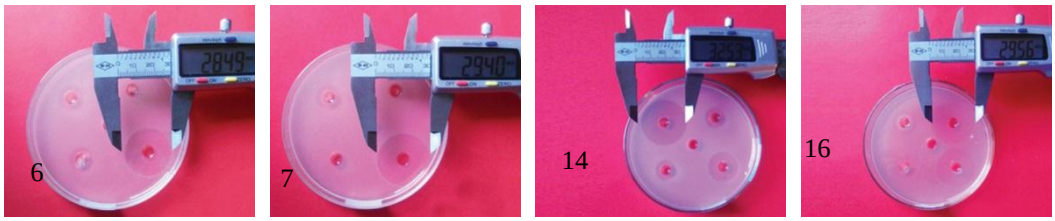

Figure 6 Antibacterial activity of isolated fungi against *Agrobacterium tumefaciens*

Table 6 Antifungal Activity of Nine Selected Fungi against *Aspergillus paraciticus*

Sr No.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	20.	27.34	28.93	30.73	30.10	29.40	28.50	23.00
2	NLF-7	15.	18.16	22.96	29.96	27.14	25.56	23.12	19.00
3	NLF-9	22.	27.89	29.02	31.09	28.50	27.73	26.18	18.00
4	NLF-10	16.	19.38	20.32	25.25	17.78	16.13	14.50	-
5	NLF-12	18.	25.76	30.12	28.34	24.70	20.82	17.12	15.56
6	NLF-14	16.	17.19	24.45	32.43	19.67	19.46	17.64	16.59
7	NLF-15	15.50	18.23	20.12	25.43	17.56	16.12	15.04	-
8	NLF-16	17.13	19.28	23.43	25.76	20.84	22.76	19.43	17.21
9	NLF-18	20.44	24.01	26.05	25.22	24.78	22.02	19.82	16.00

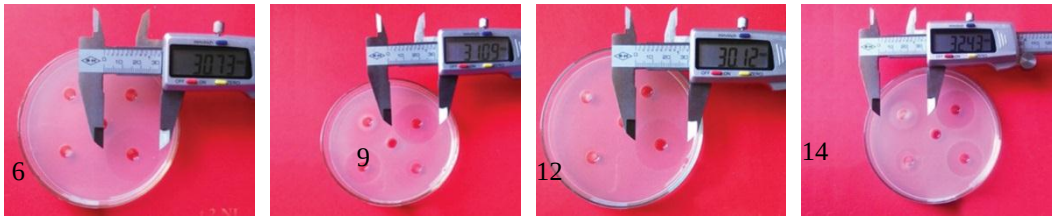

Figure 7 Antifungal activity of isolated fungi against *Aspergillus paraciticus*

Table 7 Antibacterial Activity of Nine Selected Fungi against *Micrococcus luteus*

Sr No.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	24.35	16.89	27.12	28.43	26.43	23.12	20.42	18.00
2	NLF-7	14.12	20.95	28.54	26.42	23.78	20.43	19.12	17.13
3	NLF-9	20.12	24.56	28.43	30.09	27.12	25.43	24.56	19.84
4	NLF-10	18.12	19.00	23.43	25.73	20.45	18.84	12.11	11.56
5	NLF-12	22.54	28.76	34.85	29.74	28.70	25.43	20.12	18.64
6	NLF-14	17.84	19.21	23.42	27.12	18.43	17.18	15.43	13.21
7	NLF-15	15.10	17.64	19.81	20.43	18.12	16.00	14.21	12.55
8	NLF-16	11.51	16.54	19.21	23.78	25.64	24.12	22.56	13.96
9	NLF-18	14.56	17.12	19.96	25.43	18.53	16.12	15.41	14.12

Figure 8 Antibacterial activity of isolated fungi against *Micrococcus luteus*

Table 8 Antibacterial Activity of Nine Selected Fungi against *Pseudomonas fluorescens*

SrNo.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3 days	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	20.41	25.64	30.26	28.12	26.43	24.12	22.76	19.48
2	NLF-7	15.53	17.12	18.94	20.42	23.73	20.65	18.56	16.72
3	NLF-9	17.34	19.42	23.43	29.99	22.56	20.43	18.76	17.00
4	NLF-10	18.74	20.60	26.60	24.12	22.76	20.12	18.42	16.00
5	NLF-12	15.64	18.67	25.27	23.00	21.56	19.56	17.43	16.72
6	NLF-14	19.93	21.43	23.56	26.00	24.12	20.56	19.00	17.76
7	NLF-15	13.97	15.82	16.36	17.84	18.03	19.67	16.43	15.00
8	NLF-16	14.54	16.76	18.43	20.76	17.12	15.43	14.12	-
9	NLF-18	21.43	25.64	26.00	27.77	23.43	20.56	18.80	17.00

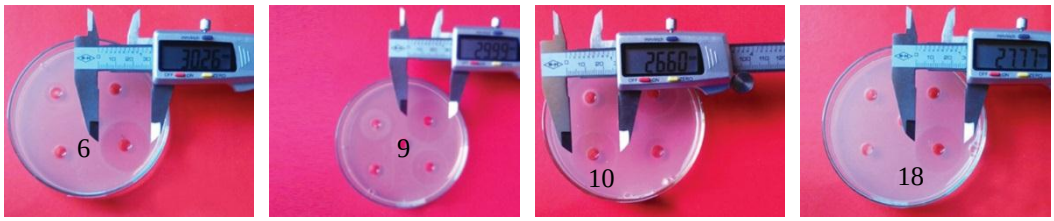

Figure 9 Antibacterial activity of isolated fungi against *Pseudomonas fluorescens*

Discussion and Conclusion

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

A total of twenty fungi were isolated from four soil samples collected at Beikthano Ancient City, Taungdwingyi Township, Magway Region. The fungi NLF-1, 2, 3, 4, & 5 were isolated from Gokkone village (Loam), NLF-6, 7, 8, 9 & 10 from Shwe Young Taw (sandy loam), NLF-11, 12, 13, 14 & 15 from Nan Twin Taw Ya monastery, (sandy loam) NLF-16, 17, 18, 19 & 20 from Beikthano station (sandy loam). All these fungi were different according to their morphological characters. NLF-1, 2, 6, 11, 12, 16 & 17 were white, greenish yellow, pale orange, pale brown, gray and white in the surface and reverse color was white, cream, brown, gray and pale yellow on the LCA medium.

Sharma (2010) reported that white to orange with green spores at centre and bright orange on reverse side in Potato Dextrose Agar (PDA) and colourless on both side Low Carbon Agar (LCA) medium. NLF-3, 4, 5, 7, 8, 9, 10, 13, 14, 15, 18, 19 & 20 were white, green, black, pale orange, greenish white and brownish yellow in the surface and reverse color were pink, white, gray, pale yellow, greenish yellow, cream, black green on the MEA medium. Ilhan (2006) reported that colonies on MEA were soluble pigment was lemon yellow, reverse was luteus to lemon yellow in colour.

In the investigation of antimicrobial activities, twelve fungi were tested with four test organisms by using Agar Well Method. Among them, NLF-6, 7, 9, 10, 12, 14, 15, 16 & 18 showed the antimicrobial activity on *Agrobacterium tumefaciens, Aspergillus paraciticus, Micrococcus luteus* and *Pseudomonas fluorescens.*

All of them, NLF-14 showed the highest antimicrobial activity (32.53 mm and 32.43 mm) against *A. tumefaciens* and *A. paraciticus* respectively. Moreover, NLF-12 showed the best inhibition zone (34.85 mm) on *Micrococcus luteus* and NLF-6 showed the antibacterial activity (30.26 mm) against *P. fluorescens.* According to the results, NLF-12 gave the highest antibacterial activity.

Acknowledgements

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POLLEN MORPHOLOGY, PHYTOCHEMICAL TEST AND ANTIMICROBIAL ACTIVITIES OF TEA LEAVES FOUND IN WAN SAING VILLAGE, KYAING TONG

Nwe Ni Tin[1], Nang Moon Sar[2], San San Oo[3]

Abstract

From the methanolic extract of green tea leaves of *Camellia sinensis* (L) Kuntze, collected from *Wan - Saing*, a *Loi* (ethics minority in Myanmar) village within the Tract of *Kat- Pha*, Kyaing Tong District, Eastern of Shan State. Phytochemical contents of the tea leaves were analyzed and evaluated. By these analyses, carbohydrate, glycoside, phenolic compound, α - amino acid, saponin, tannin, flavonoid, steroid, terpenoid and reducing sugar are found to be present. Notably, starch and cyanogenic glycosides were not observed in the extract sample under study. In addition the values of the nutrients from the leaf extract were examined using AOAC (Analytical Official of Chemic Method). As a result the energy value was found to be the highest with the potential value of 280 % whereas carbohydrate content is second in line with the concentration of 51.33%. The concentric percent of the remaining nutrients such as moisture, ash, crude protein, crude fiber and crude fat were found to be 11.73%, 5.02%, 10.42%, 17.80% and 3.70% respectively. Caffeine concentration was found as the lowest in concentration (0.27%). By agar-well diffusion method, using different kinds of solvents, the antimicrobial activity of constituents from the leaf extract were studied. Their inhibitory zones were also evaluated.

Keyword: pollen morphology, phytochemical tests, nutritional values and antimicrobial activities, Wan Saing Village, Kyaing Tong Township.

Introduction

The green tea made from leaves of Camellia *sinensis* (L.) Kuntze is the second most (after coffee) consumed beverage in the world. The tea bush grows best on well- drained acid habitat. Soil in a warm climate with ample rainfall of about 150 – 300 cm (60-200 in) per year. The tea plant in nature is a small tree but grown under cultivation it is intentionally kept under 2 m (3-5 ft) to enable pickers to harvest the young leaves. . Constant pruning stimulate the vigorous development of new shoot called "flushes". Harvesting is usually done by hand to ensure the best quality.

The quality is dependent on a number of factors: cave taken in cultivation, rain fall and elevation at which it was grown. The flavour and quality vary with the soil type, climate, age of the leaf, time of picking and method of preparation. In fact, the green tea itself is actually the dried which leaves are dull green with even texture.

There are three main types of tea: (i) green tea where the leaves are steam-dried without being allowed to ferment; (ii) black tea, where the leaves are fermented and dried; (iii) among Myanmar people there is still another type of tea consuming-pickled tea, where the tea leaves are steam- dried and fermented. As the saying goes " The king of the leaf is pickled tea leaf" consuming together with a variety of fried dried beans make the green tea the most palatable of all of food delicacies.

The significance of pollen attributes in taxonomy has been widely realized. Recently palynological data are found to be applicable critical in advance investigation on angiosperm

[1] Dr, Lecturer, Department of Botany, Kyaing Tong University.
[2] Dr, Lecturer, Department of Botany, Kyaing Tong University.
[3] Dr, Professor, Department of Botany, Kyaing Tong University

flora. Diagnoses base on pollen features have been found in agreement with those prepare on the basic of Botany (Metcalfe, 1961).

The classification of pollen is base on number position character analysis known as "NPC" system. Due to the fact that pollen morphology should have been property appraised in angiosperm systematic recent systems of classification proposed by Zimmer Mann and Cronquist. Palynological characteristic were provided and used as one of the main criteria in identification of the plant sample.

Thirteen bioactive components of green tea leaves have been detected the phytochemical test showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, α-amino acids, saponins, tannins, flavonoids, steroids, terpinoids and reducing sugar as the leaf components where as the starch and cyanogenic glycosides were totally absent.

When the nutritional value is considered, the following characteristics tendencies were observed: the energy content was found as the most abundant; caffeine was the leaf content of all with the value 0.27 in percent. The value of the remaining components were observed in the following sequence; Carbohydrate (51.33 %); Moisture (11.73%); Ash (5.02 %); Protein (10.42 %); Fiber (17.80 %); Fat (3.70 %).

Six different kinds of solvents were prepared for the detection of antimicrobial activity. This experimentation was conducted using agar – well diffusion method. The results showed that-

(i) The ethanol extract had the most efficient impact against all the six species of the microbes under study especially against *Bacillus pumilus* and *Escherichia - coli* with the maximum record inhibition zone of 21 mm.

(ii) The ethanol – water extract also showed the impact upon all the six microbes but with the inhibitory zone a bit limited in area.

(iii) The methanol extract showed to possess the inhibitory effect upon the other five microbe species except *Pseudomonous aeruginosa*.

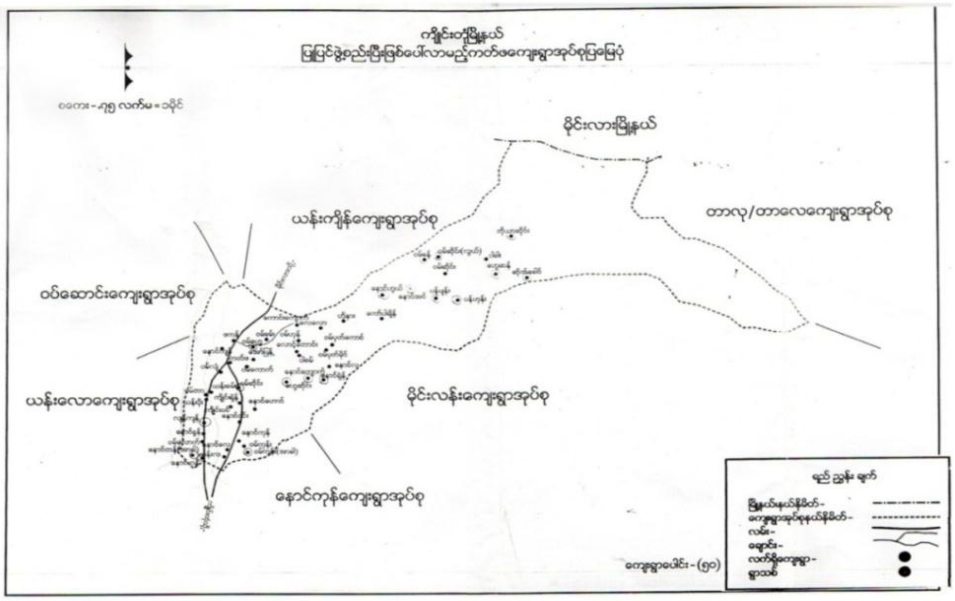
The present study was carried out to investigate the leaves of *Camellia sinensis* (L.) Kuntze, for medicinal and health for human being. It can be assume that the study result will be helpful to students or investigator who interested in morphology, nutritional value, phytochemical and antimicrobial activities.

Materials and Methods

1. Collection and plant systematic study

The green tea leaves of *Camellia sinensis* (L.) Kuntze were cultivated in *Wan-Sai*, village of *Loi* ethnics people, *Ka-Pha* tract, Kyaing Tong District. The specimens were collected during September to December, 2018 at the period of anthesis and fructification. Natural habit and flowers had been photographed. The plant material was identified and specimens are deposited in our Department Herbarium, University of Kyaing Tong.

Location map of study area



2. Collection of pollen samples

A few stamens of *Camellia sinensis* (L.) Kuntze were taken from buds (than in full bloom flowers). Collected pollens was stored in small glass vials with 1 cc of glacial acetic acid and labeled.

2 (a) Acetolysis of reference material

Polliniferous material was crushed with a glass rod, 1cc of glacial acetic acid was added and then 3 to 5 drops of concentrated sulphuric acid was added to the above. The test tube was put in a water-bath for 15 to 30 minutes. The fluid in the test tube was stirred frequently and boiled. On cooling, the mixture was diluted with distilled water to centrifuge for 15-30 minutes. This was repeated twice, decanting the water each time. After centrifuging, the distilled water was removed and glycerine jelly with saffranin was added to the polliniferous material and this was then transferred to store in air-tight bottle and labeled.

2 (b) Preparation of glycerine jelly

It is prepared by Kisser's formula (Erdtman, 1952), using 50 gms of gelatine, 150 ml of glycerine and 7 gms of phenol crystals were mixed with 175 ml of distilled water in a beaker and stirred with a glass rod. This was heated for about 3 hours in a water bath till homogenous. Then 0.05g of saffranin was finally added just before removal and stored. Only the amount of material needed during the day should be made each time.

2 (c) Preparation of microscopic slides

The sample bottle was warmed in a water bath and a drop of polllliniferous jelly was taken out with a pair of forceps and placed on the glass slide, and then covered with a cover slip. The pollen sample was examined by using electric Novex Trinocular Microscope with 40 X and imaged by taking Canon (8.0 megapixels) Digital Camera. A micrometer was used to measure the size of the grain.

3. Phytochemical Examination of leaves of *Camellia sinensis* (L.) Kuntze

The dried leaves of green tea plant *Camellia sinensis* (L.) Kuntze were cut and repeatedly washed with water. The slice samples were dried under shades for three weeks. Dried samples were ground into powder form for phytochemical investigation.

Phytochemical test of the sample has been conducted to at Food Industries Development Supporting Laboratory (FIDSL), Myanmar Food Processors and Exporters Association (MFPEA), Yangon.

The leaf powders were extracted 3 times with methanolic from the methanolic extracts, 13 major photochemical viz, alkaloids, carbohydrates, glycosides, phenolic compounds, α-amino acids, saponins, tannins, flavonoids, steroids, terpinoids, reducing sugar, starch and cyanogenic glycosides were isolated and analyzed.

4. Nutritional value investigation

The nutritional value of the leaves of *Camellia sinensis* (L.) Kuntze were identified according to analytical official of chemic methods (AOAC), at Food Industries Development Supporting Laboratory (FIDSL), Yangon.

5. Antimicrobial activities

The samples were dried and powdered. The leaf powder was subjected to examine antimicrobial activities by Ager-well diffusion method after Cruickshank, 1970.The crude sample was subjected to antibacterial screening against some pathogenic organisms. These organisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *Escherichia coli*. These investigations were conducted at the Pharmaceutical Research Department of Ministry of Industry Yangon Division.

Results

I. (a) Systematic Position of the Tea plant

Kingdom	- Plantae
Division	- Magnoliophyta
Class	- Magnoliopsida
Order	- Theales
Family	- Theaceae
Genus	- *Camellia* L.
Species	- *Camellia sinensis* (L.) Kuntze
Myanmar name	-Laphet
English name	-GreenTea
Loi name	-Larce^
Shan name	-La

I. (b) Taxononic Description

Shrubs or trees, stem and branches cylindrical, internodes 2.0 to 3.5 cm long, 2.0-4.0 mm wide, grayish-brown, white-pubescent. Leaves simple, alternate, exstipulate, petiolate; petiole terete, 4.0-7.0 mm, green, pubescent, glabrescent. Blade oblong- elliptic, leathery 5.0-16.0 cm long and 2.0-8.0 cm wide, abaxially pale green and glabrous, adaxially dark green, shiny and

glabrous midvein, serrate to serrulate along the margin acute to acuminate at the apex, with an obtuse tip, cuneate to broadly cuneate at the base. Lateral nerves 5 – 7 on each side of midvein, reticulate veins visible on both surfaces. Flowers bisexual, actinomorphic, hypogynous, white, axillary, solitary 2 – 4 cm across at anthesis, ebracteate, bracteolate, pedicellate; pedicel 5.0 -10.0 mm, recurved pubescent or glabrous, thickened toward apex. Bracteoles 2, caduceus, ovate; sepal 5-6, broadly ovate to suborbicular. Petals 5-6, white outer 1-3 petals sepaloid; inner petals obovate to broadly obovate. Ovary globose, sparsely white pubescent, 3-loculed; style linear, glabrous or base pubescent, apically 3-lobed. Capsule oblate, rarely globose, 1-3 loculed with 1-2 seeds per locule; pericarp about 1mm thick. Seeds subglobose, brown .

Flowering and fruiting period : September to December

A distant view of Tea Plantation on mountain slop

Habit

A tall tea plant understudy

Tea leaves

Flower

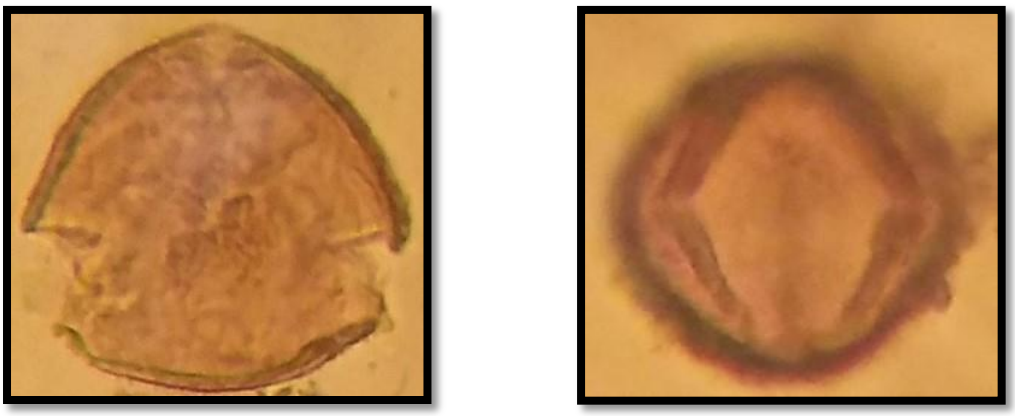
Fruits

Figure 1 Morphological characters of *Camellia sinensis* (L.) Kuntze.

2. Pollen morphology of *Camellia sinensis* (L.) Kuntze.

The present research concerns the palynology of *Camellia sinensis* (L.) Kuntze, of Family Theaceae. The palynological data is illustrated with figure as follow-

Aperture condition tricolporate, medium size, pollen shape oblate, 25-30 X 25-35µ in length and breadth; amb subtriangular; polarity isopolar; colpi longicolpate, about 20-30 x 3-5 µ in length and breadth; pori lolongate about 3.5 x 2.5 µ in length and breadth; exine thicker than nexine; sculpturing verrucate.



Polar view Equatorial view

Figure 2 Pollen Morphological characters of *Camellia sinensis* (L.) Kuntze.

3. Phytochemical examination of leaves of *Camellia sinensis* (L.) Kuntze.

Preliminary phytochemical tests of the leaves of Tea plant indicated the experimental result was shown in table (1).

Table 1 The Phytochemical examination of *Camellia sinensis* (L.) Kuntze.

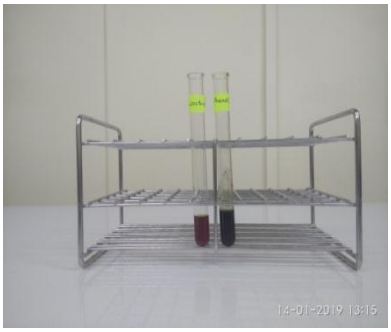
No.	Type of compound	Extract	Reagent used	Observation	Results
1.	Alkaloid	1%HCL	Mayer's reagent	Cream colour ppt.	+
			Wagner's reagent	Brown ppt.	
			Dragendorff 's reagent	Reddish brown ppt.	
			Hager's reagent	Yellow ppt.	
2.	Carbohydrate	H_2O	10% α -naphthol & H_2SO_4 (Conc:)	red ring	+
3.	Glycoside	H_2O	10% Lead acetate solution	White ppt.	+
4.	Phenol	H_2O	5% $FeCL_3$ solution	Greenish black ppt.	+
5.	α-amino acid	H_2O	Ninhydrin reagent	Pink colour	+
6.	Saponin	H_2O	H_2O	Persistent foam	+
7.	Tannin	H_2O	1% Gelatin & 10% NaCL solution	ppt.	+
8.	Flavonoid	70%EtOH	Mg ribbon & Conc; HCL	Pink colour	+
9.	Steriod	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Bluish green	+
10.	Terpenoid	Petroleum ether	Acetic anhydrite & Conc; H_2SO_4	Pink	+
11.	Reducing sugar	H_2O	Fehling's solution	Brick red ppt.	+
12.	Starch	H_2O	Iodine solution	Brown ppt.	-
13.	Cyanogenic glycoside	powder	H_2O,Conc; H_2SO_4 sodium picrate paper	No colour change	-

(+) = present (-) = absent

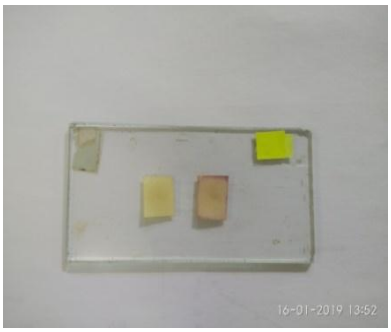
Test for Alkaloid

Test for Carbohydrate

Test for Glycoside



Test for Phenol



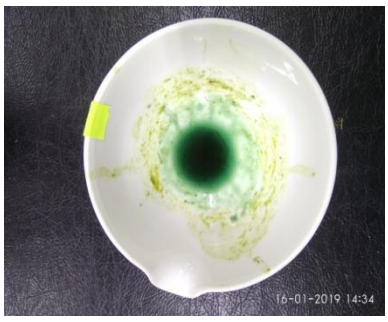
Test for α-Amino acid

Test for Saponin

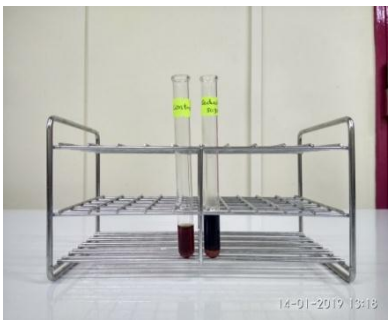
Test for Tanin

Test for Flavonoid

Test for Steroid

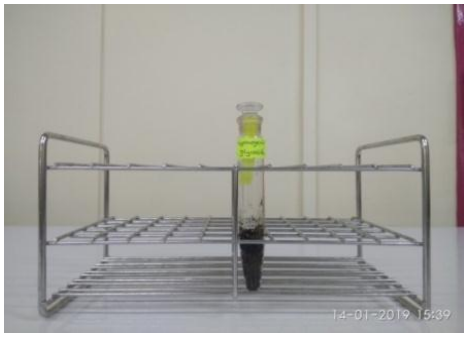


Test for Terpenoid



Test for Reducing sugar

Test for Starch




Test for Cyanogenic glycoside Thirteen kind of Phytochemical test

Figure 3 Test for Phytochemical examination of *Camellia sinensis* (L.) Kuntze.

4. Nutritional value of leaves of *Camellia sinensis* (L.) Kuntze,

Determination of nutritional value carried out at the laboratory of Food Industries Development Supporting Laboratory (FIDSL). The result of the detection showed that the energy content was found to be the most abundant, the value of carbohydrate and the remaining nutrients are as shown in table (2).

Table 2 Nutritional value of leaves of *Camellia sinensis* (L.) Kuntze

No	Type of Nutrients	Content (%) of leaves of *Camellia sinensis* (L.) Kuntze
1	Moisture	11.73 %
2	Ash	5.02 %
3	Protein	10.42 %
4	Fiber	17.80 %
5	Fat	3.70 %
6	Carbohydrate	51.33 %
7	Energy (Kcal / 100g)	280 %
8	Caffeine	0.27 %

5. Antimicrobial activities of leaves of *Camellia sinensis* (L.) Kuntze

Antimicrobial activities were conducted at the Pharmaceutical Research Department of Ministry of Industry, Yangon Division.

Table 3 Test for Antimicrobial activities of leaves of *Camellia sinensis* (L.) Kuntze

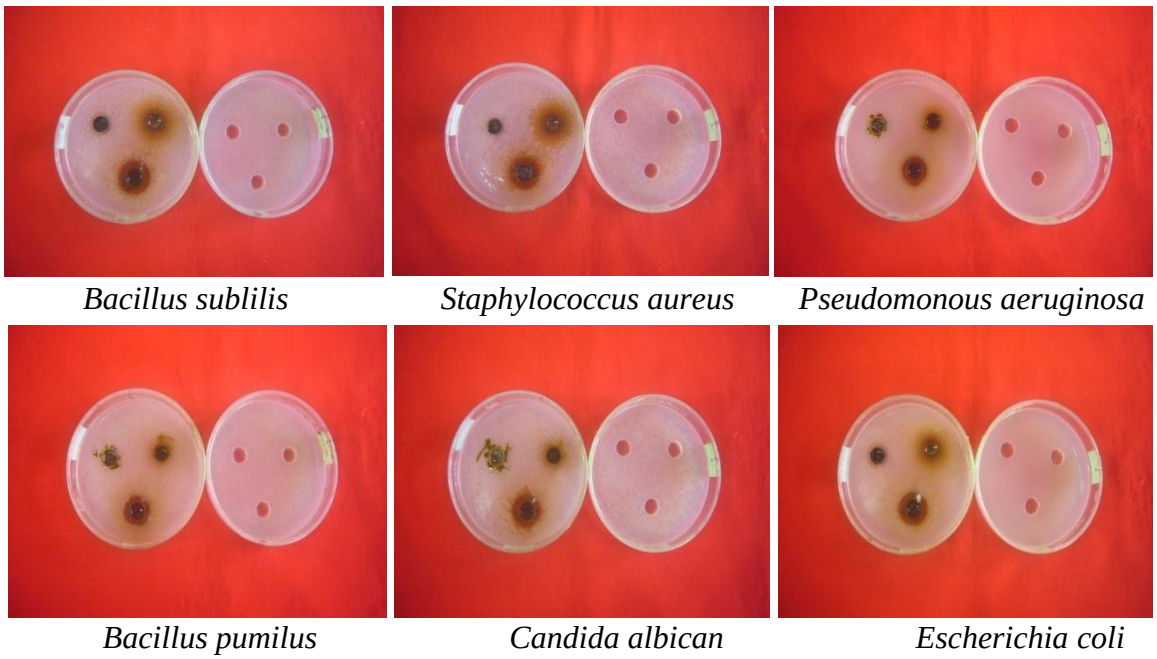
Samples	Solvents	Organisms					
		Bacillus subtilis	***Staphylo coccus aureus***	***Pseudomo-nas aeruginosa***	***Bacillus pumilus***	***Candida albican***	***E-coli***
Tea Plant	Pet-ether	-	-	-	-	-	-
	Methanol	19 mm (++)	20 mm (+++)	-	20 mm (+++)	20 mm (+++)	18 mm (++)
	Acetone	12 mm (+)	-	-	14 mm (+)	15 mm (++)	14 mm (+)
	Ethanoic acid	15 mm (++)	-	-	15 mm (++)	15 mm (++)	16 mm (++)
	Ethanol	19mm (++)	20 mm (+++)	20 mm (+++)	21 mm (+++)	17 mm (++)	21mm (+++)
	Water	17 mm (++)	15 mm (++)	17 mm (++)	16 mm (++)	17 mm (++)	16 mm (++)

Agar well – < 10 mm (inactive), 10 mm ~ 14 mm (partially active), 15 mm ~ 21 mm (active)

Organisms

Agar well – 10 mm
10 mm ~ 14 mm (+)
15mm ~ 19 mm (++)
20 mm above (+++)

(1) *Bacillus sublilis*
(2) *Staphylococcus aureus*
(3) *Pseudomonous aeruginosa*
(4) *Bacillus pumilus*
(5) *Candida albican*
(6) *Escherichia coli*



Bacillus sublilis *Staphylococcus aureus* *Pseudomonous aeruginosa*

Bacillus pumilus *Candida albican* *Escherichia coli*

Figure 4 Antimicrobial treatment of different solvent extracts of leaves of *Camellia sinensis* (L.) Kuntze

Discussion and Conclusion

Man has always sought to make his drinks more palatable to taste than is pure water. The diffusion and decoctions from variously processed plant parts; especially the tea and coffee are the most commonly used today. Tea is obtained from the tea plant, *Camellia sinensis* (L.) Kuntze. The naturally growing tea plants can be tall trees upon the height of 15-20ft. But they are usually heavily pruned and not allowed to reach more than 1.5 - 2.0 meters in height. Harvesting is traditionally done by hand to ensure the best quality. The tea plantation from which we collected the plant samples is located at the attitude of about 5000-5700 feet and above.

It is a large plantation cultivated upon steep ridges and mountain slopes with soil that is too poor for other types of agriculture can thrive on. The tea plant is propagated from seeds or from seedling. In the above said plantation apart from low shrubs for commercial purposes. There are some two hundred tall plants of about twenty feet. These plants continue growing for 50 years or more. It is said that the tea plants in their grove are propagated from seeds of a century old plants at their farm. The flavor of the processed tea is dependent on the content of the plant's phytochemical components. The stimulating effects are the result of its caffeine content.

The Phytochemical test of the leaf powder of the plant sample indicated that carbohydrate, glycoside, phenolic compound, α-amino acid, saponin, tannin, flavonoid, steroid, terpenoid and reducing sugar are present but that starch and cyanogenic glycoside were not observed.

From the view point of the nutritional value, the Energy (Kcal / 100) value was found to be the highest (280%) and the Carbohydrate (51.33 %), runs second whereas Moisture (11.73%), Ash (5.02 %), Protein (10.42 %), Fiber (17.80 %), Fat (3.70 %) and Caffeine was found to be the content the least (0.27 %) in this experiment.

To examine antimicrobial activities, leaf extract in six different kinds of solvent namely petroleum ether, methanol, acetone, ethanoic acid, ethanol and water were used by agar well diffusion method. The experiment revealed that the ethanol extract showed the maximum inhibitory effect with 21 mm inhibition zone, in ethanol extract against the *B pumilus* and *E. coli*. The ethanol-water extract showed antibacterial activity against all the six species of organism. The methanol extract showed antibacterial activity against the other five species except *Pseudomonous aeruginosa.* Acetone and Ethanoic acid extract showed antibacterial activity against other four species except *Staphylococcus aureus* and *Pseudomonous aeruginosa.* Pet ether extract do not showed the antibacterial activity.

The usefulness and applicability of pollen investigations in plant systematic should have been considered because many pollen traits are influenced by the strong selective forces evolved in various reproductive processes including pollination, dispersal and germination. In plant systematic criteria of pollen are of the pollen size, pollen shape, pollen type, structure of the pollen wall, pollen architecture, and number of aperture, aperture position and aperture shape. In this study, tricolporate, medium size, pollen shape oblate, amb subtriangular, polarity isopolar, exine thicker than nexine and sculpturing verrucate were found as the pollen charactreristics.

In this respect the authors like to suggest that the further advance investigations focusing on phytochemistry and physiology of the plant species are needed.

Acknowledgements

Our heartfelt sincere gratitude and thank go to Dr. San San Mar, Rector and Dr. Myat Nyunt, Pro-rector, University of Kyaing Tong for their permission to do this research. We would like to thank Dr San San Oo, Professor and Head and Dr Moe Moe Lwin, Professor, Department of Botany, University of Kyaing Tong for giving us all the necessary guidance and continuous encouragement in this research.

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ISOLATION, IDENTIFICATION OF BACTERIA FROM MANGROVE SOIL AND THEIR ANTIMICROBIAL ACTIVITY

Moe Moe Win[1] and Zar Zar Yin[2]

Abstract

The soil samples were collected from mangrove area at Shwe-thaung-yan Township, Ayeyawady Region. Isolation of bacteria was done by serial dilution method and cultured on Nutrient Agar (NA) medium (Atlas, 1993). As the results, total of seven bacterial strains (M-1 to M-7) were obtained and tested by agar well diffusion method with five kinds of test organisms. Four strains showed different levels of antimicrobial activities. Especially M-7 showed the best antifungal activity on *Candida albicans.* Therefore, M-7 was selected and identified by colony morphology, gram staining, spore staining, microscopical characters and biochemical reactions. According to the results, M-7 was characterized as the genus *Pseudomonas* sp.

Keywords: Mangrove Soil Bacteria, Antimicrobial activity, Identification, *Pseudomonas* sp.

Introduction

Mangrove are coastal wetlands mainly found at the intertidal zones of estuaries, deltas, creeks, lagoons, marshes of tropical and subtropical latitudes (Sahoo K, Dhal NK. 2009).

A vast suite of bacterial genera and functional types exist in mangrove soils and on above ground roots, but data are limited (Kathiresan and Bingham, 2001).

Antimicrobial agents play the most important role in the treatment of bacterial infections (Hacioglu, 2011) and wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic producing microbes (Oskay, *et al.,* 2004).

Natural products having novel structures have been observed to possess useful biological activities, soil is a natural reservoir for microorganisms and their antimicrobial products (Dancer, 2004).

The bacteriologist must separate the mixed population into the separate components or isolate the organism desired. In general bacteria are classified both on the basis of what they do and of what they look like. Observations of the morphology of the cells, of their staining properties of the colonies they form on agar, and of the physiological or biochemical behavior of pure cultures of bacteria are two important tools in the study, identification, and classification of these minute forms of life (Alongi, 1988).

Therefore, the aim and objectives of the present study were to isolate the bacteria from mangrove soil, to study the antimicrobial activities of isolated bacteria and to observe the identification of selected bacterial strain based on their colony characters, gram staining, spore staining, cell morphology and biochemical reactions.

Materials and Methods

Soil samples were collected from mangrove soil of Shwe-thaung-yan Township, Magyi tidal Creek, Ayeyawady Region. The isolation of mangrove soil samples were carried out at the (BDC), Pathein University.

[1] Lecturer, Department of Botany, Pathein University
[2] Associate Professor, Department of Botany, Pathein University

Serial dilutions of plating and streaking techniques described by Salle (1948), Collins (1964) and Pelezar and Chan (1972) were used for the isolation of bacteria from soil.

Source-UTM 17.94-8, (Geography Dept. Pathein University)

Figure 1 Location map of Shwe-thaung-yan Township in Ayeyawady Region

Gram Staining Method (Collins, 1965)

A smear of bacterial cells was prepared on a clean glass slide and the smear was then allowed to air-dry followed by a mild heat fixation. Crystal violet solution was added onto bacterial smear and incubated for one minute. The smear was washed with water Mordant Gram's iodine Solution was then added on bacterial smear and incubated for one minute. The smear was decolonized by washing with 95% ethyl alcohol and rinsed with water. Finally, safranin was used as counter for one minute and washed with water. Cell were then air dried and studied under microscope.

Spore Staining

Spore staining of isolated microbes were carried out according to the procedures described by Cruickshank 1968.

Preliminary Study on Antimicrobial Activities of Isolated Bacteria

The isolated soil bacteria were inoculated into seed medium and incubated for 1 day at room temperature. After one day, the seed culture was transferred into the fermentation medium and carried out by static culture. Then, the fermented broth was used to check the antimicrobial activity by agar well method (Collins, 1965). Agar well having (8 mm in diameter) were utilized for antimicrobial activity.

Agar Well Method (Collins, 1965)

1 day old culture test broth (0.2 mL) was added to 25 mL warm of assay medium (glucose 1.0 g, peptone 0.3 g, yeast 0.2 g, DW 100 mL, agar 1.8 g) and thoroughly mixed and poured into plate. After solidification, the agar was left to set. Cork borer was used to make the wells (8 mm in diameter). And then, the fermented broth (20 µL) was carefully added into the well 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.

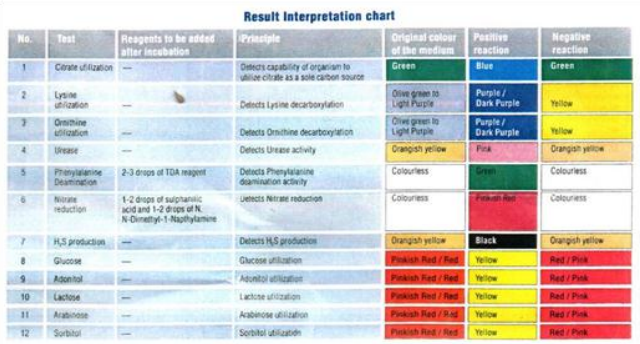
Test Organisms

Bacillus subtilis IFO 90571, *Candida albicans* NITE 09542, *Escherichia coli* AHU 5436, *Agrobacterium tumefaciens* NITE, 09678, *Bacillus pumilus* IFO 90571 were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan) and Institute of fermentation Osaka, Japan.

Identification of Isolated Bacteria

The identification of isolated bacterial strains were carried out by their colony morphology, gram staining methods (Woodland 2004 and Collins, 1965), spore staining (Cruickshank, 1968) and biochemical tests such as catalase test (Salle 1948) oxidase test (Dickey & Kelman, 1988), motility test (Prescott, 2002), aerobic/anaerobic test (Prescott, 2002), methyl red (MR) test (Bisen and Verma, 1998), Voges-Proskauer (VP) test, citrate utilization (Atlas, 1993), urea hydrolysis (Christenson, 1946), hydrogen sulphide (H_2S) (Cowan, 1975), phenylalanine (PPA) test (Atlas, 1993) and NaCl salt tolerance (2%-10%) (Atlas, 1993), carbohydrate fermentation (Cowan, 1975) using seven different sugar, starch hydrolysis (Pelezar and Chan, 1972) of using the powder of soluble starch, tapioca and wheat powder, rice, sticky rice, glue, corn and potato; using KB002Hi Assorted™ Test Kit (India) were observed. In the proteolytic activity, gelatin and casein hydrolysis, esterase activity, and potato slice test were also studied.

Figure 2 KB 002 Hi Assorted™ Biochemical Test Kit



Result Interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
2	Lysine utilization	—	Detects Lysine decarboxylation	Olive green to Light Purple	Purple / Dark Purple	Yellow
3	Ornithine utilization	—	Detects Ornithine decarboxylation	Olive green to Light Purple	Purple / Dark Purple	Yellow
4	Urease	—	Detects Urease activity	Orangish yellow	Pink	Orangish yellow
5	Phenylalanine Deamination	2-3 drops of TDA reagent	Detects Phenylalanine deamination activity	Colourless	Green	Colourless
6	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Naphylamine	Detects Nitrate reduction	Colourless	Pinkish Red	Colourless
7	H_2S production	—	Detects H_2S production	Orangish yellow	Black	Orangish yellow
8	Glucose	—	Glucose utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Adonitol	—	Adonitol utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Lactose	—	Lactose utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Arabinose	—	Arabinose utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Sorbitol	—	Sorbitol utilization	Pinkish Red / Red	Yellow	Red / Pink

Figure 3 Result interpretation chart

Results

The total of seven bacterial strains such as M-1, M-2, M-3, M-4, M-5, M-6 and M-7 were isolated from the mangrove soils of coastal area. The results showed that the colonies morphology of those isolated strains (M-1 to M-7) were small and moderate in sizes; circular, irregular, entire in margins; cream, white and pale yellow in color; raised and flat in elevation and form; shiny, pale green and dull in pigments on agar, respectively. The results of colony morphology and cell morphology for the isolated bacterial strains were shown in Table 1-2 and Figure 4, 5. The antimicrobial activities were shown in Table 3 and Figure 6-9. The biochemical tests for the selected bacterium M-7 were shown in Table 4 and Figure 10-18.

Table 1 Colony morphology of the isolated bacteria

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
M-1	Small	circular	white	flat	pale
M-2	Small	entire	cream	raised	shiny
M-3	Small	circular	white	raised	shiny
M-4	Small	circular	white	flat	shiny
M-5	Small	circular	cream	raised	shiny
M-6	Moderate	irregular	white	raised	dull
M-7	Small	circular	cream	raised	shiny

Table 2 Cell morphology of the isolated bacteria

Isolated strains	Gram staining	Cell morphology
M-1	+	cocobacilli
M-2	+	cocobacilli
M-3	-	short rod
M-4	-	short rod
M-5	-	short rod
M-6	-	short rod
M-7	-	short rod

positive = + negative = -

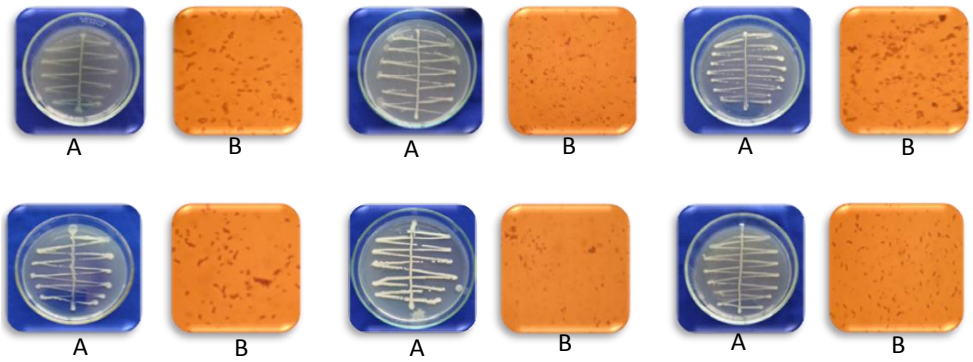


Figure 4 Cultural characteristics and cell morphology of M-1 to M-6
A = Culture (Streaks method), B = Cell morphology

Figure 5 Cultural characteristics and cell morphology of M-7

Antimicrobial activities of Isolated Bacterial strains

Seven isolated bacteria (M-1 to M-7) had antimicrobial activity and remaining 3 isolates (M-3, 4, 5) could not produce antimicrobial metabolites. These results were displayed in Figure (6-9) and Table 3. Among them, antifungal activity of isolated bacterium M-7 showed the maximum inhibitory zone against *Candida albicans.*

Table 3 Antimicrobial Activities of 7 Isolated Bacteria Against Five Test Organisms

No.	Isolated Bacteria	*Candida albicans*	*E. coli*	*Bacillus subtilis*	*Bacillus pumilus*	*Agrobacterium tumefaciens*
1	M-1	24.77 mm	23.28 mm	21.63 mm	20.03 mm	22.66 mm
2	M-2	16.60 mm	14.05 mm	15.21 mm	15.51 mm	13.35 mm
3	M-3	-	-	-	-	-
4	M-4	-	-	-	-	-
5	M-5	-	-	-	-	-
6	M-6	16.72 mm	14.48 mm	14.73 mm	15.45 mm	12.90 mm
7	M-7	**25.37 mm**	21.09 mm	19.21 mm	23.63 mm	19.24 mm

(+) = Activity present, (-) = No activity, Agar well size = 8 mm

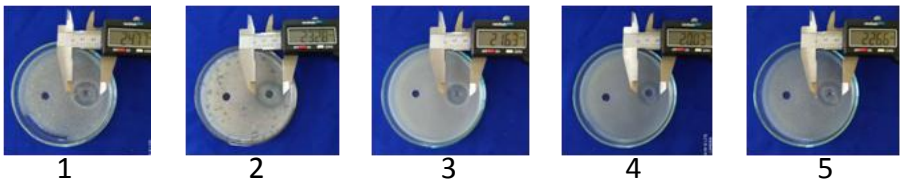


Figure 6 Antimicrobial activities of M-1 on five test organisms

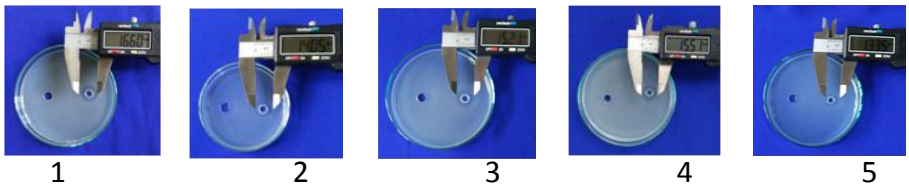


Figure 7 Antimicrobial activities of M-2 on five test organisms

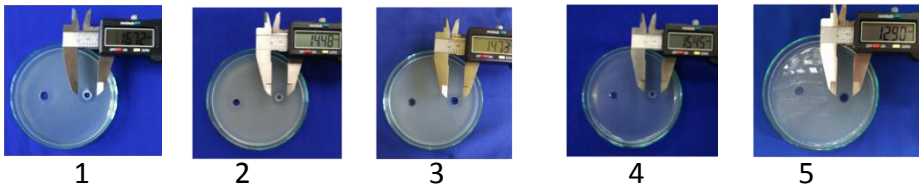


Figure 8 Antimicrobial activities of M-6 on five test organisms

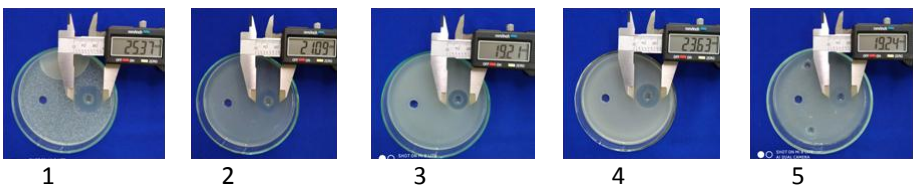


Figure 9 Antimicrobial activities of M-7 on five test organisms

1. *Candida albicans* 2. *Escherichia coli*
3. *Bacillus subtilis* 4. *Bacillus pumilus*
5. *Agrobacterium tumefaciens*

Table 4 Biochemical tests for the selected bacteria M-7

No.	Biochemical tests	Results
1	Catalase	+
2	Oxidase	-
3	Motility	+
4	Methyl Red (MR)	-
5	Voges-Proskauer (VP)	-
6	Citrate	+
7	Urea hydrolysis	+
8	Hydrogen Sulphide (H_2S)	+
9	Phenylalanine (PPA)	+
10	Salt tolerance NaCl (2% - 10%)	+
11	Carbohydrate fermentation (eight different	+
12	Starch hydrolysis	+
13	Gelatin hydrolysis	-
14	Casein hydrolysis	-

positive = + negative = -

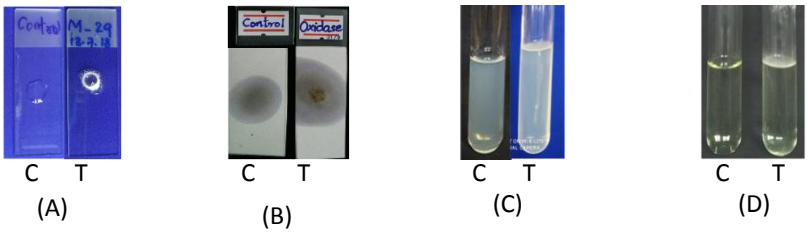


Figure 10 Biochemical tests of selected bacterium M-7 (A) Catalase Test (B) Oxidase Test (C) Motility Test (D) Aerobic/Anaerobic Test

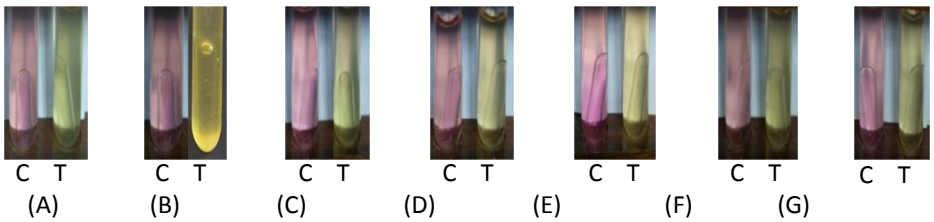


Figure 11 Carbohydrate fermentation for selected bacterium M-7

(A) Sucrose (B) Dextrose (C) Maltose (D) Arabinose
(E) Galactose (F) Xylose (G) Lactose

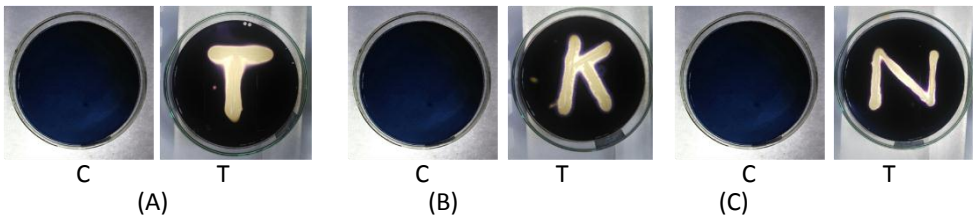


Figure 12 Starch hydrolysis tests of selected bacterium M-7
(A) Rice (B) Sticky Rice (C) Wheat

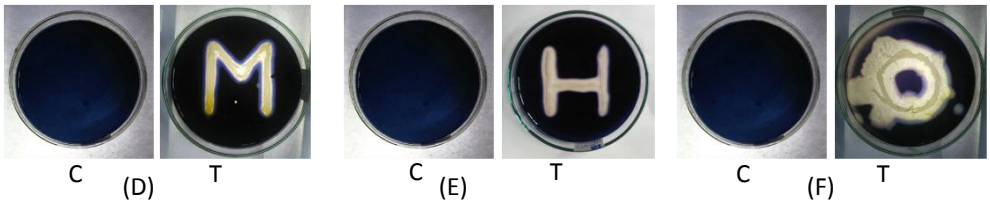


Figure 13 Starch hydrolysis tests of selected bacterium M-7
(D) Glue (E) Soluble starch (F) Potato

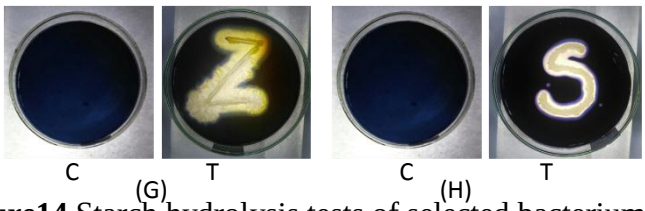


Figure14 Starch hydrolysis tests of selected bacterium M-7
(G) Corn (H) Tapioca

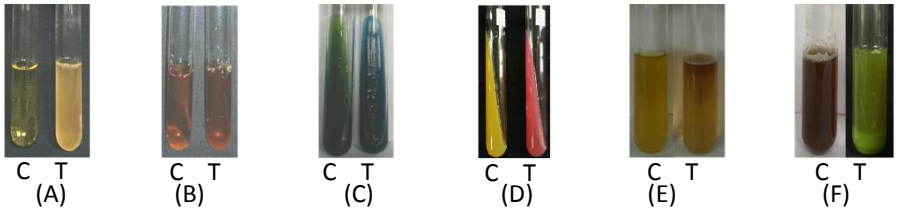


Figure 15 Biochemical tests of selected bacterium M-7
(A) Methyl Red (MR) Test (B) Voges Proskauer (VP) Test
(C) Citrate utilization Test (D) Urea hydrolysis Test
(E) Hydrogen Sulfide (H2S) production (F) Phenylalanine (PPA)

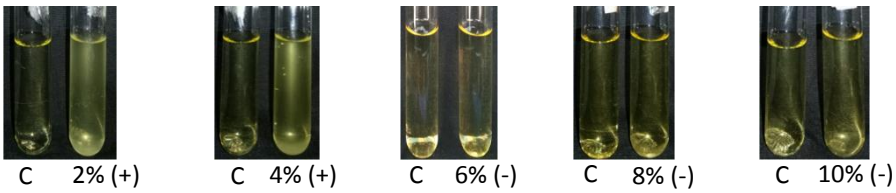


Figure 16 NaCl salt tolerance of selected bacterium M-7

Control **Treatment**

Figure 17 KB 002 Hi Assorted^{TM} Biochemical Test Kit of M-7

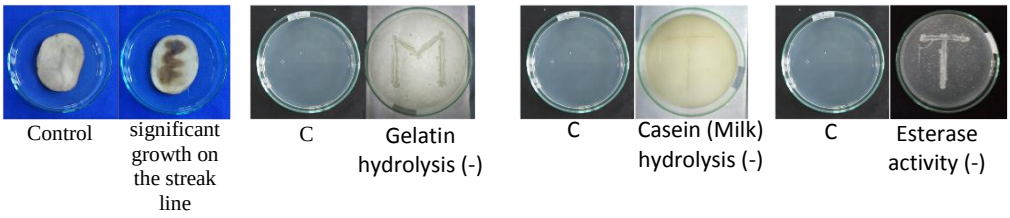


Figure 18 Biochemical test of selected bacterium M-7

Discussion and Conclusion

Mangrove ecosystems are rich in bacterial flora. Fertility of the mangrove water results from the microbial decomposition of organic matter and recycling of nutrients. Among the microbes, the bacterial population in mangroves is many-fold greater than the fungi. In tropical mangroves, bacteria and fungi constitute 91% of the total microbial biomass, whereas algae and protozoa represent only 7% and 2% respectively (Alongi D.M., 1988).

For the past 50 years antibiotics have revolutionized medicine by providing cures for formerly lie threatening diseases. Many marine free-living inhabiting marine bacterial have been shown to produce secondary metabolites that display antibacterial properties (Burgess JG *et al.*,1991).

In the present study, the total of seven bacterial strains were isolated and identified from the mangrove soil of magyi coastal area.

Seven isolated bacteria (M-1 to M-7) could display the antimicrobial activity inhibiting the five test organisms with agar well diffusion method and the remaining 3 isolates (M-3, 4, 5) could not produced antimicrobial metabolites. Among them, M-7 showed different levels of antimicrobial activities on five test organisms. M-7 showed the higher antifungal activity (25.37 mm and 23.63 mm) against *Candida albicans* and *Bacillus pumilus* than the other strains. Therefore, selection of M-7 was carried on further experiments.

Antibiotics and other bioactive compounds have been isolated from microorganisms in different environment (Charousova *et al.*, 2017; Devi *et al.*, 2017)

In the microscopical and biochemical characters, M-7 was gram-negative and short rod, catalase positive, aerobe, acid was produced in the sugar (dextrose, fructose, sucrose, maltose, arabinose, galactose, xylose and lactose) fermentation and gas was produced in the sugar sucrose, dextrose, galactose and xylose.

And then, M-7 can hydrolyze various starch sources (rice, sticky rice, wheat, soluble starch, tapioca powder, glue, potato and corn) respectively, H_2S was produced and motility present, Methyl red (MR) and Voges-Proskauer (VP) tests were negative, Phenylalanine (PPA) positive, citrate utilization positive, oxidase test negative, urease test positive, gelatin and casein hydrolysis negative; M-7 can tolerate in 2% and 4% NaCl salt concentration except 6%, 8% and 10% ; grown on potato slice.

These characters were performed according to the Bergey's Manual of Determinative Bacteriology by Breed *et al.*, 1957. Based on the obtained results of different biochemical tests, M-7 was classified as belonging to the genus *Pseudomonas* sp.

The genus *Pseudomonas* is the most heterogeneous and environmentally significant known bacterial group and includes mobile gram-negative aerobic rods, extended in all nature and characterized by its high metabolic versatility given by a complex enzymatic system. In addition, *Pseudomonas* species tend to be predominant among the bacteria associated with plants rhizosphere. Jaharamma *et al.,* 2009, Opasola *et al.,* 2011.

Juliana *et al.,* 2013 reported that in the nutrient agar, the colonies of *Pseudomonas* sp were circular, convex, entire margin, without or with pigmentation , which varied between-brown and pale yellow, some shinny.

It was concluded that the present study was the isolation bacteria from mangrove soil samples and the antimicrobial activity of isolated bacteria on five test organisms. In order to identify the selected bacterial strain M-7, biochemical tests were carried out and this bacterium can be noted as the *Pseudomonas* sp.

Acknowledgements

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CULTURAL CHARACTER AND ANTIMICROBIAL ACTIVITIES OF ISOLATED FUNGI FROM SOIL SAMPLES OF BELU-GYUN, CHAUNG-ZON TOWNSHIP, MON STATE

Mya Min Min Myo[1], Zaw Lin Aung[2]and Kay Thi Mya[3]

Abstract

Soil samples from twenty different locations of Belu-Gyun, Chaung-Zon Township, Mon State were collected. These soil samples were utilized in the isolation of 37 fungal strains. They were designated as MM-1 to MM-37. Among these fungi colonies, MM-34 was found to give the highest antibacterial activity of 36.35 mm clear zone against *Micrococcus luteus.* So, MM-34 was preferred to conduct further investigation concerning the optimal fermentation conditions to produce antibacterial metabolite. In the fermentation experiments with MM-34, it was observed that the 84 hours of ages, 15% sizes of inoculums were suitable for highest antibacterial activity against *Micrococcus luteus.* The result revealed that maximum production of antimicrobial metabolite (27.27 mm clear zone) was obtained when medium was supplemented with glucose. Culture containing yeast extract showed the highest antibiotic production (27.54 mm clear zone). FM-3 gave maximum yield of antibacterial activity (30.02 mm clear zone) against *Micrococcus luteus.*

Keywords: soil fungi, antibacterial activity

Introduction

Soil is the most abundant ecosystem on Earth, but the vast majority of organisms in soil are microbes. There may be a population limit of around one billion cells per gram of soil, but estimate the number of species vary widely from 50,000 per gram to over a million per gram of soil. Soil sustains an immerse diversity of microbes, which to a large extent, remains unexplored. Bacteria including actinomycetes and fungi are most preferably used as screening sources from various habitats. Fungi are well known as prolific producers of biologically active natural products (Hara Kishore *et al.,* 2007).

The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem. 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 (Ainsworth, 1995).

Fungi grow best in environments that are slightly acidic. 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 (Asnaashari, *et al.,* 2012).

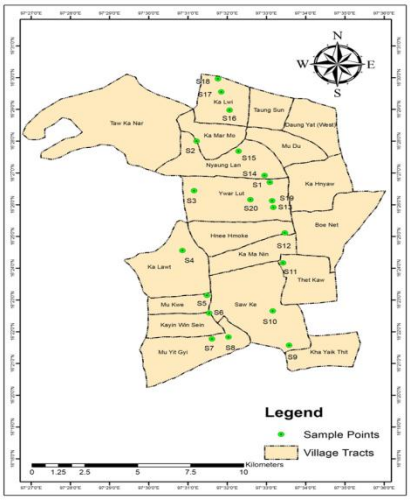
Antibiotic have an important role in human health. Most of the naturally occurring antibiotics have been isolated from soil microorganisms. Antibiotics represent the medically important group of secondary metabolites, which have been useful in our battle against infectious disease. The proper cultivation and transfer of inoculums are essential for the production of both primary and secondary metabolites. Media used in the cultivation of microorganisms must

[1] Lecturer, Department of Botany, Pathein University
[2] Lecturer, Department of Botany, Pakokku University
[3] Professor and Head, Department of Botany, Pathein University

contain all elements in a form suitable for the synthesis of cell substances and for the production of metabolic products (Dale, 1984; Balows *et al.*, 1992). Therefore, the fermentation conditions such as substrates, inoculums cultivation and transfer have to be optimized for the production of primary and secondary metabolites.

The aim and objectives of this research were to study the various isolated fungi, age and size of the inoculum of fungus MM-34 and to investigate the effect of carbon and nitrogen utilization on the fermentation.

Materials and Methods



Twenty Soil samples were collected from the twenty different places of Belu-Gyun, Chaung-Zon Township, Mon State
Source: Department of Geography, Pathein University
Figure 1 Map of Collected Soil Samples from Belu-Gyun

Collection of Soil Samples

Twenty soil samples were collected from the twenty different places of Belu-Gyun, Chaung-Zon Township, Mon State. Soil samples were utilized for the isolation of fungi and the soil samples were put into plastic bag under aseptic condition. The soil type as its pH was analyzed by Department of Agriculture (Land Use) Insein.

Table 1 Some characters of soil samples

Sr No.	Soil No.	Collected Area	pH	Texture	Sr No.	Soil No.	Collected Area	pH	Texture
1	Soil-1	Ywar Lut	4.13	Silt loam	11	Soil-11	Thet Kaw	3.92	Sandy loam
2	Soil-2	Ka Mar Mo	3.60	Loamy sand	12	Soil-12	Hnee	4.74	Sandy loam
3	Soil-3	Ywar Lut	3.55	Sandy loam	13	Soil-13	Ywar Lut	4.77	Loam
4	Soil-4	Ka Lawt	3.67	Sandy loam	14	Soil-14	Nyaung	4.52	Sandy loam
5	Soil-5	Mu Kwe	4.11	Sandy loam	15	Soil-15	Nyaung	4.48	Silt loam
6	Soil-6	Kayin Win	4.11	Sandy clay	16	Soil-16	Ka Lwi	3.68	Loamy Sand
7	Soil-7	Mu Yit Gyi	4.12	Loam	17	Soil-17	Ka Lwi	3.93	Sandy loam
8	Soil-8	Mu Yit Gyi	3.42	Sandy loam	18	Soil-18	Ka Lwi	3.89	Sandy loam
9	Soil-9	Saw Ke	4.51	Sandy Clay	19	Soil-19	Ywar Lut	4.74	Sandy Clay
10	Soil-	Saw Ke	4.10	Loam	20	Soil-20	Ywar Lut	4.04	Sandy loam

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

Isolation medium		**Transfer Medium**	
Glucose	- 1.0 g	Glucose	- 1.2 g
Yeast extract	- 0.7 g	Yeast extract	- 0.8 g
KH_2PO_4	- 0.01 g	KH_2PO_4	- 0.01 g
KNO_3	- 0.02 g	$MgSO_4$	- 0.01 g
Agar	- 1.8 g	Agar	- 1.8 g
Distilled water	- 100 mL	Distilled water	- 100 mL

Isolation of fungi from soil samples

The isolation of soil fungi were carried out by feeding method (Hayakawa, and Kobayashi, 2005)

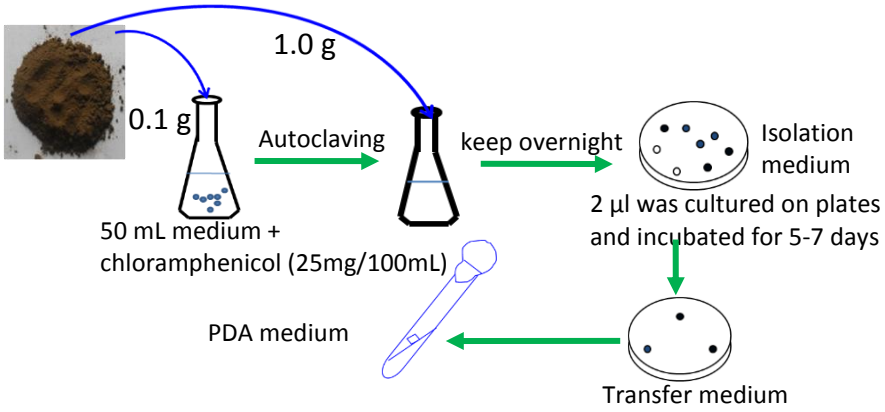


Figure 2 Feeding method (Hayakawa, and Kobayashi, 2005)

Medium used for antimicrobial activity test (Ando, 2004)

Seed Medium		**Fermentation Medium**		**Assay Medium**	
Glucose	- 1.0 g	Glucose	- 1.5 g	Glucose	- 0.8 g
Yeast extract	- 0.8 g	Yeast extract	- 0.8 g	Peptone	- 0.7 g
K_2HPO_4	- 0.01 g	Peptone	- 0.4 g	KNO_3	- 0.2 g
KCl	- 0.1 g	$MgSO_4$	- 0.001 g	Agar	- 1.8 g
DW	- 100 mL	K_2HPO_4	- 0.01 g	DW	- 100 mL
		KCl	- 0.1 g		
		DW	- 100 mL		

Screening of antimicrobial activity of isolated soil fungi by paper disc diffusion assay (Tomita, 1988)

The isolated fungi were grown at room temperature for 5 days. The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Seed culture (20%) was transferred into the fermentation medium and incubated at room temperature for 10 days. 20µL of fermented broth was put on paper disc. After drying, placed on assay plate containing test organism and incubated for 24-36 hours. Six pathogenic microorganisms were utilized for antimicrobial activity.

Table 2 Test Organisms utilized for antimicrobial activities

No.	Test Organisms	Disease
1	*Aspergillus flavus* IFO3290	Aspergillosis
2	*Bacillus subtilis* KY-327	Fever
3	*Micrococcus luteus* NITE83297	Skin disease
4	*Escherichia coli* AHU5436	Diarrhoea
5	*Pseudomonas fluorescens* IFO94307	Rice disease
6	*Salmonella typhi* AHU7943	Typhoid fever and food poison

Effect of age of inoculum on the fermentation of antibacterial activity by isolated soil fungus MM-34

Five days old culture of the selected fungus MM-34 was inoculated into seed medium and then transfer to fermentation medium. Age of culture with 36 hrs, 48 hrs, 60 hrs, 72 hrs, 84 hrs, 96 hrs and 108 hrs were utilized for fermentation. The antibacterial activity was carried out by paper disc diffusion assay with 8 mm paper disc.

Effect of size of inoculum on the fermentation medium of antibacterial activity of isolated soil fungus MM-34

In this study, 5%, 10%, 15%, 20%, 25%, 30% and 35% of 84 hrs of seed culture were utilized for the fermentation. Fermentation was carried out by paper disc diffusion assay with 8 mm paper disc.

Effect of carbon sources utilization on fermentation of antibacterial activity by isolated soil fungus MM-34

To study the effect of different carbon sources utilization on fermentation, glucose, sucrose, soluble starch, potato powder, tapioca powder, corn powder, rice powder were used. 2 g of each carbon sources were added to the basal medium individually.

Effect of nitrogen sources utilization on fermentation of antibacterial activity by isolated soil fungus MM-34

To study the effect of different nitrogen sources utilization on fermentation, yeast extract, peptone, KNO_3, NH_4Cl, NH_4SO_4, NH_4NO_3 and meat extract were used, 1 g of each nitrogen sources were added to basal medium individually.

Basal fermentation medium used in carbon sources utilization		**Basal fermentation medium used in nitrogen sources utilization**	
Yeast extract	1.0 g	Glucose	2.0 g
$MgSO_4$	0.001 g	$MgSO_4$	0.001 g
KNO_3	0.1 g	D/W	100 mL
D/W	100 mL		

Medium Selection for Fermentation

Based on the results of carbon and nitrogen sources utilization on fermentation, 6 fermentation media were composed. Fermentation was undertaken with 84 hrs age and 15% size of inoculum with six different media.

FM-1		**FM-2**		**FM-3**	
Glucose	2.0 g	Glucose	2.0 g	Potato	2.0 g
Yeast extract	1.0 g	Peptone	1.0 g	Yeast extract	1.0 g
$MgSO_4$	0.001 g	$MgSO_4$	0.001 g	$MgSO_4$	0.001 g
D/W	100 mL	D/W	100 mL	D/W	100 mL

FM-4		**FM-5**		**FM-6**	
Potato	2.0 g	Glucose	1.0 g	Glucose	1.0 g
Peptone	1.0 g	Potato	1.0 g	Potato	1.0 g
$MgSO_4$	0.001 g	Yeast extract	1.0 g	Peptone	1.0 g
D/W	100 mL	$MgSO_4$	0.001 g	$MgSO_4$	0.001 g
		D/W	100 mL	D/W	100 mL

Results

Isolation of fungi from soil samples

In the study of isolation of fungi, a total of 37 soil fungi were isolated from soil samples collected at 20 different places of Belu-Gyun, Chaung-Zon Township, Mon State.

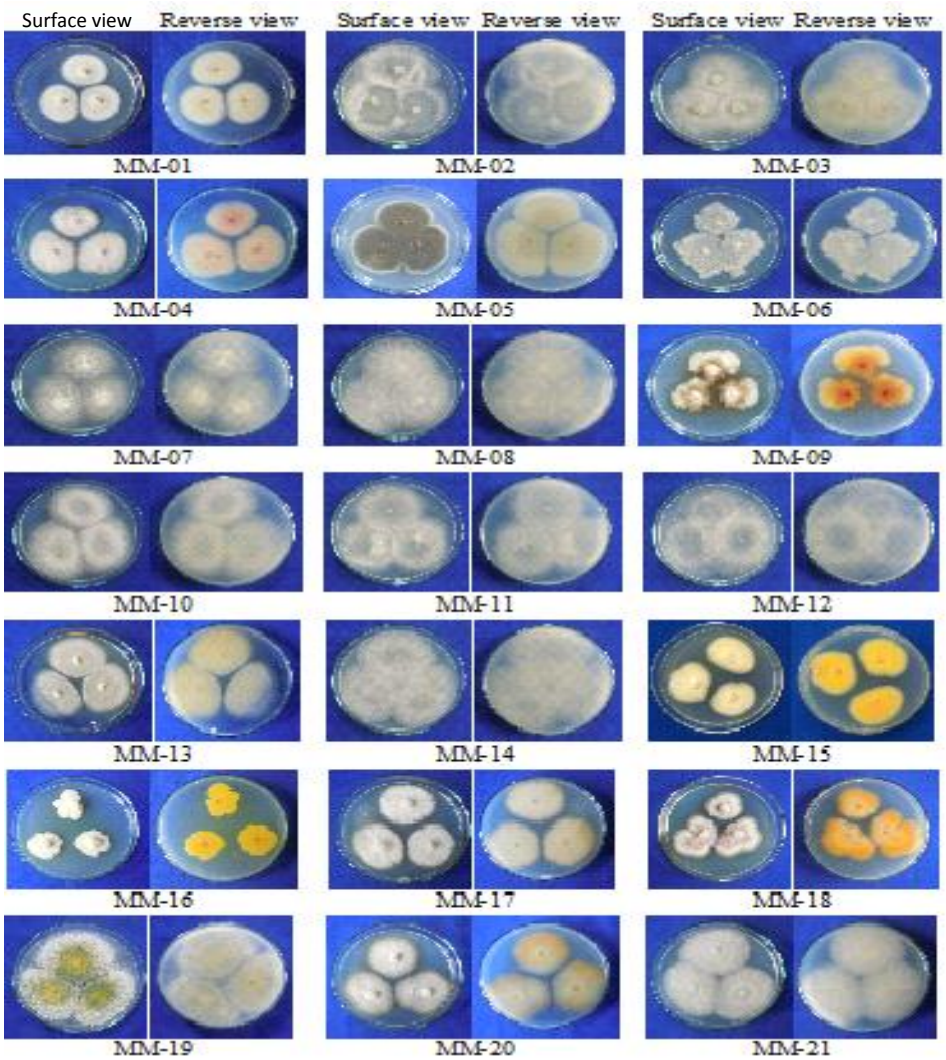


Figure 3 Morphology of isolated soil fungi MM-01 to MM-21(7 Days old culture)

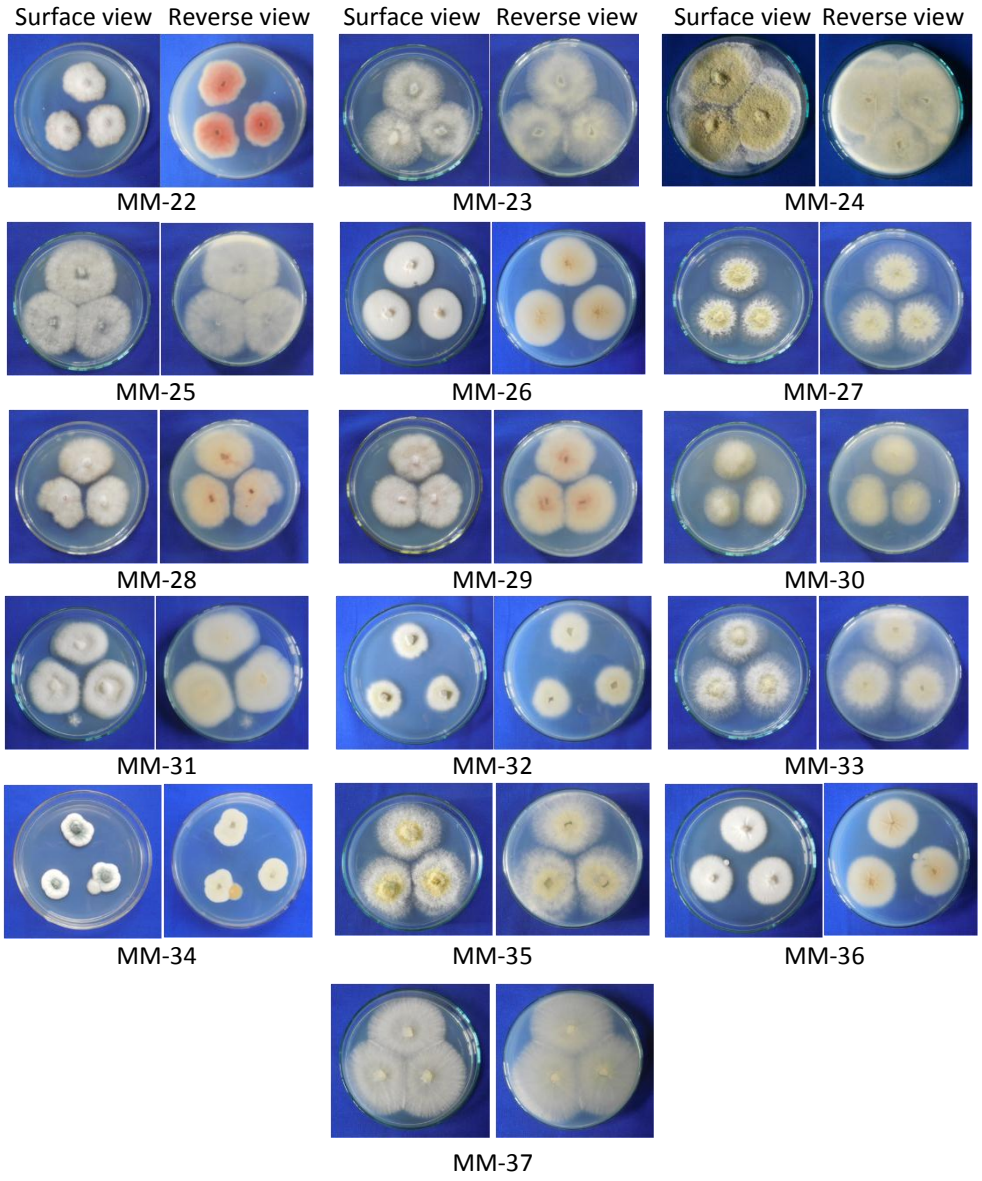


Figure 4 Morphology of isolated soil fungi MM-22 to MM-37 (7 Days old culture)

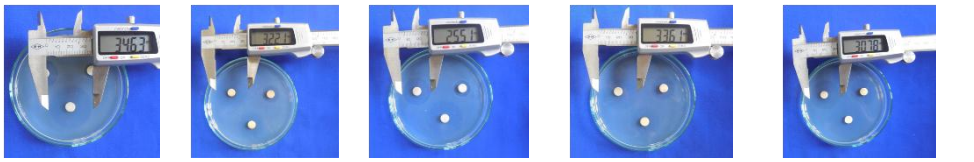
Screening of antimicrobial activity of isolated soil fungi by paper disc diffusion assay

The study of biological properties of these fungi, MM-10 and MM-21 showed antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Pseudomonas fluorescens.* Soil fungus MM-34 exhibited antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus* and *Escherichia coli.* Among them MM-34 showed the highest antibacterial activity against *Micrococcus luteus* (36.35 mm) (Table 3 and Figure 5, 6, 7).

Table 3 Antimicrobial activities producing fungi and their activities

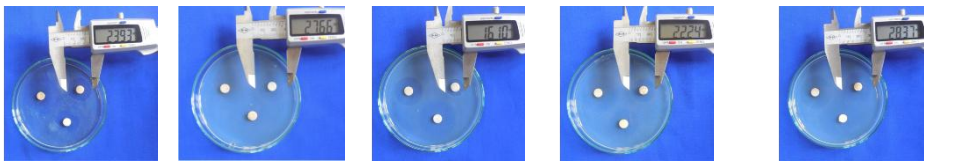
Isolated fungi	Antimicrobial activities on test organisms (inhibitory zone, mm)					
	Aspergillus flavus	*Bacillus subtilis*	*Micrococcus luteus*	*Escherichia coli*	*Pseudomonas fluorescens*	*Salmonella typhi*
MM-10	**34.63**	32.21	25.51	33.61	30.78	-
MM-21	23.93	27.66	16.10	22.24	**28.37**	-
MM-34	20.11	26.67	**36.35**	28.58	-	-

(-) = No activity



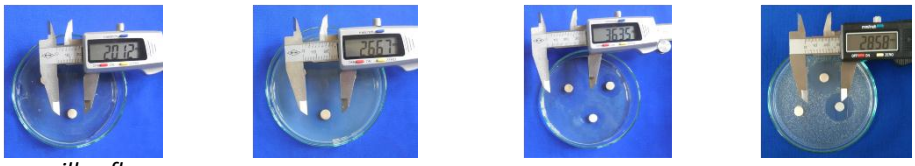
Aspergillus flavus *Bacillus subtilis* *Micrococcus luteus* *Escherichia coli* *Pseudomonas fluorescens*

Figure 5 Antimicrobial activity of isolated soil fungus MM-10



Aspergillus flavus *Bacillus subtilis* *Micrococcus luteus* *Escherichia coli* *Pseudomonas fluorescens*

Figure 6 Antimicrobial activity of isolated soil fungus MM-21



Aspergillus flavus *Bacillus subtilis* *Micrococcus luteus* *Escherichia coli*

Figure 7 Antimicrobial activity of isolated soil fungus MM-34

Study on the effects of ages of culture on the fermentation of antibacterial activity of isolated soil fungus MM-34

In this study, age of culture (36, 48, 60, 72, 84, 96, 108 hrs) were utilized for the fermentation. In the experiment it was observed that 84 hrs age of seed culture was suitable for the fermentation (Table 4, Figure 8).

Table 4 Effects of age of inoculum on the fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Seed culture (Time, hrs)	Activity (Clear zone, mm)
36	13.57
48	16.84
60	18.59
72	19.69
84	**21.98**
96	20.33
108	18.15

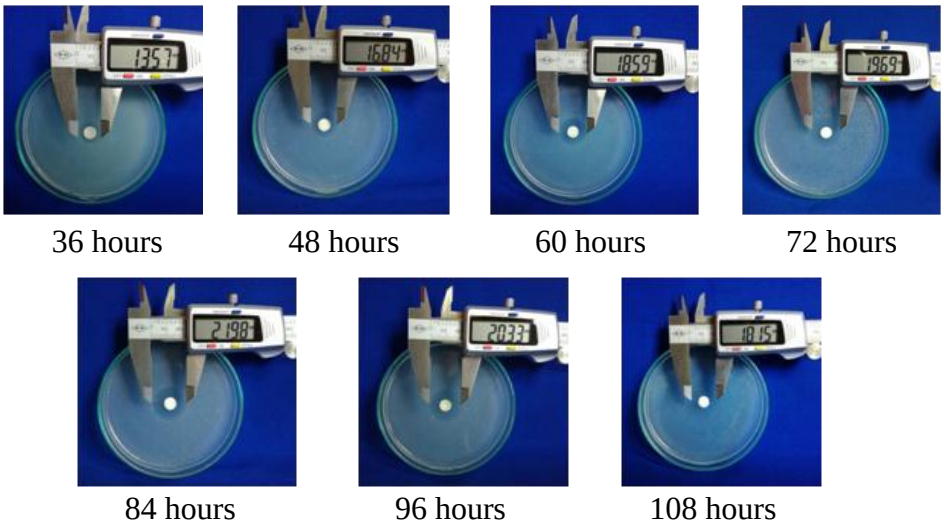


Figure 8 Effects of age of inoculum on the fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Study on the effect of size of inoculum on the fermentation of antibacterial activity of isolated soil fungus MM-34

In the study of size of inoculum, different size of inoculum (5%, 10%, 15%, 20%, 25%, 30% and 35%) were tested, 15% isolated fungi size of inoculum concentration was the best for fermentation (Table 5, Figure 9).

Table 5 Effects of size of inoculum for fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Size of inoculum	Activity (clear zone mm)
5 %	21.65
10 %	21.00
15 %	**25.62**
20 %	23.70
25 %	23.04
30 %	23.21
35 %	25.57

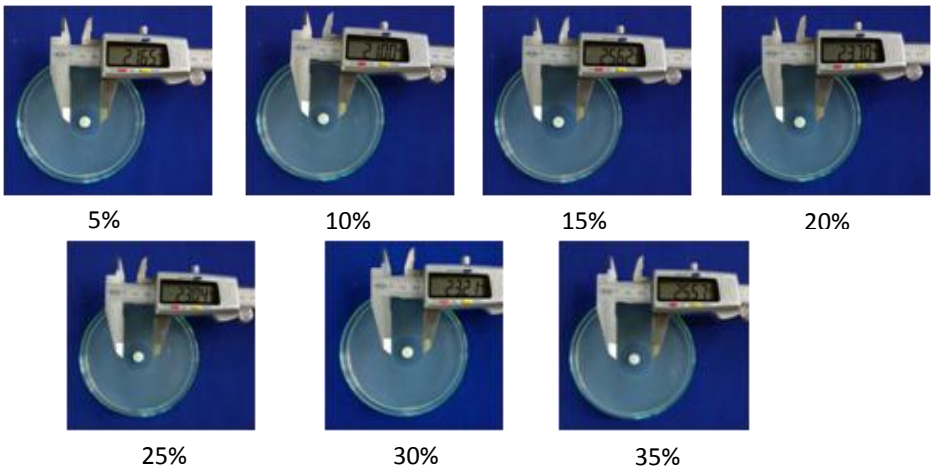


Figure 9 Effect of size of inoculum on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Study on the effect of carbon source utilization on fermentation of antibacterial activity of isolated soil fungus MM-34

In the study of carbon source utilization on fermentation, glucose, sucrose, soluble starch, potato powder, tapioca powder, corn powder, rice powder were used. Among them, glucose was found to be the best for the fermentation. (Table 6, Figure 10).

Table 6 Effects of carbon sources utilization on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Carbon sources	Inhibitory zone, mm
Glucose	**27.27**
Sucrose	23.90
Soluble starch	25.40
Potato powder	25.71
Tapioca powder	25.22
Corn powder	25.22
Rice powder	24.57

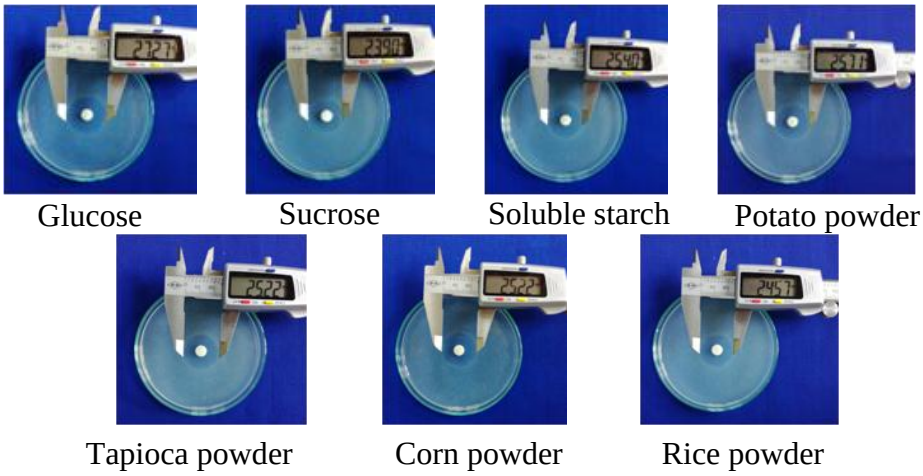


Figure 10 Effect of carbon sources utilization on fermentation of antibacterial activity of isolated soil fungus MM-34

Study on the effect of nitrogen source utilization on fermentation of antibacterial activity of isolated soil fungus MM-34

In this study, different nitrogen sources (yeast extract, peptone, KNO_3, NH_4Cl, NH_4SO_4, NH_4NO_3 and meat extract) were utilized for the fermentation. In the experiment it was observed that yeast extract was most suitable for the fermentation. (Table 7, Figure 11)

Table 7 Effects of nitrogen utilization on fermentation of antibacterial activity of isolated soil fungus MM-34 against *Micrococcus luteus*

Nitrogen sources	Inhibitory zone, mm
Yeast extract	**27.54**
Peptone	19.32
KNO_3	13.97
NH_4Cl	13.90
NH_4SO_4	14.70
NH_4NO_3	13.73
Meat extract	15.08

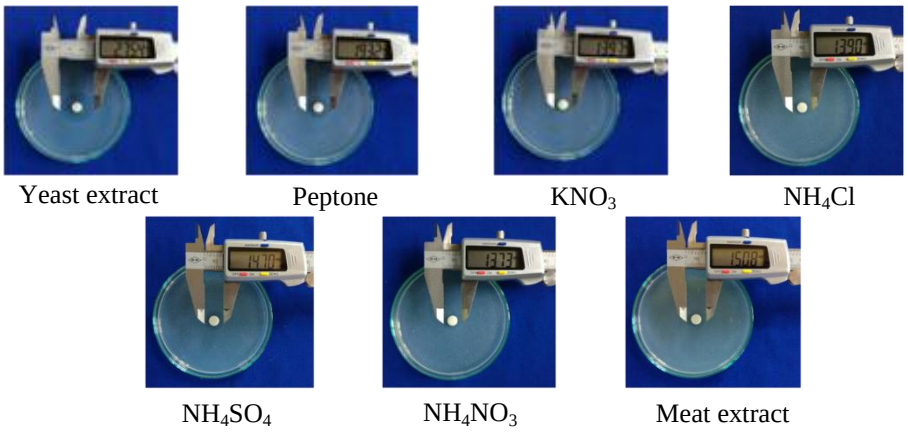


Figure 11 Effect of nitrogen utilization on fermentation of antibacterial activity of isolated soil fungus MM-34 against *Micrococcus luteus*

Medium selection for fermentation

In order to select the best fermentation medium for maximum antibiotic production, six different fermentation media FM-1 to FM-6 were tested. Fermentation medium FM-3 gave maximum yield of antibacterial metabolites against *Micrococcus luteus* (Table 8, Figure 12).

Table 8 Effects of media on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Medium	Activity (Clear zone, mm)
FM-1	16.56
FM-2	19.06
FM-3	**30.02**
FM-4	22.39
FM-5	27.01
FM-6	22.51

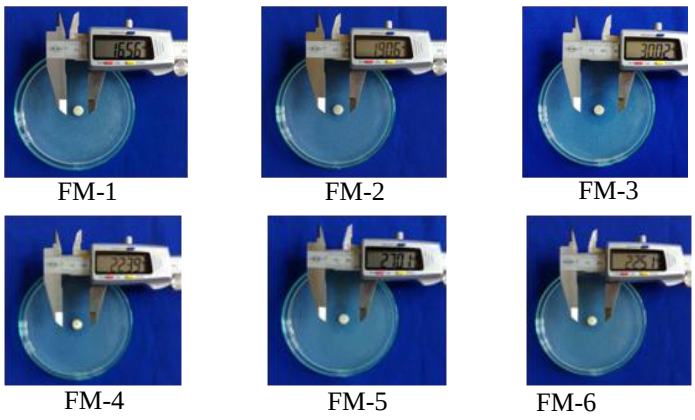


Figure 12 Effects of media on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Discussion and Conclusion

In the course of investigation, 37 fungi were isolated from twenty different soil samples which were collected from Belu-Gyun, Chaung-Zon Township, Mon State. Feeding method was used in the isolation of soil fungi. Isolated 37 fungi their morphological character and reverse color were found to be different. Among 37 soil fungi, MM-10, MM-21 and MM-34 showed antimicrobial activity. During the study of biological properties of these fungi, MM-10 and MM-21 exhibited antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus, Escherichia coli* and *Pseudomonas fluorescens.* Soil fungus MM-34 showed antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus* and *Escherichia coli*. Soil fungus MM-10 was isolated from soil sample No.6. MM-10 showed the highest antifungal activity against *Aspergillus flavus* (34.63 mm). Soil fungus MM-21 was isolated from soil sample No.13. MM-21 showed the highest antibacterial activity against *Pseudomonas fluorescens* (28.37 mm). Soil fungus MM-34 was isolated from soil sample No.19. MM-34 exhibited the highest antibacterial activity against *Micrococcus luteus* (36.35 mm). Therefore, this strain MM-34 was selected for further investigation such as age of inoculum, size of inoculum, the effect of different carbon and nitrogen sources and medium selection of fermentation. This isolated soil fungus MM-34 was collected from Ywar Lut, Belu-Gyun, Chaung-Zon Township, Mon State.

From the present result, it was observed that the most suitable parameter such as 84 hrs age of inoculum and 15% size of inoculum were optimized to produce the antibacterial activity during fermentation. The different carbon and nitrogen source supplement to the culture broth strongly influenced the growth and biosynthesis of active metabolite by MM-34. On studying the effect of different carbon sources, the results indicated that glucose affected the antibacterial production (27.27 mm clear zone). This finding was coincide with those of Buchanan, *et al.,* (1984) where simple sugar such as glucose, fructose, sucrose, glycerol enhance growth as well as secondary metabolite production by microorganisms rather than complex carbon sources like starch, galactose, xylose, mannitol, etc.

Other than the carbon, the source of nitrogen is important for the production of antibiotic substances. MM-34 produced the antibacterial metabolites with most of nitrogen sources, but yeast extract was most suitable for antibacterial metabolite production (27.54 mm clear zone). Similar results were shown by Mathan *et al.,* (2013) where yeast extract promoted the secondary metabolite biosynthesis. The results were in good agreement with early studies by Kalyani *et al.,* (2016) have shown that yeast extract as nitrogen source, promoted the biosynthesis of secondary metabolites. Likewise, Zain *et al.,* (2009) reported that yeast extract showed the best secondary metabolite production. Similarly, Alatwani R.H (2016) reported that yeast extract was found to be a best and most suitable nitrogen source for the optimum production of bioactive metabolites. The results of antibacterial tests indicated that maximum antibacterial production was obtained in culture supplemented with glucose as carbon source and yeast extract as nitrogen source.

In order to select the best fermentation medium for maximum antibiotic production, six different fermentation media FM-1 to FM-6 were tried. FM-3 medium was the best fermentation condition of isolated soil fungus MM-34 for the production of antibacterial activity (30.02 mm clear zone).

The choice of a good fermentation medium is virtually as important to the success of an industrial fermentation as is the selection of an organisms to carry out the fermentation (EL-

Tayeb *et al.*, 2004). The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production (Stanbury *et al.*, 1997). The result of the present work agree with EL-Tayeb *et al.*, 2004 and Stanbury *et al.*, 1997.

In conclusion, the isolated soil fungus MM-34 was selected for further investigation and to find out the nature of metabolites those can kill the test organism.

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 and Dr Nilar Myint, Pro-rectors, Pathein University, for their suggestion and advices. I wish to express most sincere gratitude to Dr Kay Thi Mya, 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 Wah Wah Lwin, Professor, Department of Botany, Pathein University, for her encouragement and suggestion for this paper. Many thanks are due to my supervisor, Dr Zaw Lin Aung, Lecturer, Department of Botany, Pakokku 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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APPENDIX

DEPARTMENT OF AGRICULTURE (LAND USE)
SOIL ANALYTICAL DATA SHEET

ဒေါ်မြမင်းမင်းမျိုး (၁.၁၂.၂၀၁၇)

Division - မွန်ပြည်နယ်
Township - ဘီလူးကျွန်း

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

Sr No.	Sample plot	Moisture %	pH Soil : Water 1 : 2.5	Texture				SOIL INTERPRETATION OF RESULTS	
				Sand %	Silt %	Clay %	Total %	pH Soil : Water 1 : 2.5	Texture
	မြေနမူနာ								
1	Soil-1	1.32	4.13	26.30	63.90	8.30	98.50	Extremely acid	Silt Loam
2	Soil-2	2.99	3.60	74.50	20.10	4.30	98.90	Extremely acid	Loamy Sand
3	Soil-3	1.84	3.55	59.75	21.15	17.45	98.35	Extremely acid	Sandy Loam
4	Soil-4	2.48	3.67	52.55	39.50	6.90	98.95	Extremely acid	Sandy Loam
5	Soil-5	1.26	4.11	61.10	18.30	19.35	98.75	Extremely acid	Sandy Loam
6	Soil-6	1.13	4.11	57.80	17.40	23.75	98.95	Extremely acid	Sandy Clay Loam
7	Soil-7	2.91	4.12	44.90	30.70	23.25	98.85	Extremely acid	Loam
8	Soil-8	0.91	3.42	73.80	18.35	6.70	98.85	Extremely acid	Sandy Loam
9	Soil-9	0.72	4.51	75.50	1.10	22.20	98.80	Strongly acid	Sandy Clay Loam
10	Soil-10	3.45	4.10	51.50	38.30	9.10	98.90	Extremely acid	Loam

ခင်ဝင်းမာ
ဒု-ညွှန်ကြားရေးမှူး
ဓါတ်ခွဲခန်းဌာနခွဲ
မြေအသုံးချရေးဌာနခွဲ

DEPARTMENT OF AGRICULTURE (LAND USE)
SOIL ANALYTICAL DATA SHEET

ဒေါ်မြမင်းမင်းမျိုး (၁.၁၂.၂၀၁၇)

Division - မွန်ပြည်နယ်
Township - ဘီလူးကျွန်း

Sheet No. 2
Sr No. S 11-20 / 17-18

Sr No.	Sample plot	Moisture %	pH Soil : Water 1 : 2.5	Texture				SOIL INTERPRETATION OF RESULTS	
				Sand %	Silt %	Clay %	Total %	pH Soil : Water 1 : 2.5	Texture
	မြေနမူနာ								
11	Soil - 11	1.36	3.92	71.90	22.50	4.50	98.90	Extremely acid	Sandy Loam
12	Soil - 12	3.85	4.74	56.15	33.65	8.55	98.35	Strongly acid	Sandy Loam
13	Soil - 13	1.64	4.77	37.05	38.30	22.70	98.05	Strongly acid	Loam
14	Soil - 14	1.02	4.52	54.15	36.45	7.90	98.50	Strongly acid	Sandy Loam
15	Soil - 15	2.22	4.48	13.00	71.75	14.15	98.90	Extremely acid	Silt Loam
16	Soil - 16	1.99	3.68	78.95	18.35	1.00	98.30	Extremely acid	Loamy Sand
17	Soil - 17	0.96	3.93	75.50	12.90	9.95	98.35	Extremely acid	Sandy Loam
18	Soil - 18	1.75	3.89	72.30	24.10	2.30	98.70	Extremely acid	Sandy Loam
19	Soil - 19	1.51	4.74	58.05	17.50	22.85	98.40	Strongly acid	Sandy Clay Loam
20	Soil - 20	1.53	4.04	44.55	48.40	5.35	98.30	Extremely acid	Sandy Loam

ခင်ဝင်းမာ
ဒု-ညွှန်ကြားရေးမှူး
ဓါတ်ခွဲခန်းဌာနခွဲ
မြေအသုံးချရေးဌာနခွဲ

CULTURAL CHARACTERISTICS AND ANTIMICROBIAL ACTIVITIES OF ENDOPHYTIC FUNGI FROM LEAVES OF TWELVE MEDICINAL PLANTS

Myo Htaik Aung[1], Zaw Lin Aung[2]and Kay Thi Mya[3]

Abstract

A total of 18 endophytic fungi were screened and isolated into pure culture from the leaves of 12 medicinal plants collected from Pathein University and Mawlamyine University Campus and Taung wine area, Mawlamyine. The isolates were designated as MHA-1 to MHA-18. Among them MHA-10 which was isolated from the leaves of *Dioscorea birmanica* Prain and Burkill. (Khat-Cho) showed the highest antibacterial activity (18.90 mm clear zone) against *Bacillus subtilis.* Thus MHA-10 was selected for further study of optimal fermentation parameter to produce antibacterial metabolites. It was recorded that 84 hrs ages of inoculums, 30% size of inoculums were found to provide the best antibacterial activity in fermentation medium-1. Maximum antibacterial activity (18.03 mm clear zone) was found when fermentation media was supplemented with glucose. Among the wide variety of nitrogen sources tested, peptone proved to be the most suitable for antibacterial activity (18.95 mm clear zone) of selected fungus MHA-10 against *Bacillus subtilis.* FM-1 gave maximum antibacterial activity (20.64 mm clear zone) against *Bacillus subtilis.*

Keywords: endophytic fungi, antibacterial activity

Introduction

Natural products from microbial origin have been a consistent source of novel lead molecules and recently several endophytes have been shown to possess the potentials to synthesize novel bioactive compounds that have found great use for drug discovery (Okoye, *et al.,* 2015).

It is estimated that there might be as many as one million different endophytic fungal species, however, only a handful of them have been described, which means investigating the metabolites of these endophytes can increase the chance of finding novel bioactive natural products (Petrini, 1991). Endophytes are microorganisms which live in close association with living plant tissues in a symbiotic relationship. Fungi form a major portion of the endophytic population (Strobel *et al.,* 2004).

Isolation of endophytic fungi from medicinal and other plants to produce biologically active agents for biological utilization on a large commercial scale is possible because they can easily culture in laboratory instead of harvesting plants and affecting the environmental biodiversity. Medicinal plant acts as a richest source of endophytic fungi.

Scientists have exploited endophytic fungi very much attention for detection of bioactive compounds in the form of antimicrobial activity, anti-cancer activity etc. (Yu *et al.,* 2010).

The aim and objectives of the present study were to isolate the endophytic fungi from the leaves of 12 medicinal plants, to determine the antimicrobial activities of these fungi and to optimize the fermentation conditions of the selected fungus.

[1] Lecturer, Department of Botany, Pathein University
[2] Lecturer, Department of Botany, Pakokku University
[3] Professor and Head, Department of Botany, Pathein University

Materials and Methods

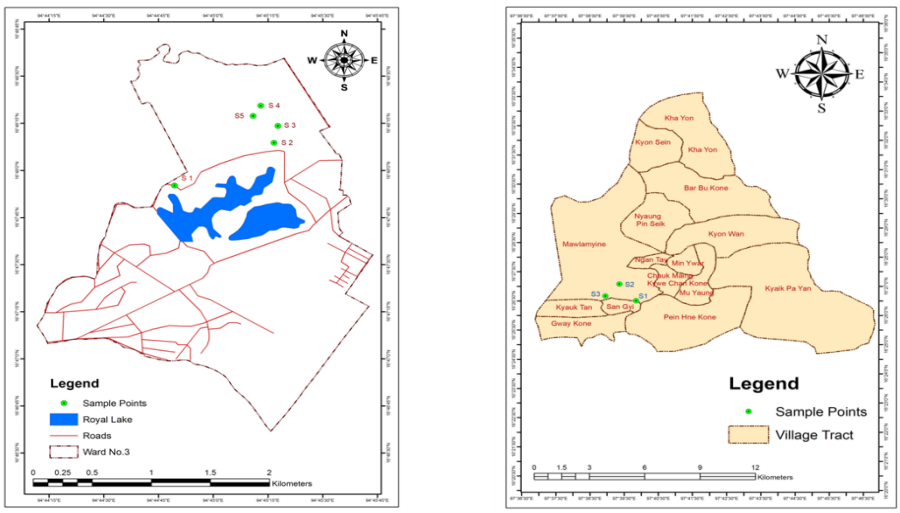
Isolation of Endophytic Fungi from Leaves of Medicinal Plants

Leaf samples were washed thoroughly in running tap water and air dried. The materials were then surface sterilized by immersing them 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 rinsed in sterile distilled water for 1 min and blot-dry. Then the leaf samples were dissected and place on petri-plates containing fungi isolation media.

Media Used for Isolation of Endophytic Fungi (Ando, 2004)

Isolation medium		**Transfer medium**	
Glucose	1.0 g	Glucose	1.2 g
Yeast extract	0.5 g	Yeast extract	0.7 g
$MgSO_4$	0.01 g	$MgSO_4$	0.01 g
K_2HPO_4	0.01 g	KNO_3	0.1 g
Agar	1.8 g	Agar	1.8 g
Distilled water	100 mL	Distilled water	100 mL

After autoclaving, chloramphenicol (25 mg/100mL) was added to the medium



(a) (b)

Source: Geography Department, Pathein University

Figure 1 (a) Map of Pathein University Campus and
(b) Map of Mawlamyine Showing Locations from
where Plant Samples were Collected

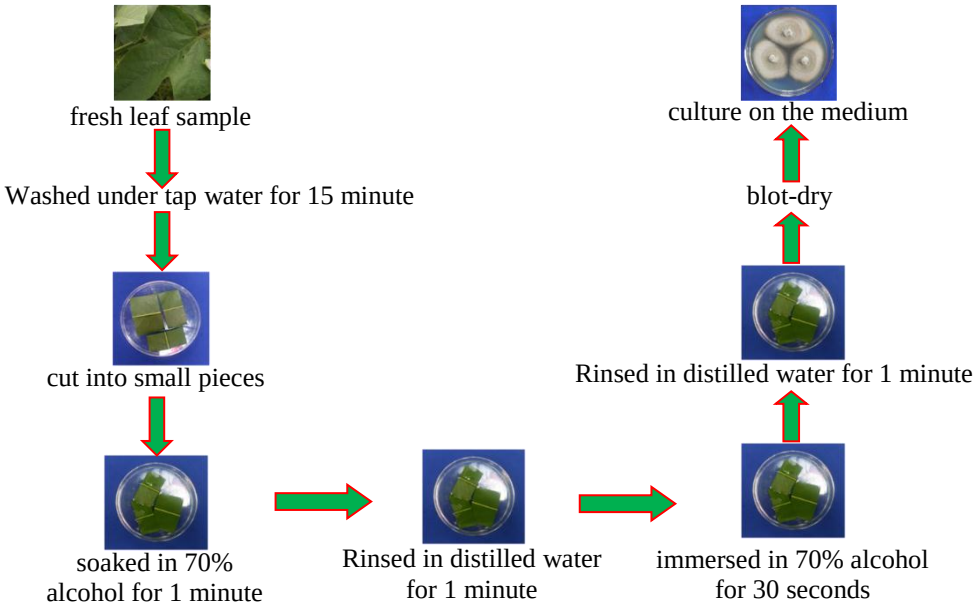


Figure 2 Procedure for isolation of endophytic fungi (Ando, 2004)

Table 1 Medicinal plants used for the isolation of the endophytes

No.	Botanical Name	Myanmar name	Family	Collected area
1	*Chrozophora rottleri* Geiseler Juss.	Joe-sar-kauk	Euphorbiaceae	Pathein University Campus
2	*Momordica cochinchinensis* (Lourein) Sprengel	Taw Sa byit	Cucurbitaceae	Pathein University Campus
3	*Gloriosa superba* L.	Si mi dauk	Colchicaceae	Mawlamyine
4	*Passiflora foetida* L.	Taw Su Ka	Passifloraceae	Pathein University Campus
5	*Spilanthes iabadicensis* A.H. Moore.	Shar-hton	Asteraceae	Mawlamyine
6	*Vernonia patula* (Dryand.) Merr.	Not known	Asteraceae	Pathein University Campus
7	*Cayratia japonica* (Thunberg) Gagnepain	Yin-naung	Vitaceae	Mawlamyine
8	*Dioscorea birmanica* Prain and Burkill	Khat-cho	Dioscoreaceae	Mawlamyine University Campus
9	*Hyptis capitata* Jacq.	Not known	Lamiaceae	Pathein University Campus
10	*Plantago major* L.	Akyawta-htaung	Plantaginaceae	Mawlamyine
11	*Tiliacora triandra* (Colebr.) Diels	Not known	Menispermaceae	Mawlamyine
12	*Kaempferia parviflora* Wall ex Baker	Nanwin-net	Zingiberaceae	Mawlamyine

Medium Used for Antimicrobial Activity Test (Ando, 2004)

Seed Medium		**Fermentation Medium**		**Assay Medium**	
Glucose	- 1.0 g	Glucose	- 1.5 g	Glucose	- 0.8 g
Yeast extract	- 0.5 g	Yeast extract	- 1.0 g	Peptone	- 0.7 g
MgSO4	- 0.001 g	MgSO4	- 0.001 g	KNO3	- 0.2 g
KNO3	- 0.1 g	KNO3	- 0.1 g	Agar	- 1.8 g
DW	- 100 mL	DW	- 100 mL	DW	- 100

Screening of Antimicrobial Activities of Effective Endophytic Fungi by Paper Disc Diffusion Assay (Tomita, 1988)

The isolated fungi were grown at room temperature for 5 days. The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Seed culture (20%) was transferred into the fermentation medium and incubated at room temperature for 10 days. 20 µL of fermented broth was put on paper disc. After drying, placed on assay plate containing test organism and incubated for 24 hours. In the present study, six microorganisms were used for antimicrobial activity.

Table 2 Test Organisms utilized for antimicrobial activities

No.	**Test Organisms**	**Disease**
1	*Aspergillus flavus* IFO3290	Aspergillosis
2	*Bacillus subtilis* KY-327	Fever
3	*Micrococcus luteus* NITE83297	Skin disease
4	*Escherichia coli* AHU5436	Diarrhoea
5	*Pseudomonas fluorescens* IFO94307	Rice disease
6	*Salmonella typhi* AHU7943	Typhoid fever and food poison

Effect of Age of Inoculum on the Fermentation of antibacterial activity by Isolated Endophytic Fungus MHA-10

Five days old culture of the selected fungus MHA-10 was inoculated into seed medium and then transfer to fermentation medium. Age of culture with 48 hrs, 60 hrs, 72 hrs, 84 hrs, 96 hrs, 108 hrs and 120 hrs were employed for fermentation. The antibacterial activity was checked by paper disc diffusion assay method.

Effect of Sizes of Inoculum on the Fermentation of Antibacterial activity by Isolated Fungus MHA-10

In this study 6%, 12%, 18%, 24%, 30% and 36% of 84 hrs of seed culture were employed for the fermentation. The antibacterial activity was undertaken by paper disc diffusion assay method.

Effect of Carbon Source Utilization on the Fermentation of Antibacterial Activity by Isolated Fungus MHA-10

To evaluate the effect of various carbon sources on the fermentation of antibacterial activity by isolated endophytic fungus MHA-10, different carbon sources such as glucose, sucrose, soluble starch, potato powder, tapioca powder, corn powder and rice powder were supplemented separately into the fermentation medium. 2 g of each carbon sources were added into basal medium.

Effect of Nitrogen Source Utilization on the Fermentation of Antibacterial Activity by Isolated Fungus MHA-10

To evaluate the effect of various nitrogen sources on the fermentation of antibacterial activity by isolated endophytic fungus MHA-10, different nitrogen sources such as yeast extract, peptone, KNO_3, NH_4Cl_2, NH_4SO_4, NH_4NO_3 and meat extract were utilized. 1 g of each nitrogen sources were added into basal fermentation medium.

Study on Medium Optimization for Fermentation

In the present study, 6 fermentation media was undertaken. Fermentation was carried out with 84 hrs age and 30% size of inoculums with six different media.

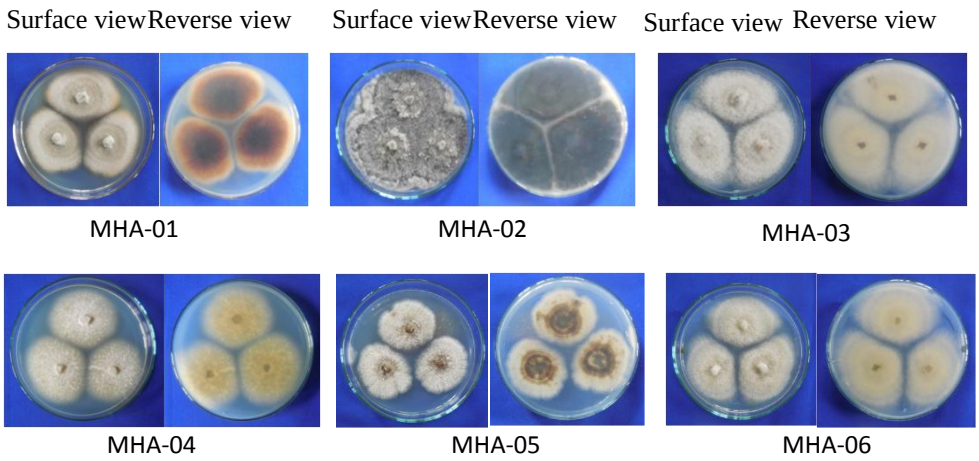
FM-1		**FM-2**		**FM-3**	
Glucos e	2.0 g	Glucose	2.0 g	Glucose	2.0 g
Yeast extract	1.0 g	Peptone	1.0 g	Yeast extract	0.5 g
$MgSO_4$	0.001 g	MgSO4	0.001 g	Peptone	0.5 g
KNO_3	0.1 g	KNO3	0.1 g	MgSO4	0.001 g
D/W	100 mL	D/W	100 mL	KNO3	0.1 g
				D/W	100 mL

FM-4		**FM-5**		**FM-6**	
Glucose	2.0 g	Glucose	2.0 g	Glucose	1.5 g
Yeast extract	0.7 g	Yeast extract	0.3 g	Yeast extract	0.5 g
Peptone	0.3 g	Peptone	0.7 g	Peptone	0.5 g
$MgSO_4$	0.001 g	$MgSO_4$	0.001 g	$MgSO_4$	0.001 g
KNO_3	0.1 g	KNO_3	0.1 g	KNO_3	0.1 g
D/W	100 mL	D/W	100 mL	D/W	100 mL

Results

Isolation of Endophytic Fungi from Leaves of Medicinal Plants

In the present investigation, 18 endophytic fungi were isolated from leaves of 12 medicinal plants.



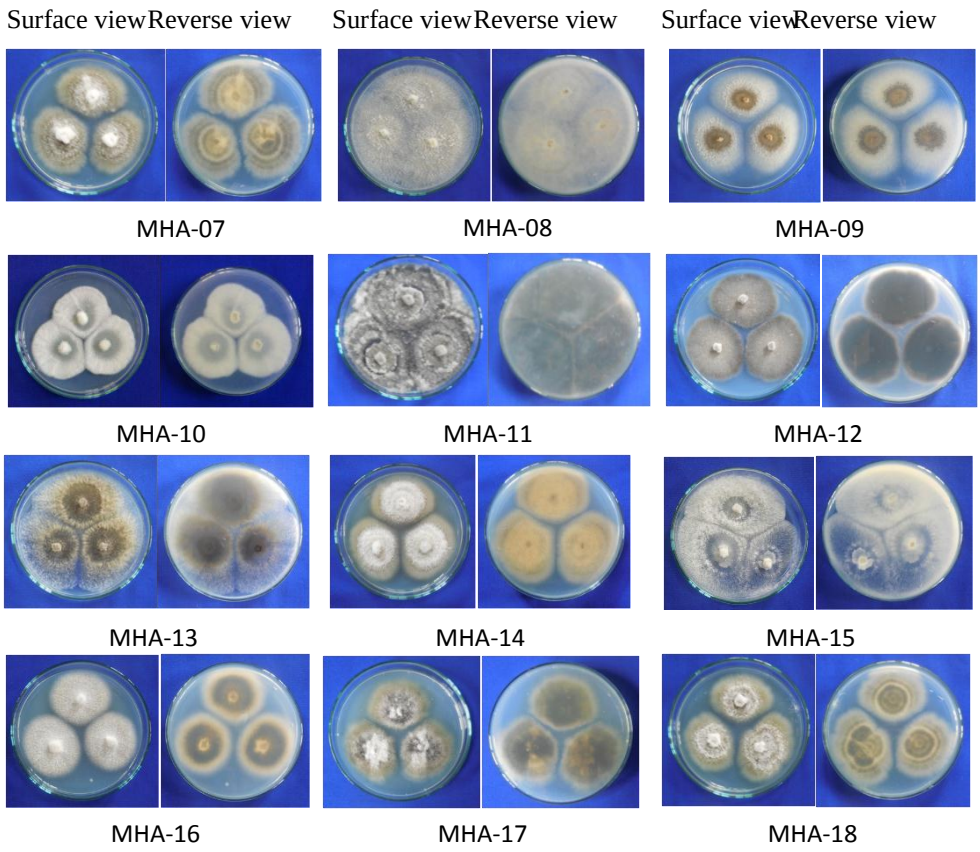


Figure 3 Morphology of isolated soil fungi MHA-01 to MHA-18

(7 Days old culture)

Table 3 Endophytic fungi isolated from the leaves of 12 medicinal plants

No.	Botanical Name	Isolates	Designated Number of endophytic fungi
1	*Chrozophora rottleri* Geiseler Juss.	1	MHA-01
2	*Momordica cochinchinensis* (Lourein)	1	MHA-02
3	*Gloriosa superba* L.	2	MHA-03, MHA-04
4	*Passiflora foetida* L.	1	MHA-05
5	*Spilanthes iabadicensis* A.H. Moore.	1	MHA-06
6	*Vernonia patula* (Dryand.) Merr.	1	MHA-07
7	*Cayratia japonica* (Thunberg) Gagnepain	2	MHA-08, MHA-09
8	Dioscorea birmanica Prain and Burkill	2	MHA-10, MHA-11
9	Hyptis capitata Jacq.	2	MHA-12, MHA-13
10	Plantago major L.	2	MHA-14, MHA-15
11	Tiliacora triandra (Colebr.) Diels	2	MHA-16, MHA-17
12	Kaempferia parviflora Wall ex Baker	1	MHA-18
	Total endophytic fungi	18	

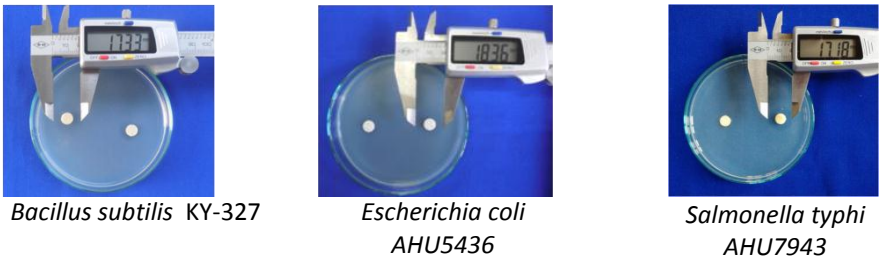
Screening of Antimicrobial Activities of Effective Endophytic Fungi by Paper Disc Diffusion Assay

In this study, two endophytic fungi, (MHA-09 and MHA-10) showed the antimicrobial activities on *Bacillus subtilis*, *E. coli* and *Salmonella typhi*. The endophytic fungus MHA-10 showed the highest activity on *Bacillus subtilis* (18.90 mm, inhibitory zone). This fungus MHA-10 was isolated from the leaf of *Dioscorea birmanica* Prain and Burkill.

Table 4 Antimicrobial activities of isolated fungi on six test organisms

Isolated fungi	Antimicrobial activities on test organisms (inhibitory zone, mm) (4 to 9 days)					
	Aspergillus flavus	***Bacillus subtilis***	***Micrococcus luteus***	***Escherichia coli***	***Pseudomonas fluorescens***	***Salmonella typhi***
MHA-09	-	17.33	-	18.36	-	17.18
MHA-10	-	18.90	-	17.64	-	18.74

(-) = No activity



Bacillus subtilis KY-327 | *Escherichia coli* AHU5436 | *Salmonella typhi* AHU7943

Figure 4 Antimicrobial activity of isolated fungus (MHA-09) against three test organisms

Bacillus subtilis KY-327 | *Escherichia coli* AHU5436 | *Salmonella typhi* AHU7943

Figure 5 Antimicrobial activity of isolated fungus (MHA-10) against three test organisms

Effect of Age of Inoculums on the Fermentation of antibacterial activity by Isolated Endophytic Fungus MHA-10

According to the table 5, it was shown that 84 hrs ages of inoculums was the best for fermentation.

Table 5 Effects of age of culture on fermentation of antibacterial activity by isolated endophytic fungus MHA-10

Seed culture (Time, hrs)	Activity (clear zone mm)
48	13.72
60	15.51
72	17.28
84	**18.80**
96	17.69
108	16.73
120	14.05

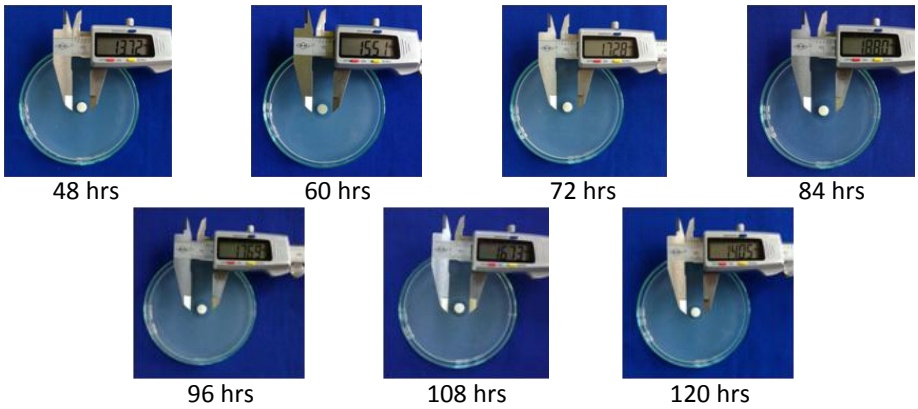


Figure 6 The effect of age of inoculum on fermentation of antibacterial activity by isolated endophytic fungus MHA-10 against *Bacillus subtilis*

Effect of Size of Inoculum on Fermentation of Antibacterial Activity by Isolated Endophytic Fungus MHA-10

According to the table 6, it was observed that 30% size of inoculums was the best for fermentation.

Table 6 Effects of size of inoculum on fermentation of antibacterial activity by isolated endophytic fungus MHA-10 against *Bacillus subtilis*

Size of inoculum	Activity (clear zone mm)
6 %	9.94
12 %	10.03
18 %	11.03
24 %	14.48
30 %	**17.06**
36 %	15.04

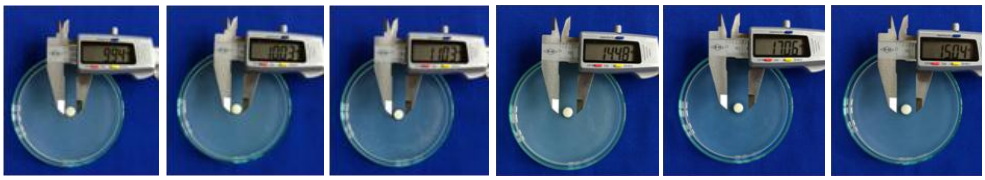


Figure 7 The effect of size of inoculum on fermentation of antibacterial activity by isolated endophytic fungus MHA-10 against *Bacillus subtilis*

Effect of Carbon Source Utilization on the Fermentation of Antibacterial Activity by Isolated Fungus MHA-10

Among the carbon source tested, glucose showed the highest antibacterial activity (18.03 mm clear zone) followed by sucrose (14.39 mm clear zone) against *Bacillus subtilis*, lowest activity was exhibited by corn powder (10.07 mm clear zone). (Table 7, Figure 8).

Table 7 Effects of carbon utilization on fermentation of antibacterial activity by isolated fungus MHA-10 against *Bacillus subtilis*

Carbon sources	Activity (clear zone mm)
Glucose	**18.03**
Sucrose	14.39
Soluble starch	12.79
Potato powder	13.46
Tapioca powder	10.19
Corn powder	10.07
Rice powder	12.88

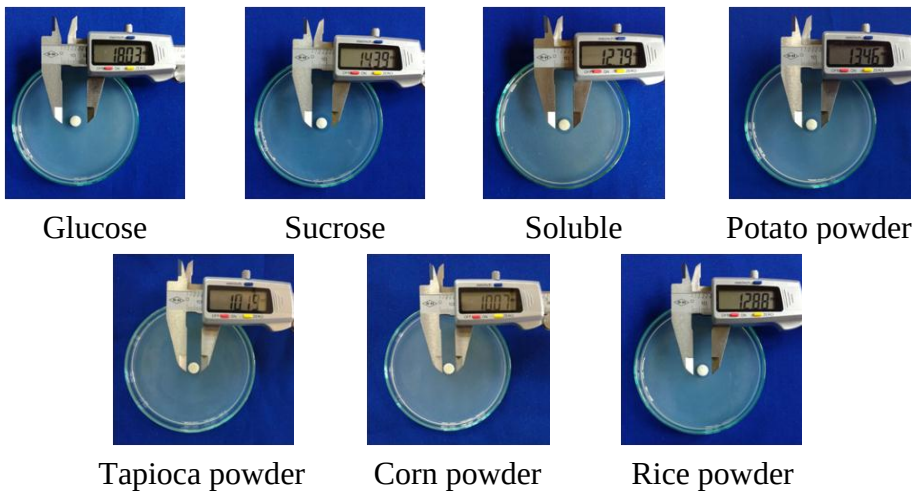


Figure 8 Effects of carbon utilization on fermentation of antibacterial activity by isolated endophytic fungus MHA-10 against *Bacillus subtilis*

Effect of Nitrogen Source Utilization on the Fermentation of Antibacterial Activity by Isolated Fungus MHA-10

Among the nitrogen source tested, peptone showed the highest antibacterial activity (18.95 mm clear zone) followed by yeast extract (16.34 mm clear zone) against *Bacillus subtilis*. Lowest activity was exhibited by potassium nitrate (KNO_3) (10.08 mm clear zone). (Table 8, Figure 9).

Table 8 Effects of nitrogen utilization on fermentation of antibacterial activity by isolated fungus MHA-10 against *Bacillus subtilis*

Nitrogen sources	Activity (clear zone mm)
Yeast extract	16.34
Peptone	**18.95**
KNO_3	10.08
NH_4Cl	14.19
NH_4SO_4	15.63
NH_4NO_3	14.43
Meat extract	15.28

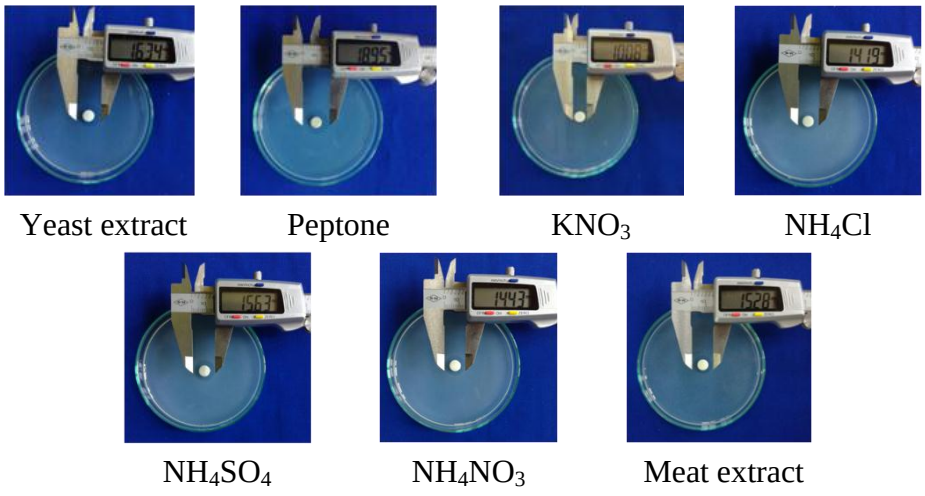


Figure 9 Effects of nitrogen utilization on fermentation of antibacterial activity by isolated endophytic fungus MHA-10 against *Bacillus subtilis*

Effect of Medium on Fermentation

FM-1 gave maximum antibacterial activity (20.64 mm clear zone against *Bacillus subtilis*).

Table 9 Effects of media on fermentation

Medium	Activity (clear zone mm)
FM-1	**20.64**
FM-2	13.77
FM-3	16.78
FM-4	13.17
FM-5	14.04
FM-6	14.45

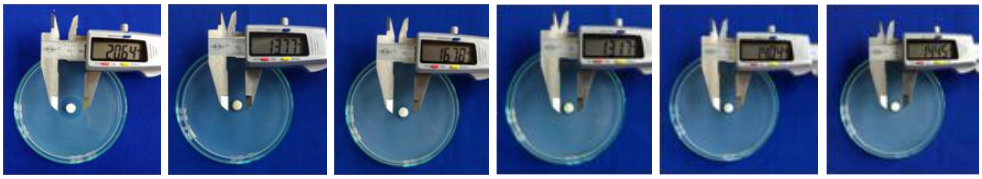


Figure 10 Effects of media on fermentation against *Bacillus subtilis*

Discussion and Conclusion

A total 18 endophytic fungi were isolated from leaves of 12 medicinal plants. Endophytic fungus MHA-01 was isolated from *Chrozophora rottleri* Geiseler Juss. Fungus MHA-02 was isolated from *Momordica cochinchinensis* (Lourein) Sprengel. Endophytic fungi MHA-03 and MHA-04 were isolated from *Gloriosa superba* L. Budhiraja *et al.,* (2012) isolated 22 endophytic fungi from different parts of *Gloriosa superba* L. Endophytic fungus MHA-05 was isolated from *Passiflora foetida* L. Kanjana *et al.,* (2019) isolated endophytic fungus *Chaetomium globosum* from *Passiflora foetida* L. Endophytic fungus MHA-06 was isolated from *Spilanthes iabadicensis* A.H. Moore. Endophytic fungus MHA-07 was isolated from *Vernonia patula* (Dryand.) Merr. Endophytic fungi MHA-08 and MHA-09 were isolated from *Cayratia japonica* (Thunberg) Gagnepain. Endophytic fungi MHA-10 and MHA-11 were isolated from *Dioscorea birmanica* Prain and Burkill. Endophytic fungi MHA-12 and MHA-13 were isolated from *Hyptis capitata* Jacq. Endophytic fungi MHA-14 and MHA-15 were isolated from *Plantago major* L. Farias *et al.,* (2018) isolated *Colletotrichum gloesporioides* from leaves of *Plantago major* L. Endophytic fungi MHA-16 and MHA-17 were isolated from *Tiliacora triandra* (Colebr.) Diels. Senadeera *et al.,* (2012) isolated endophytic fungus *Dothideomycete* sp. from *Tiliacora triandra* (Colebr.) Diels. Endophytic fungus MHA-18 was isolated from *Kaempferia parviflora* Wall ex Baker. Jankong (2004) isolated 36 endophytic fungi from *Kaempferia parviflora* Wall ex Baker.

The endophytic fungi MHA-10 has shown greater antibacterial activity against *Bacillus subtilis.* The maximum production of the antibacterial metabolites by MHA-10 was achieved by optimizing various parameters like age (84 hrs) and size (30%) of inoculums, and fermentation

media (FM-1) were found to be optimum for the maximal production of bioactive metabolite. Jain, 2010 reported that the variations in the fermentation environment often result in alteration in antibiotic production. Among the different carbon sources tested, glucose was the best carbon source for antibacterial activity. Similar results were shown by Tanseer and Anjum (2011) where glucose promoted the secondary metabolite production. The results were in good agreement with Haque *et al.,* (2014). Among the different nitrogen sources tested, peptone was the best nitrogen source for antibacterial activity. Peptone has been observed to be the best nitrogen sources by Reddy *et al.,* (2011). Peptone has been found to favour antibiotic production by Praveen (2008), Tanseer and Anjum (2011). These findings will assist in formulating a suitable culture medium for production of the antibacterial compound from endophytic fungus MHA-10.

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A PRELIMINARY STUDY ON THE ANTIMICROBIAL ACTIVITIES OF ISOLATED SOIL FUNGI FROM THA-BEIK-KYIN AREA

Mai Bawi Nuam Thein [1], Zaw Lin Aung[2] and Kay Thi Mya[3]

Abstract

In this present study, seven different soil samples were collected in the Tha-Beik-Kyin area of Mandalay region for the isolation of soil microorganisms. After that soil fungi were isolated from different soil samples at Biological Resource and Biotechnology Development Center (BRBDC) of Botany Department in Pathein University. Eleven fungi were isolated from these seven different soil samples by using physical treatment dilution method on low carbon medium. Then, the isolated fungi were photographed and the studies were conducted on their respective morphological characters. Following that, antimicrobial activities of the fungi were tested by the paper disc diffusion assay method. In the study of antimicrobial activities, 8 different test organisms were utilized. According to the results, three isolated strains (MAI 01, MAI 06, and MAI 07) showed activity against the test organisms. Among them, strain MAI-07 showed the highest antibacterial activity against *Bacillus subtilis* (25.70 mm). In the fermentation studies, 84 hr age and 15% size of inoculums were optimized for the fermentation. Carbon sources (glucose, glycerol) and nitrogen sources (yeast extract, polypeptone) showed the best activities on the fermentation.

Keywords: *Bacillus subtilis*, antimicrobial activities

Introduction

Microorganisms have been traditionally used to produce a variety of important substances for the pharmaceutical and food industries (Demain, 2000). Antibiotics are microbial products or their derivatives that can kill susceptible microorganisms or inhabit their growth. The fungi have been widely studied for their bioactive metabolites and have proven to be a rich and promising source of noble anticancer, antibacterial, antiflammatory and anti-viral agents (Rajalakshmi and Mahesh, 2014).

Microbial secondary metabolites continue to be a chemically diverse source for the discovery and development of pharmaceutical agents and also biochemical probes to study human disease process (Tamotsu *et al.,* 2002). The main basic for the therapy of microbial (bacterial and fungal) infection is provided by antibiotics (Khan, 2004). Microorganisms have been traditionally used to produce a variety of important substances for the pharmaceutical and food industries (Demain, 2000). The aim and objectives are to contribute the valuable knowledge for applied microbiology, to isolate useful soil fungi, to study their antimicrobial potentials and to optimize the fermentation of selected fungus for further study.

Materials and Methods

Samples collection and isolation of soil fungi

Seven different soil samples were collected from Thabeik-kyin Area, Mandalay Region (Figure 1). The collected soil samples were air dried at room temperature. Then the isolation of fungi was carried out by physical treatment dilution method as shown in Figure 2.

[1] Assistant Lecturer, Department of Botany, Pathein University
[2] Lecturer, Department of Botany, Pakokku University
[3] Professor and head, Department of Botany, Pathein University

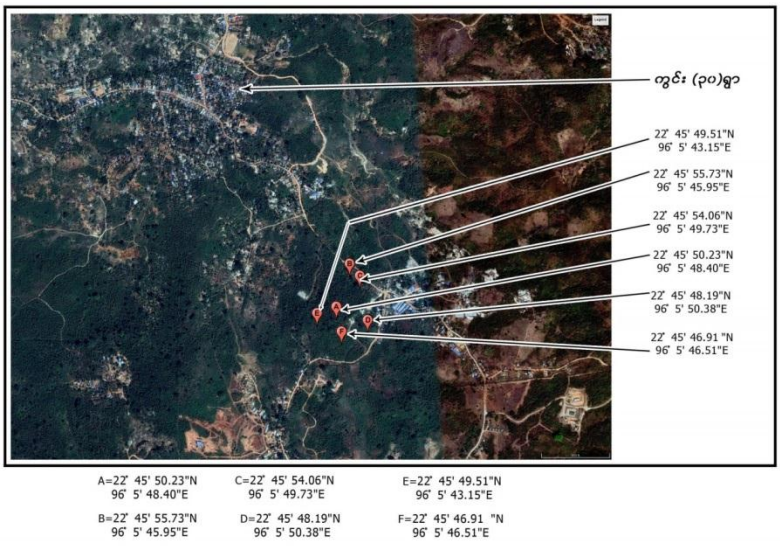

Figure 1 Soil samples collected at different places of Tha-beik-kyin

Map Source: Google Earth

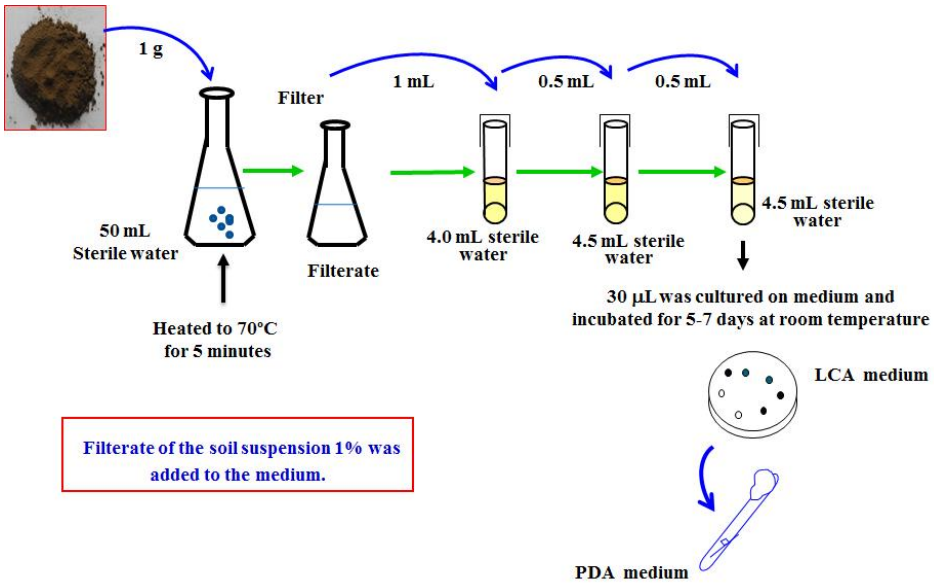

Figure 2 Physical treatment dilution method (Hayakawa and Kobayashi, 2005)

Preliminary study on antimicrobial activities of isolated fungi

Preliminary study for antimicrobial activities was carried out by paper disc diffusion assay (Tomita, 1988). The isolated fungi were grown on medium for 7 days at room temperature for sporulation. Then that fungal stain was inoculated into seed medium (Glucose 2 g, Sucrose 0.3 g, Yeast Extract 0.3 g, KNO_3 0.1 g, K_2HPO_4 0.01 g, DW 100 mL at pH 6.5) for 3 days and incubated for 3 days at room temperature. Fermentation (Glucose 2.5 g, Yeast Extract 1g, NZ Amine Type A 0.5 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.1 g, DW 100 mL at pH 6.5) was undertaken at room temperature for 10 days. Twenty µL of fermented broth was put onto the paper disc (8 mm) and placed on assay plates containing test organism and incubated for 24 to 36 hrs (Figure 3). Test organism utilized in the study of antimicrobial activities were *Aspergillus*

flavus IFO 3290, *Bacillus subtilis* KY-327, *Candida albicans* NITE 09542, *E. coli* AHU 5436, *Micrococcus luteus* NITE 83297, *Pseudomonas fluorescens* NITE 52847 and *Salmonella typhi* AHU 7943. These test organisms were supported by NITE (National Institute of Technology and Evaluation), Japan, 2005.

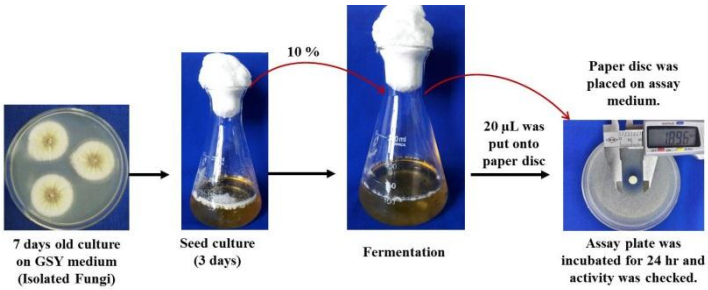

Figure 3 Preliminary study for antimicrobial activity against test organisms

Microbial growth kinetics of MAI-07

The strain MAI-07 was inoculated into the medium and incubated for 144 hrs. The culture sample was checked in 12 hrs interval for the growth at 2000 rpm for 30 minutes and packed cell volume% (PCV%) was calculated (Omura, 1985 and Crueger and Crueger, 1989).

Effects of ages and size of inoculums on the fermentation

According to the results of microbial growth kinetics of MAI-07 (66, 72, 78, 84, 90 and 96 hrs) seed culture were utilized for the fermentation (Figure 4). Based on the results of the ages of inoculums of MAI-07 (5%, 10%, 15%, 20%, 25% and 30%) of 84 hr seed cultures were employed for the fermentation. Fermentation was carried out 6 days and antibacterial activity was tested by paper disc diffusion assay method (Figure 5).

Effects of carbon and nitrogen sources on the fermentation

In this study, carbon sources such as glucose, glycerol, sucrose, soluble starch and potato powder and nitrogen sources such as yeast extract, polypeptone, KNO_3, rice ban and meat extract were employed. Fermentations were incubated at room temperature for 6 days. Each 100 mL fermentation medium was prepared for different sources test. The antibacterial activities were checked by paper disc diffusion assay method. Carbon source (2) g and nitrogen source (0.5) g was added to 100 mL of their basal fermentation medium (Yeast Extract 0.5 g, NZ Amine Type A 0.5 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.01 g) and (Glucose 2 g, Soluble starch 0.5 g, K_2HPO_4 0.001 g, $MgSO_4.7H_2O$ 0.001 g, $CaCO_3$ 0.01 g) at pH 6.5 respectively.

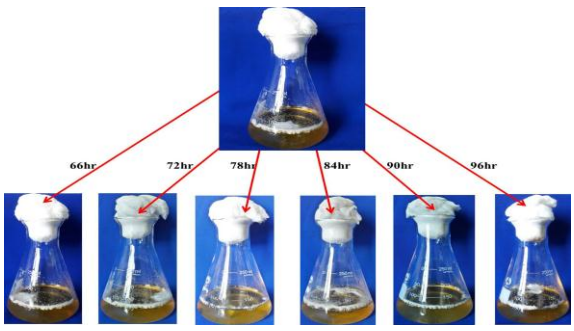

Figure 4 Effects of ages of inoculum of MAI-07 on the fermentationb

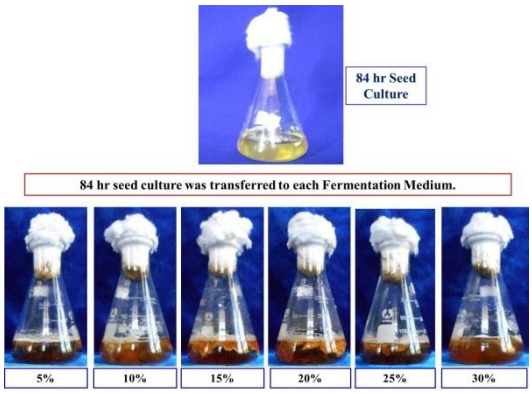

Figure 5 Effects of sizes of inoculum of MAI-07 on the fermentation

Results

Isolation of fungi from soil samples

In the isolation of soil fungi, eleven different fungi were isolated from seven different kinds of soil samples collected at Tha-Beik-kyin Area, Mandalay Region. Colony morphologies of isolated soil fungi were shown in Figures 6.

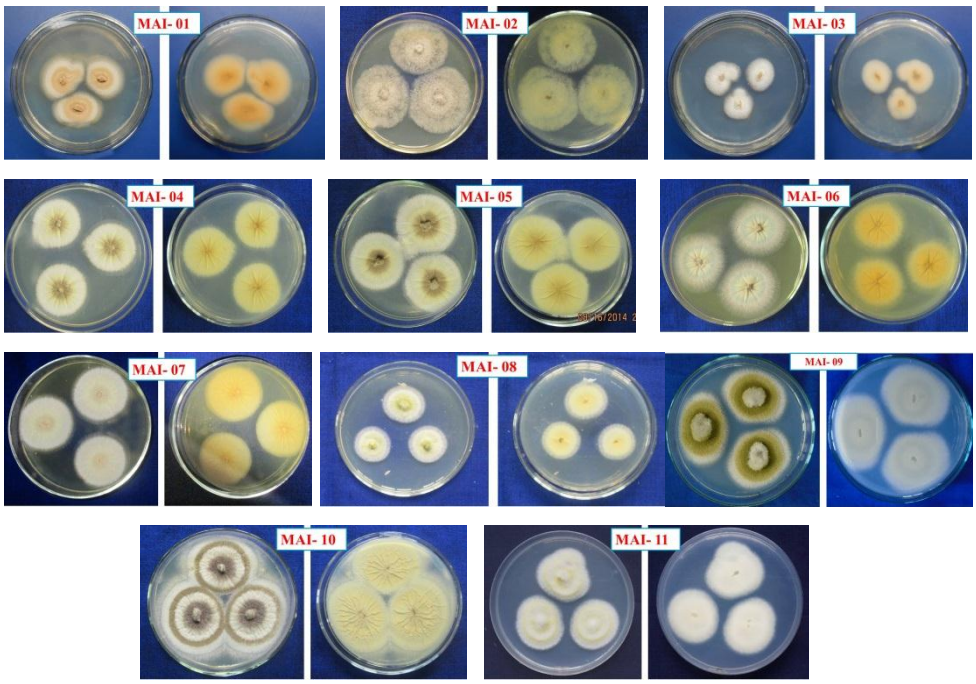

Figure 6 Colony morphologies of isolated fungi

Preliminary study on antimicrobial activities

Preliminary study on antimicrobial activities of isolated fungi was carried out by paper disc diffusion assay method. In this investigation, three strains (MAI-01, MAI-06 and MAI-07) showed activity against test organisms. MAI-01 against *E. coli* (17.54 mm), MAI-06 against *Salmonella typhi* (13.35 mm), MAI-07 against *Bacillus subtilis* (25.70 mm). Strain MAI-07 was selected for further studies because it showed the highest antibacterial activity against *Bacillus*

subtilis KY-327 (25.70 mm). Antimicrobial activities of isolated fungi were shown in Table 1 and Figure 7.

Table 1 Antibacterial activity of isolated fungi against test organisms

Soil No.	Isolated Fungi	Antibacterial activity
1	**MAI-01**	***E. coli* (17.54 mm)**
	MAI-02	No Activity
2	MAI-03	No Activity
	MAI-04	No Activity
3	MAI-05	No Activity
4	**MAI-06**	***Salmonella typhi* (13.35 mm)**
5	**MAI-07**	***Bacillus subtilis* (25.70 mm)**
	MAI-08	No Activity
6	MAI-09	No Activity
7	MAI-10	No Activity
	MAI-11	No Activity

(A) (B) (C)

Figure 7 Antibacterial activities of isolated fungi against test organisms

(A). Antibacterial activity of MAI-01 against *Escherichia coli* AHU5436

(B). Antibacterial activity of MAI-06 against *Salmonella typhi* AHU7943

(C). Antibacterial activity of MAI-07 against *Bacillus subtilis* KY-327

Microbial growth kinetics of MAI-07

In the growth kinetics of fungus MAI-07, it was found that growth phase was between 48 hr and 96 hr. According to Crueger and Crueger (1989), it was considered that ages of inoculum (66, 72, 78, 84, 90 and 96 hr) were suitable for the optimization of fermentation (Table 2 and Figure 8).

Table 2 Microbial growth kinetics of MAI-07

Culture Time (hr)	PCV of 5 mL	PCV %
24	0.2	4
36	0.25	5
48	0.35	7
60	0.55	11
72	0.85	17
84	1.1	22
96	**1.25**	**25**
108	1.25	25
120	1.2	24
132	1.1	22
144	1.0	20

*Packed Cell Volume (PCV)

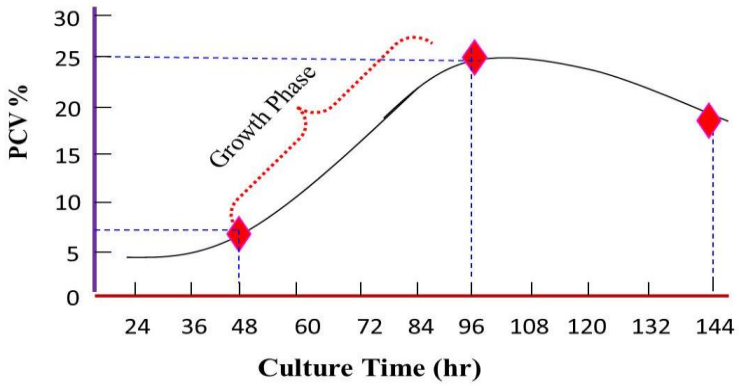

Figure 8 Microbial growth kinetics of MAI-07

Effects of ages of inoculum on the fermentation

According to the results of microbial growth kinetics of MAI-07, (66, 72, 78, 84, 90 and 96 hr) seed culture were utilized for the fermentation. It was considered that 84 hr seed culture showed the best activity on *B. subtilis* than others seed culture (Table 3 and Figure 9).

Table 3 The effects of ages of inoculum on the fermentation

Ages of culture (hr)	Activity (Inhibitory Zone, mm)
66	15.68
72	18.73
78	27.56
84	**29.85**
90	28.29
96	20.12

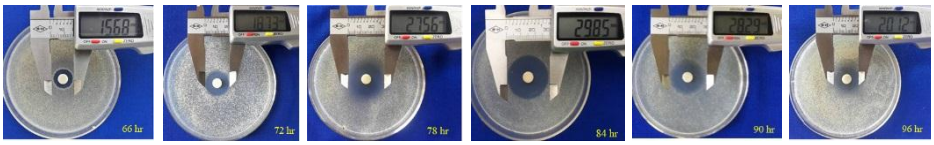

Figure 9 The effects of ages of inoculum on the fermentation

Effects of sizes of inoculum on the fermentation

Based on the results of the ages of inoculum of MAI-07, (5%, 10%, 15%, 20%, 25% and 30%) of 84 hr seed cultures were utilized for the fermentation. It was considered that 15% size of inoculum showed the best activity on *B. subtilis* (Table 4 and Figure 10).

Table 4 Effects of sizes of inoculum on the fermentation

Sizes of culture (%)	Activity (Inhibitory Zone, mm)
5	14.95
10	15.70
15	**30.31**
20	19.11
25	18.96
30	16.51

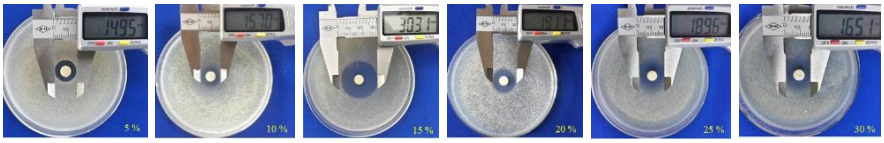

Figure 10 Effects of sizes of inoculum on the fermentation

Effects of different carbon sources utilization on the fermentation

In the studies of different carbon sources utilization on the fermentation of MAI-07, glucose and glycerol showed the best activities on *B. subtilis*(Table 5 and Figure 11).

Table 5 Effects of different carbon sources utilization on the fermentation

Carbon Source	Activity (Inhibitory Zone, mm)
Glucose	**23.5**
Sucrose	18.6
Soluble starch	20.4
Potato powder	20.6
Glycerol	**21.7**

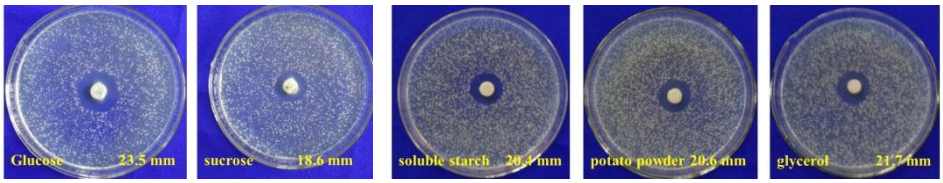

Figure 11 Effects of different carbon sources utilization on the fermentation

Effects of different nitrogen sources utilization on the fermentation

In the studies of five different nitrogen sources utilization for the fermentation of MAI-07, yeast extract and polypeptone showed the best activities on *B. subtilis* (Table 6 and Figure 12).

Table 6 Effects of different nitrogen sources utilization on the fermentation

Nitrogen sources	Activity (Inhibitory Zone, mm)
Yeast extract	**22.5**
Meat extract	19.5
Rice bran	18.2
KNO_3	19.4
Polypeptone	**21.3**

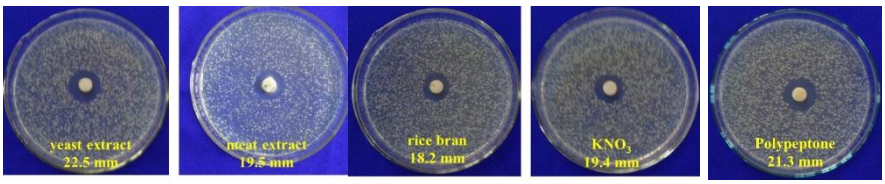

Figure 12 Effects of different nitrogen sources utilization on the fermentation

Discussion and Conclusion

In this study, soil was collected from seven different places of Tha-Beik-Kyin area of Mandalay region. After that the isolation and screening of effective soil fungi were investigated. In this investigation, a total of eleven fungi were isolated from seven different soil samples at Tha-Beik-kyin area. The isolation of soil fungi was carried out by physical treatment dilution method with low carbon agar medium. In this investigation, three strains MAI-01, MAI-06 and MAI-07 were showed the activity against test organisms. MAI-01 showed against *E. coli*, MAI-06 showed against *Salmonella typhi* and MAI-07 showed against *Bacillus subtilis.* Strain MAI-07 showed the highest antibacterial activity against *Bacillus subtilis* KY-327 (25.70 mm). Fungi are remarkable organisms that readily produce a wide range of natural products often called secondary metabolites. In many cases, the benefits of these compounds confer on the organisms is unknown. Secondary metabolite production usually commences late in the growth of the microbe, often upon entering the stationary or resting phase (Bu'lock, 1961).

Fermentation time is a very important factor, which affect the yield and quality of metabolites (Chen, 2003). Mansi and Charlie, 2003 reported that in search of favorable fermentation conditions, the types of fermentation used as well as its size, durations, and

nutrients profile will depend critically on the nature of the microbial products. Therefore fermentation optimization was undertaken to produce the antibacterial metabolite in high yield. The fermentation conditions were investigated for the production of antibacterial metabolite against *Bacillus subtilis.*

Based on the growth kinetics of MAI-07, 84 hrs age and 15% size of inoculums were optimized for the production of metabolite. In the studies of different carbon and nitrogen sources utilization on the fermentation, carbon sources (glucose, glycerol) and nitrogen sources (yeast extract, polypeptone) showed the best activities. According to Ronald, 1988, the composition of the fermentation medium must include the nutrients essential to support the growth of the microbial strain and the formation of the desired product. Essential nutrients for microbial growth include sources of carbon, nitrogen and phosphorous.

In conclusion, it was found that there is high potential to discover useful antibiotic producing from the site with some possibly novel strain for applied microbiology. Therefore more work can be conducted for further study.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Si Si Hla Bu, Rector, University of Pathein for providing me an opportunity to do this work. I also extended my thank to Professor Dr Nilar Myint and Dr Than Htun, Pro-Rectors, University of Pathein, for their valuable instruction and guidance. I would like to record my special thanks to Professor Dr Kay Thi Mya, Head of Botany Department, University of Pathein and Professor Dr Wah Wah Lwin, Department of Botany, University of Pathein for their suggestion and kind understanding during this study. I wish to express my heartiest gratitude to my supervisor Dr Zaw Lin Aung, Lecture, Department of Botany, University of Pakkoku, for his valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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ISOLATION AND ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES FROM CHAUNG-THA AREA AND BIOCHEMICAL CHARACTERIZATION OF SELECTED *STREPTOMYCES* (TR-2)

Hnin Thiri Lwin[1], Zar Zar Yin[2] and Kay Thi Mya[3]

Abstract

A total of 13 actinomycetes were isolated from two samples sea-water and sludge, Chaung-Tha area, Ayeyarwady region. Among them, 8 strains were obtained from sludge sample and 5 strains from sea water. In the elevation, the isolated strains were circular, raised, convex, umbonate and flat. In the margin and textures, all strains were entire and rough. The colony size of isolated strains were large, small and moderate. The aerial mass colour of all strains was greenish blue, white and centre greenish blue, white in periphery. Their substrate colour was yellow. The antimicrobial activity of all strains were screened by agar well diffusion method on ten test organisms. Of these 13 strains, eight strains showed the antimicrobial activity. Among them, TR-2 showed the highest antifungal activity on *Candida albicans.* Therefore, TR-2 was selected for biochemical characterization. Positive results were found in methyl red test, citrate utilization test, casein test, mannitol salt broth test, potato plug test, nitrate reduction, catalase and urease test. But, Voges proskauer test, hydrogen sulfide test, gelatin hydrolysis test, esterase activity test, oxidase test and motility test were negative. According to the results, TR-2 was classified as the possible genus *Streptomyces* sp.

Keywords: Sea water, sludge, streptomyces, antimicrobial activity

Introduction

Marine ecosystems represent 95% of the biosphere and coastal regions are particularly promising, because of the rightly adapted species found in these harsh environments (Ireland *et al.*, 1993). The oceans represent a virtually untapped resource for discovery of even more novels compounds with useful activity. So far, more than 10000 bioactive molecular have been discovered from marine sources with hundreds of new compound still being discovered every year (Proksch *et al.*, 1997).

It is also reported that marine actinomycetes are useful and suitable source of new bioactive natural product (Nevine *et al.*, 2002).

Mangrove forests are highly productive ecosystems which comprise of unique woody plant communities and located in tropical and subtropical coastal area (Hong, 2009 and Hunadanamra, 2013). According to Ara, *et al.*, 2013, mangroves form unique saline environments under the influence of tidal flow, hence the muddy alluvial soil due to the intermittent flooding. Mangrove ecosystems are nutritionally versatile as they are highly rich in organic matter, nitrogen and sulfur content which can be used by living microorganisms. Thus, it is believed that mangrove ecosystems have the potential of becoming new reservoir for highly diverse actinomycetes as demonstrated by the isolation of *Micromonospora rifamycinica* (Huang, *et al.*, 2003) and *Verrucosispora wenchangensis* (Xie, *et al.*, 2012).

Streptomyces is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae (Kampfer *et al.*, 1991). Over 500 species of *Streptomyces* bacteria have been described by Euzeby (Euzeby 2008). Streptomycetes have genomes with high GC content and these are gram-positive (Madigan and Martinko 2003).

[1] PhD Candidate, Department of Botany, Pathein University
[2] Associate Professor, Department of Botany, Pathein University
[3] Professor and Head, Department of Botany, Pathein University

Many species belonging to the genus *Streptomyces* are well known as biocontrol agents that inhibit or lyase several soil borne and air borne plant pathogenic fungi (Sousa *et al.,* 2008). The genus Streptomyces comprises a large group of microorganisms with some characteristic features compared to most other bacteria such as their complex fungi-like life cycle and earthy odor. Furthermore, they are ubiquitous in nature and show a higher diversity in colour of colonies secreted pigments, etc. compared to other bacteria (Good fellow *et al.,* 1983).

The genus *Streptomyces* is morphologically highly diverse. Colour of substrate and aerial mycelium, configuration of spore chains and spore ornamentation are used as taxonomic markers. All of them are determined using cultivation on standard media and fixed incubation times (Shirling and Gottlieb, 1966). The aim of this study is to isolate the actinomycetes from Chaung-Tha area, to screen the antimicrobial activity of actinoymcetes and to study biochemcial characterization of selected Streptomyces (TR-2).

Materials and Methods

Study area and collection of plant samples

Two different samples such as sea water and sludge were collected from Chaung-Tha area, Ayeyawady Region in the month of June, 2016. The sludge sample was taken from top 6 cm soil profile and sea water sample was collected in depth 2 feet. The isolation of actinomycetes were carried out by serial dilution method and utilized on six different media.

Isolation of mangrove microorganisms (Salle, 1948)

In order to isolate, an appropriate amount (1 gm) of soil was put into a conical flask containing 99 mL of distilled water to make a soil water dilution ratio of 1:100. The flask was then shaken for about 30 minutes in order to make the soil particles free from each other. This dilution solution was then serially diluted into 10^{-1} to 10^{-5} dilution in separate test tube and 1 mL each of the above dilution was separately transferred into sterile petridishes under aseptic conditions. The sterilized medium in conical flask was cooled down to about 45ºC and separately poured into each of the petridish containing the respective soil dilutions. The inoculated plates were shaken clock-wise and anitclock-wise direction to make uniform distribution of the inoculums.

Isolation of pure culture from Plate to Slants (Atlas, 1993)

For pure culture from plate to test tube, about 100 mL of culture media were put into test tube. These test tube were plugged with cotton wool and sterilized by autoclaving. The sterilized media were cooled down. Each of the separate colonies on petridish was taken out to streak on the slant medium on the slant medium to obtain pure cultures.

Media used for the isolation of Actinomycetes

Kuster's agar medium (Balagurunathan and Subramanian, 1992) (Glycerol 10 g, Casein 0.3 g, KNO_3 3 g, NaCl 2 g, $MgSO_4$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01 g, Agar 16 g, DW 1000 mL, Sea water 50%/L), Actinomycetes isolation agar medium (Sodium caseinate 2 g, L-asparagine 0.1 g, Sodium propionate 4 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.1 g, Ferrous sulphate 0.001 g, Agar 15 g, DW 1000 mL, Sea water 50%/L), Yeast extract malt extract agar (ISP-2) (Shirling and Gottlieb, 1966) (Yeast extract 4 g, Malt extract 10 g, Dextrose 4 g, Agar 20 g, DW 1000 mL, Sea water 50 %/ L), Potato dextrose agar medium (Potato 200 g, Dextrose 20 g, Agar

20 g, DW 1000 mL), Modified nutrient agar medium (Glucose 5 g, Peptone 5 g, Beef extract 3 g, NaCl 5 g, Agar 15 g, DW 1000 mL) and Starch casein agar medium (Wellington and Cross, 1983) (Starch 10 g, Casein powder 1 g, Agar 15 g, DW 1000 mL, Sea water 50 %/ L).

Morphological Characteristics and Staining Reactions of isolated actinomycetes

Gram staining

A drop of sterile distilled water was place on clear grease-free slide and a small loop of isolated actinomycetes was smeared on the slide and allows it to dry. The smear was forced by passing dried slide 3 or 4 times rapidly over a flame. The slide was covered with crystal violet strain and allow it to act for 30-60 seconds. Then, the slide was rinsed with distilled for a few seconds. The slide was covered with fresh iodine solution and allowed it to act for about 30 -60 seconds. The alcohol drop was added until no more color flows out from the smear for 10 -20 seconds and washed with distilled water. Then the slide was air dry. The stained slide was examined under the oil immersion objective of the microscope.

Screening for antimicrobial activities (NITE, 2005)

The isolated actinomycetes were grown on ISP-2 medium at room temperature for 7 days. After incubation period, these strains inoculated into the fermentation medium (glycerol 2 g, peptone 5 g, yeast extract 3 g, malt extract 3 g, $CaCO_3$ 2.5 g, DW 1000 mL) the seed medium (glucose 1 g, starch 1 g, peptone 0.75 g, meet extract 0.75 g, NaCl 0.3 g, DW 1000 mL) for 3 days at room temperature. After three days, the seed medium (3%) was transferred into the fermentation medium (glycerol 2 g, peptone 5 g, yeast extract 3 g, malt extract 3 g, $CaCO_3$ 2.5 g, DW 1000 mL) and carried out for 3-10 days and evaluated the antimicrobial activity by agar well diffusion method.

Screening of antimicrobial activity by agar well method (Collins, 1965)

One day old culture test broth (0.2 mL) was added to 25 mL warm assay medium (glucose 10 g, peptone 3 g, KNO_3 1 g, DW 1000 mL, agar 18 g) and thoroughly mixed and poured into plate. After solidification, the agar was left to set. Cork borer was used to make the wells (8 mm in diameter). Then, the fermented broth (20 µL) was carefully added into the well 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.

Test organisms

Agrobacterium tumefaciens NITE 09678, *Aspergillus paraciticus* IFO 5123, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542, *Micrococcus luteus* NITE 83297, *Salmonella typhi* AHU 7943, *Escherichia coli* AHU 5436, *Pseudomonas fluorescens* IFO 94307, *Staphylococcus aureus* AHU 8465 and *Saccharomyces cerevisiae* NITE 52847 were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan).

Scope for isolation and identification of marine actinomycetes

Morphological and biochemical characteristics of selected strain TR-2

Morphological

- Gram staining
- Cell morphology

Biochemical tests

- Sugar fermentation of carbohydrate test (Cowan, 1975)
- Catalase test (Salle, 1948)
- Oxidase test (Dubey, 2002)
- Hanging slied test (Dubey and Maheshwari, 2002)
- Urea hydrolysis test (Christenson, 1946)
- Nitrate reduction test (Harrigan and Mc Cance, 1966)
- Mannitol sal**t** broth test (Prescott 2002)
- Methyl red test (Bisen and Verma, 1998)
- Citrate test (Atlas, 1993)
- Hydrogen sulfide Test (Cowan, 1975)
- Voges proskaucer test (Cruickshank, 1963)
- Soluble starch hydrolysis (Pelezar and Chan, 1972)
- Rice powder hydrolysis (Pelezar and Chan, 1972)
- Wheat powder hydrolysis (Pelezar and Chan, 1972)
- Tapioca powder (Pelezar and Chan, 1972)
- Sticky rice powder (Pelezar and Chan, 1972)
- Arginine hydrolysis (Dubey and Maheshwari, 2002)
- Gelatin hydrolysis test (Dubey and Maheshwari, 2002)
- Casein hydrolysis test (Aneja, 1996)
- Esterace hydrolysis test (Prescott, 2002)
- Melanin production test (Shirling and Gottlieb, 1966)
- Potato slice test (Atlas, 1993)
- Salt tolerance test (Atlas, 1993)

Cover slip insertion method (William *et al.*, 1989)

Adequate magnification used to establish the presence or absence of spore chains and to observe with the magnification. By the standard protocol of cover slip culture technique, the plates were prepared and after the incubation of 7 to 10 days it was observed. During this method of spore morphological study, ISP 2 medium plates were prepared. After solidification, by a sharp scalpel from the central portion of the plate, medium should be scooped out making a rectangular area. Then, three sterile cover slips were placed on the hollow rectangular space. Slowly Actinomycetes spores have to be inoculated at the edge of the cover slips touching the medium. The plates must be incubated at 28 ± 2ºC for 5 days and examined periodiacally taking out the cover slips.

Results

A total of 13 strains such as TR-1 to 13, were isolated from sea water and sludge samples collected from Chaung-Tha. All 13 strains had shown white, greenish blue and brownish green color. The form, elevation and margin of these strains were circular, raised and entire, rough in texture, small, moderate and large in colony size. The aerial mass colour of all strains was greenish blue, white and centre greenish blue, white in periphery. After six days, the aerial mass colour of strains TR-4 and TR-8 white that turned into brown and white in colour of TR-12 turned into blue after five days. The spore chain of all strains were straight, flexous, rectiflexibiles, single conidia, ovoid, open spiral, spirals and fragmenting branched aerial hyphae. The spores of isolated strains were globose, ovoid and polytrichous.

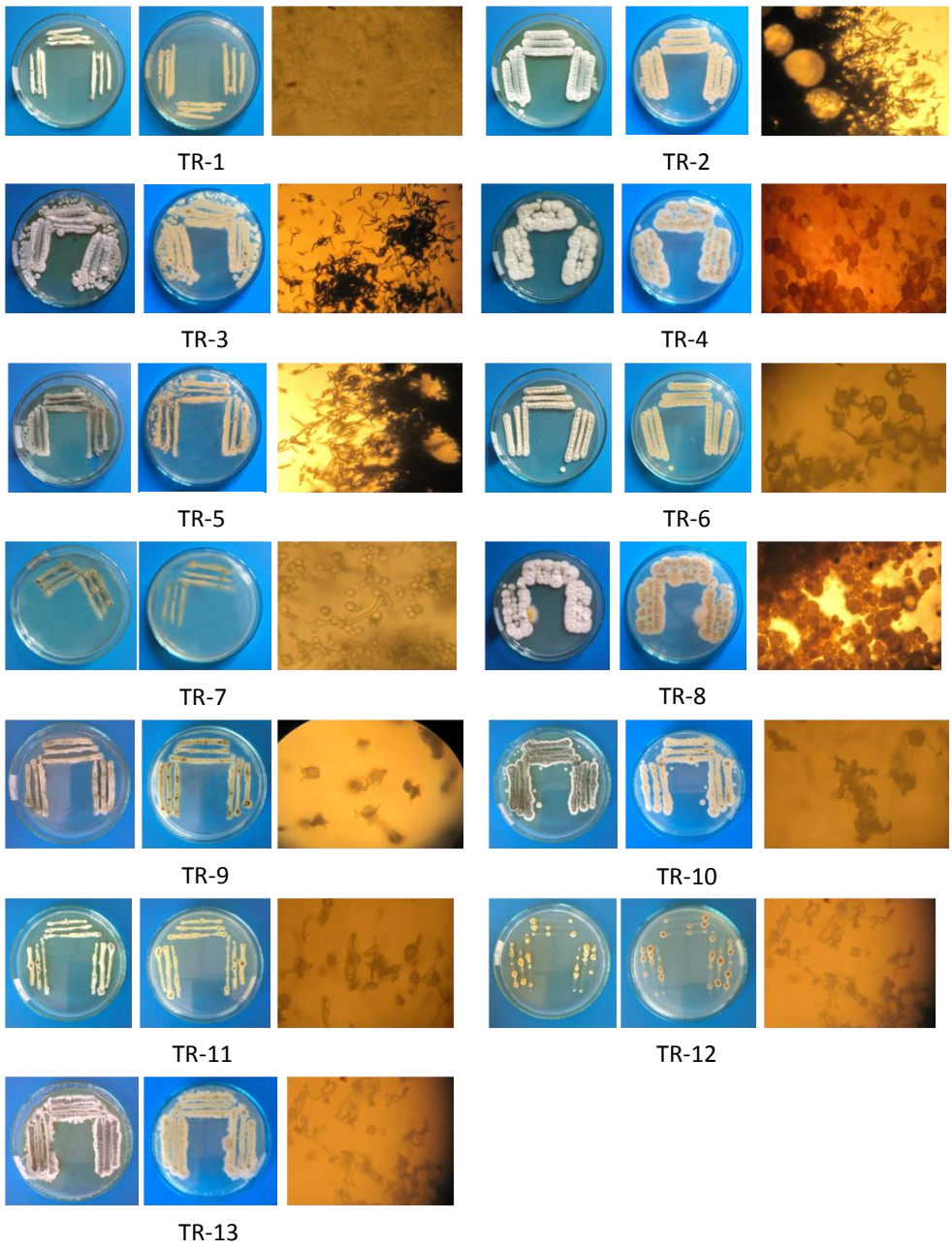


Figure 1 Colony morphology and Cell shape of isolated strains TR 1-13

Colony morphology

All 13 strains had shown white, greenish blue and brownish green color. In these strains, TR-2, 5 and 10 had water drop. In elevation, TR-1-3, 6-11 were raised, flat in TR-12 and 13, TR-4 was convex and TR-5 was umbonate. TR-1, 2, 4 and 8 were large, TR-3, 5, 9-13 were small and TR-6 and 7 were moderate in colony size. In form and texture, all strains were circular and rough as shown in Table 1.

Table 1 Morphological characters of isolated actinomycetes

No	Isolated strains	Form	Elevation	Magin	Pigment		Colony size	Texture
					Front colour	Reverse colour		
1.	TR-1	Circular	Raised	Entire	Greenish blue	Yellow	Large	Rough
2.	TR-2	Circular	Raised	Entire	Centre greenish blue, white in periphery (Water drop present)	Yellow	Large	Rough
3.	TR-3	Circular	Raised	Entire	Centre greenish blue, white in periphery	Yellow	Small	Rough
4.	TR-4	Circular	Raised	Entire	White- Brown After 6 days	Yellow-Red	Large	Rough
5.	TR-5	Circular	Raised	Entire	Greenish blue (Water drop present)	Yellow	Small	Rough
6.	TR-6	Circular	Raised	Entire	Centre brownish green, white in periphery	Brown	Moderate	Rough
7.	TR-7	Circular	Raised	Entire	Greenish blue	Orange	Moderate	Rough
8.	TR-8	Circular	Raised	Entire	White- Brown After 6 days	Green-Red After 5 days	Large	Rough
9.	TR-9	Circular	Raised	Entire	Centre greenish blue, white in periphery	Brown	Small	Rough
10.	TR-10	Circular	Raised	Entire	Centre white, dark green in periphery	Yellow	Small	Rough
11.	TR-11	Circular	Raised	Entire	Centre greenish blue, white in periphery	Yellow-Orange After 5 days	Small	Rough
12.	TR-12	Circular	Raised	Entire	White- Blue After 5 days	Orange	Small	Rough
13.	TR-13	Circular	Raised	Entire	Centre greenish blue, white in periphery	Yellow	Small	Rough

Small < 2 mm (diameter)
Medium between 2 mm & 5 mm (diameter)
Large > 5 mm (diameter)

Spore chain morphology

In the spore surface morphology, TR- 1, 2, 3, 7 & TR-11-13 were globose, ovoid in TR-4, 5, & 8, polytrichous in two strains (TR- 6 & 9) and peritrichous in TR-10. TR-2, 3, 5 & 11 were flexous and TR- 10, 12 & 13 were open spiral and spirals in the spore chain. Another isolates were straight (TR-1), fragmenting branched aerial hyphae (TR-4), rectiflexibiles (TR-6 & 9), single conidia (TR-7) and ovoid (spore production with sporangia) in TR- 8 as shown in Table 2.

Table 2 Morphologies of Spores chains and spores features of isolated actinomycetes

No	Isolated strains	Spore Chain	Morphological feature of spores
1.	TR- 1	Straight	Globose
2.	TR- 2	Flexous	Globose
3.	TR- 3	Flexous	Globose
4.	TR- 4	Fragmentating branched aerial hyphae	Ovoid
5.	TR-5	Flexous	Ovoid
6.	TR- 6	Rectiflexibiles	Ovoid
7.	TR- 7	Single conidia	Globose
8.	TR- 8	Ovoid (Spore production with sporangia)	Ovoid
9.	TR- 9	Rectiflexibiles	Polytrichous
10.	TR- 10	Open spiral	Peritrichous
11.	TR- 11	Flexous	Globose
12.	TR- 12	Spirals	Globose
13.	TR- 13	Spirals	Globose

Antimicrobial activities of isolated actinomycetes strains

All strains were tested for antimicrobial activities with ten test organisms. Among them, the strain TR-2 was selected for further investigation according to the results of maximum inhibition against *Candida albicans*,NITE 09542 than the other.

Table 3 Antifungal activities of selected strain TR-2 on *C. albicans*

Fermentation period (day)	Antifungal activitiy (mm) and Test organisms									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	-	14.02	18.35	21.01	-	-	-	21.08	20.10	18.32
4	-	17.45	19.23	**24.52**	-	-	-	23.05	21.54	20.56
5	-	16.08	19.18	21.43	-	-	-	20.87	20.01	20.31
6	-	16.00	18.56	21.14	-	-	-	20.35	19.21	19.21
7	-	15.32	17.42	18.27	-	-	-	20.22	19.05	18.05

1. *Agrobacterium tumefaciens*
2. *Aspergillus paraciticus*
3. *Bacillus subtilis*
4. *Candida albicans*
5. *Micrococcus luteus*
6. *Salmonella typhi*,
7. *Escherichia coli*,
8. *Pseudomonas fluorescens*,
9. *Staphylococcus aureus*
10. *Saccharomyces cerevisiae*

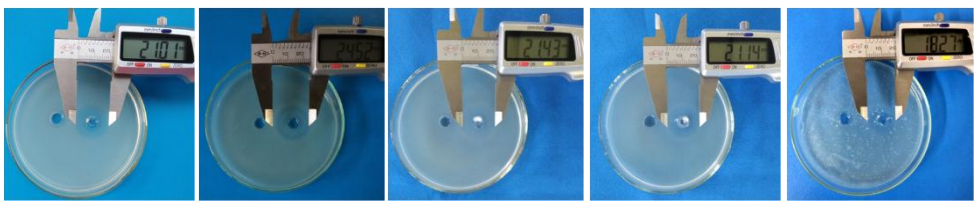


Figure 2 Antifungal activity of isolated strain TR-2 against *Candida albicans*

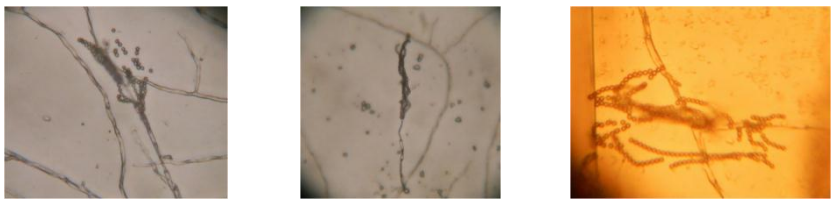
Table 4 Biochemical tests of selected strain TR-2

No.	Test	Result
1	Cell morphology	Slender hyphae spore chain, straight and long
2	Gram staining reaction	Gram positive
3	Catalase test	+
4	Oxidase test	-
5	Motility test	-

(+) positive (-) negative

Front view Reverse view Single colony of selected strain TR-2

Figure 3 Colony morphology and cell shape of selected strain TR-2



Glabrous spore Rectiflexibiles type Gram staining (+)

Figure 4 Microscopical characters of selected strain TR-2

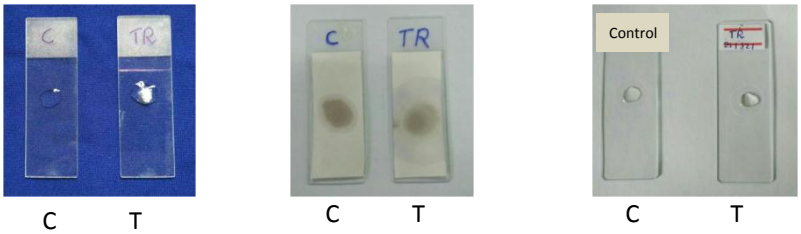


Figure 5 Biochemical tests for selected strain TR-2 **(A)** Catalase test **(B)** Oxidase test **(C)** Motility test (Hanging slide)

Table 5 Colony morphology of TR-2 on three different culture media

Cultural media	Surface color (Aerial mycelium)	Reverse color (Substrate
ISP-2 (yeast-malt extract agar)	White changed into Blue	Yellow
ISP-5 (Glycerol Asparagine	Greenish	Yellow
ISP-6 (Peptone Iron medium)	White	Yellowish

Figure 6 Colony morphology of TR-2

Biochemical characterization of selected strain TR-2

Acid was produced in glucose, galactose, maltose, sucrose, fructose, xylose, lactose and destrose and gas not produced.

Positive results were found in methyl red test, citrate utilization test, casein test, mannitol salt broth test, potato plug test, nitrate reduction, catalase and urease test. But, VP test, hydrogen sulfide test, gelatin hydrolysis test, arginine test, esterase activity test, oxidase test and motility test were negative. In salt tolerance test, the optimum growth of the strain TR-2 was observed at 6% NaCl.

Table 6 Sugar fermentation of selected strain TR-2

No.	Various sugar	Response	Acid
1	Glucose	Yellow colour change in	+
2	Galactose	Yellow colour change in	+
3	Maltose	Yellow colour change in	+
4	Sucrose	Yellow colour change in	+
5	Fructose	Yellow colour change in	+
6	Xylose	Yellow colour change in	+
7	Arabinose	No change in colour	-
8	Lactose	Yellow colour change in	+

+ = positive - = negative

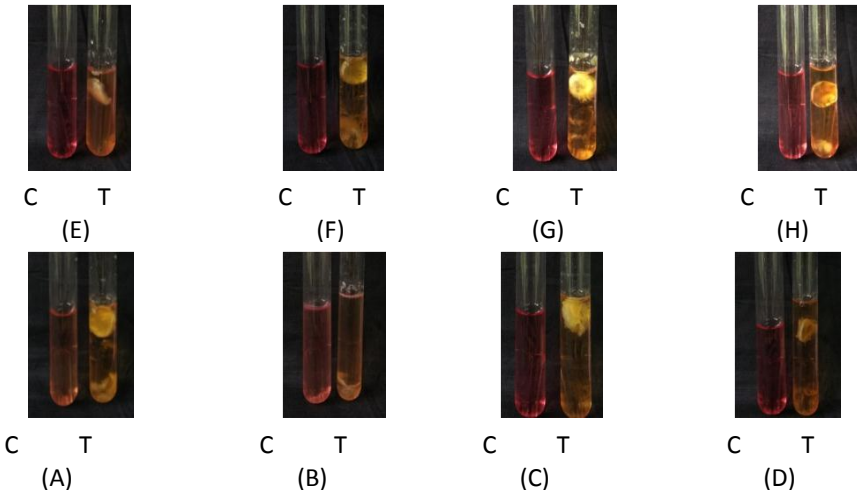


Figure 7 Sugar fermentation of selected strain TR-2 **(A)** Glucose **(B)** Fructose **(C)** Maltose **(D)** Galactose **(E)** Lactose **(F)** Dextrose **(G)** Sucrose **(H)** Xylose

Table 7 Biochemical tests for selected strain (TR-2)

No.	Reaction	Response	Result
1	Citrate utilization test	The colour of medium changes from green to blue	Positive
2	Methyl red test	No colour change from methyl red to yellow	Positive
3	Voges Proskaucer	Test no colour change medium	Negative

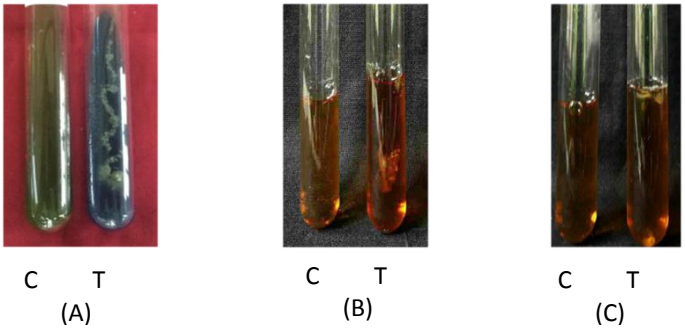


Figure 8 Biochemical test for selected strain TR-2 **(A)** Citrate utilization test **(B)** Methyl red (MR) test **(C)** Voges proskaucer (VP) test

Sodium chloride tolerance test

The selected strain TR-2 can grow well in the sodium chloride (2%, 4%, 6% and 8%) except 10% NaCl.

Table 8 Sodium chloride tolerance test of TR-2

Sodium chloride (%)	Result
NaCl 2%	++
NaCl 4%	++
NaCl 6%	++
NaCl 8%	++
NaCl 10%	+

\+ poor growth ++ moderate growth

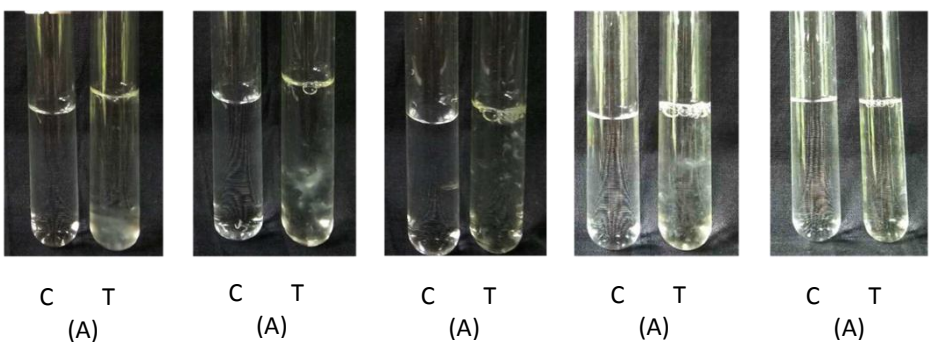


Figure 9 NaCl tolerance test of selected strain TR-2 **(A)** 2% **(B)** 4% **(C)** 6% **(D)** 8% **(E)** 10%

Table 9 Biochemical tests for selected strain TR-2

Sr. No.	Reaction	Response	Result
1	Mannitol salt broth	The colour of medium changed from yellow to pink	Positive
2	Urea hydrolysis	Colour changed from yellow to deep pink	Positive
3	H_2S production	No black colour change in the medium	Negative

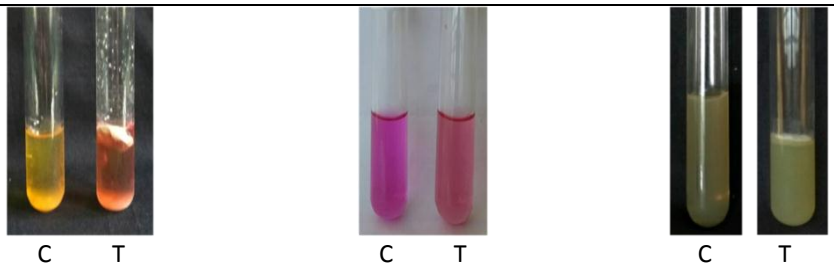


Figure 10 Biochemical test for selected strain TR-2 **(A)** Mannitol salt broth **(B)** Urea hydrolysis **(C)** H_2S production

Table 10 Starch hydrolysis test for selected strain TR-2

No.	Sources of strach	Result
1	Soluble starch	++
2	Wheat flour	++
3	Tapioca powder	+
4	Sticky rice	++
5	Rice powder	++

++ maximum hydrolysis
+ minimum hydrolysis

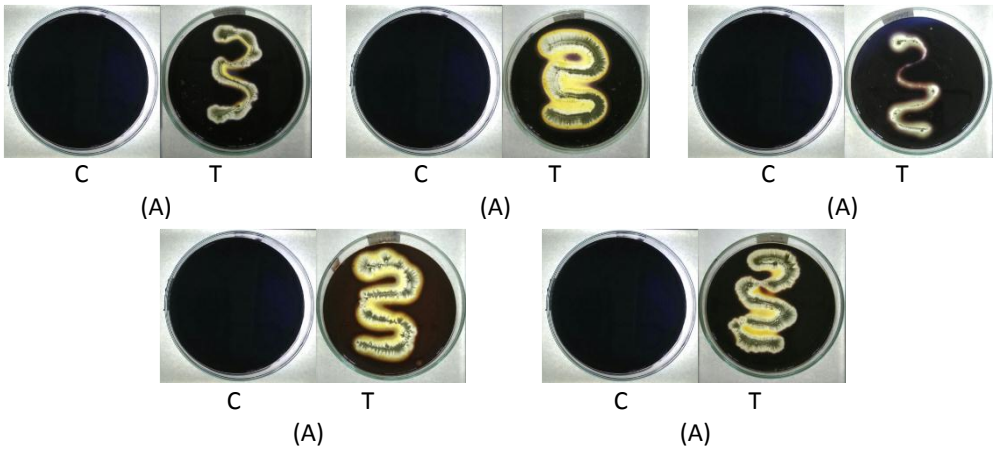


Figure 11 Starch hydrolysis test for selected strain TR-2 **(A)** Soluble starch **(B)** Wheat flour **(C)** Tapioca **(D)** Sticky rice **(E)** Rice powder

Table 11 Biochemical tests for selected strain TR-2

Sr. No.	Reaction	Response	Result
1	Casein test	Clear zone is found around the growth zone	Positive
2	Potato slice test	Growth on the streak line of potato	Positive

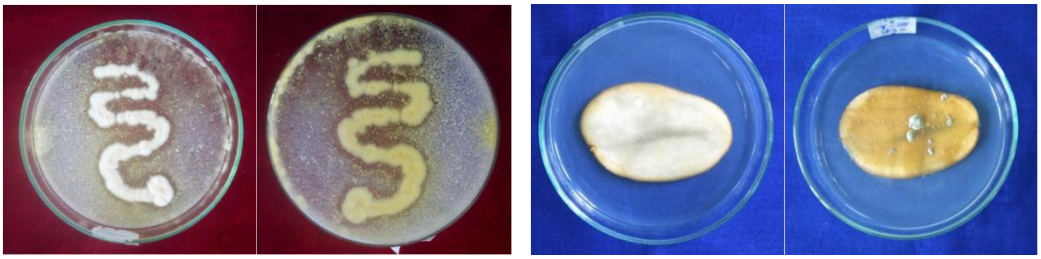


Figure 12 Biochemical test for selected strain TR-2 **(A)** Casein test **(B)** Potato slice test

Discussion and Conclusion

During the study of the isolation of actinomycetes, two samples were carried out by serial dilution method. Of these 13 strains, five strains were got from sea water sample and eight strains from sludge sample. Six different media were utilized only one strain was got from Kuster's agar medium and twelve strains were collected from (ISP-2) medium. Gil, *et al.*, 2009 suggested that nutrient availability is one of the main factors that determine the growth of actinomycetes. Most actinomycetes can use wide variety of compounds such as glucose, starch, proteins and amino acids as their energy source; unlike other bacterial groups that only favour simple carbon and nitrogen compound. Therefore, ISP 2 medium was effective for the isolation of actinomycetes among the other media.

In the identification of TR-2, colony morphology, spore chain and shape, cultural characters and biochemical characteristics were studied. Spore chains were straight and long, rectiflexbiles type, spore wall glabrous. Waksman and Henrici, 1943 described that the streptomyces were spores chain straight and long, rectiflexibiles type, retiaculiaperti type, spira type, spore wall glabrous, hairy. These results were the same of TR-2. Carbohydrate utilization properties are one of the important biochemical activities of microorganisms to identify and classify them (Dielz, 1988 and Holt, *et al.*, 2000). The selected strain TR-2 produced acid from carbohydrate such as glucose, galactose, maltose, sucrose, fructose, xylose, lactose and dextrose. Al-saadi, *et al.*, 2013, suggested that the actinomycetes were positive for citrate and starch hydrolysis test. Similarly, TR-2 was also positive for citrate and starch hydrolysis test. Methyl red (MR) test were positive and Voges Proskaucer (VP) test negative, casein and urea hydrolysis positive, mannitol salt broth positive. TR-2 can grow in NaCl salt (2% to 10%) and potato slice.

These results were similar to the previous research of Waksman and Henrici 1943, in the Bergey's Manual of Determinative Bacteriology and Selman and Waksman (Volume I and II of the Actinomycetes). Based on the obtained results, selected strain TR-2 was classified as the

possible genus *Streptomyces* sp. Streptomycetes has been exploited to produce a wide range of antibiotics. But many Streptomyces species also produce pigments. Actinotiodin is a biological pigment produced by *Streptomyces*. It can be applied as an antibiotic compound against Gram-positive bacteria and also as an indicator compound in laboratory agents.

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 and Dr Nilar Myint, Pro-Rectors, Pathein University, for their valuable instruction and guidance. I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, Pathein University and Professor Dr Wah Wah Lwin, 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, Pathein University, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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COMPARATIVE POLLEN MORPHOLOGY OF TEN *GREWIA* SPECIES

Ohmar Htwe[1], Soe Myint Aye[2], Tin Tin Khaing[3]

Abstract

Pollen morphology of 10 species belonging to the genus *Grewia* of subfamily Tilioideae were studied. All the specimens were collected from Mandalay and Magway Regions during 2018. Plant identification was carried out and pollen grains were studied. Pollen grains of all investigated species were monad. The aperture types of pollen grains of all species were found as colporate, except *Grewia villosa* Willd which is colpate. The shapes of pollen grains were found as subprolate in *Grewia columaris* Smith, *Grewia parviflora* (Burge) Handen-Mazzetti, and *Grewia villosa* Willd and the remaining seven species were prolate. The sizes of pollen grains were medium and large. Among the studied species, the smallest pollen grains was found in *Grewia columaris* Smith and the largest pollen grains was in *Grewia laevigata Vahl.*ex Juss. The taxonomic descriptions of each species were described with their inflorescences. The polar view and equatorial view of the pollen for each species were presented. Moreover, the comparative pollen morphological characters were presented in table.

Keywords: pollen morphology, genus *Grewia*, acetolysis technique, key to identification

Introduction

Palynology is the study of the pollen grains and spores of plants. Spores and pollen grains have a number of morphological and ultrastructural features. These palynological features have been important in inferring phylogenetic relationships of plants. In addition, the features of spores and pollen grains can often be used to identifiy a particular plant taxon. For this reason, palynological studies are used extensively to examine the fossil record, a field called paleo-palynology. It is also applicable in genetic study, forensic science in tracing history of vegetation, which consists of individual species, community and climate change study. It is also used in the field agriculture, forestry, archaeology and plant geography (Aftab & Perveen 2006).

Malvaceae are found throughout the world, growing in many different environments and climates. Malvaceae had been divided into four subfamilies: Sterculioideae, Tilioideae, Malvoideae and Bombacoideae. Genus *Grewia* consists of 90 species distributed in tropical and subtropical regions. In Myanmar, 24 species of genus *Grewia* of Tilioideae were presented in Checklist of Myanmar (2003).

The genus *Grewia* consists of about 280-300 species of trees, shrubs or climbers, distributed from Madagascar, tropical Africa northwards and southeastwards to the Himalayas, China and Taiwan, India, Sri Lanka, Myanmar, Thailand, Indo-China, Malesia, Western Pacific and the northern parts of Australia (Chung *et al*. 2003).

People always depend on plants for their various needs. The fruits of *Grewia* species are edible, tasty and loved by birds. This species make a good screen for the forest, being very attractive with its green leaves and bright yellow flowers. The pollen morphology of *Grewia* species will still lacking to be studied in Myanmar. So, some species of genus *Grewia* will focus to be studied.

[1] Assistant Lecturer, Department of Botany, Pakokku University
[2] Pro-rector, Department of Botany, Myitkyina University
[3] Associate Professor, Department of Botany, Pakokku University

The aim and objectives of the genus *Grewia* is to identify and classify the *Grewia* species, to attain the morphological variation in the pollen of genus *Grewia*, to accomplish the taxonomic value and to contribute to a better understanding of palynological characters of these members.

Materials and Methods

(A) Data Collection and Plant Identification

The specimens were collected from Mandalay and Magway Regions during 2018. The photographic records were taken during flowering time and field noted were taken specifically. Identification of collected specimens was carried out by comparing to key and description referring to floristic literature: Hooker (1878), Dassanayake (1991), Flora of China Vol.12 (2008). All the fresh pollen were collected from the anthers of mature flowers. The collected pollens of each species was stored in glass vial containing 1cc of glacial acetic acid and then labeled.

(B) Acetolysis of Materials

The pollen samples were acetolysed by Erdtman method (1960). The pollen samples in a glass vials were put into a test tube, then crushed with a glass rod. Acetolysis solution was mixed by 9 part of glacial acetic acid and 1 part of concentrated sulphuric acid. 1cc of acetolysis mixture was poured into the test tube containing the pollen sample and stirred with a glass rod. The test tube was heated in a water bath 70℃-80℃ for 25-30 minutes. The test tube was allowed to cool, and the sample diluted with distilled water and centrifuged for 20-30 minutes at 3000 rpm. This was repeated twice decanting the water each time. Dilute glycerine solution was added to the residue, then transferred and stored in air tight glass vial and labeled.

Results

1. *Grewia acuminata* Jussieu, Ann. Mus. Natl. Hist. Nat. 4:91.1804. (Figure 1 A)

Myanmar name : Thayaw

English name : Unknown

Flowering period : July to September

Perennial shrubs; stems and branches terete, coarsely stellate. Leaves simple, alternate; stipules linear, densely pubescent; blades linear-oblong, cordate at the base, serrate along the margin, acuminate at the apex, 3 basal nerved, dark green above with stellate tomentose, pale green beneath with densely stellate tomentose. Inflorescences axillary cymes, 2- to 3-flowered per leaf axil; stellate tomentose. Flowers bisexual, actinomorphic, pentamerous, hypogynous, creamy white; bracts linear, caducous. Sepals 5, free, ovate oblong, stellate tomentose without, glabrous within, persistent. Petals 5, ovate, creamy white, glabrous. Stamens numerous, free; filaments filiform; anthers dithecous, basifixed, yellow, dehiscing by longitudinal slit. Carpels 2, ovary superior, oblongoid, bilocular, two ovules in each locule on the axile placentae; styles slender, stigma 2-lobed, glabrous. Fruits drupaceous, subglobose, tetragonous, stellate tomentose.

Pollen morphology (Figure 1 B, C)

Tricolporate, prolate, medium, 38 – 39 × 28 – 29 µm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 31.2 – 33.7 × 2.5 – 3.5 µm in length and breadth; pori

lolongate, 7 – 8 × 5 – 6 µm in length and breadth; exine 1.8 – 2.5 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrocate, 5.3 – 6.2 µm width; muri simplibaculate, 0.3-0.5 µm wide.

2. *Grewia columaris* Smith in Reces, Cyclop. 17. 1811. (Figure 1 D)

Myanmar name : Unknown

English name : Unknown

Flowering period : July to August

Perennial shrubs; stems and branches terete, stellate tomentose. Leaves simple, alternate; stipules subulate, densely pubescent; blades elliptic ovate, cordate at the base, serrate along the margin, acuminate at the apex, 3 basal nerved, dark green above with stellate tomentose, pale green beneath with densely stellate tomentose. Inflorescences axillary cymes, 1- to 2-flowered per leaf axil; stellate tomentose. Flowers bisexual, actinomorphic, pentamerous, hypogynous, creamy white; bracts ovate to narrowly linear, caducous, densely stellate tomentose. Sepals 5, free, ovate oblong, recurved, stellate tomentose without, glabrous within, persistent. Petals 5, ovate, glabrous. Stamens numerous, free; filaments free, unequal, pale yellow, glabrous; anthers dithecous, basifixed, yellow, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, bilocular, two ovules in each locule on the axile placentae; styles slender; stigma dilated, glabrous. Fruits drupaceous, subglobose, tetragonous, stellate tomentose.

Pollen morphology (Figure 1 E, F)

Tricolporate, subprolate, medium, 37.5 – 38.7 × 31.2 – 32.5 µm in length and breadth; amb rouded triangular; colpi ¾ way up to the pole, 29.0 – 30.2 × 5.0 – 6.2 µm in length and breadth; pori lolongate, 7.5 – 8.2 × 5.0 – 6.0 µm in length and breadth; exine 3.7 – 5.0µm thick, sexine as thick as nexine; sculpturing reticulate; lumina heterobrocate, 2.8 – 3.5 µm width; muri simplibaculate, 1.0 – 1.5 µm wide.

3. *Grewia damine* Gaertn., Fruct. 12:113.t.106.f. 7. 1790. (Figure 1 G)

Myanmar name : Unknown

English name : Unknown

Flowering period : April to May

Perennial small tree; stems and branches terete, stellate pubescent when young. Leaves simple, alternate; stipules linear lanceolate; densely pubescent; blades elliptic ovate, cordate at the base, serrate along the margin, acute at the apex, 3 basal nerved, glabrous above, finely tomentose below, base slightly oblique. Inflorescences axillary cymes, many-flowered per leaf axil; stellate tomentose. Flowers bisexual, actinomorphic, pentamerous, hypogynous, creamy white; bracts ovate to narrowly linear, caducous, densely stellate tomentose. Sepals 5, free, ovate oblong, recurved, stellate tomentose without, glabrous within, persistent. Petals 5, ovate, glabrous. Stamens numerous, free; filaments free, unequal, pale yellow, glabrous; anthers dithecous, basifixed, yellow, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, densely appressed villous, bilocular, two ovules in each locule on the axile placentae; styles slender, densely hairs; stigma discoid, glabrous. Fruits depressed globose, 2-lobed when dry, black with brown dots when dry, almost glabrous when mature, stellate tomentose.

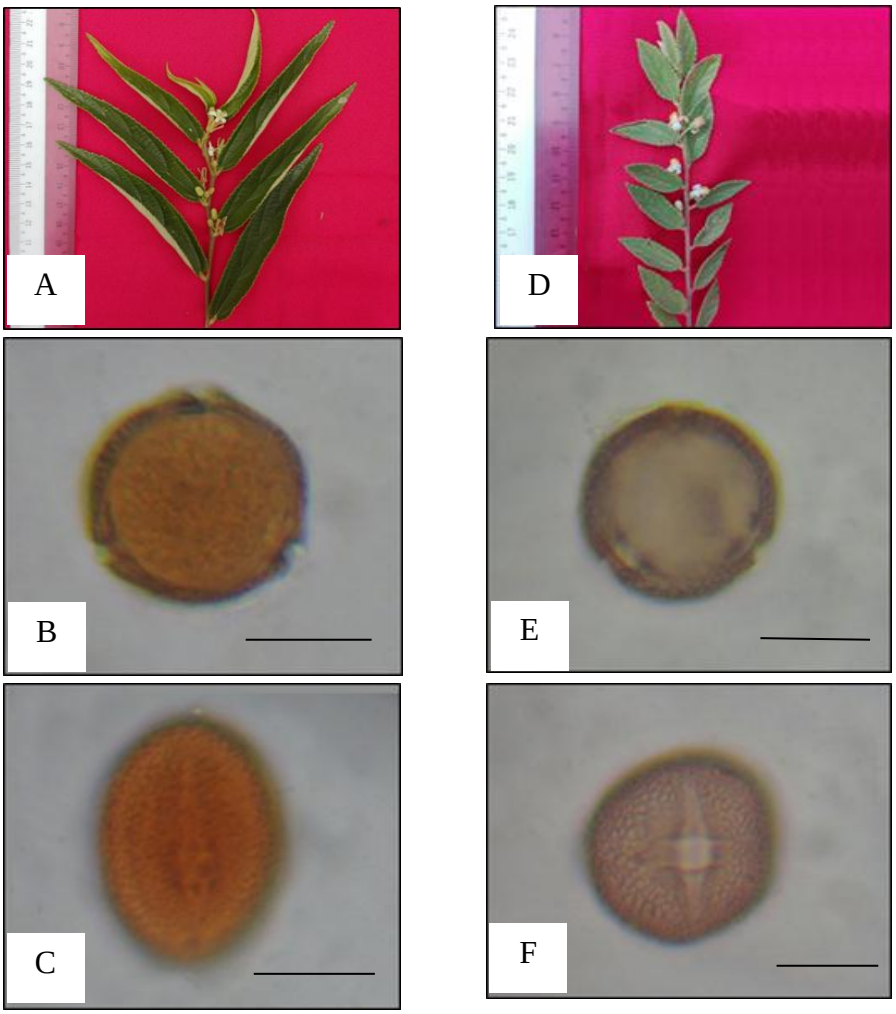


Figure 1 A. Inflorescences of *Grewia acuminata* Juss.
B. Polar view pollen of *G. acuminata* Juss.
C. Equatorial view pollen of *G. acuminata* Juss.
D. Inflorescences of *Grewia columaris* Smith.
E. Polar view pollen of *G. columaris* Smith.
F. Equatorial view pollen of *G. columaris* Smith.

Pollen morphology (Figure 1 H, I)

Tricolporate, prolate, medium, 45.2 – 46.2 × 28.7 – 31.2 µm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 40.2 – 41.2 × 1.2 – 1.8 µm in length and breadth; pori lolongate, 8.7 – 10.0 × 6.5 – 7.5 µm in length and breadth; exine 1.8 – 2.5 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrocate, 3.5 – 4.0 µm width; muri simplibaculate, 0.3 – 0.4 µm wide.

4. *Grewia flava* DC., Cat. Pl. Horti Monsp. 113. 1813. (Figure 1 J)

Myanmar name : Unknown

English name : Unknown

Flowering period : May to June

Perennial multi-stemmed shrubs; stems and branches terete, stellate. Leaves simple, alternate, often upright; stipules caducous; densely pubescent; blades oblanceolate to obovate, cuneate at the base, serrate along the margin, obtuse at the apex, 3 basal nerved, base symmetric, densely tomentose. Inflorescences axillary cymes, solitary or in few flowered per leaf axil; stellate tomentose. Flowers bisexual, actinomorphic, pentamerous, hypogynous, creamy white; bracts ovate to narrowly linear, caducous, densely stellate tomentose. Sepals 5, free, linear lanceolate, recurved, stellate tomentose without, glabrous within, persistent. Petals 5, ovate, glabrous. Stamens numerous, free; filaments free, unequal, pale yellow, glabrous; anthers unequal, dithecous, basifixed, yellow, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, bilocular, two ovules in each locule on the axile placentae; styles slender, densely hairs; stigma lobed, glabrous. Fruits depressed globose, 2-lobed when dry, reddish brown when ripe, glabrescent.

Pollen morphology (Figure 1 K, L)

Tricolporate, prolate, medium, 48 – 48 × 35 – 36 µm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 38.7 – 39.8 × 4.0 – 5.0 µm in length and breadth; pori lalongate, 2.0 – 2.5 × 3.0 – 3.7 µm in length and breadth; exine 2.5 – 3.7 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrocate, 1.5 – 1.8 µm width; muri simplibaculate, 0.3 – 0.4 µm wide.

5. *Grewia laevigata* Vahl., Symb. Bot. 1:34. 1790. (Figure 1 M)

Myanmar name : Kyet tayaw, Tayaw nyo, Yaw

English name : Unknown

Flowering period : July to September

Perennial trees; stems and branches terete, glabrous. Leaves simple, alternate; stipules linear subulate, caducous; pubescent; blades oblong, cuneate at the base, serrate along the margin, sharply acuminate at the apex, 3 basal nerved, glabrous, base symmetric, dark green above, pale green beneath, densely tomentose. Inflorescences axillary cymes, solitary or 2- to 3-flowered per leaf axil; stellate tomentose. Flowers bisexual, actinomorphic, pentamerous, hypogynous, creamy white; bracts linear subulate, caducous, stellate tomentose. Sepals 5, free, linear lanceolate, stellate tomentose without, glabrous within, persistent. Petals 5, oblong, glabrous. Stamens numerous, free; filaments free, unequal, pale yellow, glabrous; anthers dithecous, basifixed, yellow, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, tetralocular, two ovules in each locule on the axile placentae; styles slender, densely hairs; stigma lobes, glabrous. Fruits drupaceous, globose, 1- to 4-lobed when dry, black when ripe.

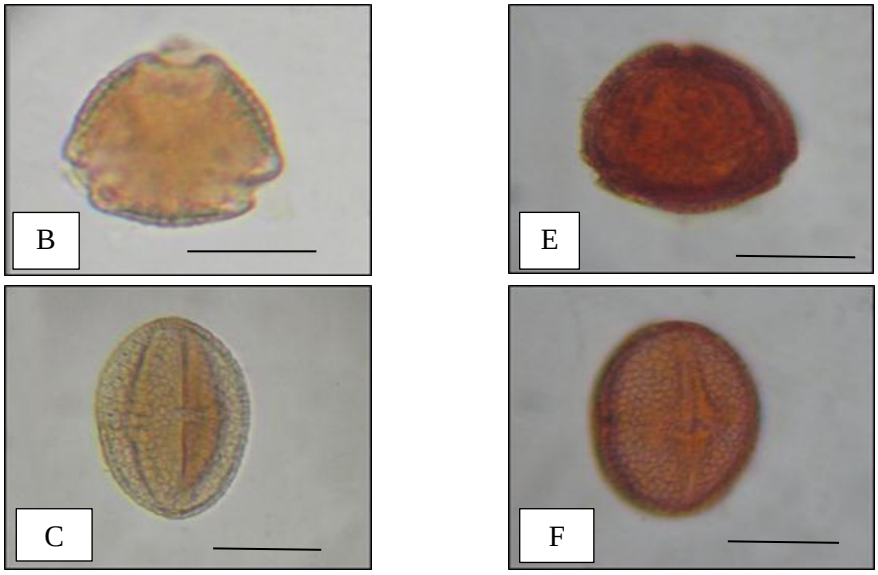


Figure 2 A. Inflorescences of *Grewia diamine* Gaertn.
B. Polar view pollen of *G. diamine* Gaertn.
C. Equatorial view pollen of *G. diamine* Gaertn.
D. Inflorescences of *Grewia flava* DC.
E. Polar view pollen of *G. flava* DC.
F. Equatorial view pollen of *G. flava* DC.

Pollen morphology (Figure 1 N, O)

Tricolporate, prolate, large, 66.2 – 67.5 µm × 49.2 – 50.2 µm in length and breadth; amb circular; colpi ¾ way up to the pole, 52.0 – 56.5 × 2.0 – 2.5 µm in length and breadth; pori lolongate, 11 – 12 × 9 – 10 µm in length and breadth; exine 3.7 – 4.2 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate; lumina heterobrocate, 2.0 – 2.7 µm width; muri simplibaculate, 0.8 – 1.0 µm wide.

6. *Grewia parviflora* (Burge) Handen-Mazzetti, Symb. Sin 7: 612. 1933. (Figure 2 A)

Myanmar name : Unknown

English name : Unknown

Flowering period : May to July

Perennial small trees; stems and branches terete, glabrous. Leaves simple, alternate; stipules linear subulate; blades ovate orbicular, cuneate at the base, serrate along the margin, acute at the apex, 3 basal nerved, glabrous above, base symmetric, dark green above, pale below, tomentose. Inflorescences axillary cymes, 2- to 3-flowered per leaf axil; stellate tomentose. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white; bracts linear subulate, caducous, stellate tomentose. Sepals 5, free, linear lanceolate, recurved, stellate tomentose without, glabrous within, persistent. Petals 5, ovate, glabrous. Stamens numerous, free; filaments free, unequal, pale yellow, glabrous; anthers dithecous, basifixed, yellow, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, tetralocular, two ovules in each locule on the axile placentae; styles slender, densely hairy; stigma lobed, glabrous. Fruits drupaceous, globose, 2- to 4-lobed when dry.

Pollen morphology (Figure 2 B, C)

Tricolporate, subprolate, medium, 42.5 – 43.7 × 36.2 – 37.5 µm in length and breadth; amb circular; colpi longicolpate, 39.5 – 40.0 × 1.2 – 1.8 µm in length and breadth; pori lolongate, 9 – 10 × 7 – 8 µm in length and breadth; exine 2.5 – 3.0 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrocate, 1.8 – 2.3 µm width; muri simplibaculate, 0.8 – 0.9 µm wide.

7. *Grewia retusifolia* Pierre, Fl. Forest. Cochinch. t. 168. 1888. (Figure 2 D)

Myanmar name : Unknown

English name : Unknown

Flowering period : June to August

Perennial shrubs; stems and branches terete, stellate hairy. Leaves simple, alternate; stipules caducous; pubescent; blades linear ovate, oblique at the base, serrate along the margin, obtuse at the apex, 3 basal nerved, densely pubescent on both surfaces, pale below. Inflorescences axillary cymes, solitary or 2- to 3-flowered per leaf axil; peduncles very short, pale green, pubescent. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white; pedicel short; bracts small, caducous, pubescent. Sepals 5, free, linear lanceolate, white, pubescent, persistent. Petals 5, ovate, glabrous. Stamens numerous, free; filaments free, unequal, pale green, glabrous; anthers dithecous, basifixed, green, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, tetralocular, two ovules in each locule on the axile placentae; styles slender, densely hairs; stigma 4-lobes, glabrous. Fruits drupaceous, globose, 4 lobed when dry.

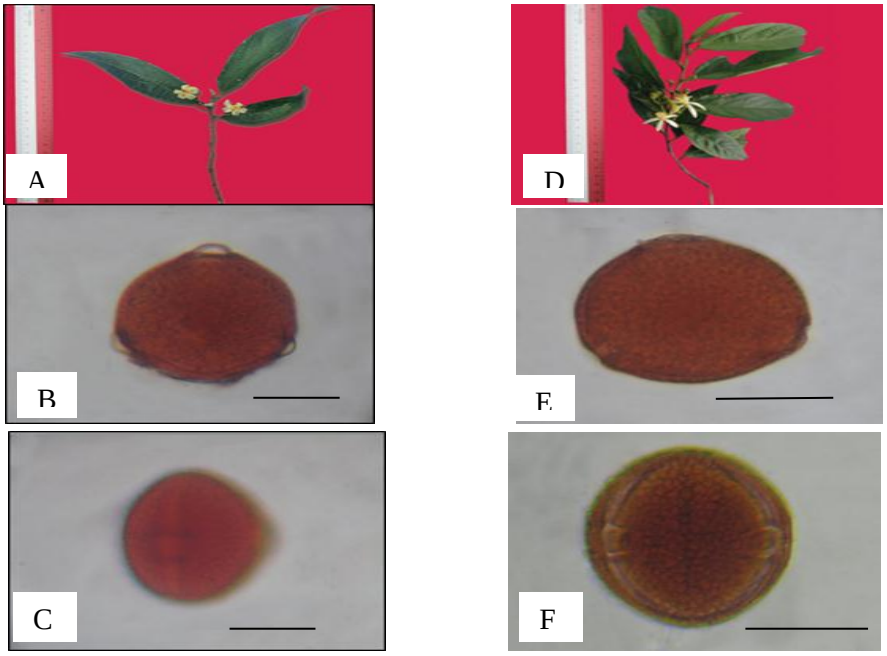


Figure 3 A. Inflorescences of *Grewia laevigata* Vahl.
B. Polar view pollen of *G. laevigata* Vahl.
C. Equatorial view pollen of *G. laevigata* Vahl.
D. Inflorescences of *Grewia parviflora* (Burge) Handen-Mazzetti.
E. Polar view pollen of *G. parviflora* (Burge) Handen-Mazzetti.
F. Equatorial view pollen of *G. parviflora* (Burge) Handen-Mazzetti.

Pollen morphology (Figure 2 E, F)

Tricolporate, prolate, medium, 41.2 – 42.3 × 30.0 – 31.2 µm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 31.2 – 32.5 × 2.5 – 3.7 µm in length and breadth; pori lolongate, 6.2 – 7.5 × 5.0 – 6.2 µm in length and breadth; exine 3.0 – 3.7 µm thick, sexine as thick as nexine; sculpturing reticulate; lumina heterobrocate, 0.7 – 0.8 µm width; muri simplibaculate, 0.2 – 0.4 µm wide.

8. *Grewia sapida* Roxb. ex DC., Prodr.1: 512. 1824. (Figure 2 G)

Myanmar name : Unknown

English name : Unknown

Flowering period : April to May

Perennial shrubs; stems and branches terete, stellate pubescent. Leaves simple, alternate; stipules linear lanceolate, small; pubescent; blades ovate to suborbicular, cuneateat the base, serrate along the margin, obtuse to acute at the apex,stellate pubescent, pale below. Inflorescences axillary cymes, solitary or 2- to 3-flowered per leaf axil; pubescent. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white; pedicel short; bracts small, pubescent. Sepals 5, free, linear lanceolate, pubescent, persistent. Petals 5, ovate, glabrous. Stamens numerous, free; filaments free, equal; anthers dithecous, basifixed, green, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, tetralocular, two ovules in each locule on the axile placentae; styles slender, densely hairs; stigma 4-lobes, glabrous. Fruits drupaceous, globose, 4 lobed, brown.

Pollen morphology (Figure 2 H, I)

Tricolporate, prolate, medium, 42.5 – 43.7 × 30.0 – 31.2 µm in length and breadth; amb circular; colpi ¾ way up to the pole, 37.5 – 38.7 × 5.0 – 6.2 µm in length and breadth; pori lalongate, 6.5 – 7.0 × 7.5 – 8.0 µm in length and breadth; exine 2.5 – 3.0 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrocate, 1.8 – 2.5 µm width; muri simplibaculate, 0.7 – 0.8 µm wide.

9. *Grewia titifolia* Vahl., Symb. Bot. 1: 35. 1790. (Figure 2 J)

Myanmar name : Pin tayaw, Tayaw

English name : Unknown

Flowering period : July to August

Perennial trees; stems and branches terete, stellate tomentose. Leaves simple, alternate; stipules linear lanceolate; pubescent; blades ovate to suborbicular, cordate at the base, serrate along the margin, shortly acute at the apex, stellate pubescent, pale green. Inflorescences axillary cymes, many-flowered per leaf axil; pubescent. Flowers bisexual, actinomorphic, pentamerous, hypogynous, greenish yellow; bracts small, pubescent. Sepals 5, free, linear lanceolate pubescent, persistent. Petals 5, ovate, glabrous. Stamens numerous, free; filaments free, unequal, pale green, glabrous; anthers dithecous, basifixed, green, dehiscing by longitudinal slit. Carpels 2, ovary superior, ovoid, bilocular, two ovules in each locule on the axile placentae; styles slender, densely hairs; stigma 4-lobes, glabrous. Fruits drupaceous, globose, 1 or 2 lobed, brown.

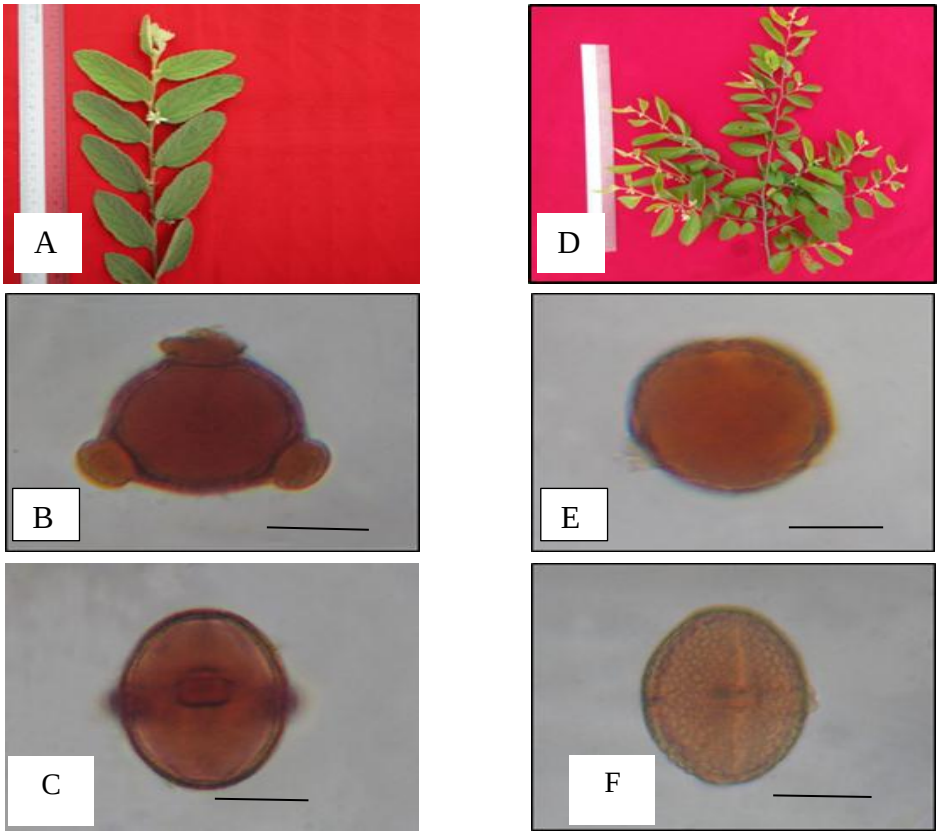


Figure 4 A. Inflorescences of *Grewia retusifolia* Pierre.
B. Polar view pollen of *G. retusifolia* Pierre.
C. Equatorial view pollen of *G. retusifolia* Pierre.
D. Inflorescences of *Grewia sapida* Roxb. ex DC.
E. Polar view pollen of *G. sapida* Roxb. ex DC.
F. Equatorial view pollen of *G. sapida* Roxb. ex DC.

Pollen morphology (Figure 2 K, L)

Tricolporate, prolate, medium, 45.5 – 46.0.5 × 33 – 34 µm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 36 – 38 × 4 – 5 µm in length and breadth; pori lalongate, 6.3 – 6.8 × 7.2 – 7.8 µm in length and breadth; exine 2.8 – 3.5 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrocate, 2.1 – 2.5 µm width; muri simplibaculate, 0.7 – 0.9 µm wide.

10. *Grewia villosa* Willd., In Nov. Act. Nat. Cur. Berol. 205. 1803. (Figure 2 M)

Myanmar name : Unknown

English name : Unknown

Flowering period : June to August

Perennial shrubs; stems and branches terete, stellate tomentose when young. Leaves simple, alternate; stipules caducous; densely pubescent; blades ovate, cordate at the base, serrate along the margin, shortly acute at the apex, stellate pubescent, pale green. Inflorescences axillary cymes, 2- to 3-flowered per leaf axil; pubescent. Flowers bisexual, actinomorphic, pentamerous,

hypogynous, reddish yellow; bracts small, pubescent. Sepal 5, free, lanceolate, reddish yellow, pubescent, persistent. Petals 5, linear lanceolate, glabrous. Stamens numerous, free; filaments free, unequal, glabrous; anthers dithecous, basifixed, green, dehiscing by longitudinal slit. Carpels 2, ovary superior, globose, bilocular, two ovules in each locule on the axile placentae; styles slender, densely hairs; stigma 4-lobes, glabrous. Fruits drupaceous, globose, 1 or 2 lobed, yellow brown or red.

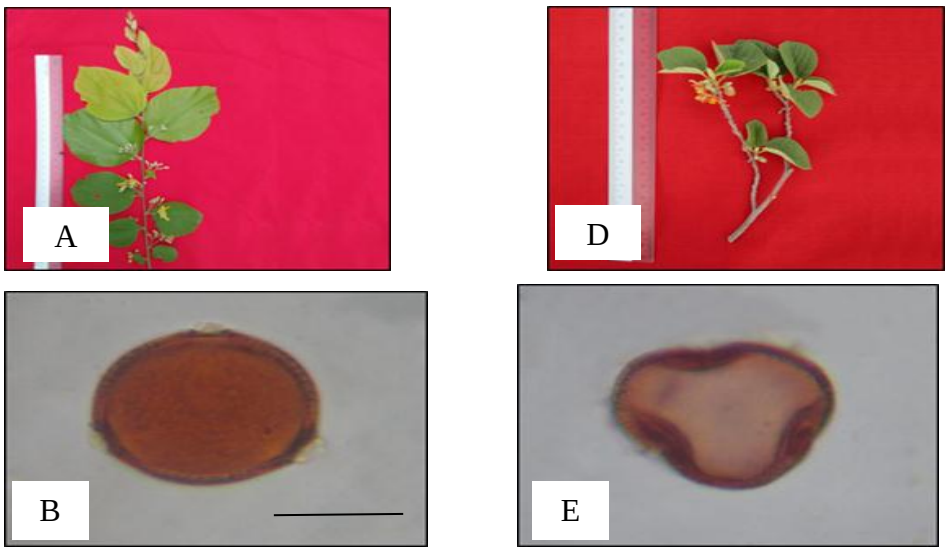
Pollen morphology (Figure 2 N, O)

Tricolpate, subprolate, medium, 38.7 – 40.0 × 33.7 – 35.0 µm in length and breadth; amb rounded triangular; colpi longicolpate, 36 – 38 × 7 – 8 µm in length and breadth; exine 1.2 – 2.0 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrocate, 1.0 – 1.5 µm width; muri simplibaculate, 0.3 – 0.4 µm wide.

Table Pollen Morphological Characters of Ten *Grewia* species

No.	Scientific Name	Aperture Type	Shape of EV	Size of PG	Pori Shape
1	*Grewia acuminata* Juss.	CP	Pro	M	Lo
2	*Grewia columaris* Smith.	CP	Subpro	M	Lo
3	*Grewia diamine* Gaertn.	CP	Pro	M	Lo
4	*Grewia flava* DC.	CP	Pro	M	La
5	*Grewia laevigata* Vahl.	CP	Pro	L	Lo
6	*Grewia parviflora* (Burge) Handen-Mazzetti.	CP	Subpro	M	Lo
7	*Grewia retusifolia* Pierre.	CP	Pro	M	Lo
8	*Grewia sapida* Roxb. ex DC.	CP	Pro	M	La
9	*Grewia titiifolia* Vahl.	CP	Pro	M	La
10	*Grewia villosa* Willd.	C	Subpro	M	-

EV = Equatorial View PG = Pollen GrainsCP = Colporate
Pro = Prolate Subpro = Subprolate L = Large
M = Medium La = Lalongate Lo = Lolongate
C = Colpate



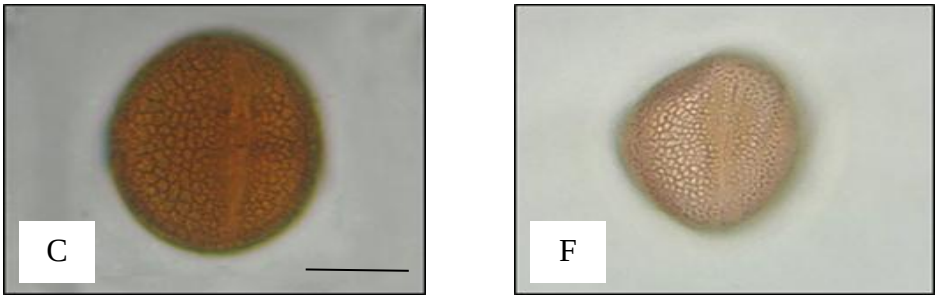


Figure 5 A. Inflorescences of *Grewia titiifolia* Vahl.
B. Polar view pollen of *G. titiifolia* Vahl.
C. Equatorial view pollen of *G. titiifolia* Vahl.
D. Inflorescences of *Grewia villosa* Willd.
E. Polar view pollen of *G. villosa* Willd.
F. Equatorial view pollen of *G. villosa* Willd.

Discussion and Conclusion

The present research work deals with the study on taxonomy and pollen morphology of genus *Grewia* of Tilioideae. The collected species were *Grewia acuminata* Juss., *G. columaris* Smith., *G. diamine* Gaertn., *G. flava* DC., *G. laevigata* Vahl., *G. parviflora* (Burge) Handen-Mazzetti., *G. retusifolia* Pierre., *G. sapida* Roxb. ex DC., *G. titiifolia* Vahl., and *G. villosa* Willd. In the present study, different morphological characters were observed. *Grewia diamine* Gaertn, *Grewia laevigata* Vahl, *Grewia parviflora* (Burge) Handen-Mazzetti, *Grewia titiifolia* Vahl were trees and the other species were shrubs. In the shape of leaf, the linear-oblong was occured in *Grewia laevigata* Vahl and *Grewia acuminata* Juss, and the remaining species were occured as ovate to linear ovate and orbicular. In the inflorescences of *Grewia diamine* Gaertn and *Grewia titiifolia* Vahl, the flowers were numerous but those of the others are solitary to 3-flowered. All of the studied species were simple leaves, stipulate and leaf arrangement of these were alternate. The flowers were bisexual, actinomorphic, pentamerous and hypogynous in all studied species. All the studied species have superior ovary and the placentation was all axile.

Pollen morphology was studied based on aperture type, shape, size and sculpturing pattern. In this research, the types of pollen grain were found as monad. The species of colpate pollen grains was found in *Grewia villosa* Willd and the remaining species were colporate pollen grains. Sharma (1968) proposed that colporate character is considered as an advanced character over colpate and porate condition as still advanced.

In equatorial view, pollen shape is described by the P/E ratio (P- polar axis, E- equatorial diameter). In this study, the shape of pollen grains were found as prolate and subprolate. In polar view, amb was circular and rounded triangular. The size of pollen grains were medium and large. The sculpturing patterns of pollen grains of all studied species were reticulate.

In the present study, the pollen grains of *Grewia* sp., are prolate, subprolate; tricolporate; colpi ¾ way up to the pole; sexine thicker than nexine; sculpturing reticulate. Preveen (2004) proposed that the pollen grains of *Grewia* sp., are prolate to subprolate; tricolporate; colpi ¾ way up to the pole; sexine thicker than nexine; sculpturing reticulate. Chung *et al.* (2003) proposed that the pollen grains of *Grewia* species are single, isopolar, radially symmetric, tricoloporate,

and rounded-tringular in equatorial outline (ambs). The pollen grains are medium to large. Therefore, these characters are the same as present result.

In conclusion, these pollen characters will support in identification and classification. All of these interesting pollen characters are undoubtedly important and beneficial for the future researchers. Therefore, this research is provided the knowledge of pollen morphology of genus *Grewia*.

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TAXONOMIC STUDY ON TEN WILD MUSHROOMS FROM HOPONE AND HSIHSEING TOWNSHIPS IN SOUTHERN SHAN STATE

Ohnmar Htwe[1] and Soe Myin Ayet[2]

Abstract

The taxonomic studies on wild mushrooms from Hopone and Hsihseing Township, Southern Shan State have been undertaken. The study area is located between N' 20º 48' - 20º 53' and E' 97º 10' - 97º 15'. The wild mushrooms were collected from June to September, 2018. The 10 species of 8 genera belonging to 7 families and 4 order were collected, preserved, classified, identified and described. The collected species were identified as *Helvella crispa* (Scop.) Fr., *Auricularia auricular-judage* (Bull.) Wettst., *Amanita caesarea* (Scop.) Pers., *Calvaria aurea* (Fr.) Quel., *Pleurotus lignatilis* (Pers.) Rehdead & Ginns., *Pleurotus pulmonarius* (Fr.) Quel., *Schizophyllum commune* (Fr.) Fr., *Lactarius determinus* Groger., *Lactarius volemus* Fr., *Russula cyanoxantha* (Schaeff.) Fr. and *Russula delica* Fr. The growing habitats of *Auricularia auricular-judage* (Bull.) Wettst. and *Schizophyllum commune* (Fr.) Fr. were on the decayed woods and the others were on the soil. An artificial key to the studied species was constructed and presented.

Keywords: Taxonomic study, wild mushrooms, Hopone and Hsihseing Township, Southern Shan State, an artificial key

Introduction

Mushrooms are fungi, generally considered to be lower forms of life, belonging to Kingdom Fungi. There are about 45,000 known species of fungi and about 2000 of them are considered edible. Of these, less than twenty five species are widely accepted as an item of food and only about a dozen of them have been commercially cultivated. The term mushroom is generally used to denote the edible fleshy fungi. The poisonous ones are called the toadstools. Both of them represent a short stage in the life cycle of fungi. To the Scientists, the word 'Mushroom' refers to both epigeous and hypogeous macroscopic fruiting bodies of fungi (Nair 1990).

Among the fungi, the common mushrooms are the puffballs, club fungi, coral fungi, hedgehod fungi, truffles, trembling fungi, morels, stinkhorns, tube-bearing fungi and lastly, the gilled fungi or agarics. All fungi, whether bacteria, yeasts or agarics, have in common an important characteristic feature that lack of chlorophyll. This remarkable substance that makes green the leaves of trees and herbs, also enables them to utilize for their nutrition, the simple elements of air, water and earth. Fungi, on the other hand, possessing no chlorophyll, must, like animals, depend for their nourishment upon living or dead organic matter. Loam, decaying wood and dead leaves support the majority of mushrooms. Gilled mushrooms, or agarics as they are called, are plants that belong to the botanical group known as fungi. Mushrooms of one kind or another are to be found at almost every season but they occur in greatest abundance after showery weather in the months of July, August, and September (Thomas 1948).

In Myanmar, mushrooms of Karen State was studied by Ku Yin Myint (1983). In 1987, Thida Saint presented by mushrooms of Taunggyi and Kalaw areas. In 2010, Kyi Kyi Win studied on the systematic studies of mushrooms in Pyay District and phytochemical investigation of *Dictyophora indusiata* (pers) Fish. In 2014, Khin Sandi Pyone Cho presented the taxonomic study on mushrooms growing in Mandalay. In 2015, Aye Aye Maw presented taxonomic studies on wild mushrooms from Monywa District. Although many researchers had done the wild mushrooms flora in Myanmar, the taxonomic

[1] Assistant Lecturer, Department of Botany, Pakokku University
[2] Pro-rector, Department of Botany, Myitkyina University
[3] Associate Professor, Department of Botany, Pakokku University

studies on wild mushrooms have not been undertaken in Southern Shan State. Therefore, this study was carried out for this research work.

The aim and objectives of this study were to collect, classify and identify the morphological characteristics of the wild mushrooms from Hopone and Hsihseing, to study their detailed taxonomic characteristics and distribution, and to fulfill the scientific information in the compilation of the mushroom flora in Myanmar.

Materials and Methods

The naturally growing wild mushrooms were collected from Hopone and Hsihseing Townships during the month from June to October, 2015. The wild mushrooms were observed on grassland, meadows, decomposing organic matter, hollow tree stumps, rotten tree trunks and shrub forests. The specimens were collected from different localities and habitats, and locations of specimens were determined by using a Global Positioning System (GPS) device.

All the specimens were recorded with photographs to get their actural habit and noted the fruiting characteristics. The collection, preservation and the spores print technique were followed to Krieger & Schaffer (1967) and Pacioni (1981). To prepare the spore print, the fleshy mature specimens were selected. The stipe was removed by cutting it off as close as possible to the point of attachment of cap. It is obtained by placing a cap with the hymenium facing down on a sheet of white, black paper or a piece of glass-slide. A blow can serve as a cover. After a few hours, a layer of the spores was deposited. The real colour of the spores was determined by this way.

The collected specimens were preserved in Formalin-Acetic acid-Alcohol (FAA) by the ratio of 5:5:90. Some dried specimens were placed plastic bags and plastic bottles. The classification and identification of collected specimens were done by referring the literature such as Thomas (1948), Krieger & Schaffer (1967), Coker & Couch (1969), Pacioni (1981), Keizer (1998), Roger Phillips (2006). An artificial key to all the studied species were also constructed and presented. The herbarium specimens were numbered and deposited at the herbarium of Mandalay University for references and other scientific studies.

Results

Ten species of 8 genera belonging to 7 families and 4 orders were collected from Hopone and Hsihseing Township, Southern Shan State. The morphological and spores characters of those species were classified and identified. The list of collected species and their comparable morphological characteristics were presented in Table 1 and Table 2.

Table 1 List of collected wild mushrooms from Hopone and Hsihseing Townships

Class	Sub-Class	Order	Family	No.	Scientific Name
Ascomycetes	Hymenoasco-mycetidae	Pezizales	Helvellaceae	1.	*Helvella crispa* (Scop.) Fr.
Basidio-mycetes	Heterobasido-mycetidae	Auriculareales	Auriculariaceae	2.	*Auricularia auricular-judage* (Bull.) Wettst.
	Homobasidio-mycetidae	Agaricales	Amanitaceae	3.	*Amanita caesarea* (Scop.) Per.
			Calvariaceae	4.	*Calvaria aurea* (Fr.) Quel.
			Pleurotaceae	5.	*Pleurotus lignatilis* (Pers.) Rehdead & Ginns
				6.	*Pleurotus pulmonarius* (Fr.) Queld.
			Schizophyllaceae	7.	*Schizophyllum commune* (Fr.) Fr.
		Russulales	Russalaceae	8.	*Lactarius volemus* Fr.
				9.	*Russula cyanoxantha* (Schaeff.) Fr.
				10.	*Russula delica* Fr.

Table 2 Comparable morphological characteristics of wild mushrooms from Hopone and Hsihseing Townships

No.	Scientific Name	Growing Habitat	Edible/ Inedible	Cap			Gills / Pores	
				Colour	Shape	Umbonate	Colour	Attachment
1.	*Helvella crispa* (Scop.) Fr.	soil	edible	white	saddle-shaped	absent	-	-
2.	*Auricularia auricular-judage* (Bull.) Wettst.	decayed wood	edible	brown	ear-shaped	-	-	-
3.	*Amanita caesarea* (Scop.) Pers.	soil	edible	yellowish	convex	absent	yellow	free
4.	*Calvaria aurea* (Fr.) Quel.	soil	edible	egg yellow	cauliflower-like	-	-	-
5.	*Pleurotus lignatilis* (Pers.)Rehdead & Ginns	soil	edible	creamy-white	depressed	absent	white	decurrent
6.	*Pleurotus pulmonarius* (Fr.) Quel.	soil	edible	white-cream	shell-shaped	absent	white - cream	decurrent
7.	*Schizophyllum commune* (Fr.) Fr.	decayed wood	edible	grayish-white	fan-shaped	absent	grayish-white	-
8.	*Lactarius volemus* Fr.	soil	edible	brownish-orange	depressed	absent	pale-yellow	decurrent
9.	*Russula cyanoxantha* (Schaeff.) Fr.	soil	edible	wine coloured	depressed	absent	pale-cream	decurrent
10.	*Russula delica* Fr.	soil	edible	white-pale yellow	funnel-shaped	absent	white-pale cream	decurrent

Table 3 Comparable morphological characteristics of wild mushrooms from Hopone and Hsihseing Townships

No.	Scientific Name	Stipe				Spore			
		Shape	colour	hollow/ solid	annulus or ring	Colour	shape	texture	size
1.	*Helvella crispa* (Scop.) Fr.	calvate	white	hollow	absent	white	elliptic	smooth	16-21×10-14 µm
2.	*Auricularia auricular-judage* (Bull.) Wettst	-	-	-	absent	white	sausage-shaped	smooth	16-18×6-8µm
3.	*Amanita caesarea* (Scop.) Per	equal	yellow	solid	present	white	elliptic	smooth	10-14×6-11 µm
4.	*Calvaria aurea* (Fr.) Quel.	-	-	-	-	deep-ochraceous	oblong	roughened	9-12×4.5-7 µm
5.	*Pleurotus lignatilis* (Pers.) Rehdead & Ginns	equal	white	solid	absent	white	elliptic	smooth	6-7.5×3-4.5µm
6	*Pleurotus pulmonarius* (Fr.) Quel.	equal	white	solid	absent	white	cylindrical	smooth	7.5-11×3-4 µm
7.	*Schizophyllum commune* (Fr.) Fr.	-	-	-	-	grayish-white	elliptic	smooth	3-4×1-1.5 µm
8.	*Lactarius volemus* Fr.	equal	brownish orange	solid	absent	white	globose	smooth	9-11 µm
9.	*Russula cyanoxantha* (Schaeff.) Fr.	equal	white	solid	absent	white	elliptic	low-wort	7-9×6-7 µm
10	*Russula delica* Fr.	equal	whitish-cream	hollow	absent	white-pale cream	ellipsoid	reticulate	8-11×6.5-8.5 µm

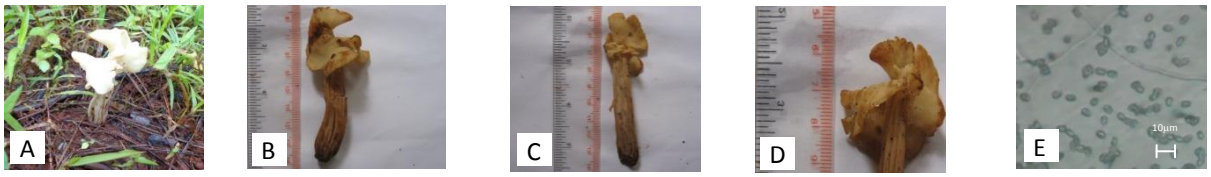

Figure 1 *Helvella crispa* (Scop.)Fr.
(A. Growing habitat, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

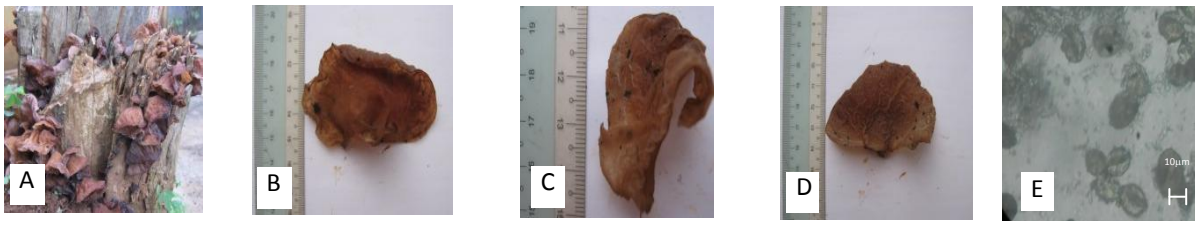

Figure 2 *Auricularia auricular-judage* (Bull.)Wettst.
(A. Growing habitat, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

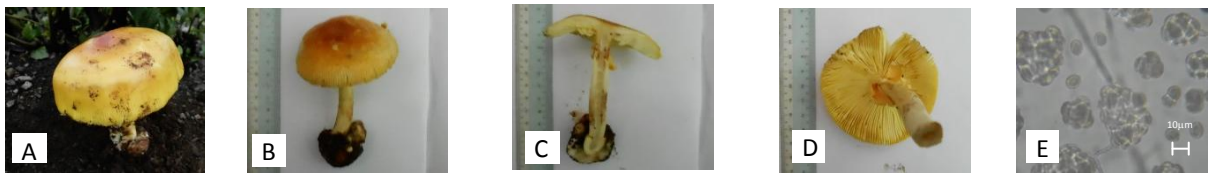

Figure 3 *Amanita caesarea* (Scop.) Per.
(A. Growing habita, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

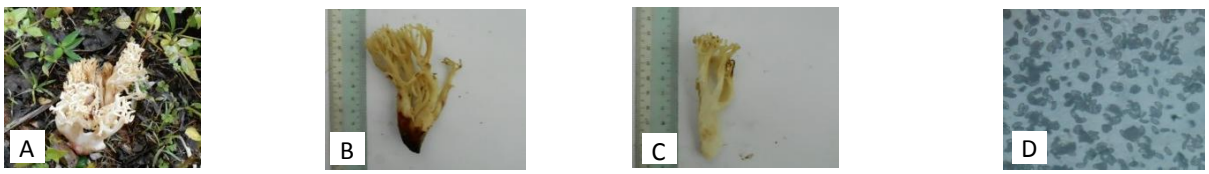

Figure 4 *Calvaria aurea* (Fr.) Quel.
(A. Growing habitat, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Spores)

Figure 5 *Pleurotus lignatilis* (Pers.) Redhead & Ginns.
(A. Growing habitat, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 6 *Pleurotus plumonarius* (Fr.) Quel.
(A. Growing habitat, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

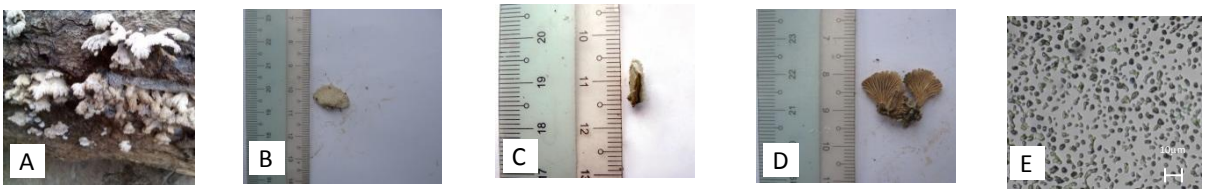

Figure 7 *Schizophyllum commune* (Fr.) Fr.
(A. Growing habitat, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

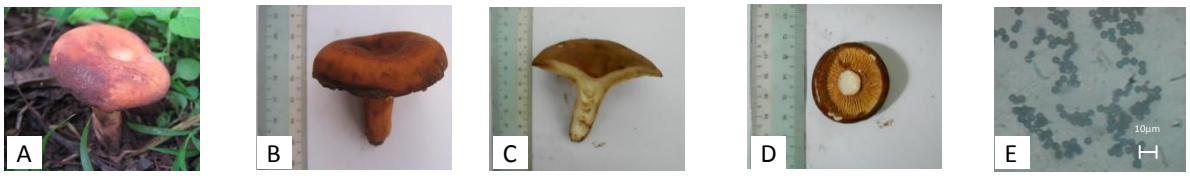

Figure 8 *Lactarius volemus* Fr.
(A. Growing habitat, B. Fruiting body in ilateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 9 *Russula cyanoxantha* (Schaeff.) Fr.
(A. Growing habitat, B. Fruiting body in ilateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

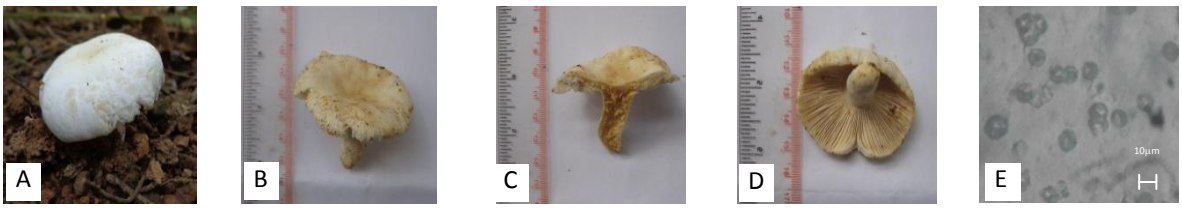

Figure 10 *Russula delica* Fr.
(A. Growing habitat, B. Fruiting body in lateral view,
C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

An Artificial Key to the Studied Species

1. Stipe hollow ---------- 2
1. Stipe solid ---------- 3

2. Cap saddle-shaped; Spores elliptic, smooth; Stipe calvate ----------
---------- 1. *Helvella crispa*

2. Cap funnel-shaped; Spores ellipsoid, reticulate; Stipe equal ----------
---------- 10. *Russula delica*

3. Spores roughened or low-wort ---------- 4

3 Spores smooth---------- ---------- 5

4. Cap egg-yellow, cauliflower; Spores oblong----------
---------- 4. *Calvaria aurea*

4. Cap wine-coloured, depressed; Spore elliptic----------
---------- 9. *Russula cyanoxantha*

5. Spore sausage-shaped or cylindrical ---------- 6
5. Spore elliptic or globose ---------- 7

6. Cap tan-brown, ear-shaped ----------2. *Auricularia auricular-judage*

6.Cap white-cream, shell-shaped----------6. *Pleurotus plumonarius*

7. Gills free or radiating ---------- 8

7. Gills decurrent ---------- 9

8 Cap yellow, convex; Spores white ----------3. *Amanita caesarea*

8. Cap grayish-white, fan-shaped; Spores grayish-white ----------

----------7. *Schizophyllum commune*

9. Stipe white; Spores elliptic; Gills white ----------5. *Pleurotus lignatilis*

9. Stipe brownish-oranged; Spores globose; Gills pale yellow ----------

----------8. *Russula virescens*

Discussion and Conclusion

In the present study, the taxonomic studies on ten species of wild mushrooms from Hopone and Hsihseing Township in Southern Shan State were undertaken. The fresh specimen of wild mushrooms from Hopone and Hsihseing Township were collected from June to October, 2015. They were included in 8 genera and 7 families.

In Hopone Township, the distributed species in Hopone Mountain were *Helvella crispa* (Scop.) Fr., *Auricularia auricular-judage* (Bull.) Wettst., *Amanita caesarea* (Scop.) Pers., *Calvaria aurea* (Fr.) Quel., *Pleurotus lignatilis* (Pers.) Rehdead & Ginns., *Schizophyllum commune* (Fr.) Fr., *Lactarius volemus* Fr., and *Russula cyanoxantha* (Schaeff.) Fr. In Hsihseing Township, *Pleurotus pulmonarius* (Fr.) Quel. and *Russula delica* Fr. were found in Lweput village.

The growing habit of fruiting bodies are very interesting. Eight species of wild mushrooms are growing in the soil. *Auricularia auricular-judae* (Bull.) Wettst. and *Schizophyllum commune* (Fr.) Fr. were growing on the decayed woods.

Various cap shapes were also observed in this study areas. In *Pleurotus lignatilis* (Pers.) Rehdead & Ginns., *Lactarius volemus* Fr. and *Russula cyanoxantha* (Schaeff.) Fr., the shaped of the cap were depressed. *Russula delica* Fr. was found in funnel shaped. *Schizophyllum commune* (Fr.) Fr. was possess the fan-shaped. The convex shaped of the cap can be found in *Amanita caesarea* (Scop.) Pers. The shell-shaped of the cap can be found in *Pleurotus pulmonarius* (Fr.) Quel. The ear-shaped of cap can be found in *Auricularia auricular-judage* (Bull.) Wettst. Saddle - shaped of the cap can be found in *Helvella crispa* (Scop.) Fr. *Calvaria aurea* (Fr.) Quel. possess the cauliflower-like of the cap.

The stipe shapes of 6 species were equal in *Amanita caesarea* (Scop.) Pers., *Pleurotus lignatilis* (Pers.) Rehdead & Ginns., *Pleurotus pulmonarius* (Fr.) Quel., *Lactarius volemus* Fr., *Russula cyanoxantha* (Schaeff.) Fr. and *Russula delica* Fr. *Helvella crispa* (Scop.) Fr. was calvate. The stipe were absent in *Auricularia auricular-judage* (Bull.) Wettst., *Calvaria aurea* (Fr.) Quel. and *Schizophyllum commune* (Fr.) Fr. The stipe of 2 species were hollow in *Helvella crispa* (Scop.) Fr. and *Russula delica* Fr. The other 5 species were solid. *Amanita caesarea* (Scop.) Pers. possess the ring on the stipe. In the other species, the ring is absent.

In the colour of spores, *Calvaria aurea* (Fr.) Quel. possess deep ochraceous colour. The spores of grayish-white can be found in *Schizophyllum commune* (Fr.) Fr. The spores of *Russula delica* Fr. was white to pale-cream. The other 7 species were white. The real colour of spores was determined by the spores print. These findings were agreed with Moore (2014). All of the studied species were edible. These findings were agreed with Groves (1979).

Some wild mushroom species from these Hopone and Hsihseing Township areas were also found in Karen State,, Mon State, Taungyi and Kalaw areas, Pyay District and Monywa Distirict. These are *Amanita caesarea* (Scop.) Pers., *Auricularia auricular-judage* (Bull.) Wettst., *Schizophyllum commune* (Fr.) Fr., *Lactarius volemus* Fr., *Russula cyanoxantha* (Schaeff.) Fr. and *Russula delica* Fr. in Karen State (Ku Yin Myint 1983); *Schizophyllum commune* (Fr.) Fr., in Thaton District, Mon State (Thandar Soe 2013); *Helvella crispa* (Scop.) Fr., *Auricularia auricular-judage* (Bull.) Wettst., *Amanita caesarea* (Scop.) Pers., *Schizophyllum commune* (Fr.) Fr., *Lactarius volemus* Fr., *Russula cyanoxantha* (Schaeff.) Fr. and *Russula delica* Fr. in Taungyi and Kalaw areas (Thida Saint 1987); *Auricularia auricular-judage* (Bull.) Wettst., *Amanita caesarea* (Scop.) Pers., *Schizophyllum commune* (Fr.) Fr., and *Russula delica* Fr. in Pyay District (Kyi Kyi Win 2010) and *Auricularia auricular-judage* (Bull.) Wettst., *Amanita caesarea* (Scop.) Pers., *Schizophyllum commune* (Fr.) Fr., *Lactarius volemus* Fr. and *Russula delica* Fr. in Monywa District (Aye Aye Maw 2015).

Therefore, it would be concluded that the present study was one of the systematic records of wild mushrooms to be used by researchers in various fields of studies. This study will be provided the partial fulfillment of the information on the wild mushrooms distribution in Hopone and Hsihseing Township, in Southern Shan State and will be beneficial to accomplish the mushroom flora in Myanmar.

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ISOLATION OF SOIL FUNGI FROM MUDON TOWNSHIP, MON STATE AND THEIR ANTIMICROBIAL ACTIVITY

Myat Myat Phyo[1] and Zar Zar Yin[2]

Abstract

In this research work, soil samples were collected from five different places of Mudon Township including Kwaiwan, Kawkhite, Tarpaton, Tharyargone and Kyauktalone village, during July 2018. Soil fungi were isolated by the serial dilution method from these samples and cultured on Blaskeslee's Malt Extract Agar (BMEA Medium), Czapek- Dox Agar (CZA Medium), Malt Extract Agar (MEA Medium), Dichloram Rose Bengal – Chloramphenicol Agar (DRBC Medium), Glucose Ammonium Nitrate Agar (GAN Medium), Potato Glucose Agar (PGA Medium) and incubated for 3-7 days at room temperature. A total of 41 fungal strains were isolated and the surface color of all strains were white, brown , greenish brown, and their reverse color were cream, black, greenish brown, yellowish brown respectively. In the colony morphology, the isolated fungi were small, medium and large in size. The margins of isolated fungi were entire convex, raised, and the form of isolated fungi circular and irregular. Moreover, physicochemical properties of soil from different locations of Mudon Township were analyzed. All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, six strains showed different level of antimicrobial activity. MP- 7 exhibited the highest antibacterial activity (25.05 mm) against and MP- 25 also showed the moderate activity (23.50 mm) on *Bacillus pumilus* at 5 days. MP- 6 gave the strong antibacterial activity (20.03 mm) against *Bacillus Subtilus* at 6 days. Especially, MP- 41 showed the moderated antimicrobial activity against all test organisms.

Keywords: Soil fungi, antimicrobial activity

Introduction

Microorganisms in soil are important because they affect soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Soil is considered one of the most suitable environments for microbial growth, for that the microorganism which have been isolated from the soil. Numerous antibiotics have been isolated from a variety of microorganism; however, studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria (Cavalcanti, *et. al.*, 2006).

Soil are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids and other invertebrates as well as plants and algae. These soil dwellers provide food or nutrients that support organisms that live above and below ground. Soils also play critical roles in buffering and filtering freshwater ecosystems. Consequently, soils are extremely important to human societies (Dominati, 2010). The number and species of microbes in soil vary directly in response to environmental conditions such as nutrient availability, soil texture and type of vegetation cover (Atlas, *et. al*. 1998).

Natural products from microorganisms have been the most successful source that has found many applications in the fields of medicine, pharmacy and agriculture. Most of the antibiotics in current use for the treatment of various infectious diseases are microbial products (Tawiah, *et. al*., 2012).

[1] Assistant Lecturer, Department of Botany, Mawlamyine University
[2]Associate Professor, Department of Botany, Pathein University

Fungi are an important component of the soil microbiotatypically constitution more of the soil biomass than bacteria depending on soil depth and nutrient conditions (Ainsworth & Bisby, 1995). Fungi represent a very important biological resource with an estimated 1.5 million species in the world. The tropics are generally recognized as embracing the greatest variation on earth and in the case of plants about two-thirds (180,000 species) are believed to occur there (Raven, 1988).

Therefore, soil sample is the most effective and popular materials for especially isolating a number of fungi (Ando, 2004). Wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic production microbes (Oskay.M, 2004).

Soil is a naturally occurring loose mixture of mineral and organic particles, still remains the most important target for most researchers in their efforts to discover novel antibiotics which have pharmaceutical values (Nejad, 2013).

Therefore, the aim of the research work was to produce antimicrobial compounds by isolated fungi from five different places soil in Mudon Township. To achieve this aim, the present work has been done according to the following objectives - to collect soil samples from five places of Mudon Township, to isolate soil fungi from these soil samples, to study the cultural characteristics of isolated soil fungi on six different media, to investigate the colony morphology of isolated fungi and to determine the preliminary antimicrobial activity of isolated fungi.

Materials and Methods

Method for collection of soil samples

The soil samples were collected from five different places in various location of Mudon Township, during July 2018. The soil samples were collected from different places (up to 15 cm depth) into sterilized polythene bags after removing the surface soil for the isolation of fungi and brought to the laboratory of Botany department at Mawlamyine University.

Physicochemical analysis of Soil Samples

The collected soil samples were characterized for its physicochemical properties. Physiochemical parameters include organic nitrogen, phosphorous, potassium oxide, pH, temperature, moisture and texture. Temperature and color of the soil samples was recorded on the spot. The other physicochemical parameters of the soil samples were analyzed at Land Use, Perennial Crops Research & Development center (Mawlamyine).

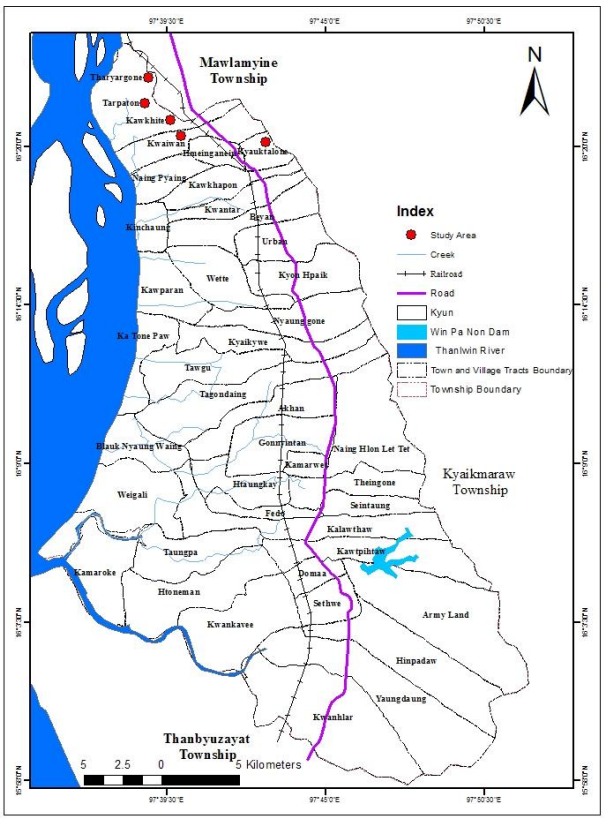
Isolation of fungi from the soil samples

The soil micro fungi were isolated by serial dilution method (Dubey, 2002) on different media such as Blaskeslee's Malt Extract Agar (BMEA Medim), Czapek- Dox Agar (CZA Medium), Malt Extract Agar (MEA Medium), Dichloram Rose Bengal- Chloramphenicol Agar (DRBC Medium), Glucose Ammonium Nitrate Agar (GAN Medium), Potato Glucose Agar (PGA Medium).

Table 1 Collected Soil samples from five different places at Mudon Township

Soil sampleNo.	Place	Location
1	Kwaiwan	N 16˚ 19. 353′ E 97˚ 41. 544′
2	Kawkhite	N 16˚ 20.35′ E 97˚ 41. 269′
3	Tarpaton	N 16˚ 20.643′ E 97˚ 40.965′
4	Tharyargone	N 16˚ 20.819′ E 97˚ 40.741 ′
5	Kyauktalone village	N 16˚ 19.732′ E 97˚ 42.082′

Collected Soil Sample Area



Source: Department of Geography, Mawlamyine University

Figure 1 Map of collected soil sample area (Mudon Township)

Serial Dilution Method (Dubey, 2002)

1 g of soil sample was introduced into a conical flask containing 99 ml of distilled water. The flask was than 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 separate test tubes and 0.5 ml each of the above dilution was separately transferred into sterile petridishes under aseptic condition. The sterilized medium in conical flask was cooled down to about 45º C and separately poured into each of the petridish containing the respective soil dilutions. The inoculated plates were shaken clock-wise and anti-wise direction for about 5 minutes so as to make uniform

distribution of the fungi inoculums. When the agar was solidified, the inoculated plate were inverted and incubated at 27ºC- 30ºC for 3-7 days. Isolated pure fungi were preserved into slant culture containing BMEA Medium for further experimentations.

Agar Well Method (Collins, 1965)

Isolated strains were tested by agar well method for the 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 broths (20 µL) were incubated at room temperature for 24- 28 hours. After 24- 48 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones had seen 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.

Test Organisms

The test organisms used for the experiment were *Escherichia coli* AHU5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus* IFO 90571, *Candida albicans* NITE 09542, *Pseudomonas fluorescens* IFO94307, *Staphlylococcus aureus* AHU8465, *Agrobacterium tumefaciens* NITE 09678 and *Malassezia furfur*. The organisms were obtained from National Institute of technology and Evaluation (NITE, Japan) and Pharmaceutical Research Department, Yangon, Myanmar.

Results

In present research work, 41 fungal strains were isolated from five different samples collected from Mudon Township. The results of the physicochemical properties of soil samples showed that soil environments of Kwaiwan, Tarpaton and Tharyargone village were sandy loam and the soil sample form Kawkhite and Kyauktalone village were sandy clay loam.

The pH values of the soil samples show that Kwaiwan, Kawkhite, Tarpaton, Tharyargone and Kyauktalone village were moderately acidic with pH of 6.43, 6.41, 6.42, 6.16 and 6.52 respectively. The temperature of soil environments of Mudon Township at the time of this investigation (rainy season) revealed that the soil environment of Mudon Township had temperature range between 20ºC and 28ºC with great variation in present moisture content (1.18-2.99), organic nitrogen (0.09- 0.15), phosphorus (2.40 -11.88), potassium dioxide (4.16 - 12.73). The color of soil samples were red, black and brown. These results were shown in Table 2.

Table 2 Physico-chemical Properties of Soil Samples collected from five different places of Mudon Township

Sample No	Place	Soil color	Texture	pH	T(C^o)	Moisture (%)	Organic N (%)	Nutrients	
								P (ppm)	K_2O (mg)
1	Kwaiwan	Black	SL	6.43	20.7	1.91	0.13	11.88	12.73
2	Kawkhite	Brown	SCL	6.41	28.8	2.41	0.09	5.94	4.16
3	Tarpaton	Brown	SL	6.42	20.75	1.85	0.15	5.94	6.61
4	Tharyar gone	Brown	SL	6.16	20.85	1.18	0.09	5.88	5.09
5	Kyaukta lone	Red	SCL	6.52	20.75	2.99	0.09	2.40	6.19

**SL- Sandy Loam, SCL- sandy clay loam,

N- Nitrogen, P- Phosphorous, K_2O- Potassium oxide

In the present research work, 41 fungal isolates were obtained fifteen strains from Kwaiwan, sixteen strains from Kawkhite, seven strain from Kyauktalone, two strains from Tharyargone and each one strain from Tarpaton. In the present research was used by six culture media. A total of 41 isolated fungi, 17 strains were isolated from BMEA Medium, 13 strains from DRBC Medium, 5 strains from PDA Medium, 4 strains from MEA Medium, each 1 strain from CZA and each 1 strain from GAN Medium. These results were shown in Table 3. The isolated fungi were designated as MP- 1 to MP- 41.

Table 3 Isolation of fungi by using six different media and soil s

Sample No	Places	BMEA Medium	DRBC Medium	PDA Medium	MEA Medium	CZA Medium	GAN Medium	Total
1	Kwaiwan	MP-1, 3,9, 10, 14,	MP- 4, 5, 6, 7, 11, 12, 13, 15	MP- 2	MP- 8	-	-	15
2	Kawkhite	MP- 20, 27, 29, 30, 31	MP- 16, 21, 26,	MP- 17, 18, 19	MP- 23, 24, 25	MP- 22	MP- 28	16
3	Tarpaton	-	MP- 32	-	-	-	-	1
4	Tharyargone	MP- 33	-	MP- 34	-	-	-	2
5	Kyauktalone	MP- 35, 36, 37, 38, 40, 41	MP- 39	-	-	-	-	7
		17	13	5	4	1	1	41

Morphology and Photomicrograph of Isolated Soil Fungi

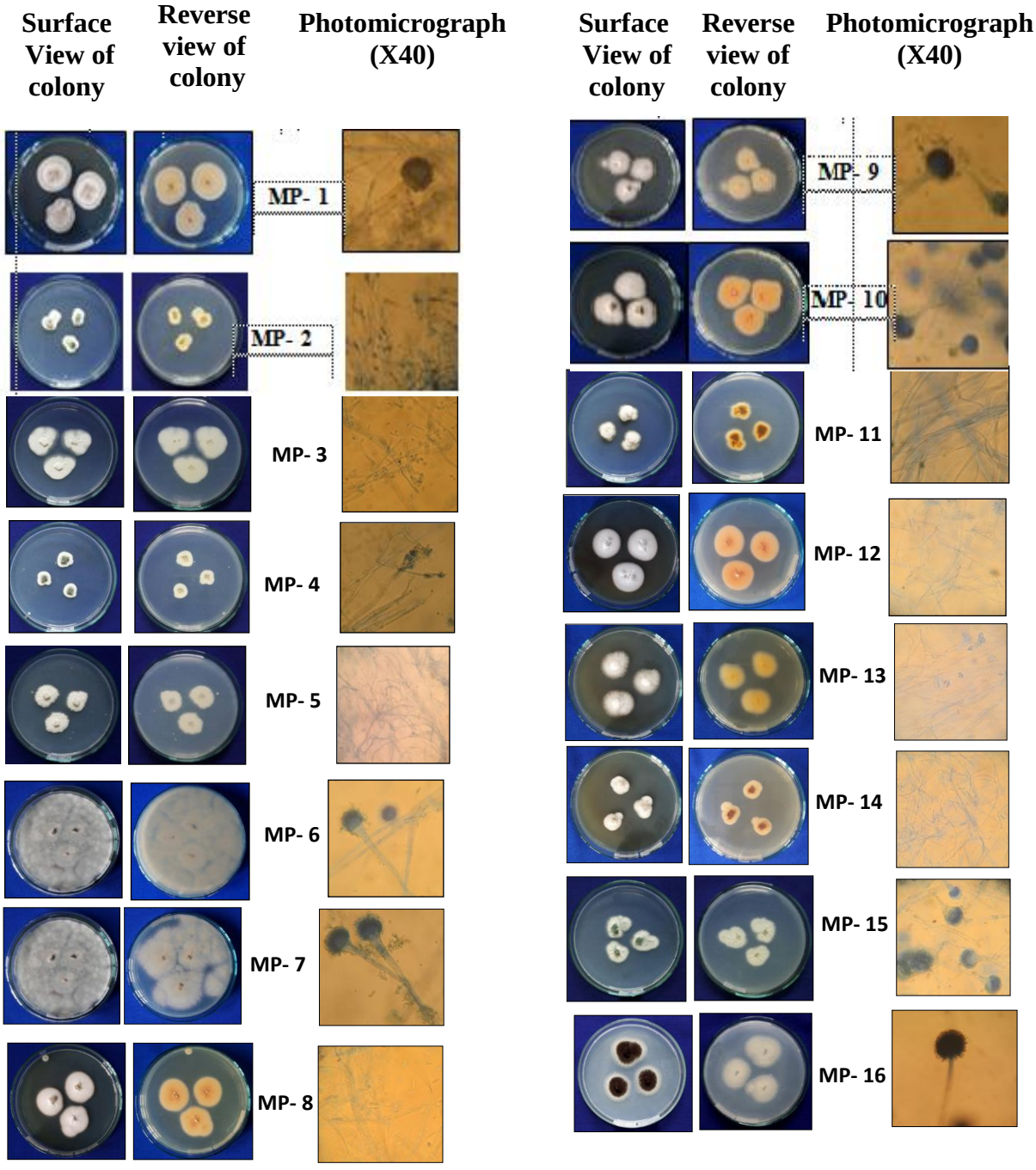


Figure 2 Morphology and microscopical characters of isolated fungi MP-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16

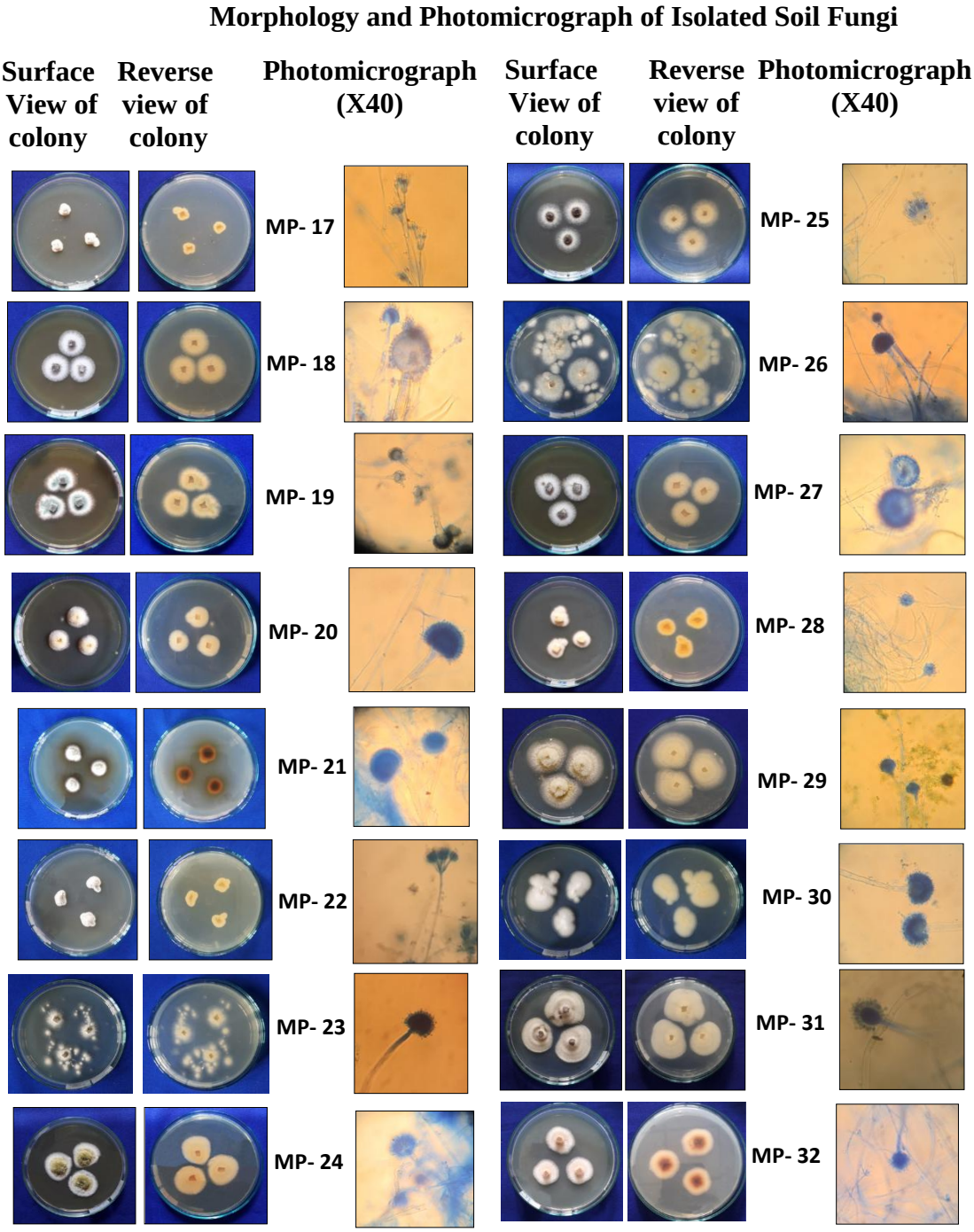


Figure 2 Morphology and microscopical characters of isolated fungi MP-17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32

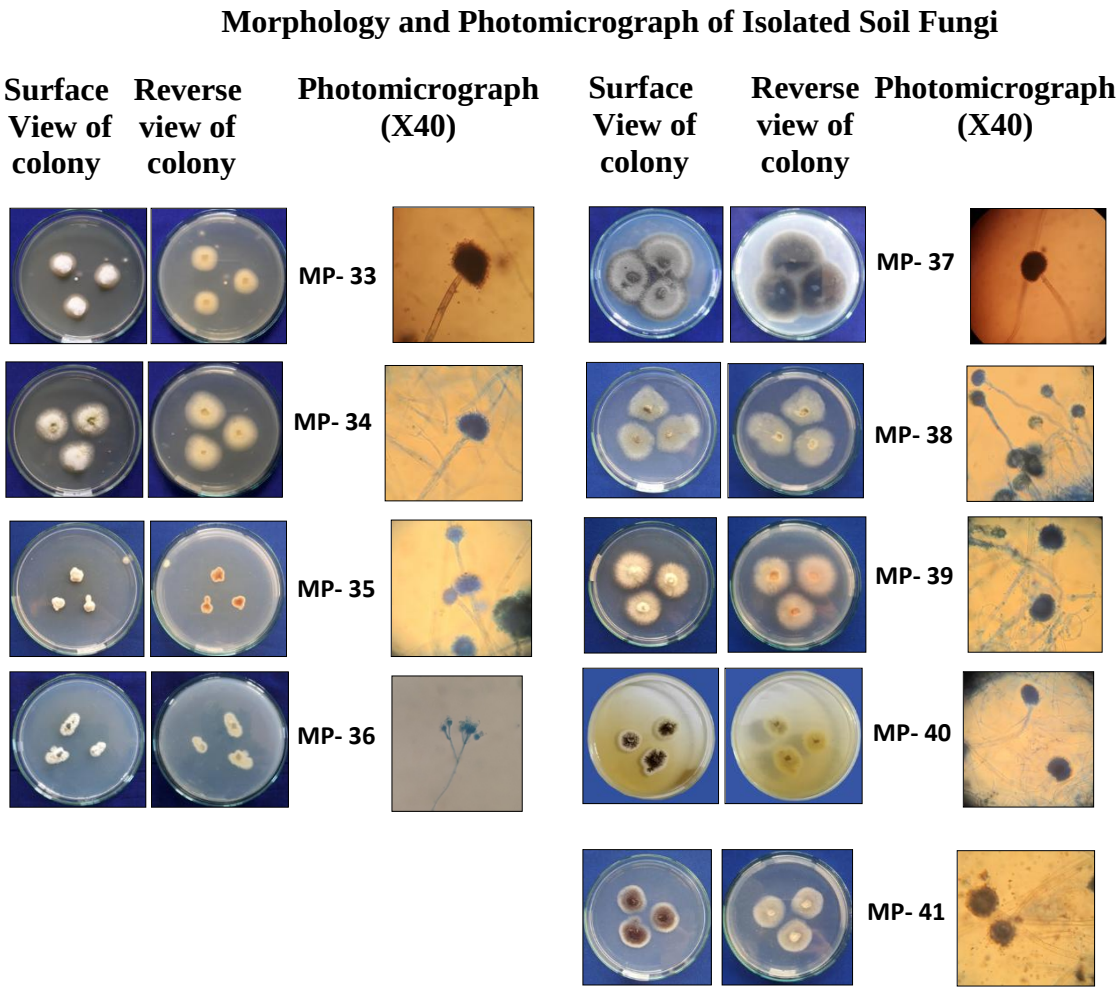


Figure 3 Morphology and microscopical characters of isolated fungi MP-33, 34, 35, 36, 37, 38, 39 40 and 41

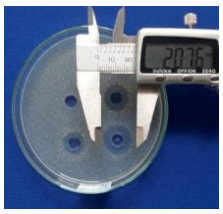
All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, six strains showed different level of antimicrobial activities. In 4 days old culture, MP- 7 showed activities are (20.76 mm) and MP- 26 showed (23.50 mm) activities against *Escherichia coli* at 5 days. In 4 days, MP- 6 (20.03 mm) and MP- 7 showed (16.99 mm) activities are against *Bacillus subtilus* at 6 days. MP- 7 showed activity (25.05 mm) at 5 days and MP- 26 (20.53 mm) against *Bacillus pumilus* at 4 days fermentation period. In 6 days, MP- 6 showed (14. 76 mm) and MP- 26 (15.29 mm) against *Candida albican* at 5 days. MP- 26 (14.75 mm) and MP- 41 showed (13.33 mm) against *Pseudomonas fluorescens* at 6 days. In 5 days, MP- 25 (13.20 mm) and MP- 41 showed (16. 30 mm) against *Straphylococcus aureus.* MP- 7 (15. 34 mm) and MP- 41 (16.07 mm) showed against *Agrobacterium tumefaciens.* In 5 days, MP-7 (15.77 mm) and MP-26 showed against *Malassezia furfur.*

Among them, MP - 7 exhibited the highest antibacterial activity (25.05 mm) against *Bacillus pumilus* at 5 days and MP- 25 also showed the moderate activity (23.50 mm) on *Bacillus pumilus* at 5 days. MP- 6 gave the strong antifungal activity (20.03 mm) against *Bacillus subtilus* at 4 days. Especially, MP- 41 showed the moderated antimicrobial activity against all test organisms.

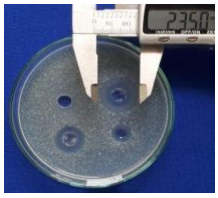
Table 4 Antibacterial activity of six fungal strains against *Escherichia coli*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	15.04	15.71	21.99	20.39
2	MP- 7	20.23	20.76	23.35	24.08
3	MP- 25	+	13.56	18.28	16.70
4	MP- 26	-	20.12	**23.50**	20.72
5	MP- 33	-	11.31	11.87	14.32
6	MP- 41	16.47	14.73	16.99	20.15

(+)present (-) absent well size=8mm



MP- 7 against *E coli* at 4 days of fermentation period



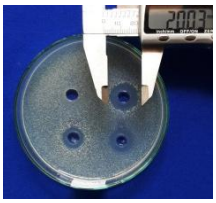
MP-26 against *E coli* at 5 days of fermentation period

Figure 4 Antibacterial activity of selected fungal strains against *Escherichia coli*

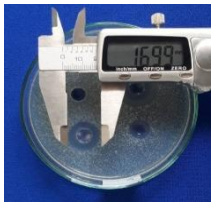
Table 5 Antibacterial activity of six fungal strains against *Bacillus subtilis*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	**20.03**	13.81	15.94
2	MP- 7	15.44	16.75	13.43	16.99
3	MP- 25	+	14.71	14.16	13.52
4	MP- 26	-	19.77	15.96	15.13
5	MP- 33	-	19.09	11.25	12.64
6	MP- 41	17.60	12.95	18.05	19.12

(+)present (-) absent well size=8mm



MP- 6 against *Bacillus subtilis* at 4 days of fermentation period



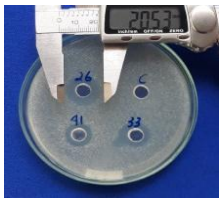
MP- 7 against *Bacillus subtilis* at 6 days of fermentation period

Figure 5 Antibacterial activity of selected fungal strains against *Bacillus subtilis*

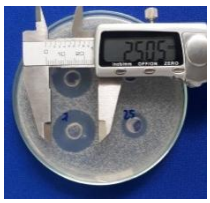
Table 6 Antibacterial activity of six fungal strains against *Bacillus pumilus*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	17.74	19.77	23.26	21.78
2	MP- 7	20.47	19.69	**25.05**	23.86
3	MP- 25	13.62	15.40	15.49	14.89
4	MP- 26	-	20.53	20.51	21.11
5	MP- 33	-	18.30	+	13.11
6	MP- 41	18.30	16.27	14.46	20.36

(+)present (-) absent well size=8mm



MP- 7 against *Bacillus pumilus* at 5 days of fermentation period



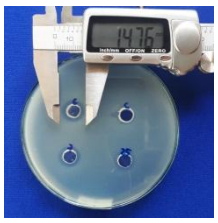
MP- 26 against *Bacillus pumilus* at 4 days of fermentation period

Figure 6 Antimicrobial activity of selected fungal strains against *Bacillus pumilus*

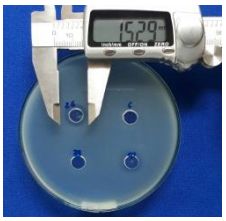
Table 7 Antifungal activity of six fungal strains against *Candida albicans*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	+	11.48	14.76
2	MP- 7	13.62	+	13.27	14.17
3	MP- 25	12.60	+	13.79	11.65
4	MP- 26	-	+	**15.29**	+
5	MP- 33	-	+	+	12.23
6	MP- 41	12.44	+	14.46	15.60

(+)present (-) absent well size=8mm



MP- 6 against *Candida albicans* at 6 days of fermentation period



MP- 26 against *Candida albicans* at 5 days of fermentation period

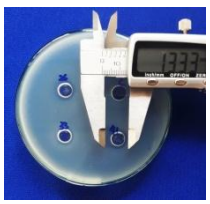
Figure 7 Antifungal activity of selected fungal strains against *Candida albicans*

Table 8 Antibacterial activity of six fungal strains against *Pseudomonas fluorescens*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	+	+	+
2	MP- 7	+	+	11.92	13.47
3	MP- 25	+	+	+	12.52
4	MP- 26	-	+	**14.75**	12.05
5	MP- 33	-	+	+	+
6	MP- 41	-	12.01	13.18	13.33

(+)present (-) absent well size=8mm

MP- 26 against *Pseudomonas fluorescens* at 5 days of fermentation period



MP- 41 against *Pseudomonas fluorescens* at 6 days of fermentation period

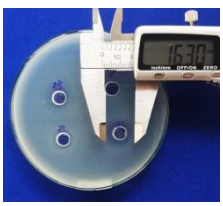
Figure 8 Antibacterial activity of selected fungal strains against *Pseudomonas fluroescens*

Table 9 Antibacterial activity of six fungal strains against *Straphylococus aureus*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	11.82	+	+
2	MP- 7	+	12.18	11.96	+
3	MP- 25	-	12.64	13.20	+
4	MP- 26	-	11.84	13.93	12.59
5	MP- 33	-	+	+	+
6	MP- 41	+	12.16	16.07	**16.30**

(+)present (-) absent well size=8mm

MP- 25 against *Straphylococus aureus* at 5 days of fermentation period



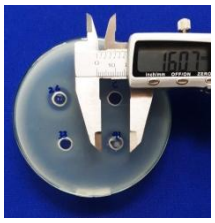
MP- 41 against *Straphylococus aureus* at 6 days of fermentation period

Figure 9 Antimicrobial activity of selected fungal strainag ainst *Straphylococus aureus*

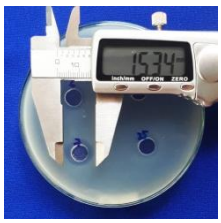
Table 10 Antibacterial activity of six fungal strains against *Agrobacterium tumefaciens*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	+	11.28	14.45
2	MP- 7	+	+	14.75	15.34
3	MP- 25	-	12.45	+	13.22
4	MP- 26	-	13.05	15.54	13.84
5	MP- 33	-	+	+	13.04
6	MP- 41	+	13.63	16.07	15.51

(+)present (-) absent well size=8mm



MP-41 against *Agrobacterium tumefaciens* at 5days of fermentation period



MP-7 against *Agrobacterium tumefaciens* at 6 days of fermentation period

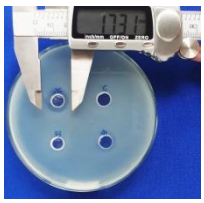
Figure10 Antimicrobial activity of selected fungal strains *Agrobacterium tumefaciens*

Table 11 Antifungal activity of six fungal strains against *Malassezia furfur*

No	**Isolated Fungi**	**Fermentation Period (days) and Inhibitory zone (mm)**			
		3 days	4 days	5 days	6 days
1	MP- 6	+	+	12.89	14.41
2	MP- 7	15.86	+	15.77	14.12
3	MP- 25	+	13.63	15.41	+
4	MP- 26	-	+	**17.31**	13.41
5	MP- 33	-	+	+	+
6	MP- 41	+	14.46	12.88	17.12

(+)present (-) absent well size=8mm

MP- 7 against *Malassezia furfur* at 5 days of fermentation period



MP- 26 against *Malassezia furfur* at 5 days of fermentation period

Figure11 Antimicrobial activity of selected fungal strains against *Malassezia furfur*

Discussion and Conclusion

Soil is a naturally occurring loose mixture of mineral and organic particles, still remains the most important target for most researchers in their efforts to discover novel antibiotics which have pharmaceutical values (Nejad *et. al.*, 2013). Scientist are continuously searching for novel antibiotic producing microbes because drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics (Kumar et al., 2010).

Physicochemical analysis showed that pH of the soil is acidic and is rich with both macro and micro nutrients which is favorable for the growth of fungi. Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content and moisture (Rangaswami , 1998).

In the present study, physicochemical properties of soil from Mudon Township were analyzed. The color of soil samples was red, black and brown.The temperature of soil environment of Mudon Township at the time of this investigation of July, 2018 (rainy season) revealed that these places had temperature range between 20 ° C and 28 ° C with great variation

in present moisture content (1.18- 2. 99 %) organic nitrogen (0.09- 0.15 %), phosphorous nutrient (5.88- 11.88 ppm) and potassium oxide nutrient (4.16- 12. 73 mg).Physicochemical analysis of five different soils from Mudon Township.

Ramann *et al*., 1899 also reported that due to the accumulation of more litter in scrub and deciduous forest more percentage of fungi are present in the soil for the purpose of recycling of dead organic matter. It is known that the bacteria thrive well in neutral and alkaline soils, whereas fungi show the best activity under acidic conditions.

The surface color of other strains were brown, greenish brown, and their reverse color were black, greenish brown, yellowish brown respectively. In the colony morphology, the isolated fungi were small, medium and large in size. The margins of isolated fungi were entire and the elevation of isolated fungi were flat, convex, raised, and the form of isolated fungi circular and irregular.

All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, six strains showed different level of antimicrobial activity. MP- 7 exhibited the highest antibacterial activity (25.05 mm) against *Bacillus pumilus* at 5 days and MP- 25 also showed the moderate activity (23.50 mm) on *Bacillus pumilus* at 5 days. MP- 6 gave the strong antibacterial activity (20.03 mm) against *Bacillus subtilus* at 4 days. Especially, MP- 41 showed the moderated antimicrobial activity against all test organisms.

For the human health and nutrition fungi are well known to produce both beneficial and deleterious natural agents and continue to be explored as useful sources of natural antimicrobial agents. In comparison to plants, microorganisms are highly diverse but narrowly explored (Chioma et al., 2016). It was concluded that the present research was to isolate the fungi from different soil samples and to study the antimicrobial activity of isolated fungi on eight test organisms. Further study will be focused on the fermentation conditions of selected fungus and extraction of antimicrobial compounds.

Acknowledgements

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ISOLATION AND CHARACTERIZATION OF *RHIZOBIUM* FROM ROOT NODULES OF *ARACHIS HYPOGAEA* L. (GROUNDNUT)

May Thazin Aung[1], Phyu Phyu Oo[2]

Abstract

The present study was to isolate the beneficial nitrogen fixing bacteria *Rhizobium* from root nodules of *Arachis hypogaea* L. (Groundnut). Plant sample and rood nodules sample were collected from cultivated field of Munkhrain Quarter, Myitkyina Township in Kachin State. This study was done at Research Center, University of Myitkyina from January to May 2019. The five selected *Rhizobium* strains (MTZA 1, MTZA 2 , MTZA 3, MTZA 4, MTZA 5) were subjected to culture on Yeast Mannitol agar (YMA) and Yeast Mannitol Broth (YMB). The method of Vincent (1970) was used for isolation of *Rhizobium* from root nodules. *Rhizobium* strains were medium sized, rod shaped, Gram negative and no spore. Colonies were sticky appearance, circular, varying from flat to raise shaped, margin entire and milky white colour. The *Rhizobium* strains were aerobic, motile, turbidity and the positive chemical reaction was observed in Catalase, Lactase, Glucose Peptone agar (GPA), Triple Sugar Iron Agar and Antibiotic test. The negative chemical reaction was observed in Gelatin Hydrolysis and Methylene blue test.

Keywords: *Rhizobium, Arachis hypogaea* L. isolation, characterization, biochemical tests.

Introduction

The groundnut, *Arachis hypogaea* L., a highly nutritious food, is rich in protein, minerals and vitamins. The groundnut contain fat 34 - 54% and very important in crop rotation system as they help in biological nitrogen fixation. Groundnut, *Arachis hypogaea* L., a member of family Fabaceae is usually nodulated by rhizobia of genus *Bradyrhizobium* demonstrated by Van Rossum *et al.* (1995).

Plants in Fabaceae through their symbiotic relationship with certain gram-negative soil bacteria, collectively known as rhizobia help to fix atmospheric nitrogen to obtain nutrients from the plant and producing nitrogen in process called nitrogen fixation (or) Biological Nitrogen Fixing (BNF) (Herridge *et al.* 2008).

Rhizobia is the group of soil bacteria that infect the roots of legumes to form root nodules. Rhizobia are found in the soil and after infection, produce nodules in the legumes where they fix nitrogen gas (N_2) from the atmosphere turning it into a more readily useful form of nitrogen. From here, the nitrogen was exported from the nodules and used for growth in the legume. Once the legume dies, the nodule breaks down and releases the rhizobia back into the soil where they live individually or rein fact a new legume host (Herridge *et al*. 2008).

Nitrogen fixation used biological agents is mostly associated with legumes and contributes to the sustainable agricultural development (Marta *et al.* (2014). This is a very good example of a symbiotic relationship accounting to the beneficial plant microbe interaction (Francisco *et al.* 2016).

Biological nitrogen fixation (BNF) is the cheapest and environmental friendly procedure in which nitrogen fixing microorganisms interacting with leguminous plants, fix aerobic nitrogen into soil (Md. Jakaria *et al.* 2013). Atmospheric nitrogen fixation is carried out by microorganisms to fix forms to nitrogen, such as ammonia and nitrate to be used by the plants.

[1] Assistant Lecturer, Department of Botany, University of Myitkyina
[2] Lecturer, Department of Botany, University of Myitkyina

Restoring, maintaining and increasing soil fertility are major agricultural priorities in many parts of the developing countries where soils are inherently poor in plant nutrients and the demand for grain food and raw materials in increasing rapidly. Sustainable production of crops cannot be maintained by using chemical fertilizers only. Nutrients used to be added from other sources such as organic manure and biofertilizer for providing soil fertility (Masharof *et al.* 2012).

In recent years, there is increasing in fertilizers cost, so increases crop cost. In addition, chemical fertilizers have harmful effect on the environment. Therefore a process known as inoculation or biofertilization instead developed. Rhizobial inoculants are known as an alternative to the use of industrial nitrogen fertilizers and mean to maintain or improve soil fertility (Peoples *et al.* 1995, Alver *et al.* 2003 and Chalk *et al.* 2006).

The aims and objectives of this research was to isolate the *Rhizobium* from root nodules of *Arachis hypogaea* L. from Munkhrain Quarter, Myitkyina Township, Kachin State and to identify the bacteria by using morphological characters and some biochemical tests.

Materials and Methods

Study area and collection of root nodules of *Arachis hypogaea* L.

The sample of plant, soil and root nodules of *Arachis hypogaea* L. were collected from cultivated field of Munkhrain quarter, Myitkyina Township, Kachin State. (Figure 1)

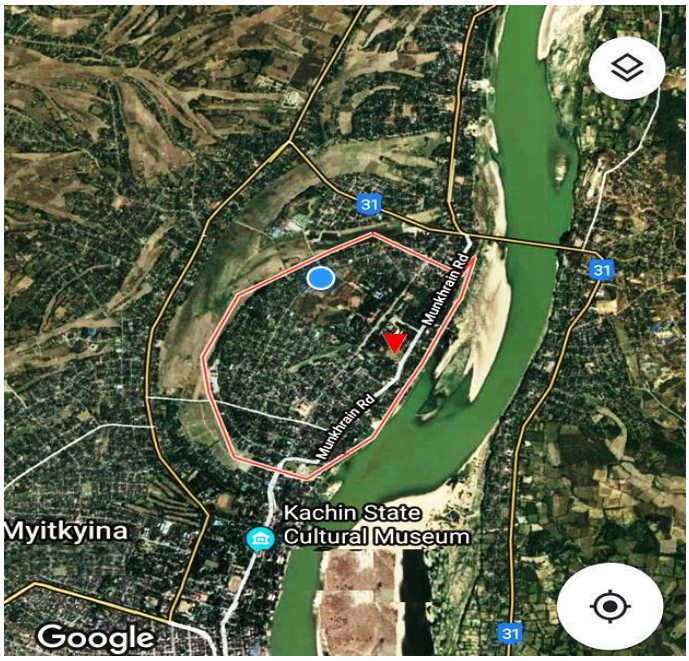

Figure 1 Location map of the study area

Source from Internet

Identification of plant sample and soil sample

The collected plants were subjected to identify with the help of literatures (Hooker 1879; Backer 1964; Dassanayake 1981). The pH value of Yeast Mannitol Broth (YMB) medium was also detected with the help of portable pH meter. The experiments of isolation and biochemical

tests were carried out at the Department of Botany, Research Center, University of Myitkyina, from January to May 2019.

Isolation of pure culture of *Rhizobium* strain isolation media, Vincent (1970)

In the isolation procedure, Yeast Mannitol Broth (YMB) and Yeast Mannitol Agar (YMA) were used as basal isolation and culture media. The composition of YMB & YMA was as follows.

Table 1 The composition of Yeast Mannitol Broth (YMB)

Mannitol	10 g
Yeast Extract	500 mg
K_2HPO_4	500 mg
$MgSO_4.7H_2O$	200 mg
NaCl	100 mg
Distilled water	1 liter
pH	6.8

Table 2 The composition of Yeast Mannitol Agar (YMA)

Mannitol	10 g
Yeast Extract	500 mg
K_2HPO_4	500 mg
$MgSO_4.7H_2O$	200 mg
NaCl	100 mg
Agar	17 g
Distilled water	1 liter
pH	6.8

In the case of Yeast Extract Agar (YMA) preparation, 17 g/1 of agar was dissolved into YMB and sterilization was done by autoclaving 15 lb/sq at 121°C for 15 minutes. 5 µL of nodule-bacterial suspension was dropped on to the culture plate with YMA medium and then were spread and incubated at 27 – 30° C in incubator single colony on YMA medium plates, selected from old cultures after 3 - 5 days. All strains were sub-cultured onto the YMA media to obtain pure culture for all experiments. Finally culture plates containing isolated bacterial strain were stored at 4°C in refrigerator for further experimental works (Figure 2).

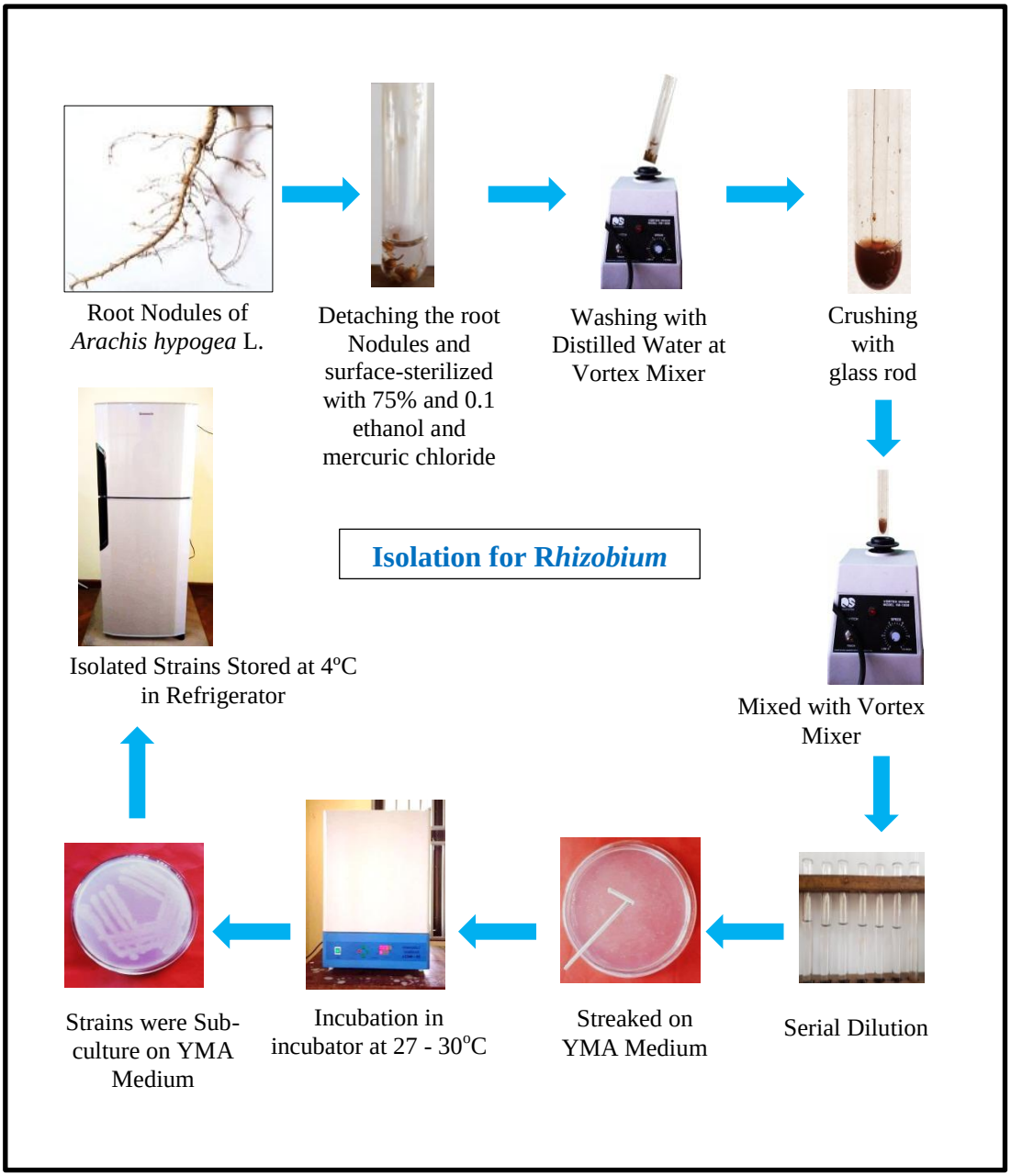

Figure 2 Isolation procedure of *Rhizobium* from root nodules

Gram staining methods of *Rhizobium* strains

The staining procedure was carried out according to the methods described by Santra *et al.* (1998).

Place a loopful of sample on a clean glass slide at angel on it. Prepare a smear/ thin film on a clean glass slide by dragging the slide over it. Air dry and heat fix the smear by passing through flame. Immerse the smear in crystal violet for one and a half minutes. Wash the slide with water and then immerse in Gram's iodine for one to one and a half minutes. At this time all cells appear violet. Wash with water and decolorize by shaking the slide gently for 10 - 15 seconds in acetone / alcohol fill the violet color comes off the slide. Immediately wash with

water and subsequently counter stain with safranin for 30 seconds. Finally, once wash the slide with water, blot dry and examine under the oil immersion lens of a microscope.

Biochemical and physiological characters of isolated five *Rhizobium* strains

The biochemical tests were carried at 28°C for 48 hours old culture. All the tests were carried out with three replicates.

Aerobic Test

Different test tube of broth cultures are prepared and then one is inoculated at room temperature. Different culture of agar test tube is prepared as usual and isolated strains are made as sub-culture and incubated at room temperature. The patterns of growth are checked daily and the oxygen requirement of the isolated strain were recorded (Prescott *et al.* 2002).

Motility Test

Motility test was tested according to the procedure described by Prescott *et al.* 2002. The composition of medium contains Gelatin 10g, sodium chloride 5.0 g, beef extract 3g, 4.0g and distilled water 1000 ml. This medium was sterilized by autoclaving at 15 psi pressure 121°C for 15 minutes and the isolated strains were inoculated into stabbing medium ¾ of the way to the bottom of the tube. During growth, motile bacteria will migrate from the line of inoculation to form a dense turbidity in the surrounding medium and non motile bacteria will grow only along the line of the inoculation.

Turbidity Test

YMB cultures medium and *Rhizobium* strain were prepared. This medium was sterilized by autoclaving at 15 lb pressure and 121^{o}C for 15 minutes and the isolated strain were inoculated into the tube of YMB medium. Incubate at the 30^{o}C for the pattern of growth is checked after two days and examine (MacFaddin 2000).

Catalase Test

Place a few drops 3% hydrogen peroxide (H_2O_2) solution onto each slide culture and watch for immediate signs of bubbling, which represented positive test; absence of bubbles indicated a negative test (MacFaddin 2000).

Glucose Peptone Agar GPA Test

GPA test was performed to determine the capability of the *Rhizobium* strains to utilize glucose as the sole carbon for its growth medium. Glucose peptone agar (40g/L glucose, 5 g/L peptone, 15 g/L agar, distilled water 1000 ml) was also used (Deora & Singal 2010).

Lactase Test

Lactase test was performed to determine capability of *Rhizobium* to utilize lactose present in medium (10 g/L lactose, 5 g/L peptone, 3 g/L beef extract, 15 g/L agar, distilled water 1000 ml) as the sole carbon source for its growth (Kucuk *et al.* 2006).

Methylene Blue Test

Methylene Blue Test was performed to check the growth of the isolates. In this test, methylene blue dye (1 mL) was added to the YMB (10 mL) and inoculated with *Rhizobium.* Inoculation was given at 30°C for 24 – 48 hours and observations were made (Gao *et al.* 1994).

Gelatin Hydrolysis Test (Liquefication Test)

The test was performed to determine capability of *Rhizobium* to produce gelatinase enzyme as use gelatin as media source. Degradation of gelatin indicates the presence of gelatinase enzyme. The actively grown cultures were inoculated in nutrient gelatin medium (5 g/L peptone, 3 g/L beef extract, 12 g/L gelatin, distilled water 1000ml) and grown for 48 hours. On subjecting the growing culture to low temperature at 4°C for 30 minutes - 60 minutes. The cultures which produce gelatinase remains liquefied while others due to the presence of gelatin become solid (Aneja 2003).

Starch Hydrolysis Test

The test was performed to determine capability of *Rhizobium* strains to use starch as carbon source. Starch agar medium (5g/1 peptone, 2g/1 beef extract, 3g/1 potato starch, 15g/ 1 agar, distilled water 1000ml, pH 7.0) were inoculated with *Rhizobium* cultures and incubated at 30^{o}C temperature for 48 hours. Iodine was used to determine capability of *Rhizobium* to use starch. Drops of iodine solution (0.1N) were spread on 48 hours old culture grown on petri-plates. Formation of blue color indicated non-utilization of starch and vice-versa (De Oliveira *et al.* 2007).

Triple Sugar Iron Agar Test

The test was performed to determine the capability of isolates to use various carbohydrates source (sucrose, glucose, lactose) as media for growth. Triple sugar medium consists of beef extract 3g/L, yeast extract 3g/L, peptone 15g/L, NaCl 5g/L, lactose 10g/L, sucrose 10g/L, dextrose 1g/L, ferrous sulfate 0.2g/L, sodium thiosulfate 0.3g/L, phenol red 0.24g/L, agar 15g/L, distilled water 1000 ml). After inoculation and incubation color was observed on the butt and the slant. On the basis of capability of organisms for use carbohydrates three possible observations were made, first after 24 hours yellow slant and red butt, second red slant and yellow butt after 48 hours whereas third after 72 hours dark red slant and dark yellow butt (Kligler 1918).

Antibiotic Resistance Test

Antibiotic resistance of the *Rhizobium* isolates was tested against to, Streptomycin and Penicillin using paper disc diffusion method (NCCLS, 1999). Cultures were inoculated by swabbing with standard inoculums according to 0.5 McFarland tube over the entire agar surface. The agar surface was allowed to dry for 3-5 minutes before applying the antibiotic discs. Antibiotic disc were placed equidistantly on 90 mm petri plate using sterile forceps. The plates were incubated aerobically at 30^{o}C for 48 hrs. Resistance to an antibiotic was detected by the inhibition zone formed around the discs. The antibiotic was used by Streptomycin (10 μg) and Penicillin (10 μg) (NCCLS 1999).

Results

Identification of *Arachis hypogaea* L.

Scientific name	-	*Arachis hypogaea* L.
Family	-	Fabaceae
Sub - Family	-	Papilionoideae
Myanmar name	-	Myae pe
English name	-	groundnut; peanut

Annual herbs; stems and branches angular, glabrous; leaves pinnately-compound, alternate, paripinnate, 4-foliotate; stipules lanceolate, acuminate at the apex; petiolue terete, leaflets ellipitic, opposite, 2-6cm by 1.0-3.5cm, green and glabrous on both surfaces, obtuse at the base, entire along the margin, rounded at the apex; petioles minute, pilose, caducous, flowers usually solitary, 1.5-1.8cm in diameter at the anthesis, yellow, bisexual, zygomorphic, pentamerous, hypogynous, bracteate, pedicellate, ebracteolate; bracts narrowly lanceolate, caducous; pedicels terete, short, pilose, calyx campanulate, 5-lobed, pale green, glabrous; tubes cylindric, 4.0-45cm long; lobes lanceolate, 0.8-1.0cm long; corolla papilionaceous; exserted; standard obovate, 0.6-1.2 cm long, yellowish with reddish lines; wings oblong, 4.8 m long, pale yellow; kneel boatshaped, 6.8 mm long, pale yellow, acute at the apex; stamens 8 or 10, monadelphous, included; filaments filiform, alternately long and short, white, glabrous; anthers dithecous, oblong, versatile, pale brown, dehiscing by longitudinal slit, ovary superior, subsessile, oblong and unilouclar, with 1 to 6 ovate ovules, the marginal placentae; styles filiform, 1.0-1.2 cm long, white glabrous; stigma simple; fruits indehiscent nut, torulose, developing underground, monoliform, commonly 1 to 3 seeded, pale brown; seeds oblongoid, with pale reddish papery coat, glabrous. (Figure 3 A, B, C, D and E)

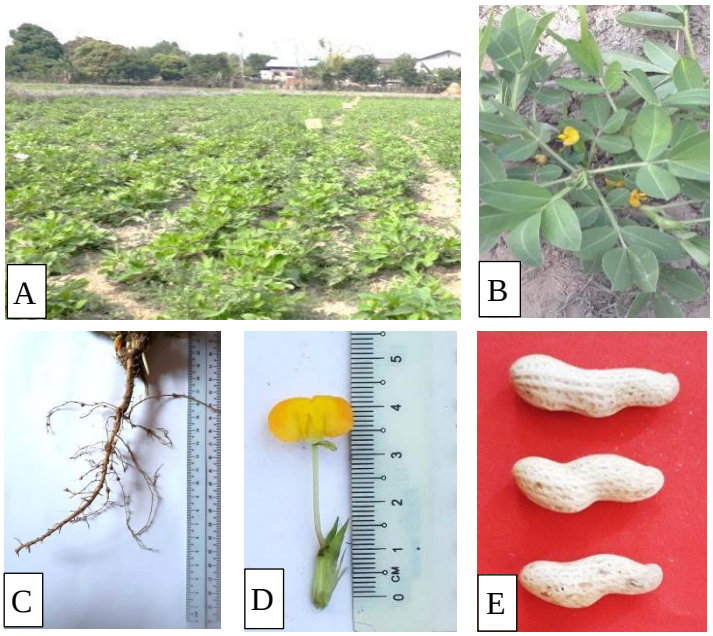

Figure 3 Identification of *Arachis hypogaea* L.

A. Cultivation field

B. Habit

C. Root nodules

D. Flower

E. Pod

Characteristics of nodule sample

Nodule types and distribution on secondary roots from the plant, as nodules may be found on the lateral roots as well as the taproot. The shape and size of the nodules recovered from the collected plants were noted. Nodules size and shape vary with the *Rhizobia* and host plant

species. Effective nodules may be found on groundnut *Arachis hypogaea* L. about 1-3 mm in diameter, most formed on tap roots and lateral roots. An active N-fixing nodule contains a protein called leghemoglobin. Its presence in the nodule can be noted by the characteristic pink to red coloration. (Figure 4. A, B, C).

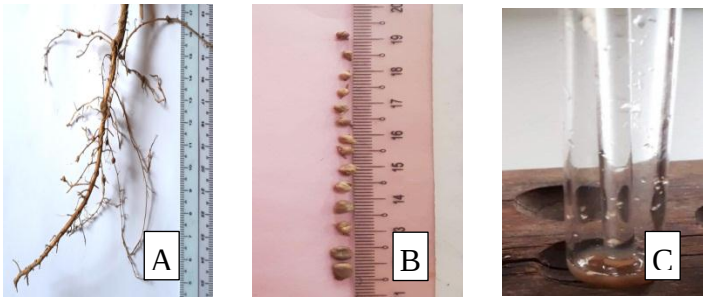

Figure 4 Morphology of root nodules

A. Roots nodules

B. Detached root nodules

C. Crushed root nodules

Morphological characters of isolated bacterial strains

Cell morphology

Rhizobium sp. or root nodule bacteria were medium sized, rod-shaped cells, 0.5-0.9 micron meter in width and 1.0-3.0 micron meter in length and they usually aerobic metabolism. Optimal growth of most strains occurs at a temperature range of 25-30^{o}C and a pH of 6.8-7.0 (Table 3, Figure 5. C).

Colony morphology

Colonies of *Rhizobium* were obtained on YMA agar medium after incubation at 30^{o}C for 2-3 days. The colonies were having sticky appearance showing the production of mucous though at lower levels. Analysis of colony morphology indicated round colonies, a smooth margin on agar surface. Colonies may be white colored till 2-3 days of growth. Typical colonies had a diameter of 2-5 mm (Table 4, Figure 5. A,B).

Table 3 Cell morphological characters of isolated *Rhizobium* strains

Characters	**Result**
Bacterium shape	Rod
Oxygen demand	Aerobic
Gram's nature	Gram negative
Consistency	Turbid
Width	0.5 - 0.9 μm
Length	1.0-3.0 μm

Table 4 Colony morphological characters of isolated *Rhizobium* strains

Characters	**Result**
Growth on media	YMA Media
Temperature	30^{o}C
Apperance	Sticky Mucous
Shape	Circular
Color	Milky White
Optical density	Opaque
Margin	Entire
Growth obtained	2-3 days
Diameter	2-5 mm

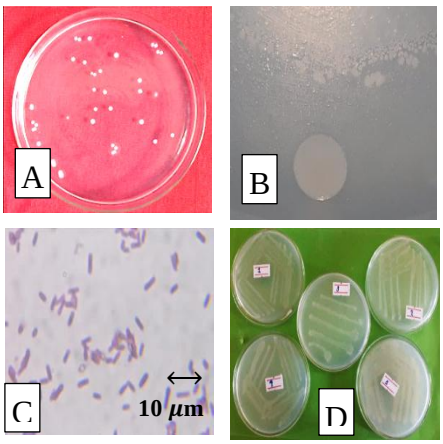

Figure 5 Colonies forming *Rhizobium* on YMA medium

A. Colonies

B. Single colony

C. Gram staining (cells)

D. Sub-culture

Biochemical test and physiology characters from five isolated *Rhizobium* Strains

According to these results, the isolated five strains were Gram negative, mucous and aerobic respiration highly positive activities in catalase, turbitity, starch hydrolysis, triple sugar iron agar, lactose and antibiotic test. Negative activities in methylene blue and gelatin hydrolysis test. (Table 5, Figure 6, 7)

Table 5 Biochemical characterization of isolated five selected *Rhizobium* Strains

Sr No.	*Rhizobium* isolates	AT	MT	TT	CT	GPAT	LT	MBT	GHT	SHT	TSIAT	AT	
												S	P
1.	MTZA 1	+	+	+	+	+	+	-	-	+	+	+	+
2.	MTZA 2	+	+	+	+	+	+	-	-	+	+	+	+
3.	MTZA 3	+	+	+	+	+	+	-	-	+	+	+	+
4.	MTZA 4	+	+	+	+	+	+	-	-	+	+	+	+
5.	MTZA 5	+	+	+	+	+	+	-	-	+	+	+	+

(+) Positive, (-) Negative reaction, AT (Aerobic test), MT (Motility test), TT (Turbidity test), CT (Catalase test), GPAT (Glucose peptone agar test), LT (Lactase test), MBT (Methylene Blue test), GHT (Gelatin hydrolysis test), SHT (Starch hydrolysis test), TSIAT (Triple sugar iron agar test), AT (Antibiotic resistance test).

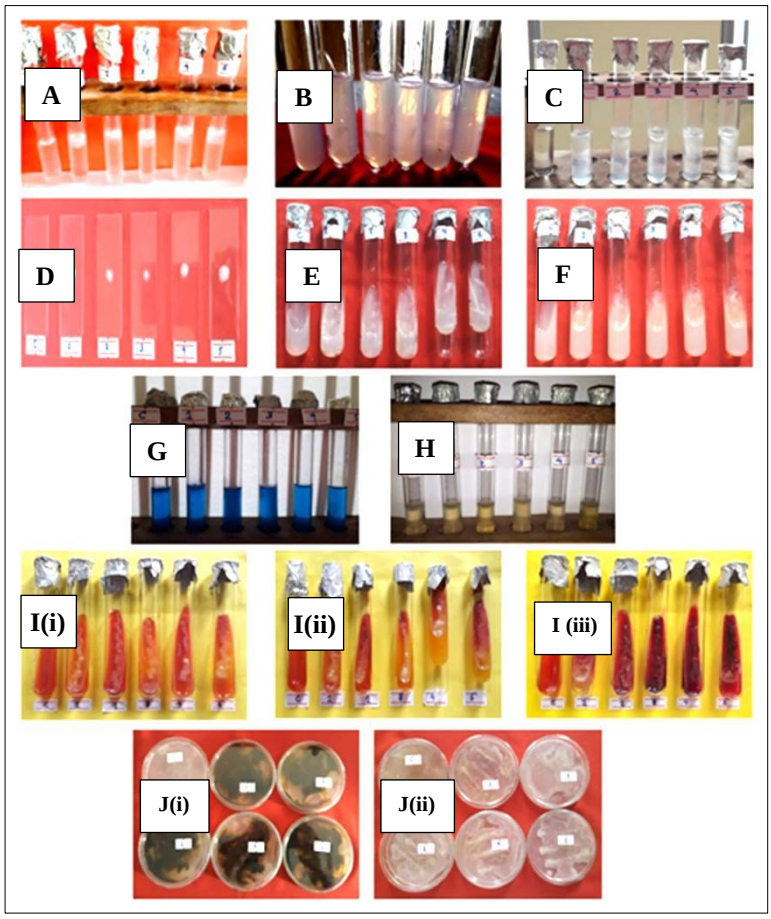

Figure 6 Biochemical tests of five *Rhizobium* strains

A. Aerobic test
B. Motility test
C. Turbidity test
D. Catalase test
E. Glucose peptone agar test
F. Lactase test
G. Methylene blue test
H. Gelatin hydrolysis test test
I(i)TSIA test (24 hour)
I(ii)TSIA test (72 hours)
I(iii)TSIA test(48 hours)
J.(i) Starch hydrolysis
J.(ii)Starch hydrolysis (after iodine)

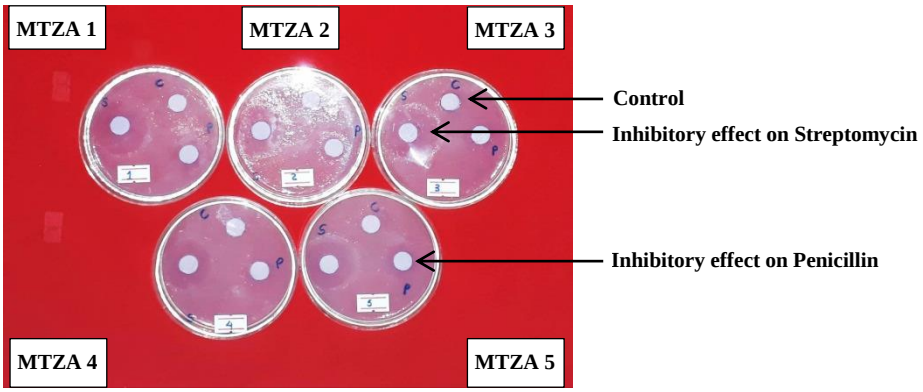

Figure 7 Antibiotic resistance test of isolated five *Rhizobium* strains Against Streptomycin and Penicillin

Discussion and Conclusion

In the present study, *Rhizobium* bacteria was isolated from root nodules of *Arachis hypogaea* L. collected from Munkhrain quarter, Myitkyina Township, Kachin State.

Arachis hypogaea L. a member of the legume family (Fabaceae) is an annual herb. The leaves are alternate and pinnate with four leaflets (two opposite pairs each leaflet is 1-7 cm long, 1-3 broad. Peanut flowers are borne in axillary clusters above the ground. The pods usually containing from one to three seeds. Each seed is covered with a thin papery seed coat. In the present work, the *Arachis hypogaea* L. were similars to those describe by Katarzyna *et al*. (2011).

According to the Sarah *et al*. (2015) the colonies were Gram negative, milky white, glisterintening and circular in shape. In the present study, five isolates showed the same colony characteristics, after 48 hours of incubation, but the colonies were not translucent. This did not agreed with Sarah *et al*. (2015).

YMA medium was used for the growth of rhizobia isolates. Similar findings were made by Kumari *et al*. (2010) for the characterization of *Rhizobium* isolates from *Indigofera* species. The morphological and cultural characteristics of isolated bacteia from nodules of *Arachis hypogaea* L. incidated that the isolated strains were grown on YMA agar plates, the colonies of isolates appeared in 2-3 days at 30^{o}C as mentioned in the results. This agreed with Burton (1967).

According to Swe Wut Hmone (2014), The bacterium was rod shaped, Gram negative, aerobic, non-spore forming and motile. It showed positive chemical reaction in aerobic, motility, catalase, glucose peptone agar (GPA), methylene blue and triple sugar iron test. In gelatin hydrolysis test, showed the negative chemical reaction. This agreed with Burton (1967). All the strains were shown positive result.

Tittsler and Sandholzer (1936) stated that the motility is observed visually by diffusing growth spreading from the line of inoculation. Certain strains of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular growths along the stab line. Non-motile organisms grow only along the line of inoculation. In the present work of the motility test result indicated that individual isolated bacteria moving in random direction showing motility. This agreed with Tittsler and Sandholzer (1936).MacFaddin (2000) stated that the cell mass is directly proportional to cell

number. Cells mass and number are also obtained by optical density method. Turbidity is developed in the liquid medium due to the prescence of cells which make cloudy appearance to the eyes. In Turbitity test of the present study, all five strains were showed positive result. In the present study of turbidity test was in agreement with MacFaddin (2000).

MacFaddin (2000) stated that the organisms containing the catalase enzyme will form oxygen bubbles when hydrogen peroxide (H_2O_2) exposed to it. In the present work, bubbles were appeared to show the positive catalase. This agreed with MacFaddin (1967). All five strains were showed positive result.

In glucose peptone agar test, all five strains were showed negative result. Wei *et al.* (2003) suggested that methylene blue was used as an agent against the growth of the microorganism. Rhizobial cells were unable to grow on medium containing 0.1% methylene blue were also suggested that one of the strain showed growth on medium containing 0.1% methylene blue. Therefore, the present research agreed with those finding by Gao *et al*. 1994 and Singh *et al*. (2008). In methylene Blue test, all five strains were shown negative result.

Kucuk *et al.* (2006) stated that the lactose is a confirmatory test for *Rhizobium* and these are able to utilize lactose as carbon source. In the present work, pure bacteria isolate was able to grow on lactose. In lactase test, five strains were shown positive result.

Hunter *et al.* (2007) stated that the gelatin hydrolysis test was observed that rhizobial cells do not produce gelatinase enzymes as medium containing gelatin solidified when kept at 4^oC for 60 minutes. Negative gelatinase activity is also a feature of *Rhizobium*. Therefore, the present research was agreed with Hunter (2007) and isolated strain may be genus *Rhizobium*. In gelatin hydrolysis test, all five strains were showed negative result. In starch hydrolysis test, all five strains were showed positive result after iodine.

According to Hajnaa (1945), yellow slants and red butt by using the utilization of glucose, lactose and sucrose in the triple sugar iron agar medium. In the present study of triple sugar iron agar test, showed yellow slants and red butt. Therefore, the present research agreed with Hajnaa (1945). In Triple Sugar Iron Agar test, of five strains positive result in 24 hours, 72 hours and 48 hours.

Hangaria *et al.* (2000) stated that the Rhizobium isolates were sensitive to tetracycline, kanamycin and streptomycin antibiotics. Detection of antibiotic resistance of the *Rhizobium* isolates was tested against Penicillin and Streptomycin using disc diffusion method NCCLS (1999). In the present study, all five strains were showed that antibiotic activities of Streptomycin and Penicillin were used to showed clear zone. Therefore, the present study was agreed with Hungaria *et al.* (2000); Sharma (2009); NCCLS (1999) and Bauer *et al.* (1966).

The present study, the five selected Rhizobium strains were isolated from root nodules of *Arachis hypogaea* L. collected from cultivated field of Munkhrain quarter, Myitkyina Township, Kachin State. *Rhizobium* sp- or root nodules bacteria were medium sized, rod-shaped cells. Colonies were obtained on YMA agar medium after incubation at 30ºC for 2-3 days. Colonies may be milky white colored till 2-3 days of growth. The isolated five strains were Gram negative, mucous and aerobic respiration, highly positive activities in catalase, turbitity, starch hydrolysis, triple sugar iron agar, lactase and antibiotic test. Negative activities in methylene blue and gelatin hydrolysis tests.

According to cell and colony morphology as well as some biochemical test, the isolated strain of bacteria were confirmed as genus *Rhizobium*. This organism is believed to increase better agricultural practices when inoculated in plant legumes. The *Rhizobium*, nitrogen fixing bacteirum is the essential feature of leguminous plants. Increased cultivation of legumes in essential for the regeneration of nutrient-deficient soils and providing nutrients to human and animals.

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We would especially thank to Dr. Soe Myint Aye, Pro-Rector, University of Myitkyina, for his valuable suggestion and enthusiastic encouragement and over all supervision of my research work. We would like to express our sincere appreciation and deep gratitude to Dr. Khin Win Naing, Professor and Head, Department of Botany, University of Myitkyina for permission to do this research work. We are also indebted to Dr. Seine Nyoe Nyoe Ko, Professor, Department of Botany, University of Myitkyina, for her valuable suggestions. We would like to express our deepest gratitude to Daw Than Than Aye, Lecturer, Myitkyina University, Dr. Yi Yi Win, Lecturer, Yadanabon University, Daw Khin Aye Win, Lecturer, Myitkyina University.

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PHYTOCHEMICAL AND ANTIMICROBIAL INVESTIGATION ON LEAVES OF *AVERRHOA BILIMBI* L.

Thet Su Hlaing[1]

Abstract

Averrhoa bilimbi L. is small tree, belongs to the family Oxalidaceae. It is locally known as Tayoke-zaung-yar. The plant was collected from Hinthada University Campus. The vegetative and reproductive parts of the fresh specimens were identified with the help of available literatures. In morphological study, the plant is small tree. The leaves are unipinnately compound and imparipinnate. The inflorescences are cauliflorous, arising in fascicles from main stem and thicker branches. The flowers are dark red-purple and hypogynous. Stamens 5+5, free and unequal. Ovary (5), axile placentation and stigma 5 fid. The fruits are berry fleshy with 5 blunt longitudinal ridges. The seeds are flattened disc like, smooth and white. The presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate were found in phytochemical investigation. According to physicochemical properties, the powdered leaves were most soluble in methanol and least soluble in chloroform. The nutrient contents of powdered leaves were examined by using David Pearson and Kjeldahl method. The presence of protein, moisture, ash, fat, fiber and carbohydrate were found in the examination. Antimicrobial activities of various crude extracts were carried out by using paper disc diffusion assay with six test organisms. Chloroform and ethyl acetate extracts indicated more effective against the test organisms. Methanol and ethanol extracts showed moderate antimicrobial activity. These results showed that *Averrhoa bilimbi* L. leaves are rich in many active constituents, nutrients and antimicrobial properties and has potential to be used as medicinal application.

Keywords : *Averrhoa bilimbi* L., phytochemical investigation, antimicrobial activity

Introduction

Medicinal plants have been used as traditional treatments for numerous human diseases in many parts of the world. *Averrhoa bilimbi* L. is long lived perennial evergreen tree widely cultivated in the gardens and fields. Flowering more or less throughout the year (Dassanayake, 1999).

It has been used in the traditional medicine for the treatment of a variety of ailments. Infusions and decoctions of the leaves are used as an antibacterial, antiscorbutic, astringent, postpartum protective medicine, in the treatment of fever, inflammation of the rectum and diabetes.

A leaf infusion is a remedy for coughs and is taken after childbirth as a tonic. The paste of leaves is used in the treatment of itches, boils, skin eruptions, bites of poisonous creatures, rheumatism, cough, cold, mumps and syphilis (Kumari, 2017).

The objectives of this study are to identify and confirm the morphological characters, to examine the solubility test and phytochemical properties, to determine the nutritional value and to evaluate the antimicrobial activity of leaves of *Averrhoa bilimbi* L.

[1] Dr, Lecturer, Department of Botany, East Yangon University

Materials and Methods

Collection and identification

The specimens of *Averrhoa bilimbi* L. were collected from Hinthada University Campus, especially during the flowering and fruiting period from February to December, 2018. After the collection, the plants were identified with the help of literatures Backer, 1963; Hooker, 1875 and Dassanayake, 1999.

Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.

The preliminary phytochemical investigation on powdered leaves were carried out to determine the presence or absence of the chemical constituents such as a alkaloid, phenolic compound, flavonoid, steroid, terpenoid, starch, reducing sugar, glycoside, saponin, tannin, α-amino acids, protein and carbohydrate according to the method of British Pharmacopoeia, 1968; Marini Bettolo, 1981; Central Council for Research in Unani Medicine, 1987; Trease and Evans, 2002 and Harborne, 2005.

Test for Alkaloid

The powdered sample (3 g) was boiled with (50 mL) of methanol and filtered. The filtrate was divided into three portions and tested with 1 % hydrochloric acid and Mayer's reagent, Wagner's reagent and Hager's reagent. The precipitate formed on addition the reagent indicates the presence of alkaloid (Central Council for Research in Unani Medicine, 1987).

Test for Glycoside

The powdered sample (2 g) was boiled with (25 mL) of methanol for about 20 minutes, allowed to cool and filtered. The filtrate was treated with 1 mL water and sodium hydroxide solution. Yellow green colouration developed within three minutes (Marini Bettolo *et al*., 1981).

Test for Phenolic Compound

The powdered sample (2 g) was boiled with (25 mL) of methanol and filtered. When the filtrate was treated with 2 mL water and 10 % ferric chloride solution, it gave black colouration, indicating the presence of phenolic compound (Marini Bettolo *et al*., 1981).

Test for Flavonoid

The powdered sample (2 g) was extracted with (25 mL) of methanol and filtered. When the methanolic extract was treated with a few drops of dilute hydrochloric acid was added followed by a small piece of magnesium. The solution was boiled for a few minutes. The appearance of pink colour indicates the presence of flavonoid (Central Council for Research in Unani Medicine, 1987).

Test for Steroid and Terpenoid

The powdered sample (2 g) was extracted (25 mL) of with methanol and filtered. When the chloroform and sulphuric acid were added, it furnished a change of colour reddish brown indicating the presence of steroid/ terpenoid (Central Council for Research in Unani Medicine, 1987).

Test for Tannin

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered. The filtrate was treated with a few drops of 5 % ferric chloride and dilute sulphuric acid. Yellow brown precipitate was produced indicating the presence of a tannin (Central Council for Research in Unani Medicine, 1987).

Test for Saponin

The powdered sample (2 g) was put into a test tube and (2 mL) of distilled water was added. The mixture was vigorously shaken for a few minutes. Observation was made to see if frothing tool place (Marini Bettolo *et al*., 1981).

Test for α-Amino acid

The powder sample (2 g) was boiled with (25 mL) of distilled water for 20 minutes and filtered. And then, a few drops of each filtrate was spotted on a filter paper using a capillary tube, allowed it to dry and spray with ninhydrin reagent. The filter paper was dried at room temperature and then kept it in oven at 110^{o}C for a few minutes, after which the pink colour appears due to the presence of α-amino acid (Marini Bettolo *et al*., 1981).

Test for Protein

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered. To this filtrate a mixture of million's reagent was added. White precipitate deposited and then turned red when heated (Trease and Evans, 2002).

Test for Reducing Sugar

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered. To this filtrate a mixture of Benedict's solution was added and boiled for a few minutes on boiling water bath. Brick red precipitate deposited, when the solution was allowed to cool (Trease and Evans, 2002).

Test for Starch

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered, 2 drops of iodine solution were added to filtrate. Observation was made to see if brown precipitate were formed (Central Council for Research in Unani Medicine, 1987).

Test for Carbohydrate

The powdered sample (2 g) was boiled with (25 mL) of distilled water for 20 minutes and filtered. The filtrate was placed into a test tube 5 % α-napthol solution was added and shaken for a few minute. The test tube was kept inclined at an angle of 45^{o} and about 1 mL of concentrated sulphuric acid was slowly introduced along the inner side of the test tube. A red ring was formed between the two layers (Trease and Evans, 2002).

Solubility test on leaves of *Averrhoa bilimbi* L.

The solubility characters such as extractive values for the various solvents were determined according to British Pharmacopoeia, 1968.

Determination of distilled water, acetone, chloroform, ethanol, ethyl acetate, methanol, petroleum ether soluble matter contents

Distilled water, acetone, chloroform, ethanol, ethyl acetate, methanol, petroleum ether soluble matter contents were determined by the methods given in the British Pharmacopoeia (1968). Five grams of powdered sample was weighed and place in a conical flask, 50 mL of each solvent was added and the flask was stoppered with a cork. The sample was soaked for 3 days. The content was filtered and then the filtrate was taken in a petridish and evaporated to dryness on a water bath at 105^{o}C. The experiments was repeated at least three times for each solvents as the above procedure mentioned. The different in weight give the soluble matter contents in each solvent.

Nutritional property analysis of powdered leaves of *Averrhoa bilimbi* L.

The nutrient content in powdered leaves of *Averrhoa bilimbi* L. were analyzed by using David Pearson and Kjeldahl method at the Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigation, North Okkalapa Township, Yangon Division, Republic of the Union of Myanmar.

Experimental analysis

The chemical composition of powdered samples was determined: dry matter, by drying at 105^{o}C to constant weight; crude fat, by Soxhlet extraction with diethyl ether; crude ash, by incineration n a muffle furnace at 580^{o}C for 8 hours; crude protein (N × 6.25) by the Kjeldahl method; carbohydrates were calculated as total carbohydrates (%) = 100 % (moisture + crude protein + crude fat + ash + crude fiber). The fibre components were determined by using the detergent method.

Antimicrobial activity of various solvent extracts from leaves of *Averrhoa bilimbi* L.

Various crude extracts of powdered leaves such as acetone, chloroform, ethyl acetate, ethanol, methanol, petroleum ether and distilled· water extracts were used for antimicrobial study. Screening of antimicrobial activity was done by paper disc diffusion assay according to Madigan and Martinko, 2005 at Microbiology Lab, University of Yangon. The six test organisms (four bacterial strains and two fungal strains) were utilized for antimicrobial activity.

Table 1 Test organisms utilized to the antimicrobial activities

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

Madigan and Martinko, 2005

Preparation of antimicrobial activity test

Paper disc diffusion assay was used according to the method described by Madigan and Martinko, 2005. The assay medium (agar 2.0 g, sucrose 1.0 g, NaCl 0.1 g, yeast extract 0.3 g, distilled water 100 ml, pH 7.0) was utilized for these test organisms. Test organisms (0.3 mL) was added to 100 mL assay medium, then poured into plates. After solidification, about 0.2 mL of crude extracts was impregnated onto the paper disc with the size of 6mm diameter on the test agar plates and these plates were incubated for 24-36 hours at 30^{o}C. After 24-36 hours, clear zones (inhibitory zones) surrounding the test discs were measured. These zones indicate the presence of the bioactive compounds which inhibit the growth of test organisms.

Results

Morphological characters

Small tree, about 10 meters high. Leaves alternate, unipinnately compound, imparipinnate, terminal leaflet larger than other, 10 to 17 pairs, oblong, 2.0 to 10.0 cm long, and 1.2-1.25 cm wide, the bases subcordate, the margins entire, the tips acuminate, petiolate and exstipulate. Inflorescences are cauliflorous, arising in fascicles from main stem and thicker branches. Flowers are dark red-purple, bracteate, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, pentamerous, cyclic, hypogynous. Calyx (5), synsepalous, petaloid (reddish green), 5-7 mm long. Petal (5), synpetalous, petaloid (dark red purple), 12-18 mm long and 4 mm wide. Stamens 5+5, the filament free and unequal, the anther two whorl, 10-12 mm long, the shorter 4-5 mm long, dithecous, extrorse, dorsifixed, longitudinal dehiscence. Ovary (5), syncarpous, pentalocular, axile placentation, one ovule in each locule, the stigma 5 fid. Fruit berry fleshy with 5 blunt longitudinal ridges, greenish yellow when ripe. Seeds, flattened disc like, smooth and white.

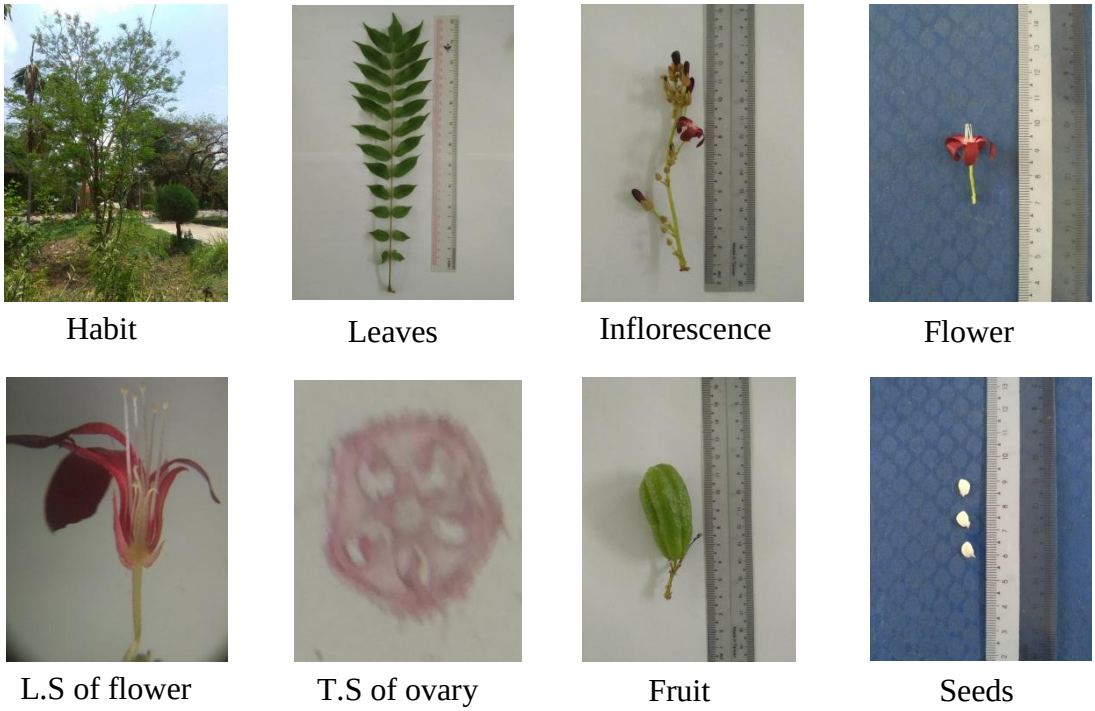


Figure 1 Morphological characters of *Averrhoa bilimbi* L.

Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.

The preliminary phytochemical test of powdered leaves of *Averrhoa bilimbi* L. indicated that the presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate. The results were shown in Table (2) and Figure (2).

Table 2 Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.

No.	Tests	Extracts	Test reagents	Observation	Results
1.	Alkaloid	Methanol	1 % HCl + Mayer's reagent	White ppt.	+
		Methanol	1 % HCl + Wagner's reagent	Brown ppt.	+
		Methanol	1 % HCl + Hager's reagent	Yellow ppt.	+
2.	Glycoside	Methanol	1 mL H_2O + NaOH	Yellow green color	+
3.	Phenolic compound	Methanol	2 mL H_2O + 10 % $FeCl_3$	Black color	+
4.	Flavonoid	Methanol	Mg coil + HCl (dil)	Pink color	+
5.	Steroid/ Terpenoid	Methanol	$CHCl_3$ + H_2SO_4 (conc.)	Reddish brown color	+
6.	Tannin	Water	5 % $FeCl_3$ + H_2SO_4 (dil)	Yellow brown ppt.	+
7.	Saponin	Water	Shaken with 2 mL H_2O	Frothing	+
8.	α-amino acid	Water	Ninhydrin reagent	Pint spot.	+
9.	Protein	Water	Millon's reagent (heated)	White ppt. turned red when heated	+
10.	Reducing sugar	Water	Benedict's solution	Brick red ppt.	+
11.	Starch	Water	Iodine	Brown ppt.	+
12.	Carbohydrate	Water	5 % α napthol sol: + H_2SO_4 (conc.)	Purple ring	+

(+) = Present

Figure 2 Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.

Solubility test on leaves of *Averrhoa bilimbi* L.

The solubility of powdered leaves were investigated to determine amount of total solids soluble in various solvents. The solubility of powdered leaves were found to be mostly soluble in methanol and least soluble in chloroform. The results were shown in Table (3) and Figure (3 and 4).

Table 3 Solubility test on leaves of *Averrhoa bilimbi* L.

No.	Solubility properties	Content in %
1.	Distilled water	5.07
2.	Ethanol	5.20
3.	Methanol	6.80
4.	Ethyl acetate	2.73
5.	Acetone	1.73
6.	Petroleum ether	2.00
7.	Chloroform	1.26

1. Distilled water
2. Ethanol
3. Methanol
4. Ethyl acetate
5. Acetone
6. Petroleum ether
7. Chloroform

Figure 3 The solubility test of various solvents of *Averrhoa bilimbi* L.

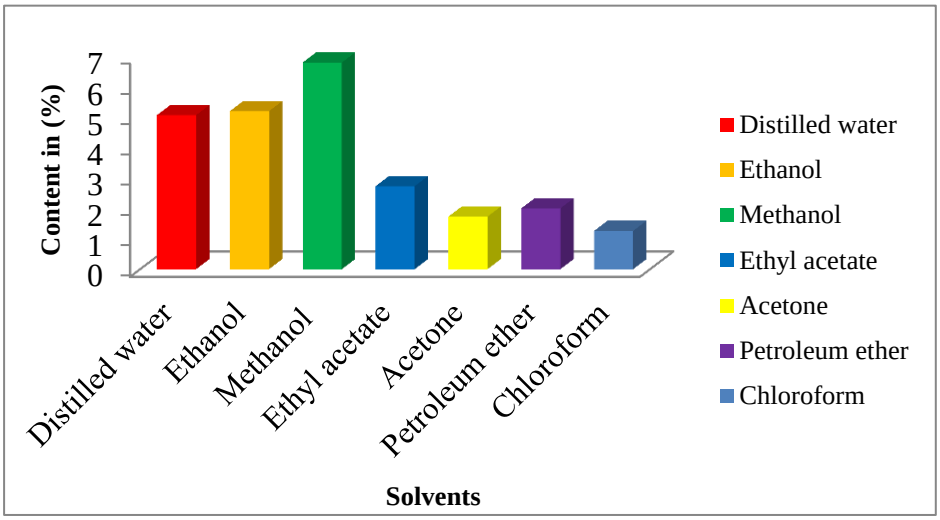


Figure 4 The solubility test of various solvents of *Averrhoa bilimbi* L.

Nutrient analysis of powdered leaves of *Averrhoa bilimbi* L.

The nutrient content in powdered leaves were determined by using David Pearson and Kjeldahl method. In this analysis yielded protein, moisture, ash, fat, fiber and carbohydrate. The results were shown in Table (4) and Figure (5) and (6).

Table 4 Nutrient content of powdered leaves of *Averrhoa bilimbi* L.

No.	Components	Values (%)
1.	Protein	12.28
2.	Moisture	9.53
3.	Ash	5.93
4.	Fat	3.34
5.	Fiber	21.95
6.	Carbohydrate	46.97

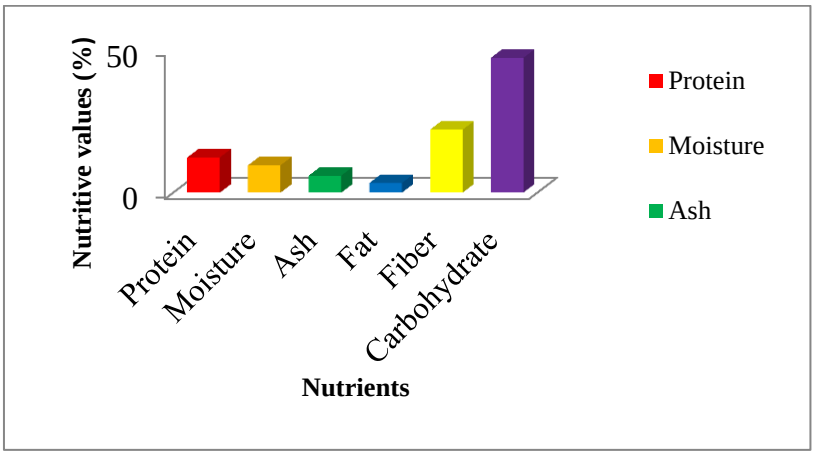
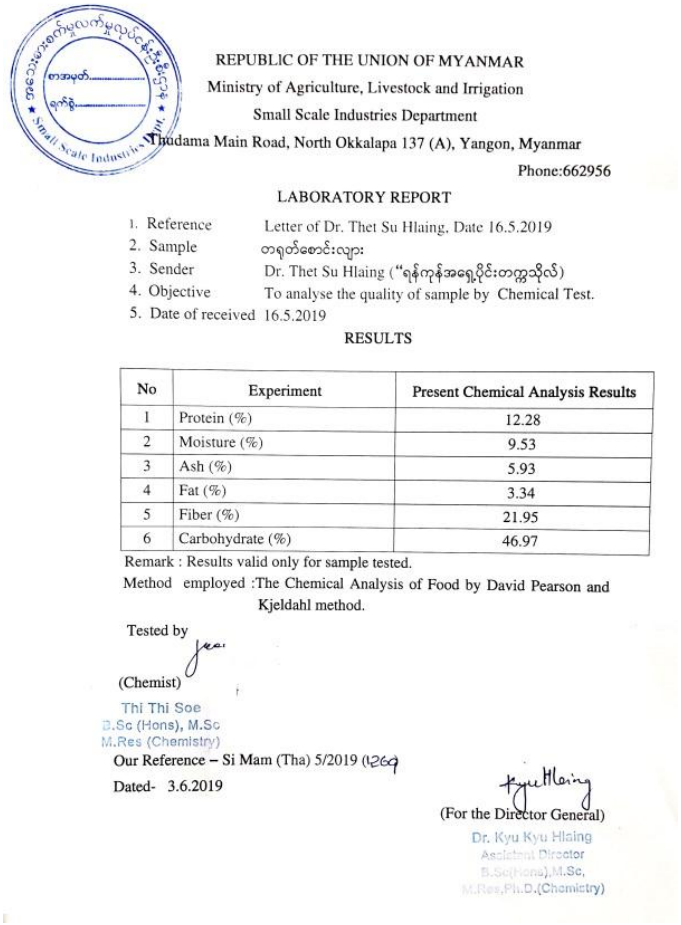


Figure 5 Nutrient composition of powdered leaves of *Averrhoa bilimbi* L.



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

LABORATORY REPORT

1. Reference — Letter of Dr. Thet Su Hlaing, Date 16.5.2019
2. Sample — တရုတ်စောင်းလှာ
3. Sender — Dr. Thet Su Hlaing ("ရန်ကုန်အရှေ့ပိုင်းတက္ကသိုလ်)
4. Objective — To analyse the quality of sample by Chemical Test.
5. Date of received — 16.5.2019

RESULTS

No	Experiment	Present Chemical Analysis Results
1	Protein (%)	12.28
2	Moisture (%)	9.53
3	Ash (%)	5.93
4	Fat (%)	3.34
5	Fiber (%)	21.95
6	Carbohydrate (%)	46.97

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

Tested by
(Chemist)
Thi Thi Soe
B.Sc (Hons), M.Sc
M.Res (Chemistry)

Our Reference – Si Mam (Tha) 5/2019 (1264)
Dated- 3.6.2019

(For the Director General)
Dr. Kyu Kyu Hlaing
Assistant Director
B.Sc(Hons),M.Sc,
M.Res,Ph.D.(Chemistry)

Figure 6 Nutrient composition of powdered leaves of *Averrhoa bilimbi* L.

Antimicrobial activity of leaves of *Averrhoa bilimbi* L.

In this investigation, chloroform, ethyl acetate, ethanol and methanol extracts observed against on *Aspergillus flavous*, *Bacillus subtilis*, *Escherichia coli* and *Xanthomonas oryzae*, Acetone, distilled water and petroleum ether extracts did not show antimicrobial activity on all tested organisms. The results were shown in Table (5) and Figure (7).

Table 5 Antimicrobial activity of various solvent extracts from leaves of *Averrhoa bilimbi* L.

Solvents / Test organisms	Acetone	$CHCl_3$	EtOAc	EtOH	MeOH	PE	DW
Aspergillus flavous	-	-	24 mm	-	-	-	-
Bacillus subtilis	-	36 mm	26 mm	-	-	-	-
Candida albicans	-	-	-	-	-	-	-
Escherichia coli	-	24 mm	16 mm	14 mm	16 mm	-	-
Pseudomonas fluorescences	-	-	-	-	-	-	-
Xanthomonas oryzae	-	30 mm	30 mm	16 mm	22 mm	-	-

Paper disc size = 6 mm

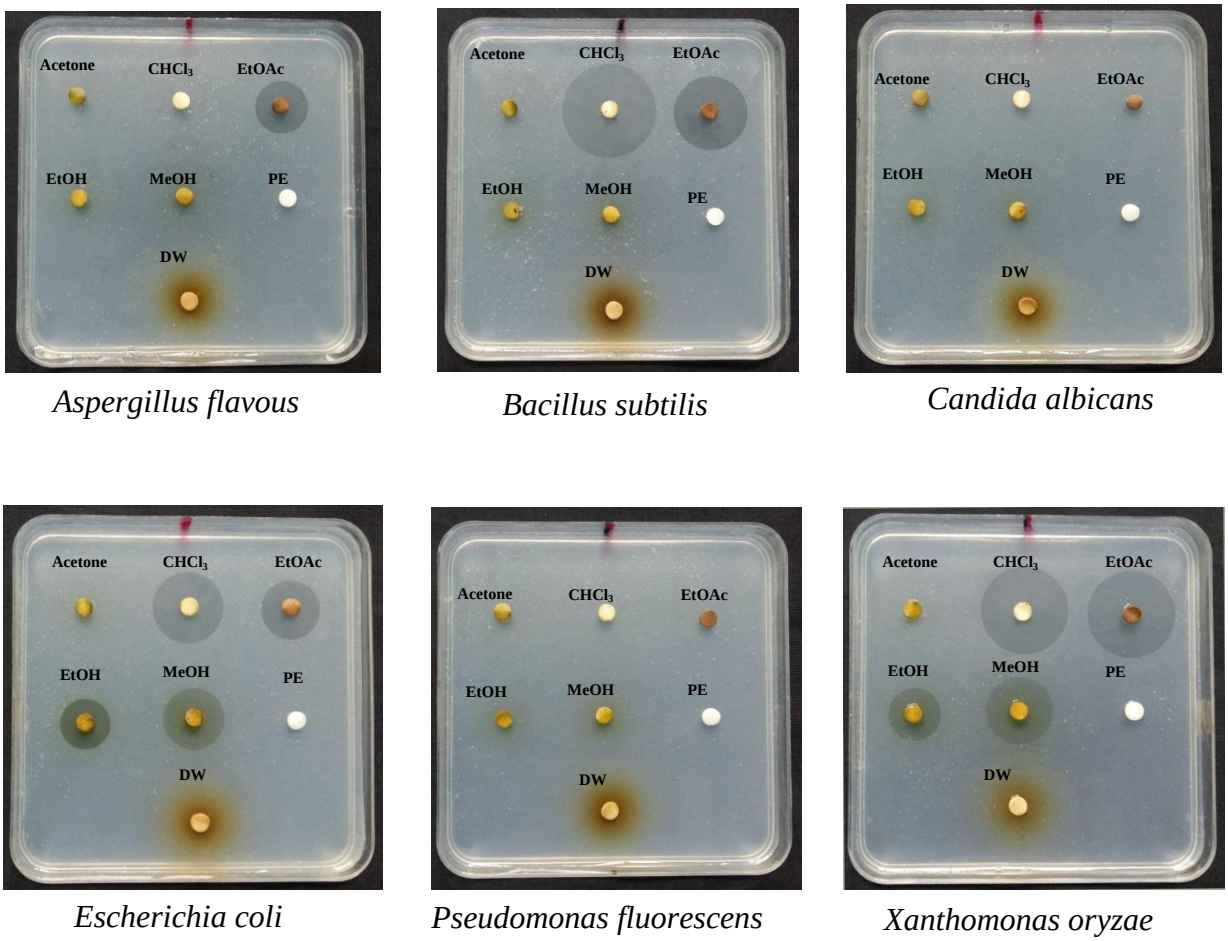


Figure 7 Antimicrobial activity of various solvent extracts from leaves of *Averrhoa bilimbi* L.

Discussion and Conclusion

In this research, morphological characters, preliminary phytochemical tests, physicochemical properties, nutritional values and antimicrobial activities on the powdered leaves had been undertaken.

In morphological study, *Averrhoa bilimbi* L. is small tree. The leaves are unipinnately compound, alternate, imparipinnate, petiolate and exstipulate. The inflorescences are cauliflorous, arising in fascicles from main stem and thicker branches. The flowers are dark red-purple, pentamerous and hypogynous. Stamens 5+5, free and unequal. Ovary (5), axile placentation and stigma 5 fid. The fruits are berry fleshy with 5 blunt longitudinal ridges. The seeds are flattened disc like, smooth and white. These characters are in agreement with those mentioned by Backer, 1963; Hooker, 1875 and Dassanayake, 1999.

Alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, α-amino acids, protein, reducing sugar, starch and carbohydrate were found in preliminary phytochemical tests.

In solubility properties, the powdered leaves were most soluble in methanol and least soluble in chloroform. This result showed that methanol should be the solvent of choice.

In the nutritional property analysis, the protein content (12.28 %), moisture (9.53 %), ash (5.93 %), fat (3.34 %), fiber (21.95 %) and carbohydrate (46.97 %) were obtained from the

powdered leaves. This data indicated that the leaves are rich source of carbohydrate, fiber, protein and are low in fat. According to the literatures, carbohydrate may serve as supplements for energy, as they have potentials to improve the health status of its users. Its fiber content can help to enhance gastrointestinal function, prevents constipation and may reduce cholesterol content. Therefore *Averrhoa bilimbi* L. has valuable nutritional components and could be a complementary nutrient.

The antimicrobial activities of various crude extracts such as acetone, chloroform, ethyl acetate, ethanol, methanol, petroleum ether and distilled water were determined by using paper disc diffusion method with six test microorganisms. These test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. In this result, chloroform, ethyl acetate, ethanol and methanol extracts showed antimicrobial activity against on *A. flavous*, *B. subtilis*, *E.coli* and *X. oryzae*. Chloroform extract exhibited a maximum inhibition zone of (36 mm) and (30 mm) against *E. coli* and *X. oryzae*. Ethyl acetate extract showed a maximum zone of inhibition against *X. oryzae* (30 mm) followed by *B. subtilis* (26 mm). Ethanol extract against *E. coli* (14 mm) and *X. oryzae* (16 mm). Methanol extract showed antimicrobial activity against on *X. oryzae* (22 mm) and *E. coli* (16 mm).

The results found in this study concerning the activity of *Averrhoa bilimbi* L. leaves extracts are in agreement with other previous works which found significant antimicrobial activity leaves methanol and ethanol extracts against *Escherichia coli* and *Xanthomonas oryzae*. The antimicrobial activities may be due to strong occurrence of active compounds i.e. saponins, tannins, alkaloids, steroids, phenols and flavonoids. Results of this finding confirmed the use of *Averrhoa bilimbi* L. as traditional medicine. This indicated that the plant have potentially antimicrobial properties and could be used in the development of novel antimicrobial agents.

Acknowledgements

I would like to express grateful to Dr. Daw San Khaing, Professor and Head, Department of Botany, East Yangon University, for her invaluable advices and kind suggestion. I am also thankful to Dr. Thida Htoo, Professor, Department of Botany, East Yangon University, for her beneficial advices and constant encouragement.

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A STUDY ON GREEN ALGAE SPECIES IN DAGON MYOTHIT (EAST) TOWNSHIP, YANGON REGION

Khin Khin Phyu[1], Htay Htay Myint[2]

Abstract

The sample species were collected from four different sites in Dagon Myothit East Township from November 2018 to May 2019. The present investigation is an attempt to record existing green algal species in study area by following proper methodology and identification procedures. All together 24 taxa of green algae belong to 16 Families, 9 Orders, 4 Classes in Division Chlorophyta were identified, described and recorded. The habitat characters of collected species were aquatic, aerial and attachment. *Bracteacoccus, Apatococcus and Chlorella* were found as aerial algae. *Characium, Oedogonium, Chaetophora, Stigeoclonium* and *Protoderma* were found attachment algae. *Chloromonas, Pandorina, Asterococcus, Pyrobotrys, Pediastrum, Scenedesmus, Gloeocystis, Microspora, Rhizoclonium, Pithophora, Mougeotia, Spirogya and Cosmarium* were found as aquatic algae. The results of this study give information about habitats characters of existing green algae in Dagon Myothit (East).

Keywords; Green algae, aquatic, aerial, attachment algae

Introduction

Algae are plants. Some consist of only a single cell, single filament, or branched filaments. Many species are capable of self – locomotion by twisting, bending, gliding, and swimming and motile spores and gametes develop in many species.

About 5000 species of green algae have been described and named. As a group of plants, they are quite varied in structure, in appearance, and in several other ways. They are usually predominantly green in color during the vegetative phase, containing chlorophyll, Xanthophyll, and carotene in about the proportion found in the seed plants. They grow chiefly in water, both fresh and marine, but occur also on and in the soil and on many other kinds of moist substrates. (Pooja, 2011).

Chlorophyceae is one of the most dominant groups of phytoplankton in integrated fishponds. Many species of Chlorophyceae are direct or indirect food for fish. For instance, Filamentous species are good for common crop and Tilapia. (Weimin 2002).

The members of Chlorophyta or green algae are abundantly found in fresh water, brackish water and marine water environments. The water body harboring them may be lentic or lotic. The lentic environment may be characterized by a static pond, more stable permanent pools, ponds and lakes in the form of natural and man-made sources. Lotic systems comprise all kinds of flowing water from a small stream to huge rivers. Besides these, any moist surface such as wet soil, rocks, tree trunks, walls of old buildings can also support growth of algae. The algal thallus ranges from unicellular mucilaginous colonies to multicellular compact forms which show considerable diversity in form and adaptation to their distinctive environment (Krishnamurthy, 2000).

Chlorococcales are economically important as food of fish. It is generally known that a number of aquatic algae from the food of fish either directly or indirectly.

Therefore, this study gives information of some epiphytic species, free floating species, subarial species of Chlorophyta in Dagon Myothit (East).

[1] Demonstrator, Department Botany, Dagon University
[2] Lecturer, Department Botany, Dagon University

Material and Methods

Study Area

Specimens were collected from 4- Sites in Dagon Myothit (East) during November 2018 to May 2019 at Latitude 16° 53´ N and Longitude 96° 17´. The site - 1 Dagon University Campus, the site - 2 is ornamental lake in 8- Quarter, the sites - 3 channels in 3 quarter and site – 4 is shallow water ponds in 5 quarter.

Collection of the Algal Specimens

The algae samples were collected from 4 sites in Dagon Myothit (East). The samples were taken from tree trunk, surface water, aquatic plants and cement wall in Dagon Myothit (East) which were measured by Global Position System (GPS), temperatures were measured by thermometer and pH of water was measured by using pH meter. The collected algal specimens were examined by using electron microscope and recorded by camera in Laboratory, Department of Botany, Dagon University.

Identification and Classification of Algae

Then they were identified on the basic of thallus shape, size, color, chloroplast, pyrenoids and sinus structure. The microscopically description and nomenclature, identification of the algal species were done according to Smith (1950), Prescott (1962), John *et al.* (2008) and other cited literatures. Moreover, the specimens were arranged by taxonomic procedures according to John *et al*. (2008).

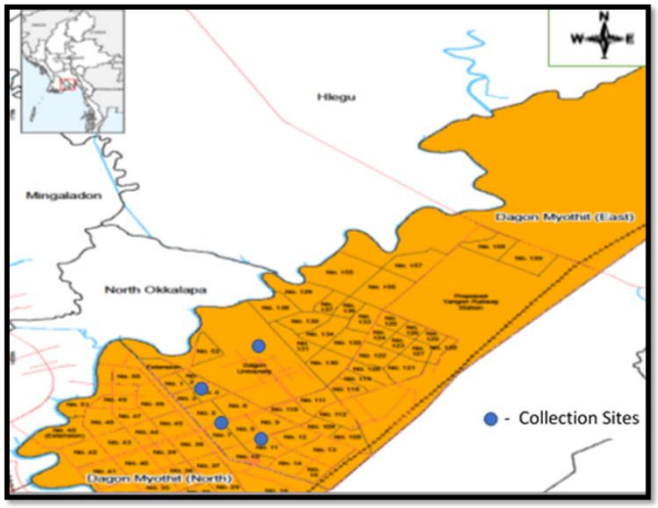


Figure 1 Location Map of Dagon Myothit (East) Area

Results

Specimens were collected from different habitats such as aquatic algae, arial algae and unusual algae from different 4- Sites in Dagon Myothit (East) during November 2018 to May 2019.The site - 1 Dagon University Campus, the site - 2 is ornamental lake in 8- Quarter, the sites - 3 channels in 3 quarter and site – 4 is shallow water ponds in 5 quarter. The study period included the cold season and the hot season. The highest average temperature of study period in Dagon Myothit East is 33 º C. The lowest average temperature of study period in Dagon Myothit East is 20 º C. In this study, a total of 24 green algae taxa collected. Among them, 21 taxa were to species level and 3 taxa were identified to genus level on the basic of their microscopically

characteristics. Systematic Enumeration of Identified algal specimens were shown in Table -1. Occurrence of Conducted Algae in Collection Sites were shown in Table -2The photomicrograph of conducted algal were showed in Plate - 1 to Plate - 4.

Table 1 Systematic Enumeration of Identified algae

Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae Volvocaceae Palmellopsidaceae Spandylomoraceae	1. *Chloromonas serbinowi* 2. *Pandorina morum* 3. *Asterococcus siderogloeus* 4. *Pyrobotrys casinoensis*
	Chlorococcales	Chlorococcaceae Characiaceae Hydrodictyaceae Scenedesmaceae	5. *Bracteacoccus cohaerans* 6. *Characium angustum* 7. *Pediastrum obtusum* 8. *Scenedesmus arcuatus* 9. *Scenedesmus ecornis* 10. *Scenedesmus plantonicus*
	Oedogoniales	Oedogoniaceae	11. *Oedogonium sp*
Ulvophyceae	Chaetophorales	Chaetophoraceae	12. *Chaetophora pisiformis* 13. *Stigeoclonium farctum* 14. *Protoderma viride*
	Sphaeropleales	Radiococcaceae Microsporaceae	15. *Gloeocystis ampla* 16. *Microspora amoena*
	Cladophorales	Pithophoraceae	17. *Rhizoclonium hieroglyphicum* 18. *Rhizoclonium riparium* 19. *Pithophora mooreana*
Trebouxiophyceae	Chlorellales	Chlorellaceae	20. *Apatococcus lobatus* 21. *Chlorella vulgaris*
Conjugatophyceae	Zygnematales Desmidiales	Zygnemataceae Desmidiaceae	22. *Mougeotia sp* 23. *Spirogya sp* 24. *Cosmarium granatum*

Table 2 Occurrence of Conducted Algae in Collection Sites

No.	Scientific Name	Site 1	Site 2	Site 3	Site 4
1.	*Chloromonas serbinowi*				✓
2.	*Pandorina morum*				✓
3.	*Asterococcus siderogloeus*			✓	
4.	*Pyrobotrys casinoensis*		✓		
5.	*Bracteacoccus cohaerans*	✓			
6.	*Characium angustum*	✓			
7.	*Pediastrum obtusum*		✓		
8.	*Scenedesmus arcuatus*			✓	
9.	*Scenedesmus ecornis*				✓
10.	*Scenedesmus plantonicus*		✓		
11.	*Oedogonium sp*	✓			
12.	*Chaetophora pisiformis*	✓			
13.	*Stigeoclonium farctum*	✓			
14.	*Protoderma viride*	✓			
15.	*Gloeocystis ampla*		✓		
16.	*Microspora amoena*		✓		
17.	*Rhizoclonium hieroglyphicum*		✓		
18.	*Rhizoclonium riparium*		✓		
19.	*Pithophora mooreana*		✓		
20.	*Apatococcus lobatus*			✓	
21.	*Chlorella vulgaris*			✓	
22.	*Mougeotia sp*				✓
23.	*Spirogya sp*		✓		
24.	*Cosmarium granatum*			✓	

Taxonomic Description of Identified algae specimens

1. *Chloromonas serbinowi* Starr & Zeikus, 1987

Cell solitary, spherical, covered by layer of mucous sheath, chloroplast cup shaped, cell 20µm in wide.

Habitat- aquatic, Occurrence - shallow water pond

2.*Pandorina morum* (O.F.Muller) Bory, 1827

Colony globose; cell pyriform; compactly arranged and enclosed by a common gelatinous envelope; 68 µm in wide.

Habitat- aquatic, Occurrence - shallow water pond

3.*Asterococcus siderogloeus* Pascher & R. Johoda Novakova, 1964

Cell ovoid, 4- celled enclosed by gelatinous envelope, chloroplast stellate, pyrenoid central, cell 30 µm wide, colony 87 µm wide.

Habitat-aquatic, Occurrence – Channel

4. *Pyrobotrys casinoensis* (Playfair) P.C. Silava, 1972

Coenobia 25µm in wide, with cells and flagella all pointing in same direction; cells pear – shaped and narrowed basally, 7.5 µm in wide, 25µm in long.

Habitat-aquatic, Occurrence - lake

5. *Bracteacoccus cohaerans* H.W. Bischoff & H.C. Bold, 1963

Cell spherical, unicellular but aggregate, chloroplast numerous, cell 8 µm wide.

Habitat- aerial, Occurrence – cement wall

6. *Characium angustum* A. Braun, 1855

Cells solitary, lance – shaped, narrowed to sharp point anteriorly, short thick stipe with a basal attaching disc, 7.5 µm in diameter, 25 µm long

Habitat- epiphytic, Occurrence – lake

7. *Pediastrum obtusum* Lucks, 1907

Colony entire; inner cells with 8 straight sides but with one margin deeply incised; peripheral cells crenate with a deep incision in the outer free margin, their lateral margins adjoined along of their length. Cells 7.5 µm wide.

Habitat- aquatic, Occurrence - lake

8. *Scenedesmus arcuatus* Lemmmermann, 1899

Coenobia are composed of 4 - 8 cells in 2 rows; cells ovoid to slightly cylindrical, with broadly round apices, cell wall usually smooth; cells 5 µm wide, 10 µm long.

Habitat- aquatic, Occurrence – Channel

9. *Scenedesmus ecornis* (Ehrenberg) Chodat, 1926

Coenobia are composed of 4 cells joined side by side, arranged linear; cells ellipsoidal, tapered at the poles, cell wall usually smooth; cells 8 µm wide, 15 µm long.

Habitat-aquatic, Occurrence – shallow water

10. *Scenedesmus planctonicus* (Korshikov) Fott, 1973

Coenobia are composed of 3 cells joined side by side, arranged linear; cells broadly ovoid, inner wall straight and outer wall convex, apices broadly rounded, cell wall usually smooth; cells 8 µm wide, 15 µm long.

Habitat- epiphytic, Occurrence - Channel

11.*Oedogonium sp* Link ex Hirn, 1900

Macrandrous; vegetative cells cylindrical, 10 µm in wide, 50 µm in long.

Habitat- epiphytic, Occurrence – Attached on *Pithophora* sp

12.*Chaetophora pisiformis* (Roth) C.A. Agardh, 1812

Plants attached, dichotomously branched filaments radiated from a common center, apical cells sharply pointed, main axis 10 µm in wide, 25µm long, cells of the branches only slightly narrower and shorter.

Habitat- epiphytic, Occurrence – aquatic plant in pond

13. *Stigeoclonium farctum* Berthold, 1878

Prostrate system pseudoparenchymatous and disc like, chloroplast and pyrenoid at same level in all radiating filaments forming the pseudoparenchymatous system, erect system absent; cells 7.5 µm wide and 1 times longer than wide.

Habitat- aerial, Occurrence - rock

14. *Protoderma viride* Kuetzing, 1894

Thallus an attached disc, irregular in outline, made up of branched filaments which are compact and parenchymatous internally but semi-radiate and spreading at the margin; terminal cells slightly narrowed; chloroplast a parietal disc with 1 pyrenoid; cells 6.25 µm in wide, 10 µm long.

Habitat- epiphytic, Occurrence – stem of aquatic plants

15. *Gloeocystis ampla* (Kuetzing) Rabenhorst 1863

Colonies spherical; cells ovoid; chloroplast parietal, without pyrenoid; Cells 5 µm wide, 6 µm long.

Habitat- aquatic, Occurrence - lake

16. *Microspora amoena* (kuetzing) Rabenhorst 1868

Filamentous, uniseriate, unbranched, cells cylindrical, not constricted at the cross walls, thick walled; chloroplast net-like, filling entire cell; 22.5 µm in wide, 50 µm long.

Habitat- aquatic, Occurrence – lake

17. *Rhizoclonium hieroglyphicum* (C. Agardh) Kutzing, 1845

Filamentous very coarse, uniseriate, unbranched, cells cylindrical to swollen, constricted at the cross walls, cell wall thickness; chloroplast granular and very dense; 15 µm in wide, 25 µm in long.

Habitat-aquatic, Occurrence – lake

18. *Rhizoclonium riparium* (Roth) Harvey 1849

Filamentous very coarse, uniseriate, unbranched, cells cylindrical, not constricted at the cross walls, cell wall slightly thick; chloroplast net-like, filling entire cell; 25 µm in wide, 1 times longer than wide.

Habitat-aquatic, Occurrence – lake

19. *Pithophora mooreana* Collins 1912

Branched macroscopic thallus; Filaments of cylindrical cells; with rounded extremity; from one to two intercalary akinetes, tonal shaped, terminal rounded; cells 75 µm in wide, 200 µm long.

Habitat-aquatic, Occurrence – lake

20. *Apatococcus lobatus* (Chodat) J.B. Petersen 1928

Cells forming 2-4 packets, clustered together; cell compressed; chloroplast lobed or without pyrenoid; cell 7.5 µm in wide.

Habitat- aerial, Occurrence – tree trunk

21. *Chlorella vulgaris* Beyerinck (Beijerinck) 1890

Cells spherical, irregular clumps; chloroplast parietal cup, without pyrenoid, cell 5-7.5 µm in wide.

Habitat- aerial, Occurrence - cement wall

22. *Mougeotia sp* C. Agardddh, 1824

Filamentous, unattached, cells cylindrical with plane end wall; chloroplast filling length of cell with several pyrenoids, filling entire cell; 27.5 µm in wide, 125 µm long.

Habitat- aquatic, Occurrence – shallow water pond

23. *Spirogya sp* Link, 1820

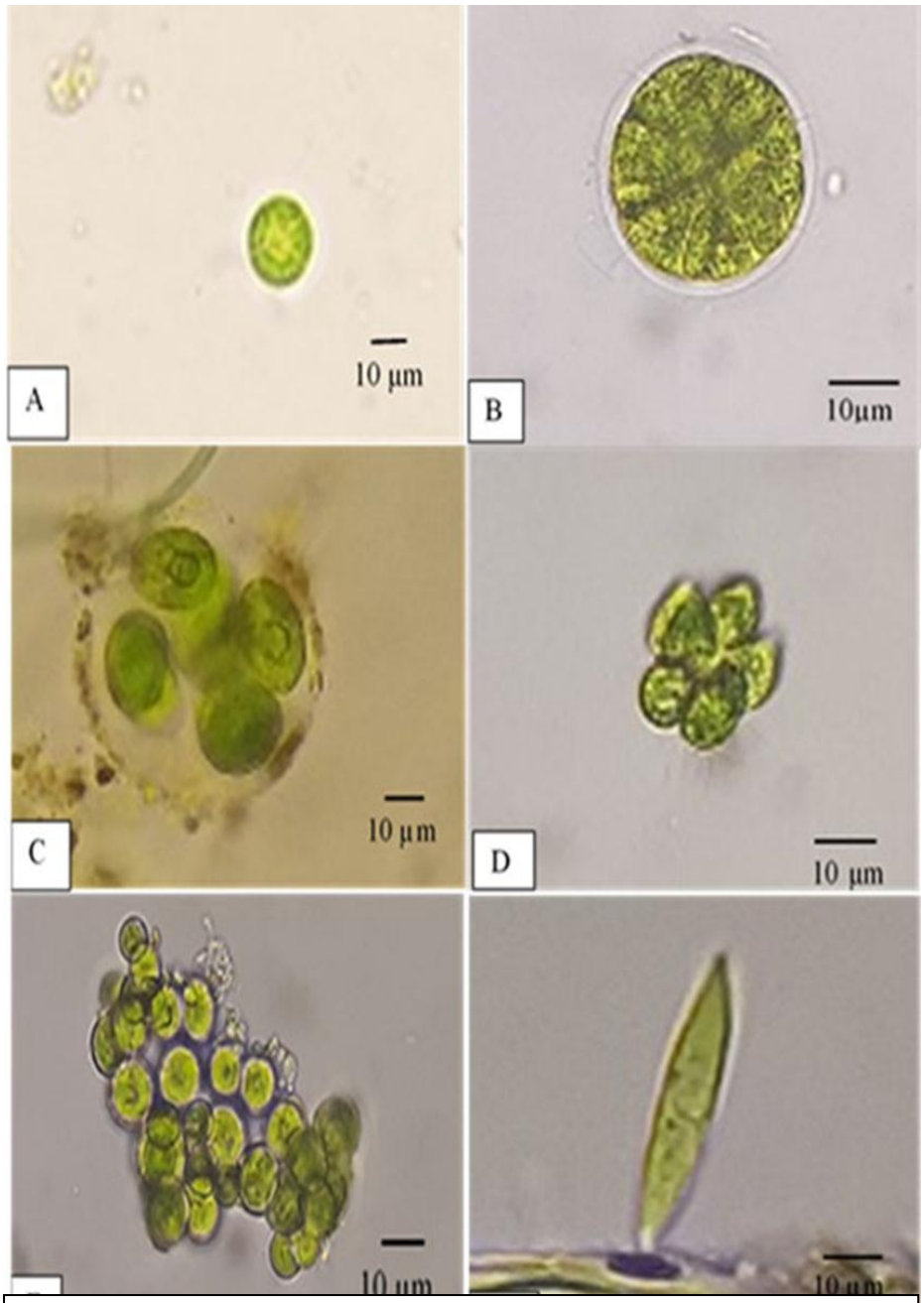
Filaments of shout cells, 37.5 µm in wide and 137.5 µm in long, with plane end walls, chloroplast solitary, making 7 turns, conjugation not occur.

Habitat – aquatic, Occurrence - lake

24.*Cosmarium granatum* Brebisson ex Ralfs, 1848

Semicells trapeziform, basal angled founded; lateral margin curving inward to broad, truncate apex, wall smooth sinus shallow and narrow. cells 30 µm wide and 40 µm long.

Habitat – aquatic, Occurrence- Channel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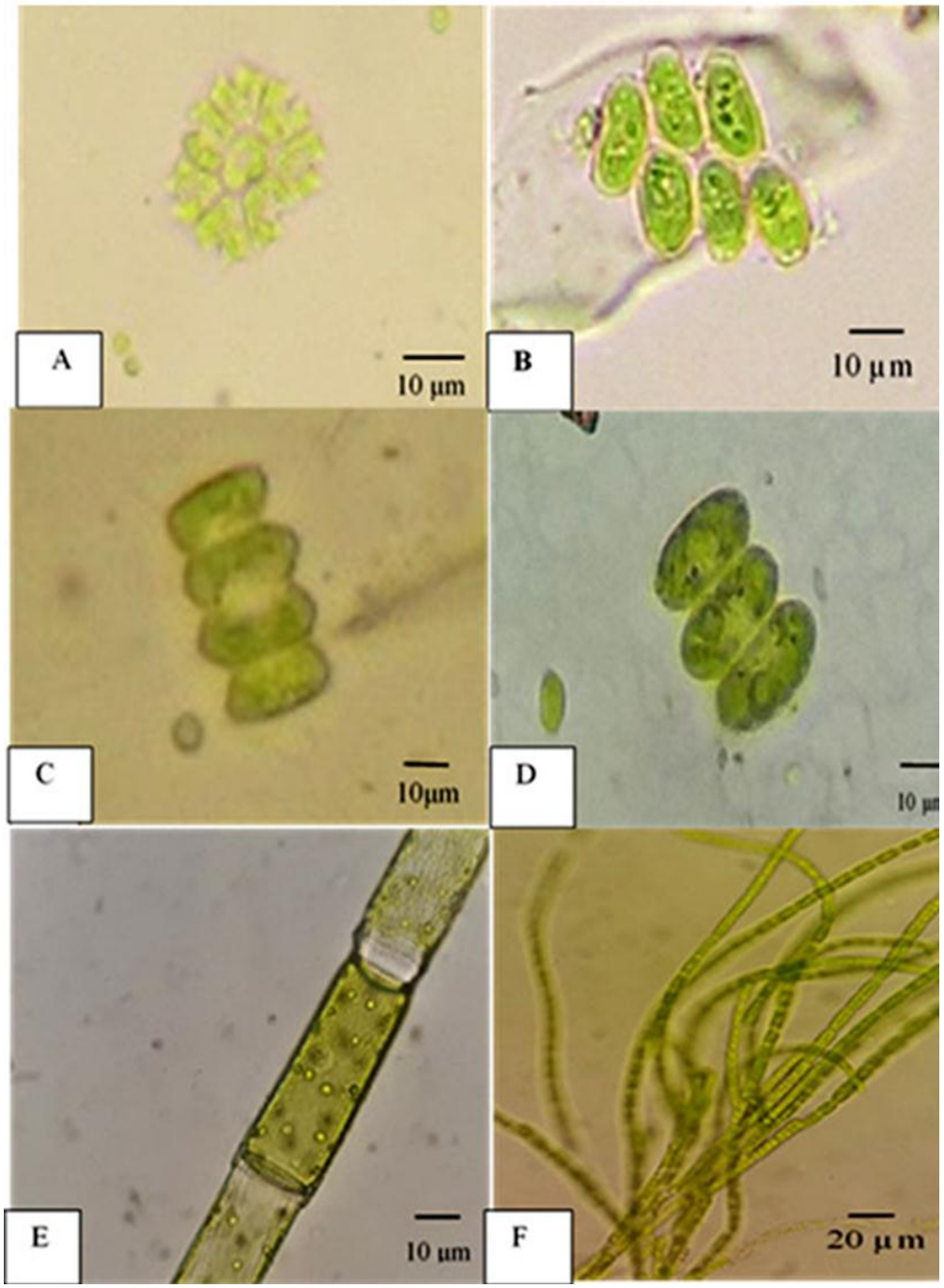
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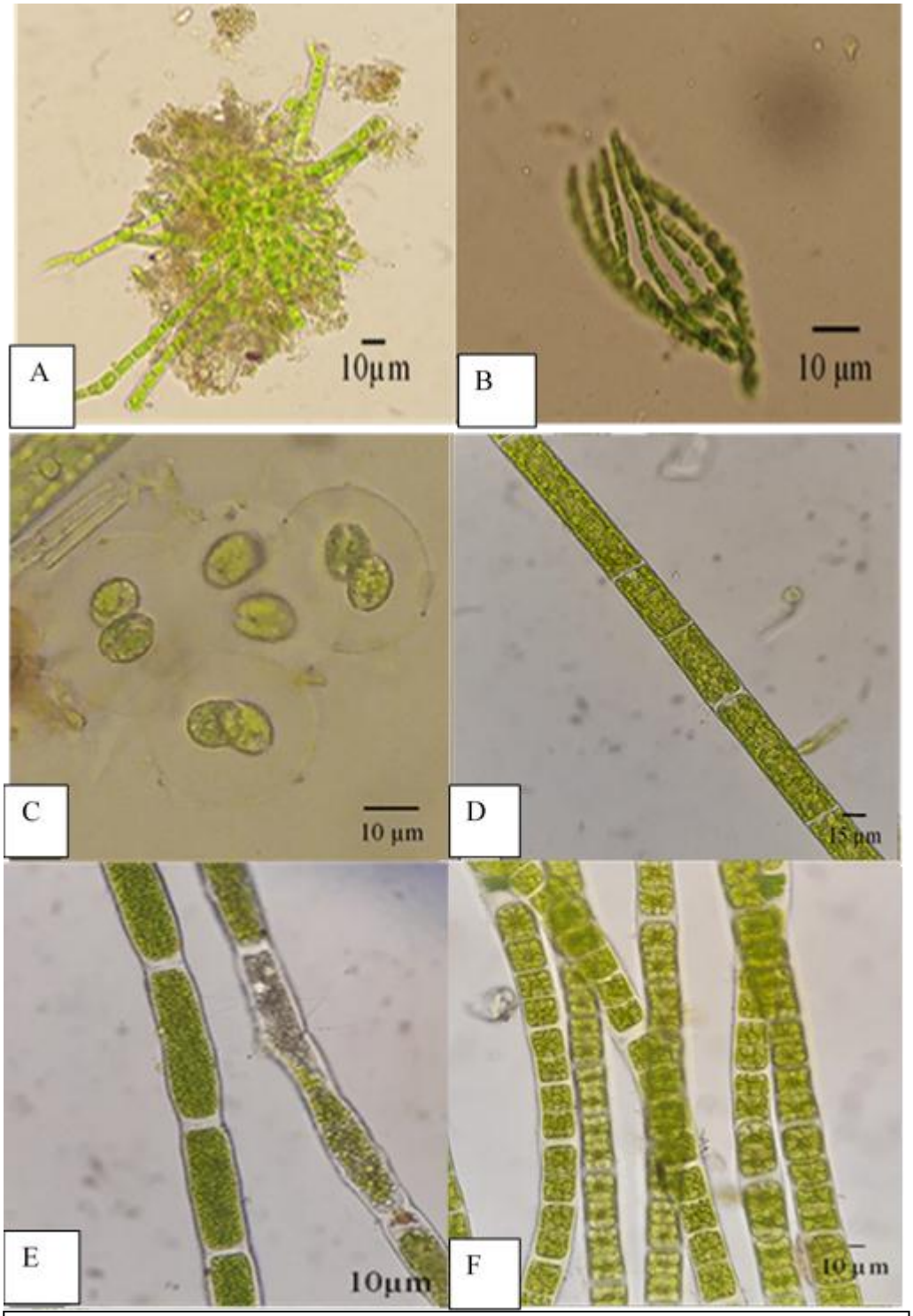
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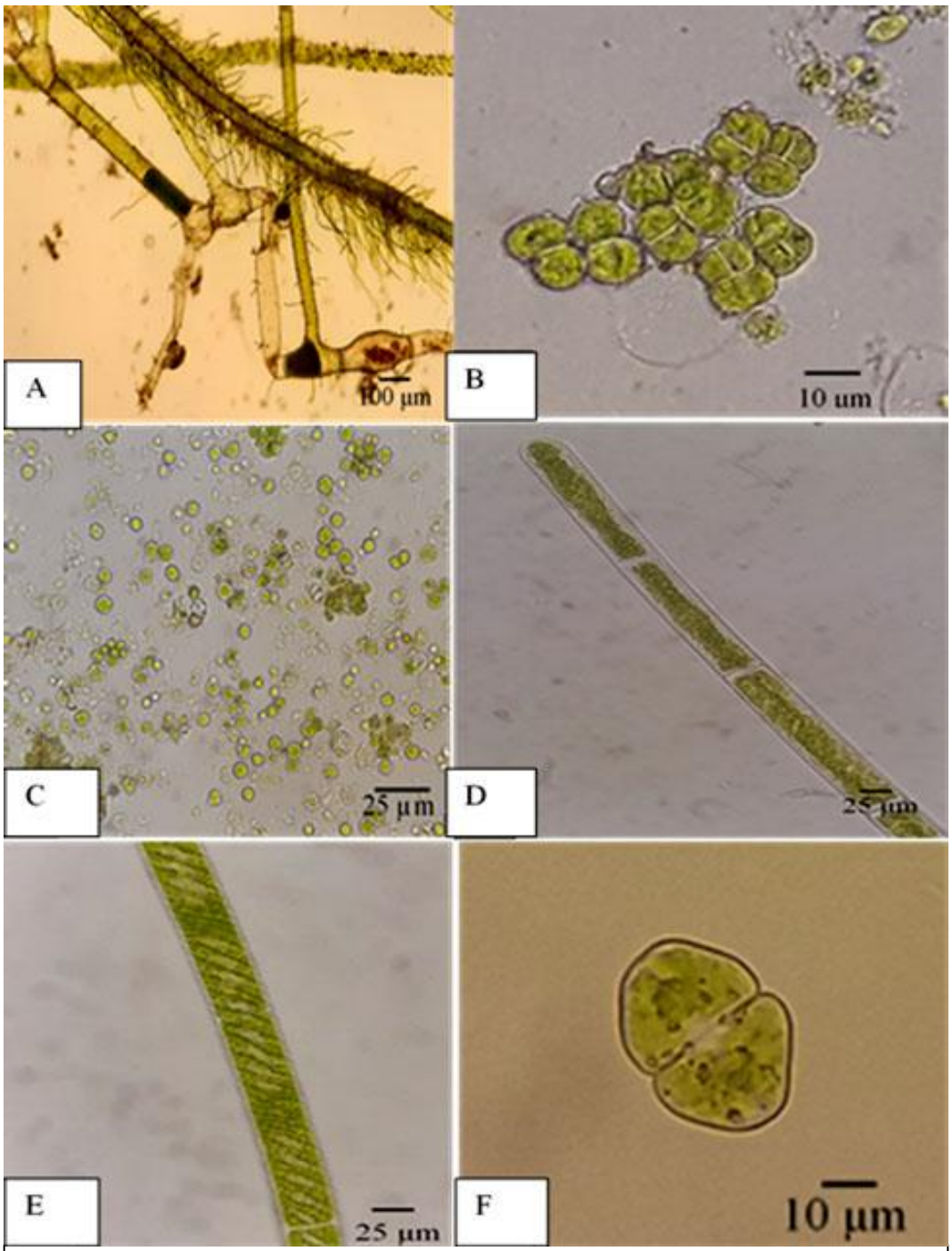
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Discussion and Conclusion

In this Study, the microscopical characters of 24 taxa belong to 16 Families, 9 Orders, 4 Classes in Division Chlorophyta were identified, described and recorded by the following literatures viz. Smith (1950), Presscott (1962), Sigee (2004) and John *et al*. (2008).

In collection site – 1, *Bracteacoccus cohaerans*, *Characium angustum*, *Oedogonium sp*, *Chaetophora pisiformis Stigeoclonium farctum*, *Protoderma viride* were found. *Pyrobotrys casinoensis*, *Pediastrum obtusum*, *Scenedesmus plantonicus*, *Gloeocystis ampla*, *Microspora amoena*, *Rhizoclonium hieroglyphicum*, *Rhizoclonium riparium and Pithophora mooreana* were found on site -2. *Asterococcus siderogloeus*, *Scenedesmus arcuatus*, *Apatococcus lobatus*, *Chlorella vulgaris* and *Cosmarium granatum* were found on site -3. *Chloromonas serbinowi*, *Pandorina morum*, *Scenedesmus ecornis* and *Mougeotia sp* were found on site -4.

Smith, 1950 stated that the habitats of algae are mainly classified into three categories viz. aerial habitats, aquatic habitats and unusual habitats. Aerial algae have been defined as algae that obtain their water wholly or in large part from moisture in the air. Strictly aerial algae are found on the bark and leaves of trees, on wood, stones, and rock and on soil surface.

According to Smith (1950), *Bracteacoccus cohaerans*, *Scenedesmus planctonicus*, *Stigeoclonium farctum*, *Apatococcus lobatus* and *Chlorella vulgaris* were found as aerial algae.

Sambamurty, 2005 stated that aquatic algae are generally growing on floating waters, ponds, lakes, pools, ditches, bogs and swamps. The algae of running water are more diversified than those of any other aquatic habitats and include a larger percentage of species restricted to the particular habitat. Finally, algae growing on unusual habitats are broadly classified into the following categories - cryophytes or snow algae, thermal algae, halophytic algae, lithophytes, epiphytes and symbiotic algae.

In this study, *Characium angustum*, *Oedogonium sp*, *Chaetophora pisiformis* and *Protoderma viride* were seen as epiphytic algae. The habitats character of *Chloromonas serbinowi*, *Pandorina morum*, *Asterococcus siderogloeus*, *Pyrobotrys casinoensis*, *Pediastrum obtusum*, *Scenedesmus arcuatus*, *Scenedesmus ecornis*, *Gloeocystis ampla*, *Rhizoclonium hieroglyphicum* , *R. riparium* , *Microspora amoena*, *Pithophora mooreana* , *Mougeotia sp* ,*Spirogya sp* , and *Cosmarium granatum* were aslo agreed with Sambamurty (2005).

The present study the green algal flora presented some existing green algal species in Dagon Myothit East township. The results emphasize the habitats characters of some green algae existing in study area. It is also hope that the present study will be proceed to identify the more species belong to Chlorophyta existing in this area.

Acknowledgements

We would like to express our deepest thanks to Professor Dr. Myat Myat Moe, Head of Botany Department, Dagon University, for her permission and support in the department.

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THE PRELIMINARY STUDY ON SOME NATIVE ORCHIDS IN EAST BAGO AREA

Moe Sandar Shein[1], Khin Swe Swe Htun[2], Moe Moe Shwe[3], Thanda Soe[4]

Abstract

The present study deals with the native orchids of Bago urban area and some reserved forest in Bago Township. Especially study sites are Bago urban area, Hpa Yar Kalay, Hpa Yar Gyi village, around Baw Net Gyi,Moe Youn Gyi Ramser site Wingabaw elephant camp and Myout Zarmani Wildlife Sanctuary of Bago Township. Bago area is hot weather and high receives of rainfall so a lot of epiphyte (fern and Orchids) they have grown well on the old big trunk. Most of the collected orchids are epiphyte, terrestrial and hydrophytes. In this recent study (7) genera and (13) species were collected in every season from study area. Collected genera namely ***Acampe, Aerides, Dendrobium, Eulophia, Habenaria,Liparis*** and ***Rhynchostylis*** were recorded with photograph have taken habitat of orchids in nature. Collected species were classified, identified and described with colour photograph of nature habitats and inflorescences. The morphological characters have been emphasized and artificial keys to the species have been constructed. GPS location system was used and also introduced conservation method for students extra curriculum.

Keyword: BagoTownship, native orchids, epiphyte, terrestrial, hydrophyte, artificial key, extra-curriculum

Introduction

The family Orchidaceae are largest family of among Angiospermae, Monocotyledonae. Some botanist estimated about 35,000 species among flowering plants. They can grow well in Temperate, Subtropical and tropical region but exception of ice capped and deserts. (Dassanayake, 1981)

The study areas are Bago Urban area, Hpa Yar Kalay, Hpa Yar Gyi village, Wingabaw elephant camp, Road side of cross road to Highway near Wingabaw village, around the Moe-Youne-Gyi Ramsar site and surrounding village of Bago Township. Some of the specimens were collected from Sa-lu reserved forest and Myauk Zarmini wildlife sanctuary. Bago Township is located on the east by Daik-U and Waw Township, on the north by Kawa Township, on the west by Hlegu Township and on the south by Kyaut-ta-ga Township and it lies between N 17° 20″ 12′ and E 96° 28′ 47″. Sa-lu reserved forest is lower tropical forest, Myauk Zarmini wildlife Sanctuary is mixed upper mix deciduous forest. It lies between N 18° 10′ 828″ and E 96° 15′ 0.81″. Genus *Habenaria, Dendrobium, Eulophia, Cymbidium, Acampe*, *Rhynchostylis* and *Aerides* have been collected in the study area in recent work.

In this recent study, (3) subfamily belong to (5) Tribes (4) Subtribe (8) genera and (14) species have been recorded form the study area including epiphyte, terrestrial and only one aquatic Orchids. The classification and taxonomic description of collected specimens are provided with coloured photographic and keys of genera and species are also constructed and GIS recorded.

[1] Dr, Associate Professor, Bago University
[2] Dr, Lecturer, Bago University
[3] Dr, Professor and Head, Bago University
[4] Dr, Assistant Lecturer, Bago University

Methodology

The specimens were collected from Eastern part of Bago District. All these specimens were colourful photographed to record their actual habitat and the nature of inflorescence. The collected specimens were classified according to Dresseler's classification (R.L. Dresseler's (1927) and identified by Seidenfaden (1992) Grant: B (1966): Nantiya Vaddhanaputi (2006) Hooker, J.D. (1954). Seidenfaden and Smitch (1965), Dassanayake, M.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 Yangon University.

Arrangement of the Subfamily, Tribe, Subtgribe and Genera in the Present Study

Class : Liliopsida (Monocotyledoneae)

Subclass : Orchidales

Family : **Orchidaceae**

Subfamily : (I) Orchidroideae

(II) Epidendroideae

(III)Vandoideae

I Subfamily : Orchidroideae

Tribe : Orchideae

Subtribe : Orchidinae

Genera : (1) ***Habenaria***

II. Subfamily : Epidendroideae

Tribe : Malaxideae

Genera : (2)***Liparis***

Tribe : Epidendrae

Subtribe : Dendrobiinae

Genera : (3) ***Dendrobium***

II. Subfamily : Vandoideae

Tribe : Cymbideae

Subtribe : Crytopodiinae

Genera : (4) ***Eulophia***

Tribe : Vandeae

Subtribe : Sarcanthinae

Genera : (5) ***Acampe***

(6) ***Aerides***

(7) ***Rhynchostylis***

The classification of Subfamilies in the study is in accordance with **Dresseler (1927)** and the key below is cited from **Seidenfaden** and **Wood, (1992)** described in "The Orchids of Indochina".

Results

In this paper(3) subfamily,(5) tribes, (4) subtribes and (7) genera and (13) species have been collected from study area.

(I) Subfamily- Orchidoideae

In this recent study only one genus *Habenaria* of subfamily Orchidroideae was collected from study area.

Subfamily	Tribe	Subtribe	Genus	Species	Myanmar Name
Orchidroideae	Orchideae	Orchidinae	***Habenaria***	*trichosantha*	မြနှင်းဖြူ မြေပေါက်သစ်ခွ

1. *Habenaria* Willd.

Terrestrial, deciduous, tuberous herbs. Foliage leaves one to several, basal lanceolate, elliptic, ovate, with one to several sterile bracts upper the foliage leaves, hairy. Inflorescence terminal, racemose few to many, small to medium sized resupinate, various coloured, sepals concave, erect always forming hood with petals , lateral sepals spreading or reflexed. Petals entire, labellum trilobed, rarely entire, clawed, spur long or short clavate, sauate, rarely absent. Colum short, anther erect or reflexed adjacent and separate rarely. Pollinia 2, rostellum trilobed. Ovary glabrous or papillose. Fruit capsule, dehiscing by longitudinal slit.

Note: In this recent study only one genus ***Habenaria*** in study area.

1.1 *Habenaria trichosantha* Lindl.

Habit

Inflorescenc

Flower

Flower parts

Terrestrial, stem short with scattered 3-4 leaves on flowering shoot. Foliage leaves elliptic ovate, acute, mucronate. Sterile bracts suberect, lanceolate acute 4-6 cm long and 2.4 cm side.

Inflorescence terminal, 5-10 cm long with 8-10 flowers, semi-dense with lanceolate floral bracts. Flower 2.2 cm across, white. Dorsal sepal erect, ovate oblong with shortly pubescent, lateral sepals reflexed, oblong ovate acute. Petals linear-furcate, acuminate, margin entire. Labellum pure white trilobed, midlobe linear - lanceolate, subacute 1.2 cm long and 1.00 cm wide, side lobes spreading with deeply cleft, filiform, laciniae, spur cylindric, green-column short.

Myanmar name - Ma-hinn-Phyu- Maypauk-Thitkhwa (မြနှင်းဖြူ မြေပေါက်သစ်ခွ)

Occurrence - Myauk Zamini Wildlife Sanctuary, (N 18˚05′ 82″ E 96˚ 13′ 08″)

Distribution - NE India, Bhutan, Myanmar, N Thailand (*www. plantillustration. org.)*

Ecology - Terrestrial. Alt 1412 m. Flowering period -August-September.

II. Subfamily Epidendroideae

In this recent study only one genus ***Dendrobium*** of subfamily Epidendroideae was collected from study area.

Subfamily	**Tribe**	**Subtribe**	**Genus**	**Species**	**Myanmar Name**
Epidendroideae	Malaxideae		*Liparis*	*Liparis sp*	None
	Epidendrae	Dendrobiinae	*Dendrobium*	*aggregatum*	ရတနာရေခက်
				crepidatum	ဂနိုင်နဘေးပေါက်
				aphyllum	လက်တံရှည်
				parcum	ကျီးခြေ
				pulchellum	ဆင်မမျက်ကွင်း
				moschatum	ဝါဆိုပန်း

2.1. *Liparis.*

Herbs, terrestrial, lithophytic or zhizomatous and leaves reduce to scales. Stem pseudobulb, sometimes with many nodes, fleshly stem when young covered by sterile bract. Leaves 1- several linear to ovate or elliptic, plicate or not, thinly textured, two leathery, basal or cauline or arising from the apex or sub terminal nodes of pseudobulb. Inflorescence erect, racemose, lexly or densely many flowers. Floral bract persistent. Flowers small to medium size, usually resupinate. Sepal spreading dorsal sepal free, lateral sepals free or sometimes fused. Petals free, often reflexed, linear nod like sepals. Lip often reflexed, ovate, oblong, entire or lobed, usually with a basal callus, without spurs. Column incurved, long with wing at apex or base. Pollinia 4 in pairs, waxy, bilaterally flattened, restellum thinly texture. Capsule subglobose.

2.1. *Liparis sp.*

Habit

Inflorescence

Flower

Herbs, terrestrial. Stem cylindric, 2-8 cm long 1.2 cm in wide, fleshy with 2-3 node enclosed by sheaths. Leaves 3-6, petiole sheath like, 15-20 cm long, plicate, blade ovate to elliptic, 2-3 cm wide, membranous, margin entire, apex subacuminate. Inflorescence terminal with 8-10 flowers, floral bracts deltoid. Flowers greenish yellow with green pedicel. Dorsal sepal linea or broadly linea, 0.2 cm long and 0.1 cm wide, margin revolute, apex obtuse, lateral sepal narrowly ovate oblong slightly oblique. Petals reflexed, parallel with lip, filiform, greenish yellow with reddish brown in margin. Lip oblong ovate, base narrowed and with 2 suboblong calli, apex truncate and emerginate. Column stout, distinctly with narrow wing. Capsule obovate.

Myanmar name - None

Occurrence -Moe-Youne- Gyi Ramsar Site, Bago township, (N 18 ˚55′- E 96˚ 25′)

Distribution - Myanmar

Ecology - Terrestrial, wetland, grow in the water. Alt 8 m. Flowering period -June-October.

(3) *Derdrobium*

This genus have more or less elongated cylindrical leafy pseudobulbs at stems, the leaves being generally bifarious, alternate and flat , they differ as in habit, so in size. The flowers are lateral and either solitary, in fasicles or in raceme. The sepals and petals all the segment of the flowers except the lip are nearly uniform in shape the general difference being that of the outer segment or sepal , two lateral sepals are larger than the other and adhere commonly to the side of the column, or usually prolonged into a blunt spur. The lip is always sessile. Pollinia 4 in pairs side by side, quite free, anther 2-celled.

Key to the species of Genus *Dendrobium*

1. Stem long---------- 2

1. Stem short ---------- 3

2. Stem slender. Inflorescence with 2-3flowers. ---------- 4

2. Stem stout. Inflorescence with many flowers. ---------- 5

3. Stem tuff, ridges. Inflorescence with many flowers. Flower golden yellow. Lip orbicular, yellow patch on the mesochile.---------- 1. ***Dendrobium aggregatum***

3. Stem stout with white velum, smooth. Inflorescence with 2-3 flowers. Flower white. Lip cordiform with yellow in the basal half, white edges with pink in front. ----------**2. *Dendrobium crepidatum***

4. Stem long and straight. Flower pale purple, about 3.5 cm across lip spathulate, pale yellow with purple vein at the base.**3. *Dendrobium aphyllum***

4. Stem branched. Flower greenish yellow, about 0.5 cm across. Lip longer than the sepal. Lip greenish yellow with small brownish purple dot on hypochile.---------- **4. *Dendrobium parcum***

5. Flower creamy white. Lip rounded with two dark blotches on each side of lip.----------**5. *Dendrobium pulchellum***

5. Flower orange yellow. Inflorescence with 4-6 flowers. Lip pouch with incurved edges, with long ciliate vein inside and two maroon blotches or each side at the base.---------- ---------- **6. *Dendrobium moschatum***

3.1. *Dendrobium aggregatum* Roxb FL iii 477.

***Dendrobium lindleyi* Steud**

***D. jenkinsii* Wall.**

Habit

Inflorescence

Flower parts

Epiphyte dwarf, evergreen species, clustered. Stems fusiform with furrow pseudobulbs about 5-6 cm long and 3.00 cm wide. Leaves solitary, oblong ovate, tip notched. Inflorescence loop drooping lateral raceme. Flowers are deep golden yellow with an orange yellow stain at the hypochile, about 300cm across. Sepal ovate obtuse spreading. Petals much broader ovate, base cuneate. Mantum subglobose. Lip shortly clawed transversely oblong from a short convolute at the base, pubescent, entire ciliolate. Column yellow. Pollen masses 4 in pairs.

Myanmar Name - Yadana Shwe Khat (ရတနာရွှေခက်)

Occurrence - HpaYar Kalay village, (N 18 ˚ 55′- E 96˚ 25′)

Distribution - Myanmar, Deccar, Sikkim, Bhutan, NE India, Thailand and China **(Seidenfaden 1992)** Arrancan, Martabow, Tenassenim Hook. f. **(Grant. B. 1966**), NW Myanmar, EW in Southern China **(Holttum, 1964)**

Ecology - Epiphyte, well grow on the old trunk of mango tree. Flowering period- February- March

3.2 .*Dendrobium crepidatum* Lindl & Paxton

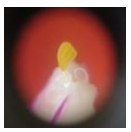
***Dendrobium lawanum* Lindl.**

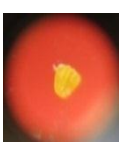
***Dendrobium roseum* Dalz Hook.**

Habit

Inflorescence

Flower parts





Pollinia

Epiphyte. Stem striate, covered with lumen like as white line, pendulous, about 1ft. Leaves oblong acute, glabrous. Raceme short with 2-4 flowers from leafless stems. Flower white expended about 3.4 cm. Sepals oblong, acute, white, tinged with pink. Petals obovate, broader than the sepals, glossy waxy texture, white tipped with purple. Colum curved. Pollinia 4. . Lip rounded cordiform pubescent with yellow in the basal half white edges with pink in front, thick edges at base unite in a transversely ridges on each side

Myanmar name - Ga-Naing-Nabay (ဂနိုင်နဘေးပေါက်)

Occurrence - Myauk Zamani Wild life Sanctuary, (N 18 ° 4′ 83″- E 96° 13′ 45″)

Distribution - Deccon Himalaya, Thailand and China (Seidafrdon, 1992), Laos, Vietnam (http://www.theplantlist.org)

Ecology - Epiphyte, well grow in tufts on the trunk of the big tree. Flowering period - March-April

3.3.*Dendrobium aphyllum* (Roxb). Fischer

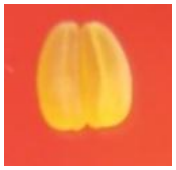
***D. pierardii* Roxb.ex Wook.**

***D. evagrnanum* Giageep, Bull.**

Habit

Inflorescence

Flower



Pollinia

Epiphyte Evergreen. Stem long and slender, pendulous, about 2-3ft .Leavers lanceolate accuminate, sessile about 6-8cm long and3.0cm wide. Inflorescence 2-3 flowers on each node. Flower marvue. Sepals 3, dorsal oblong lanceolate acute, lateral sepals oblong lanceolate acute, fuse at the base forming mentum. Petals oblong broader than the sepals. Lip spathulate, convolute at the base, slightly undulate margin with pubescescent, pale creamy yellow with purple veins in throat. Column short, pollinia masses, 4 in pairs, operculum white.

Myanmar Name - Lat Tan Shay (လက်တံရှည်)

Occurrence - Myout Zarmani Wild life Sanctuary (N 18 ° 51′ 95″- E 96° 14′ 6″)

Distribution - India to China and south to Malaya **(Seidenfaden 1992)** Eastern tropical Himalia and southward to tenasserim (**Grant.B.1966)** widely in Myanmar. India, Malaya, introduced from Myanmar (Holttum, 1964)

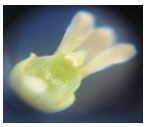
Ecology - Epiphyte, well grow on the old trunk, Flowering period- April-May

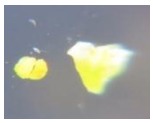
3.4. *Dendrobium parcum* Rchb.f.

***Dendrobium parcoides* Guill**

Habit

Flower





Pollinia

Epiphyte. Stem branched, wiry, about 20-30 cm long 0.4 cm wide, dark brown. Leaves linear-lanceolate 6-8.00 cm long. Raceme extremely short with 2 flowers. Flower long greenish yellow. Sepal and petals high greenish yellow. Sepal oblong obtuse with three nurved. Petals linear spathulate medium broad, obtuse, much longer than the sepals. Lip much longer than the sepals, broadly orbicular retuse apex, small purple dots on hypochile. Column short with foot. Pollnia 4 in masses.

Myanmar name - Kyee-Chey (ကြီးခြေ)

Occurrence - Myout Zarmani Wild life Sanctuary, (N 18 ° 05′ 96″- E 96° 13′ 86″)

Distribution - Myanmar, Thailand (Seidafrdon, 1992)

Ecology - Epiphyte, well grow on tree. Flowering period - February-March

3.5. *Dendrobium pulchellum* Roxb ex. Linl.Gen, and SP, Orchid, 82:FL .ind,486

***D. dalhousiearum* Wall.**

Habit

Inflorescence

Flower

Epiphyte, evergreen species. Stem stout, terete subfusiform about 20 50 cm high, marks red purple line. Leave linear oblong, base cordate, raceme drooping lateral 5-10 flowers. Flower very large rosy-creamy cold, about 6-8 cm long and wide. Sepals oblong acute, petals much broader than the sepal, mentum rounded. Lip shortly clawed orbicular oblong, tip and side densely glandular villous on epichile, two large dark crimson blotches on each side at the base of the lip. Colum short and stout, column and anther dark purple. Pollen masses 4 in pairs.

Myanmar Name - Sin –ma myat –kwin (ဆင်မမျက်ကွင်း)

Occurrence - Hpa Yar Kalay, Hpa Yar Gyi village and Wingabaw elephant camp (N 18 ˚ 56′- E 96˚ 22′)

Distribution - Myanmar, Nepal, NE India, Thailand, China and Malaysia.

(Seidenfaden1992) Native to Assam and Tenasser in Singapore Island, Malaya **(Holttum,1964).**

Ecology - Epiphyte, well grown on the trunk of Kok ko. Flowering period- May-Jun

3.6. *Dendrobium moschatum* (Buch. Ham.) Sw.

Habit

Inflorescence

Flower

Epiphyte. Stem brown slender. Leaves linear oblong, reddish green. Raceme 5-6 lax flowers arising on the top of the stem. Flower yellowish orange, large showy, about 7.00 cm long and wide. Sepals ovate-obtuse. Petals broadly rounded, larger than the sepals, glabrous. Edge of lip incurved forming a pouch, yellow with two maroom blotch at epichile and long ciliate veins, outside pubescent on incurved edge. Column long with red spot. Anther 2-celled. 4 pollinia.

Myanmar Name - Wah-so –Pan (ဝါဆိုပန်း)

Occurrence - Hpa Yar Kalay, Hpa Yar Gyi village, Bago urban area. (N 18 ˚ 56′- E 96˚ 25′)

Distribution - Himalaya, Myanmar, Thailand and China (**Seidenfaden; 1992).** Native in lower Myanmar (Holttum, 1964)

Ecology - Epiphyte, well grown on the old trunk, Flowering Period -May- June

(III) Subfamily Vandoideae

Subfamily	**Tribe**	**Subtribe**	**Genus**	**Species**	**Myanmar Name**
Vandoideae	Cymbidieae	Crytopodineae	Eulophia	*Andamanensis graminea*	ဂမုန်းသဧင် စေတီဂမုန်း
			Cymbidium	*aloifolium*	သစ်တက်လင်းနေ
	Vandeae		*Acampe*	*papillosa*	မီးမလောင်ပန်း
			Aerides	*ordorata*	စာကလေးပန်း
			Rhyncostylis	*retusa*	ကြောင်မြီးနုံ့သာ

Key to the genera of Tribe Cymbidieae

1. Terrestril,stem tuber or corn.Inflorescence erect,branched.Flower medium.Coloum short---------- ***Eulophia***

1. Epiphyte or terrestrial,psrudobulb.Inflorescence pendulous.Flower large to medium.Coloum long withl wings---------- ***Cymbidium***

Key to the genera of Subtribe Cryptopodineae

1. Inflorescence subumbel, spur short, branching, very short. Flower small and medium size ---------- ***Acampe***
1. Inflorescence longer than the leaves, unbranching, spur distinct. Flower medium.---------- (2)
   2. Inflorescence large,not like fox tail,spur forward,not flattened----------***Aerides***
   2. Inflorescence like fox tail,spur backward,laterally compressed--------- ***Rhychostylis***

4. *Eulophia* R. Br. ex Lindl.

Like the above mentioned genera in the Cymbidieae tribe. *Eulophia* has two pollinia but they are supplied with both a simple stipes and a viscidium. Nearly all species are terrestrial with tubers or short pseudobulbous stems with more than one leaf and erect lateral inflorescence of usually several flowers. More than 240 species have been described most of them in Africa from Indochina.

Key to the Species of Genus *Eulophia*

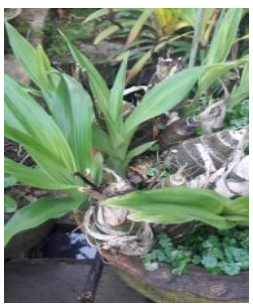
1. Leaves ovate. Inflorescence unbranched with luxflowered. Flower greenish yellow. Midlobe of lip large broadly claw with three keels on hypochile. ---------- **1. *Eulophia andamarensis***

1. Leaves lanceolate. Inflorescence branched with many flowers. Flower yellowish green with dark brown veins. Midlobe of lip forking toward epichile with 3-5 keels on hypochile, densely covered with long fat papillose. ------ **2. *Eulophia graminea***

4.1. *Eulophia andamarensis* Rchb.f.

***Eulophia keithii* Ridl., J. Linn**

***Eulophia poilanei* Gagrep., J. Bul.**



Habit

Inflorescence

Flower

Terrestrial. Leaves linea-lanceolate during glowering. Stem stout tuberous about 5-8 cm long 4.00-6.00 cm wide. Raceme lax flowered, bract small, about 2.00 ft. flower greenish yellow, 2.5-3.00 cm across. Sepals, petals oblong acute, green with brownish veins, about 1.5 cm long

0.5 cm wide. Lip trilobed, sidelobed of lip small, obtuse, midlobe large broadly claw, orbicular with three keels on hypochile ending on middle of epichile, middle one longest, spur conical, obtuse. Column foot long, operculum small. Pollinia 2 with short stipe.

Myanmar name - Ga-Mone Thazen (ဂမုန်းသဇင်)

Occurrence - Bago Urban Area and Pha-Yakalay village, (N 17 ˚ 35′ 28″- E 96˚ 36′ 40.3″)

Distribution - Andamans, Hyamans, Thailand, Malaya and Sumatra (Seidafrdon 1992). Tamasserim, Meulnur, Andaman Island. (Grants. 1964)

Ecology - Terrestrial. Flowering period - May-June.

4.2.*Eulophia graminea* Lindl.

Habit

Inflorescence

Flower

Terrestrial stem at the base tuberous with thick roots. Leaves linear-lanceolate. Scape 30-50 cm, raceme, lax-flower. Flower greenish purple. Sepals linear-lanceolate, 3-5 nerved, acuminate, petals oblong acute with 3-nerved. Lip trilobes, shorter than the sepals white with green edges. Side lobes obtuse, greenish yellow with brown stripe, midlobe large broadly elliptic to obovate, margin undulate ,three to five keels on hypochile terminating on middle of epichile, produce numerous long tubercles, the medium largest, without papillose. Spur conic, obtuse. Anther 2-celled. Pollinia 2.

Myanmar Name - Say-ti-ga-mone (စေတီဂမုန်း)

Occurrence - Bago urban area, Salu reserved forest. (N 17 ˚ 34′ 28″- E 96˚ 37′ 27.8″)

Distribution - Andamans, Myanmar, Thailand, Kalaya and Sumatra (**Seidenfaden, 992**). Myanmar, Andaman Island **(Grant's; 1966).** India to Philippines **(Holttum, 1969)**

Ecology - Terrestrial , lower tropical rain forest. Flowering Period - March-May

5. *Acampe* Lindl.

Rather large coarse plants with fleshy strap-shaped leaves, bilobulate at apex, the inflorescence shorter or rarely longer than leaves, usually branching with dense, non-resupinate flowers. Flowers fleshy, lip adnate to the short stout, footless column, a short conical sac without backward callus inside, but usually hairy and often with a longitudinal median callus. Pollinarium with a small elliptic viscidium, stipes strap-shaped to clavate, hardly twice as long as diameter of pollinia. There may be 8-9 species in the genus, but half of them are very little known and of uncertain validity. In Indochina 3 species have been recorded. (**Seidenfaden 1992**)

5.1. *Acampe papillosa* (Limell) Lindl.

***Saccolobium papillosum* Lindly, Edwards Bot Reg.18 ad-1552-1832.**

Habit

Inflorescence

Flowers

Epiphyte, stem short about 7-10 cm with distichous with bilobed ended leaves. Inflorescence subumbellate about 1-3 cm with many short branched, with dense flowers. Flower yellow with reddish brown transverse stripe about 0.6 cm across fragrant. Sepals oblong, Petals oblong smaller than the dorsal sepal. Lip trilobed, divided into hypochile and epichile, pure white with purple sport on hypochile, epichile ovate with straight with hairs. Column stout with horns and subglobose operculum. Pollinia 4, united in pairs, globose, rostellum short.

Myanmar name - Mee- Ma-Laung-Pan (မီးမလောင်ပန်း)

Occurrence - Myanmar, Bago Township, Road side of in Winkabaw village (N 17˚ 34′ 208″ - E 96˚ 36′ 22.8″)

Distribution - Bangladesh, Bhutan, NE India, Laos, Myanmar, Nepal, Thailand, Vietnum (Flora of China Vol. 25) NE Himalaya eastwards to Myanmar and Thailand. (Flora of Thailand)

Ecology - Epiphyte on the trunk of on Rode side. Alt. 11m. Flowering period - November- December.

6. *Aerides*

Stem stout and fairly long with thick and long roots. Leaves flat or terete. Inflorescence simple or branched, suberect or drooping with many flowers, moderately large, usually scented flowers. Sepals and petals similar, spreading, lateral sepals oblique at the base and connate with the prolonged base of the column. Colum short, lip join with the clow of the column, spurred or bagged, trilobed, side lobes small, centre variously shaped. Pollen 2, massed, cleft on long narrow stipe.

6.1. *Aerides falcata* Lour, FL.Coch 525. 1790

***Aerides retrofractum* Wall. MSS**

.

Habit

Inflorescence

Flower

Epiphyte. Stem about 8-10 high. Leaves a little undulate. Inflorescences pendulus, laxflowersed. Flowers white with pale violet tip, about 2.5cm across. Sepals broadly ovate, lateral sepals broadly ovate adnate to the column foot. Petals smaller than the sepals. Lips movable, trilobe, lateral lobe falcate, midlobe obovate convex, ciliated, deep rose in the middle, margin erose, apex emerginate, spur upward. Column short, anther beak at the apex. Pollinia 2, slender, waxy.

Myanmar Name - Sa ka lay pan (စကလေးပန်း)

Occurrence - Bago urban Area, Myauk Kyaung monastery, Bago township (N 17 ˚ 27′40″ - E 96˚ 46′ 78″)

Distribution - Himalaya, Nepol, Southern China to Malaya (**Holttum, 1964)** Assam, Myanmar, Thailand, (**Seidenfadern,1992**), Cambodia, NE India, Laos, Myanmar, Thailand, Vietrum (Flora of China, Vol. 25)

Ecology - Epiphyte,well grow on the old trunk. Alt. 192 m Flowering period- April-May

7. *Rhynchostylis*

Epiphyte, stem stout and thick .Leaves long sessile channeled, unequally bilobed. Inflorescence dropping or erect, densely crowded flower with long cylindrical raceme. Petals smaller than the sepals, lateral sepals adnate to the column foot, deeply saccate with backward pointing laterally flatten spur. Rostellum projection and operculum rather long-pointed. Pollinia 2, the stipes of the pollinia long. A genus of 3-4 species, in many characters much similar to *Vanda*, differing e.g. in having light green lines in the leaves.

7.1. *Rhynchostylis retusa* (L.) Blume Bijdr, 1825, 286 t, fig. 49-Gagnepain 1934

***R. praemossa* Blume**

***R. guttata* Rchd.**

Habit

Inflorescence

Flower

Epiphyte. Stem stout leafy stem. Leaves curved, channeled unequally truncate bilobed at the ends. Inflorescence axillary raceme, long pendulous, cylindrical. Flower dense white blotched with violet. Sepals ovate, lateral sepals orbicular-ovate, obtuse, petals oblong. Lip usually cuniform, rounded, entire, the lip with a compressed bluntish spur.

Myanmar Name - Kyaung-me-nant-tha (ကြောင်မြီးနုံသာ)

Occurrance - Bago urban area, widely distributed in Bago area. (N 17 ° 36′ 28.9″ - E 96° 36′ 40.3″)

Distribution - Thailand, Malaya, Indochina (**Seiden; 1992).** Ceylon, India, Malaya, Philippine **(Holttum; 1964**)

Ecology - Epiphyte, well grown on the old trunk of Kok ko. Alt. 10 m Flowering Period July to September

Discussion and Conclusion

The orchids family is very large one, it's number occurring in all part of the earth except the driest and coldest, but the great majority are found in the wetter parts of the tropics. In 1914, Schlechter estimate that about 15,000 different kinds of orchids had been described (Holttum, 1964). Some authors suggested 12,000 to 15,000 species and others as many as 35,000 species classified into (6) families namely Apostasioideae, Cypripedioideae, Spironthoideae, Orchidoideae, Epidendroideae and Vandoideae (**Dressler, 1987).** In this research paper includes three subfamilies such as Orchidoideae, Epidendroideae, Vandoideae. There are (4) Tribes (5) Subtribes, (7) Genera and (13) Species in this recent study.

In the subfamily Orchidoideae, is tribe Orchidieae and subtribe Orchidinae. in recent study only one species of ***Habenaria trichosontha*** Lindl. was collected of genus ***Habenia. H. trichosontha*** Lindl possess white flower and mid lobe of lip deeply cleft and spreading. In subfamily Epidendroideae only two genus ***Liparis*** under tribe Malaxideae and genus ***Dendrobium*** of subtribe Dendrobineae were recorded in study area. Among six species of genus ***Dendrobium, D.aggregatum*** is dwarf and tuft plants with golden yellow flowers**, *D. crepidatum*** Lindl. are green pseudobulb and whitish pink flower with rounded labellum and yellow patch at the base; ***D. aphyllum*** **(**Roxb) C.E.C Fisher is distinctly spathulate convolute lip with deep purple strip at the base; ***D. parcum*** Rohb.f. has lip such larger than the sepals, broadly orbicular retuse apex and small brownish purple spot on hypochile; ***D. pulchellum*** Roxb ex. Fid. consist of creamy white large flower, lip shortly orbicular oblong with maroon blotches on each side. ***D moschatum*** (Buch. Hom) SW. has large flowers and pouch labellum with two reddish brown blotches at the base.

In subfamily Vandoideae, genus ***Eulophia*** and ***Cymbidium*** of subtribe Cryptopodiinae was collected in recent study. Two species of genus ***Eulophia*** are ***E. andamanensis*** Rchb.f is greenish yellow flower and white broadly claw lip with three keels on hypochile and middle one is largest. ***E graminea*** Lindl possess broadly low lip with three to five keels on hypochile which keels densely covered with long flat papillose. Three genera such as genus ***Acame, Aerides, Rhynchostylist*** of subtribe Sarcanthinae are recorded in recent study. Only one species of genus ***Acampe, Acampe papillosa*** (Lindl) Lindl. possess subumbellate inflorescence and pure white labellum with purple spots on hypochile. One species of genus ***Aerides, Aerides facalta*** Lindl. consists of purple tips with flowers and distal half purple of midlobe. One species of genus ***Rhynchostylis*** is ***Rhychostylist retusa*** (L.) Blume possess drooping dense flower inflorescence and green spur transversely compressed with rounded tip. In this research paper (10) Epiphyte, (3) Terrestrial and (1) aquatic species have been recorded.

Nowaday most of the people are interested in the wild orchids for their attractive colour and shape of flowers. ***Dendrobium aphyllum, D aggregatum, D moschatum, D pulchellum*** are widely distributed in Bago Division. *D aggregatum*, ***D. aphyllum*** and **D. *moschatum*** are native in Myanmar. (**Holttum, 1964).** Among them some species of ***Eulophia graminea*** found in Kachin and Thanintharia (**Kress *et. al*, 2003**) but also found in Bago division. ***D. crepidatum*** and ***D. parcum*** found in Kachin, Chin, Shan, Mandalay, Saging, Thaninthari but this species collected from Bago Division**.** ***Acame papillosa*** recorded in Thaninthari but also collected in Bago Division especially grow well and abundant in Taungoo district and ***Rhynchostylis retusa*** found abundantly in Bago. Bago area is high rainfall and hot weather so epiphyte, Orchidaceae family is widely distributed in Bago area. Some collected species conserved in Bago University by conservation methods for student extra curiculum. Botany students are very interested in orchids conservation.

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The Study Area of Bago Townsip

COMPARATIVE MORPHOLOGICAL AND HISTOLOGICAL STUDIES OF *TALINUM TRIANGULARE* (JACQ.) WILLD. AND *TALINUM PANICULATUM* (JACQ.) GAERTN. (MYANMAR GINSENG)

Nwe Oo[1]

Abstract

Talinum triangulare (Jacq.) Willd. (waterleaf, Ceylon ginseng or leaf ginseng) and *T. paniculatum* (Jacq.) Gaertn. (fame flower, jewels of opar or ginseng jawa) belong to the family Talinaceae. Both were locally known as Myanmar-ginseng. Their leaves have been used as leafy vegetables and roots as medicinal purposes. These were look alike with each other but they have different botanical characters. The root of *T. triangulare* (Jacq.) Willd was light brown and *T. paniculatum* (Jacq.) Gaertn was dark brown. *T. triangulare* (Jacq.) Willd. can easily be distinguished from *T. paniculatum* (Jacq.) Gaertn.by its triangular peduncle and cymose inflorescence (terete peduncle and paniculate cyme in T. paniculatum (Jacq.) Gaertn). The fruits of *T. paniculatum* (Jacq.) Gaertn were colorful like jewels while the fruits of *T. triangulare* (Jacq.) Willd were creamy white with reddish spots and persistent sepals. By using the methods of Trease and Evans (2009), their common histological characters were found as the presence of paracytic stomata, starch grains, calcium oxalate crystals and mucilages in leaves, stem and root. *T. triangulare* (Jacq.) Willd. can be differentiated from *T. paniculatum* (Jacq.) Gaertn. by the presence of triangular outline young stem, presence of papillae and rounded xylem vessels.

Introduction

T. triangulare (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. are commonly known as Myanmar ginseng and belong to the family Talinaceae (formerly Portulacaceae).

T. triangulare (Jacq.) Willd. are used as leafy vegetables and it is a good source of some minerals (e,g., calcium, magnesium and potassium) and vitamins (e.g., ascorbic acid and pyridoxine). The extract from the leaves and roots is used to cure asthma, diuretic, and for the management of gastrointestinal disorders. It is also used to treat scabies, fresh cuts, high blood pressure, and anemia. Some studies reported the presence of alkaloids, flavonoids, saponins and tannins (Ikewuchi, *et al*., 2017).

The leaves of *T. paniculatum* (Jacq.) Gaertn are used as vegetables and medicinal purposes such as reproductive tonic (Thanamool, *et al*., 2013). It is also used in folk medicine to treat ulcers and microbial infections. It has secondary metabolites such as tannins, steroids and triterpenes (Dosreis, *et al*., 2015).

Some authors identified wrongly and confusedly between these two species. They reported the pharmacognostic studies of these two species but the botanically identification was incorrect. Moreover, some local people misunderstand that these two species are same with the Korean ginseng or Panax ginseng. The family of these two species was quite different with the Korean ginseng or Panax ginseng (Araliaceae) although their roots structure and medicinal properties were similar.

The misidentification of plant species and the use of unrelated or closely related inferior quality species can hinder their medicinal properties, the adverse effects of which may even kill a consumer. Therefore, this study was conducted to identify accurately these two species from morphological and histological point of views.

[1] Dr, Assistant Lecturer, Department of Botany, University of Yangon

Materials and Methods

Collection of plant species

The selected plant species were collected from Thingunkyun Township, Yangon.

Identification of plant species

Morphological study

The size, shape, color and measurement of vegetative and reproductive parts of both plant species were photographed and recorded for the morphological study. Both plant species were identified based on their morphological characters with the help of literatures available such as Backer (1963), Dassanayake (1996) etc.

Histological study

The histological characters of fresh leaves, stem and root were studied by free hand sections according to the methods of Trease and Evans (2009). The tissue distributions of lamina, midrib, stem and root of both species were studied for anatomical studies. The surface views of lamina, midrib and stem of both species were studied for cytology. The following reagents were used for histological study.

(1) Chloral hydrate solution as the clearing agent
(2) Phloroglucinol-HCl solution as the test reagent for lignified cell walls
(3) Iodine solution B.P for starch grains
(4) Conc. H_2SO_4 for crystal examination

Results

Morphological characters of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

Scientific name	:	*T. triangulare* (Jacq.) Willd.
Myanmar name	:	Myanmar ginseng
Common name	:	Waterleaf, Ceylon spinach, Leaf Ginseng
Family	:	Talinaceae
Flowering period	:	Throughout the year
Parts used medicinally	:	Leaves and roots

Perennial herbs, about 60 cm in height. **Roots** swollen and fleshy, light brown colour. Stems succulent and green at the upper, and slightly woody, dark purple or brownish and erect at the base. **Leaves** alternate, simple, subsessile, exstipulate, obovate or spatulate, 3.0 – 10.0 × 0.9 - 3.0 cm, succulent, apex emerginate, base narrowly cuneate, margin entire. **Inflorescences** terminal or axillary, cymose, peduncle triangular. **Flowers** bisexual, regular, actinomorphic, pedicellate. **Sepals** 2, aposepalous, lanceolate, 6.0 – 7.0 × 3.0 – 3.5 mm, persistent, white with green prominent veins. **Petals 5**, apopetalous, obovate (12.0 × 6.0 mm), pink. **Stamens** numerous, apostemonous, filament unequal (2.0 – 4.0 mm long), anther dorsifixed. **Carpels** (3), syncarpous, ovary ovoid, about 1.5 × 1.5 mm, free central placentation, style slender, 3.5 mm long, stigma tri fid, pinkish. **Fruits** capsule, ovoid, creamy white with reddish brown spots, many seeded, persistent sepals. **Seeds** globose, reniform (1.2 × 1.2 mm), shining black.

Scientific name	:	*T. paniculatum* (Jacq.) Gaertn.
Myanmar name	:	Myanmar ginseng
Common name	:	Fame flower, Jewels of Opar, Ginseng Jawa
Family	:	Talinaceae
Flowering period	:	Throughout the year
Parts used medicinally	:	Leaves and roots

Perennial herbs, about 50 cm in height. **Roots** swollen, fleshy, dark brown colour. **Stems** succulent and green at the upper, and slightly woody, dark purple or brownish and erect at the base. **Leaves** alternate, simple, subsessile, exstipulate, obovate or obovate-lanceolate (2.0 – 6.5 × 0.8 - 2.5 mm), succulent, apex mucronate, base narrowly cuneate, margin entire. **Inflorescences** terminal, panicle, peduncle long and terete. **Flowers** bisexual, regular, actinomorphic, pedicellate. **Sepals 2**, aposepalous, ovate (1.7 × 1.5 mm), caducous, brown. **Petals 5**, apopetalous, obovate or elliptic (3.0 × 2.0 mm), pinkish. **Stamens** numerous, apostemonous, filament unequal, 1.5 -2.0 mm long, anther dorsifixed. **Carpels** (3), syncarpous, ovary ovoid, about 0.8 × 0.8 mm, free central placentation , style slender, 1.2 mm long, stigma tri fid, pink. **Fruits** capsule, globose, colourful, many seeded. **Seeds** globose reniform (0.8 × 0.8 mm), shining black.

Figure 1 Habit of *T. triangulare* (Jacq.) Willd and *T. paniculatum* (Jacq.) Gaertn.

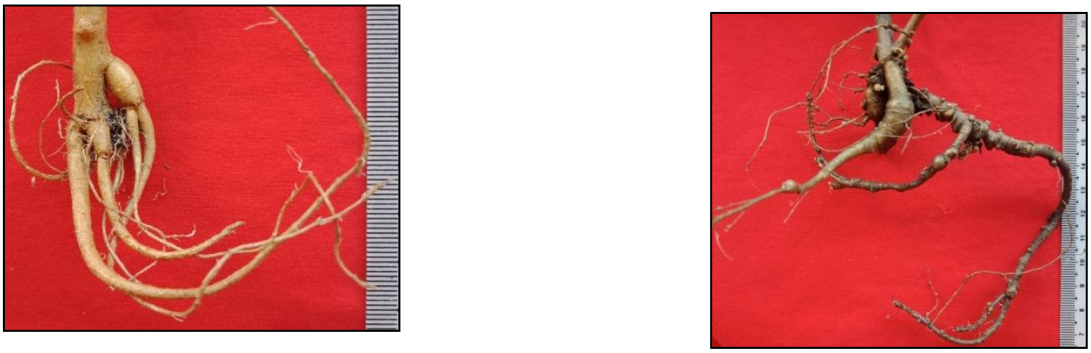

Figure 2 Root of *T. triangulare* (Jacq.) Willd.and *T. paniculatum* (Jacq.) Gaertn.

Figure 3 Various sizes of *T. triangulare* (Jacq.) Willd. leaves

Figure 4 Various sizes of *T. paniculatum* (Jacq.) Gaertn. leaves

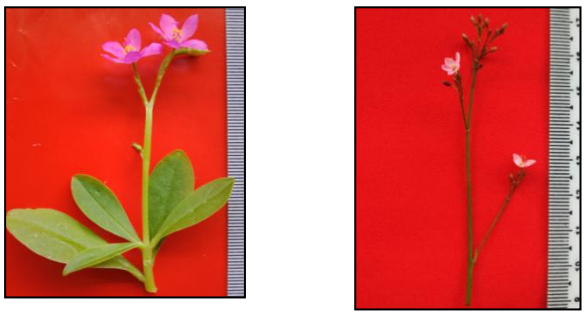

Figure 5 Inflorescence of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

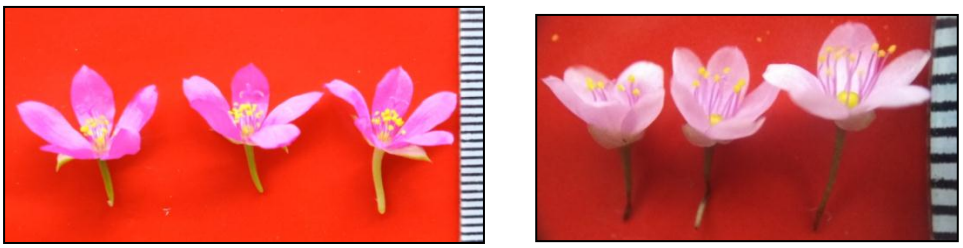

Figure 6 Flowers of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

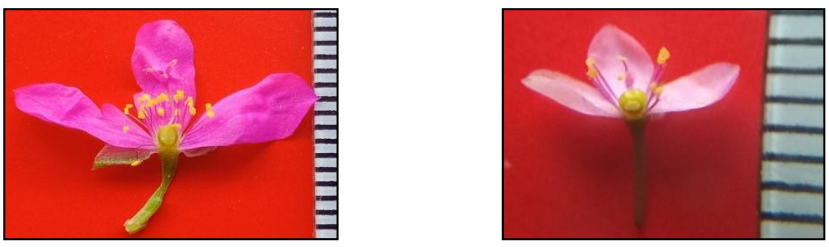

Figure 7 L.S of flower of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

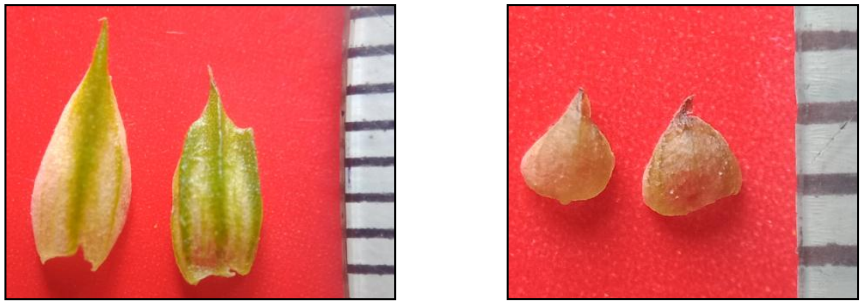

Figure 8 Sepals of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

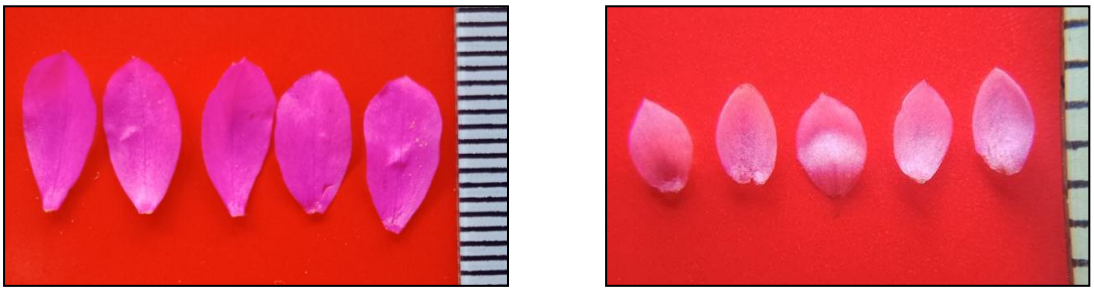

Figure 9 Petals of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

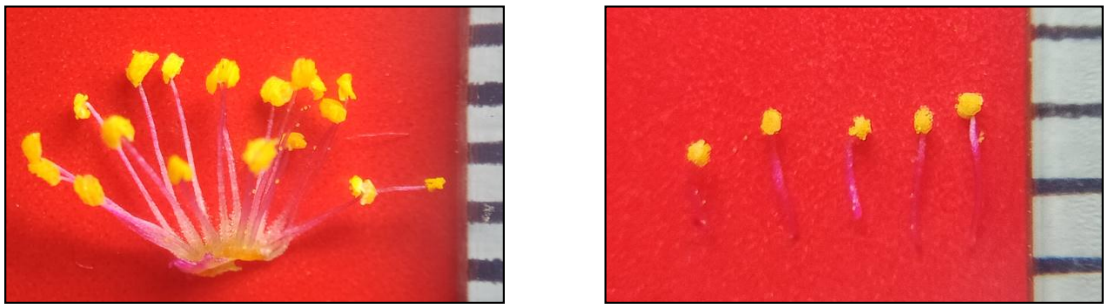

Figure 10 Stamens of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

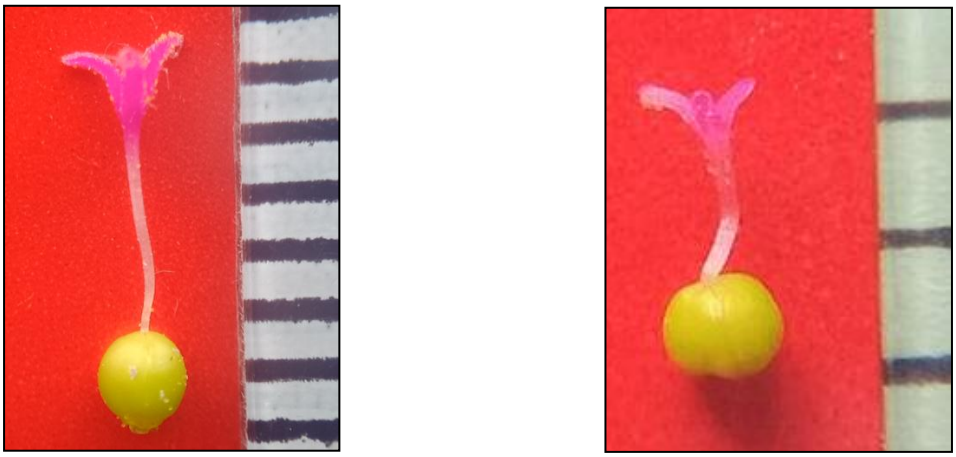

Figure 11 Gynoecium of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

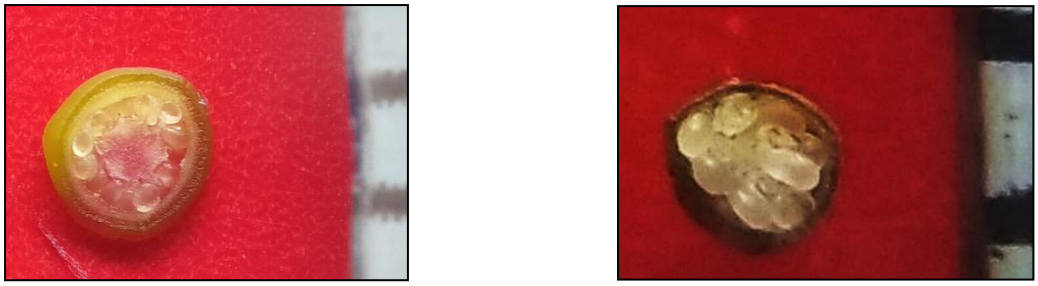

Figure 12 T.S of ovary of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn

Figure 13 Fruits of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

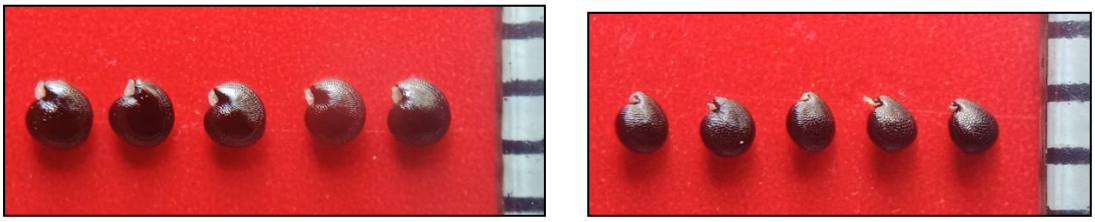

Figure 14 Seeds of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

Histological characters of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

Lamina

In surface views of lamina of both plants, epidermal cells were slightly wavy on upper surfaces. The waiver epidermal cells were present on lower surfaces of both plants. The upper epidermal cells of *T. triangulare* (Jacq.) Willd. were longer than of *T. paniculatum* (Jacq.) Gaertn. Paracytic stomata were found on both surfaces of both plants.

In transverse sections of lamina, both plants have two palisade mesophyll layers under upper epidermis and four layers of spongy mesophyll above lower epidermis. Both plants have calcium oxalate crystals in the mesophyll layers. The larger crystals were found in the mesophyll of *T. triangulare* (Jacq.) Willd.

Midrib

In both plants, the epidermal cells were elongated in surface view of lower portions. Paracytic stomata were also found.

In transverse sections of lamina of both plants, the upper portions were narrow and the lower portions were convex and rounded. The vascular bundles were collateral type. The ground tissue consists of 7 – 9 layers of parenchymatous cells.

Stem

In surface views of both plants, the epidermal cells were pigmented, thick-walled and rectangular to polygonal in shaped.

In transverse sections of the young stems, *T. triangulare* (Jacq.) Willd. was triangular in outline. The young stem of *T. paniculatum* (Jacq.) Gaertn. was more or less rounded in outline. Vascular bundles were arranged into ring in both plants. The cuticle layer of *T. triangulare* (Jacq.) Willd. was thicker than that of *T. paniculatum* (Jacq.) Gaertn.

The collenchymatous layers were more in *T. paniculatum* (Jacq.) Gaertn. The sclerenchymatous bundle cap was found only in *T. triangulare* (Jacq.) Willd. The mucilage and starch grains were abundantly present in both young stems. The larger starch grains were found in stem of *T. paniculatum* (Jacq.) Gaertn. Papillae were found only on the stem of *T. triangulare* (Jacq.) Willd.

Both old stems were circular in outline. The sclerenchymatous bundle caps were present in old stem of both plants.

Root

In transverse sections of both young roots, the vascular bundles were pentarch. The ground tissues consist of about three layers of parenchymatous cells. In old roots, starch grains were found only in *T. paniculatum* (Jacq.) Gaertn.

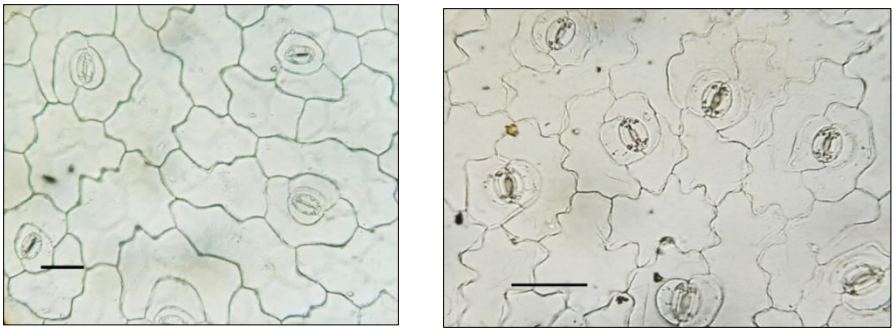

Figure 15 Upper and lower epidermis of *T. triangulare* (Jacq.) Willd. lamina; Bar = 50 μm

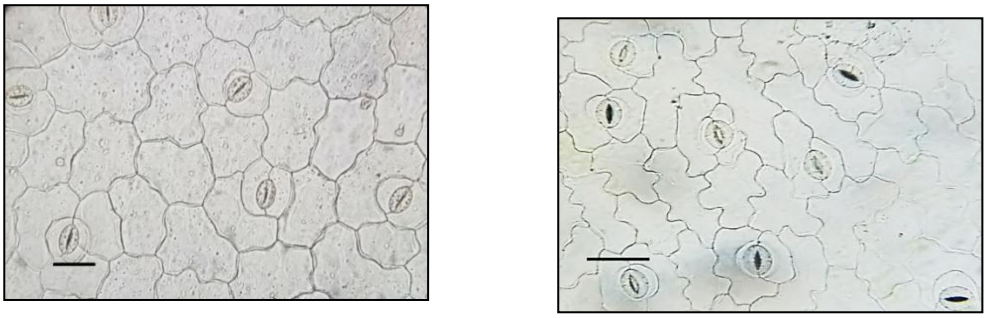

Figure 15 Upper and lower epidermis of *T. paniculatum* (Jacq.) Gaertn lamina; Bar = 50 μm

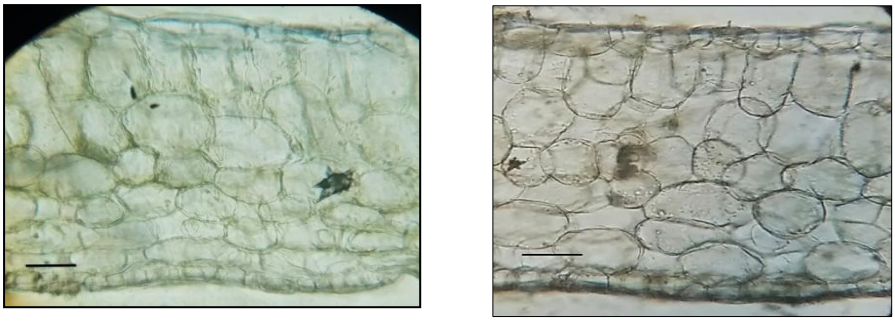

Figure 16 T.S of lamina of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.; Scale bar = 50 μm

Figure 17 Epidermis of midrib of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. Bar = 50 μm

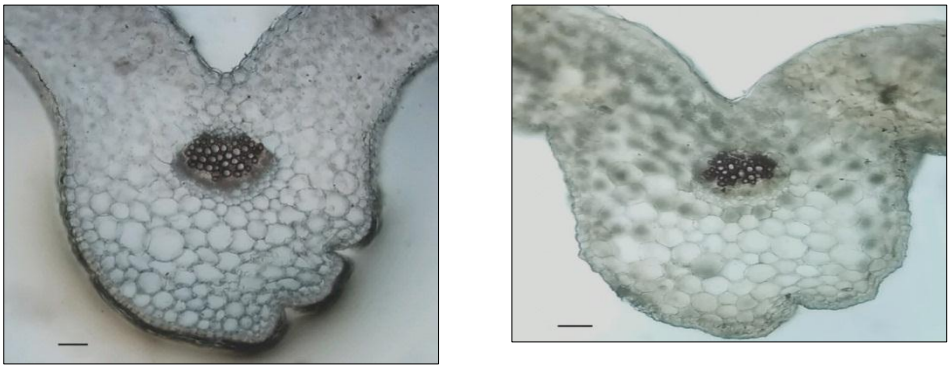

Figure 18 T.S of midrib of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. Bar = 100 μm

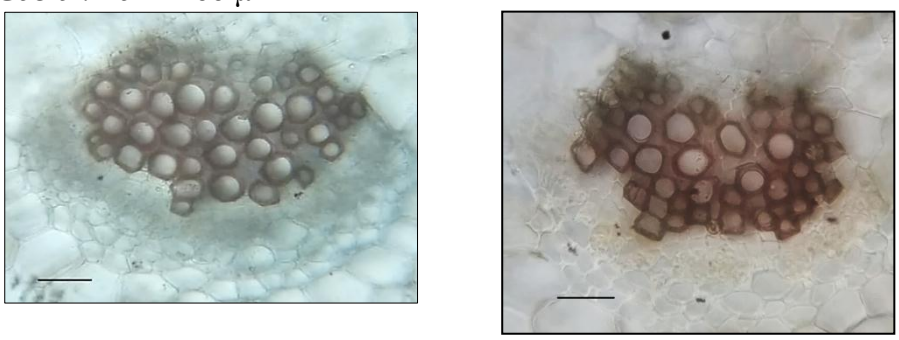

Figure 19 Vascular bundles of midrib of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. Bar = 50 μm

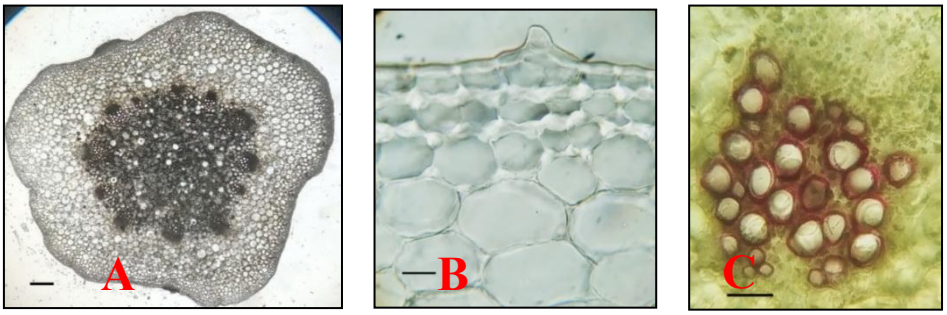

Figure 20 T.S of young stem of *T. triangulare* (Jacq.) Willd., (A) Outline; Bar = 300 µm (B) Collenchyma and parenchyma cells, (C) Vascular bundle; Bar = 50 µm

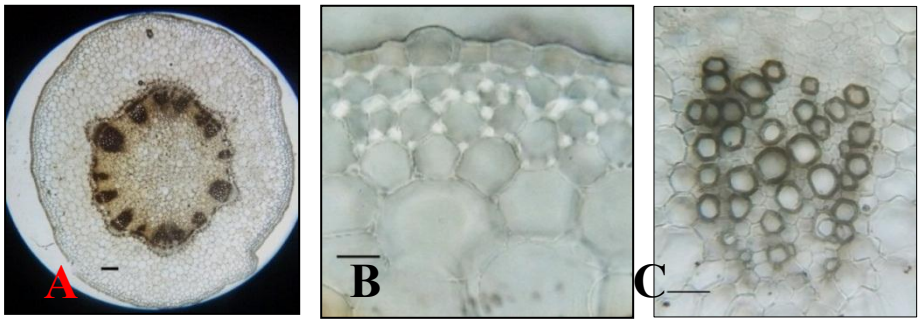

Figure 21 T.S of young stem of *T. paniculatum* (Jacq.) Gaertn., (A) Outline treated with iodine solution; Bar = 300 µm, (B) Collenchyma and parenchyma cells, (C) Vascular bundle; Bar = 50 µm

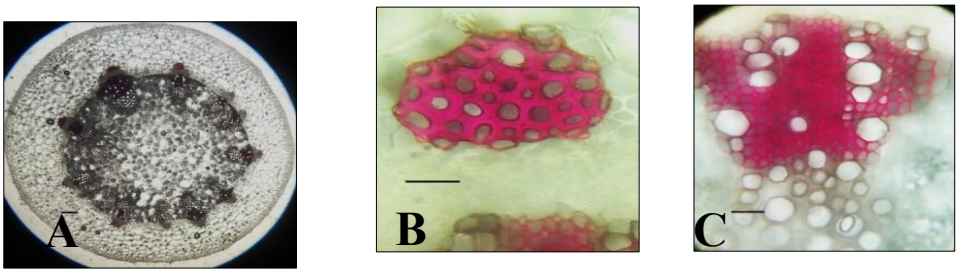

Figure 22 T.S of old stem of *T. triangulare* (Jacq.) Willd. (A) Outline; Bar = 300 µm, (B) Group of pericyclic fibers, (C) Vascular bundle; Bar = 50 µm

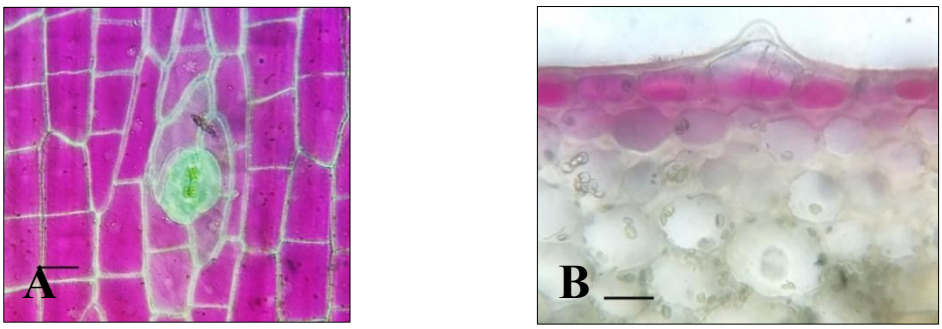

Figure 23 Epidermis of old stem of *T. triangulare* (Jacq.) Willd. (A) Surface view (B) Transverse section; Bar = 50 µm

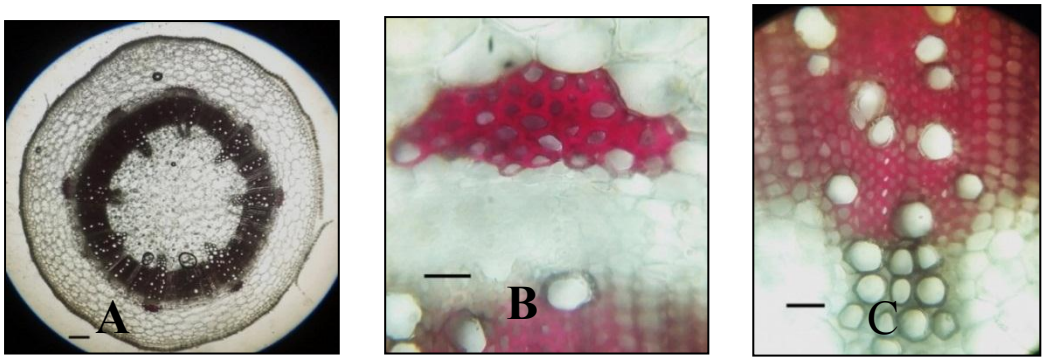

Figure 24 T.S of old stem of *T. paniculatum* (Jacq.) Gaertn., (A) Outline; Bar = 300 μm, (B) Group of pericyclic fibers, (C) Vascular bundle; Bar = 50 μm

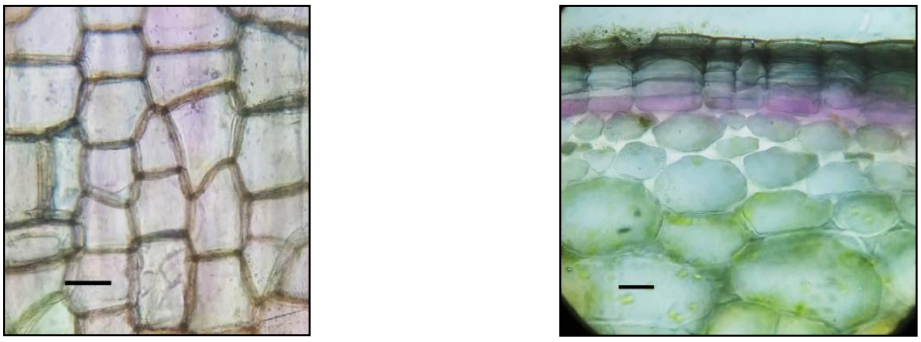

Figure 25 Periderm of old stem of *T. paniculatum* (Jacq.) Gaertn.; Bar = 50 μm

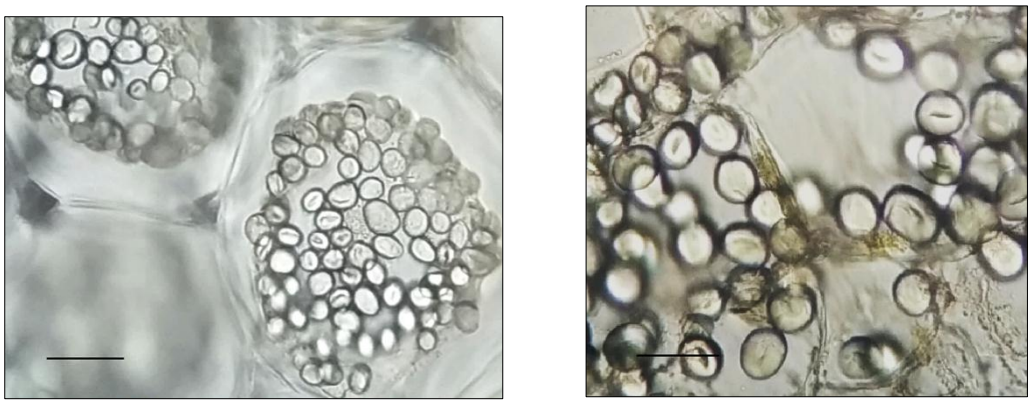

Figure 26 Starch grain found in stems of *T. triangulare* (Jacq.) Willd. And *T. paniculatum* (Jacq.) Gaertn.; Bar = 50 μm

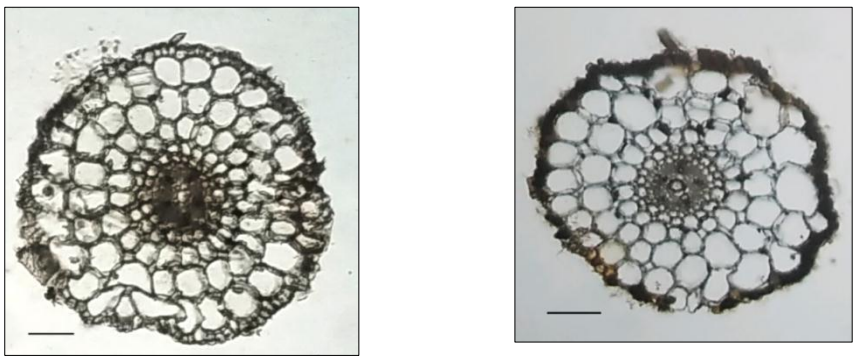

Figure 27 T.S of young root of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. Bar = 100 μm

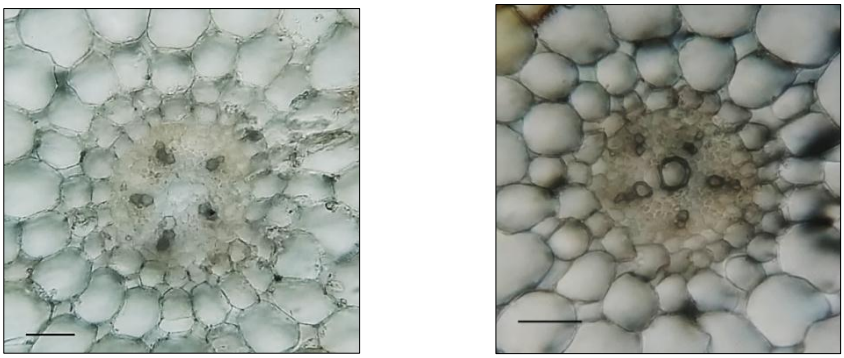

Figure 28 Vascular bundles of young root of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. Bar = 50 μm

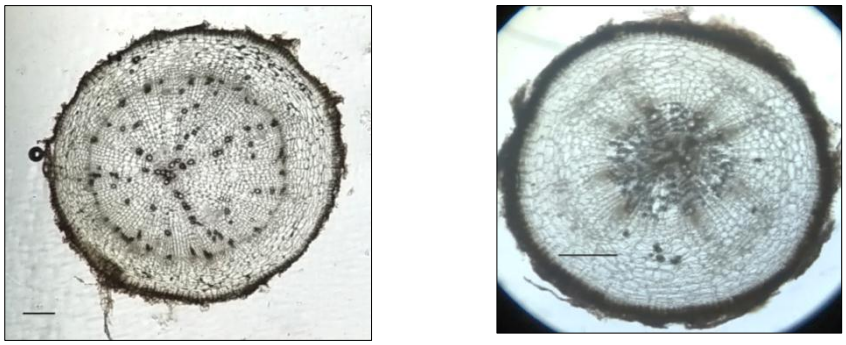

Figure 29 T.S of old root of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. Bar = 300 μm

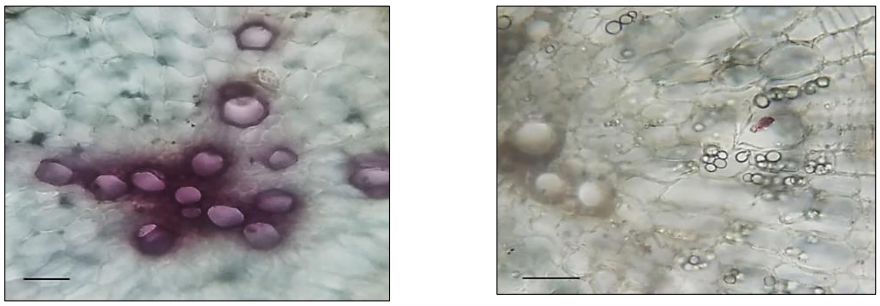

Figure 30 Vascular bundle of old root of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. Bar = 50 μm

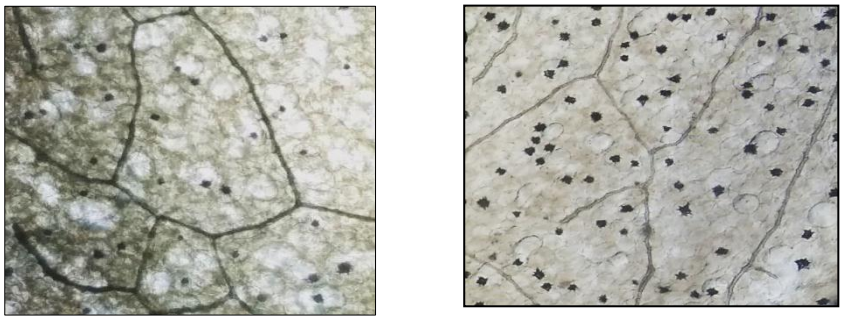

Figure 31 Veins of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn. on per square mm of leaves

Table 1 Numerical values of leaves of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

Characters	**Values**	
	***T. triangulare* (Jacq.) Willd.**	***T. paniculatum* (Jacq.) Gaertn.**
Stomatal index (upper)	13.2 – 13.8 – 16.8	9.6 – 11.3 – 12.8
Stomatal index (lower)	27.2 – 28.5 – 30.7	21.1 – 23.7 – 30.3
Palisade ratio	1.3 – 2.0 – 2.3	1.3 – 1.6 – 2.0
Vein-islets number	2.0 – 2.5 – 3.0	1.0 – 2.0 – 4.0
Vein terminations number	1.0 – 2.3 – 3.0	1.0 – 2.0 – 3.0

Table 2 Comparative morphological features of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

Features	**Species**	
	***T. triangulare* (Jacq.) Willd.**	***T. paniculatum* (Jacq.) Gaertn.**
Colour of root	Light brown	Dark brown
Leaf apex	Retuse	Acute
Inflorescence type	Cymose	Panicle cyme
Peduncle	Triangular	Terete
Sepal	Persistent, white with green prominent veins	Caducous, brown
No. of stamens	Numerous	10
Fruits	Creamy white with reddish brown spot	Yellowish green to brown

Table 3 Comparative histological features of *T. triangulare* (Jacq.) Willd. and *T. paniculatum* (Jacq.) Gaertn.

Features	**Species**	
	***T. triangulare* (Jacq.) Willd.**	***T. paniculatum* (Jacq.) Gaertn.**
Outline of young stem	Triangular	More or less rounded
Xylem vessels of young stem	Circular	Rectangular to polygonal
Papillae on stem	Presence	Absence
Starch in stem	Abundantly presence	Sparsely presence
Starch in old root	Absence	Presence

Discussion and Conclusion

According to morphological study, *T. triangulare* (Jacq.) Willd. can be distinguished from *T. paniculatum* (Jacq.) Gaertn. by its light brown color root. According to Backer (1963) and Dassanayake (1996), *T. triangulare* (Jacq.) Willd. can be differentiated from *T. paniculatum* (Jacq.) Gaertn. by its triangular flowering stem, leaves obtuse or emarginated and stamens more than 15. These characters were the same with those findings in this study.

Another differences between these two species were the shape and colour of sepals and fruits. Their flowering time was also different. Backer (1963) stated *T. triangulare* (Jacq.) Willd. as forenoon-flowering plant and *T. paniculatum* (Jacq.) Gaertn. as afternoon-flowering plant. *T. triangulare* (Jacq.) Willd. flowered around 10 am. The flowers of *T. paniculatum* (Jacq.) Gaertn. were collected at around 4 pm. These morphological characters can help in the identification and between these two related species.

In this study, the histology of both species was investigated and compared. Although the basic histological characters of these two species were relatively similar, some distinctive differences were found. *T. triangulare* (Jacq.) Willd. can be differentiated from *T. paniculatum* (Jacq.) Gaertn. by the presence of triangular outline young stem, presence of papillae and rounded xylem vessels.

Metcalfe and Chalk (1950) reported that rubiaceous stomata were present on both surfaces in certain species of *Talinum*. Clustered crystals, papillae and mucilage cells were also present. Metcalfe and Chalk (1950) also stated that the presence of mucilage make it very difficult to cut freehand sections of member of this family. The findings in this study were confirmed with those mentioned by Metcalfe and Chalk (1950).

These distinctive histological characters can be used for species identification and differentiation.The phytochemical constituents and medicinal properties of these two species will be studied in the future.

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ABUNDANCE AND DIVERSITY OF POLYCHAETES IN THE INTERTIDAL ZONE OF MYEIK COASTAL AREAS*

War War Soe[1]

Abstract

A total of 120 polychaete species belonging to 89 genera and 35 families were collected from 10 intertidal zone of Myeik Coastal Areas (Light-house, Daung-kumaw, Kyauk-phya, Pahtaw-west, Masanpa ,Maing, Panataung, Done-pale-aw, Sha-aw and Kyun-mweyar) for a period of two years from June 2010 to May 2012. The most speciose family was spionidae (12species). Species belonging to families Capitellidae, Neredidae, Orbiniidae, Glyceridae and Lumbrineridae were abundant. Polychaetes ranged from 0 to 172 individuals collected with the highest count recorded in Light- house Station (1003 individuals with 72 species) and the lowest count in Pahtaw-west Station (66 individuals with 15species). The higher composition in order of abundance was *Glycera sp., Heteromastus similis* and *Notomastus fauveli*. The highest species diversity was found in Panataung Station (3.62), near mainland and Done-pale-aw Station (3.22) away from mainland. The lowest values for species diversity were recorded in Daung-kumaw Station (2.26) and Sha-aw Station (2.73).

Keywords: Abundance, diversity, intertidal zone, Myeik Coastal Areas and polychaetes

Introduction

Polychaetes or bristle worms are a group of segmented worm and the largest group of the phylum Annelida.”Polychaete” means “many hairs, refer to the chitinous hairs that protrude from either side of their bodies, with an identical set of hairs per segment. They are one of the most abundant and diverse group in marine environments. They occur from the intertidal areas to the deepest oceanic trenches. They are mainly free-living, while some are commensal and very few are parasitic. Some species reproduced asexually and some sexually. Generally, polychaetes are separated into two large orders Errantia (free-living) and Sedentaria (living in burrows or tubes). They assist the deposition, breakdown and turnover of the organic matter in the sea bed that help to recycle nutrients to the overlying water column. They are an important link in marine food-webs. Due to the high content of protein, both the adult and the larvae of polychaetes are the main food of many economically important fishes. Polychaetes are very useful as indicator organisms for monitoring the health of marine environment (Ananthan, 2005). Some polychaete worms have been used as fish baits and were excellent live food for shrimp and fish in aquaculture with the results that maturation and breeding rates are higher.

To date over 20,000 species of polychaetes have been described and they are classified into over 70 families (Hutchings, 1984). Literatures on polychaetes of the intertidal zone in Myanmar were very rare, almost not existence. Except Yan-Kyu *et al.* (1974) conducted on Mon State; so far there was no actual record on the intertidal polychaete of Myanmar.

Previous study on polychaetes along Myeik area was conducted by Si Thu Hein (2011). This paper outlined the preliminary results on the polychaete profile within zone of Myeik Coastal areas. The main objectives of this study were:1) To know what kinds of polychaete are present in Myeik Coastal Area, 2) To state the abundance and diversity of polychaete in the study areas and 3) To establish base line data for future study.

[1] Dr, Lecturer, Department of Marine Science, Myeik University

* Best Paper Award Winning Paper in Marine Science (2019)

Materials and Methods

Study areas

The study area is situated in Myanmar southern coastal zone, Tanintharyi Division. Myeik coastal land mass is surrounded by waters mixing with marine and brackish waters. A total of ten exposed soft sediment intertidal stations were selected, seven stations off Myeik area and three stations off Thayawthadangyi-kyun and its vicinity. Regular monthly collections were made at six stations (Light-house 12° 29´N & 98°35´E, Daung-kumaw 12° 24´N & 98° 36´E, Kyauk-phya 12° 30´ N & 98° 41´E, Pahtaw-west 12° 27´N & 98° 34´E, Masanpa 12° 24´N & 98° 30´E and Maing 12° 21´N & 98° 29´E) and seasonal collections at four stations (Panataung 12° 37´N & 98° 30´E, Done-pale-aw 12° 22´N & 98° 4´E, Sha-aw 12° 25´N & 98° 6´E and Kyun-mweyar 12° 21´N & 98° 4´E) for a period of two years from June 2010 to May 2012. The Location of the study areas were shown in Figure (1)**.**

Sampling procedure

Three replicate sediment samples for 10 stations at three different tidal zones (High-tide, Mid-tide and Low-tide levels) were collected using a shovel**.** Sediment samples were taken in bucket and mixed with water. Then the mixed water passed through a hand sieve with 2mm mesh. Big animals were picked out by hand and small animals by a pair of forceps. The sieved organisms were preserved with 5% formaldehyde solution and then transferred to the laboratory for further analysis. Binocular microscope and compound microscope with digital camera were used to identify and capture the image of polychaetes. Identification of species was based primarily on Day (1967); Hutchings (1984) and Ananthan *et al.* (2005)**.** In some cases specimens could not be identified up to the species level due to damaged or unresolved taxonomic problems. Numerical abundance of each species were recorded and expressed as no./m^2.

Water temperature, salinity and soil pH were measured by thermometer, refractometer and soil pH meter in the field. Species diversity, Evenness and richness of polychaete were calculated by using the formula of Shannon-Wiener (1963) diversity index (H'), Pielou's (1966) evenness index (E') and Margalef's (1968) richness index (D').

$$H' = -\Sigma\ Pi\ In\ Pi$$

$$E' = H'/ In\ S$$

$$D' = S-1 / In\ N$$

Where, Pi = the proportional abundance of the i^{th} species (n_i / N),

n = the number of individual of the i^{th} species,

N = the total number of individuals in a transect, and

S = the total number of species

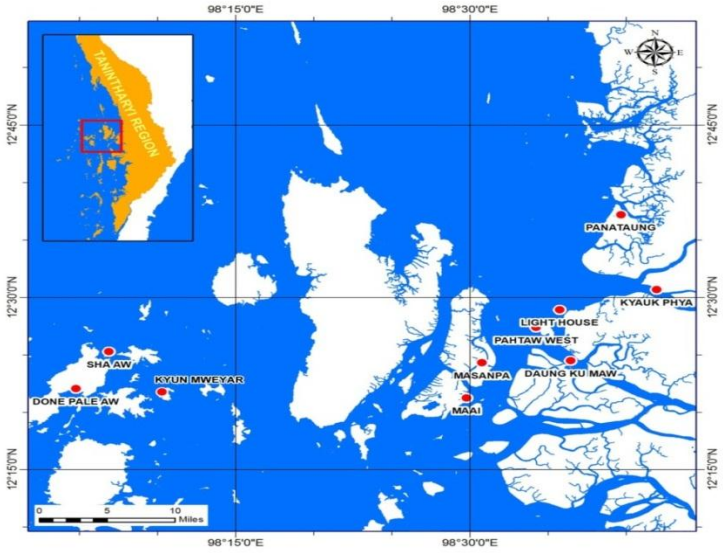

Figure 1 Map showing the specimen collecting sites in Myeik Coastal Areas.

Results

A total of 120 species of polychaetes belonging to 89 genera and 35 families were recorded. Errantia was represented by (49) species and sedentaria by (71) species. These species were from 15 families of errantia and 20 families of sedentaria.

Of the 49 species of errantia, the breakdown was as followed: 2 Amphinomidae, 1 Glyceridae, Goniadidae, Polyodontidae, Syllidae and Arabellidae each, 5 Hesionidae, 11 Nereidae, 7 Phyllodocidae, 3 Pilargidae, 4 Polynoidae, Eunicidae and Lumbrineridae, 2 Sigalionidae and Onuphidae. And the 71 species of Sedentaria: 5 species of Capitellidae, 8 species of Maldanidae and Orbiniidae each, 1 species each for Chaetopteridae, Cossuridae, Oweniidae, Magelonidae, Poecilochaetidae, Sternaspidae , Pectinariidae and Trichobranchidae, 4 species each for Cirratulidae and Serpulidae, 2 species each for Flabelligeridae and Ophellidae, Paraonidae and Ampharetidae, 3 species of Sabellidae, 12 species of Spinodae, 7 species of Terebellidae and 4 unidentified polychaete belonging to family Terebellidae, Hesionidae and probably Serpulidae.

Among them (98) species of polychaetes were found in Seven stations, near with Myeik mainland while (71) species were occurred along the Thayawthadangyi-kyun. The most speciose family was Spionidae (12 species), followed by Nereidae (11 species) .Only *Glycera sp.* occurred at all stations (406 individuals), with a density ranged from 2 to88 no./m^2 and was the most abundant at Light-house Station (88 individuals). *Heteromastus similis* was the second in abundant (307) with a density 0 to 180 no./m^2. *Notomastus fauvel* was the third abundant group (224) with an abundance of 0 to107 no./m^2. *Some were restricted in only one station such as Eurythoe sp, Syllidia sp, and Podarke sp*. The numerical abundance of polychaetes was generally high in Light- house Station (1003 individuals) for monthly data collection and Sha-aw Station (726 individuals) for five months seasonal collection. Panataung Station remained at second place in terms of abundance and species composition (628 individuals with 69 species). The composition differed at different stations. The highest species composition occurred in Light-

house Station (72 species) and Done-pale-aw Station (45 species). Pahtaw west (66 individuals with 15 species) and Kyun-mweyar (191 individuals with 27 species) Stations recorded the lowest abundance and lowest species composition.

The total abundance of polychaetes varied from 1 to 406 individuals. Near stations with Myeik mainland, polychaetes ranged from 0 to 141 individuals collected with the highest count recorded in Panataung Station (141no./m^2) in August 2011 and there was no records of polychaetes in Pahtaw-west Station (0 no./m^2) in March and August 2011. At away from mainland (Thayawthadangyi-kyun), polychaetes ranged from 31to 172 individuals collected with the highest count recorded in Sha-aw Station (172 no./m^2) and the lowest count in Kyun-mweyar Station (31 no./m^2) in September 2010. The monthly abundance and total abundance of polychaetes of ten stations was shown in Table (1). The abundance of each species from ten stations of Myeik Coastal Areas was shown in Table (2).

Family Nereidae was most abundant at Light-house Station. Capitellidae and Pilargidae were considerably abundant at Daung-kumaw and Kyauk-phya Stations. Glyceridae and Lumbrineridae were slightly higher in representation at Pahtaw-west. At Masanpa, Nereidae was very abundant. Glyceridae , Nereidae and Onuphidae showed their equal abundance at Maing Station. Maldanidae was very abundant in Panataung Station. Done-pale aw Station was dominated by Orbiniidae although Sha-aw was influenced by Capitellidae. Kyun-mweyar Station was also represented by Nereidae family.

Diversity index of polychaetes at each station were varied. The highest diversity (3.62) was recorded in Panataung Station followed by Light-house Station (3.56) and the lowest diversity (2.26) in Daung-Kumaw Station. At Thayawthadangyi-kyun, the highest diversity (3.22) was recorded in Done-pale-aw Station and the lowest diversity (2.73) in Sha-aw Station. Since Species diversity incorporates both the richness and an evenness property of the sample, the value of species richness and evenness indices mainly followed the trend of species diversity. Three diversity indices of polychaete of each station were shown in Table 3 and Figure 2.

Panataung Station is the most diversified sites and Spionidae family was the most diversified family with most representatives during the present study. On comparing the ten study areas for species evenness index high values were observed at Kyun-mweyar Staion and Panataung Station (0.86). At Sha-aw Station, the evenness in the distribution was comparatively low (0.74). So the polychaetes were not distributed with uniformity and indicating clear cut changes in the environmental conditions. The richness of the study areas varied from 2.62 to 10.55. Species richness measured by Margalef method was very low at Kyauk-phya Station (2.62).

Table 1 Monthly abundance (no./m^2) and total abundance of intertidal polychaetes in Myeik Coastal

Station	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Total
	2010												2011									2012				
Light-house	92	69	92	79	45	30	51	30	23	26	18	28	25	30	37	39	33	41	21	26	33	34	35	66	1003
Daung-kumaw	26	12	14	12	14	13	8	16	5	5	8	14	7	2	6	5	6	5	6	13	11	9	7	12	236
Kyauk-phya	19	22	12	14	27	22	14	17	28	24	21	19	18	26	14	19	11	24	11	16	18	16	12	20	444
Pathaw-west	3	3	6	1	3	2	5	2	2	0	2	2	2	5	0	2	2	1	4	3	4	5	1	6	66
Masanpa	25	18	20	27	5	15	5	9	4	8	10	24	14	10	24	7	8	12	5	1	14	11	7	20	303
Maing	25	39	27	20	18	15	11	17	17	17	13	13	17	13	23	12	12	11	15	14	16	3	25	19	412
Panataung			105				138			139					141				105						628
Done-pale-aw				110					139						81				102			54			486
Sha-aw				172					131						148				126			149			726
Kyun-mweyar				31					45						33				50			32			191

Table 2 Abundance of each polychaete species at different study areas

Sr.no	Family	Species	Light-house	Daung-Kumaw	Kyauk-Phya	Pahtaw-west	Masanpa	Maing	Panataung	Done-pale-aw	Sha-aw	Kyun-mweyar	Total
1	Amphinomidae	*Eurythoe sp.*	_	_	_	_	_	_	_	1	_	_	1
2		*Pseudoeurythoe sp.*	6	_	_	_	3	_	_	_	_	_	9
3	Glyceridae	*Glycera sp.*	88	41	75	22	48	62	51	10	7	2	406
4	Goniadidae	*Goniada sp.*	7	_	_	4	1	3	6	2	_	_	23
5	Hesionidae	*Leocrates sp.*	2	_	_	_	1	_	2	_	_	5	10
6		*Syllidia sp.*	_	_	_	_	_	_	2	_	_	_	2
7		*Telehsapia sp.*	9	6	6	2	2	3	_	_	_	_	28
8		*Ophiodromous sp.*	3	_	_	1	_	_	1	_	_	_	5
9		*Podarke sp.*	_	_	_	_	_	_	_	1	_	_	1
10	Nereididae	*Dendronereis sp.*	_	_	_	_	_	_	1	_	_	_	1
11		*Gymnonereis sp.*	29	_	_	6	_	_	28	45	56	23	187
12		*Laeonereis sp.*	4	_	_	2	_	_	4	9	8	6	33
13		*Namalycastis indicus*	4	_	_	_	1	_	_	_	_	_	5
14		*Namanereis sp.*	79	_	_	_	25	4	25	_	_	_	133
15		*Nereis sp.*1	9	1	7	_	8	7	14	8	8	_	62
16		*Nereis sp.*2	24	2	21	_	9	8	5	12	5	_	86
17		*Nereis sp.*3	129	_	_	_	12	30	1	_	_	_	172
18		*Nereis sp.*4	19	1	_	2	9	13	2	4	8	23	81
19		*Perinereis sp.*	29	_	_	_	_	_	_	_	_	_	29
20		*Ceratonereis sp.*	_	_	_	_	_	_	1	_	_	_	1

Table 2 Continued

Sr.no	Family	Species	Stations										Total
			Light-house	Daung-Kumaw	Kyauk-Phya	Pahtaw-west	Masanpa	Maing	Panataung	Done-pale-aw	Sha-aw	Kyun-mweyar	
21	Phyllodocidae	*Eteone sp.*	_	_	_	_	_	_	4	_	_	_	4
22		*Phyllodoce sp.*1	_	_	_	_	_	_	1	_	_	_	1
23		*Phyllodoce sp.*2	2	_	_	_	1	_	4	_	_	_	7
24		*Phyllodoce sp.*3	2	_	_	2	_	_	1	_	_	_	5
25		*Phyllodoce sp.*4	_	_	_	_	_	_	_	3	_	_	3
26		*Phyllodoce sp.*5	_	_	_	_	_	_	1	_	_	_	1
27		*Eulalia sp.*	4	_	_	_	1	2	_	_	_	_	7
28	Pilargiidae	*Ancistrosyllis sp.*	1	_	_	_	_	_	_	_	_	_	1
29		*Parandalia sp.*	7	15	19	_	6	7	_	_	_	_	54
30		*Pilargis sp.*	25	58	56	_	14	12	_	_	_	_	165
31	Polynoidae	*Polyeunoa sp.*	_	_	_	_	_	_	6	_	_	_	6
32		*Lepidonotus sp.*	1	_	_	_	_	_	_	1	_	_	2
33		*Iphione sp.*	_	_	_	_	_	_	_	_	_	1	1
34		*Harmothoe sp.*	_	_	_	_	_	_	_	_	1	3	4
35	Polyodontidae	*Polydontes sp.*	1	_	_	_	_	_	_	_	_	_	1
36	Sigalionidae	*Sagalion sp.*	_	_	_	_	_	_	1	_	_	_	1
37		*Sthelenalis sp.*	_	_	_	_	_	_	4	_	_	_	4
38	Syllidae	*Syllis sp.*	1	_	_	_	_	1	3	4	10	_	19
39	Capitellidae	*Heteromastus similis*	22	9	48	_	_	21	_	27	180	_	307
40		*Notomastus*	18	8	51			11	1	3	52	3	147

Table 2 Continued

Sr.no	Family	Species	Stations										Total
			Light-house	Daung-Kumaw	Kyauk-Phya	Pahtaw-west	Masanpa	Maing	Panataung	Done-pale-aw	Sha-aw	Kyun-mweyar	
41	Capitellidae	*Notomastus fauveli*	11	27	46	_	_	19	3	7	107	4	224
42		*Notomastus aberans*	6	33	21	_	11	4	13	_	_	2	90
43		*Pulliella sp.*	13	13	19	_	7	5	_	_	_	_	57
44	Maldanidae	*Axiothella sp.1*	28	_	_	_	_	_	28	6	_	10	72
45		*Axiothella sp.2*	1	_	_	_	_	_	_	_	21	_	22
46		*Euclymene quadrilobata*	13	_	_	_	_	_	38	3	_	_	54
47		*Euclymene mossambica*	_	_	_	_	_	_	6	_	_	_	6
48		*Euclymene annandalei*	15	_	_	_	_	_	33	2	13	_	63
49		*Euclymene luderitziana*	5	_	_	_	_	_	2	_	5	6	18
50		*Nicomache sp.*	_	_	_	_	_	_	15	3	_	_	18
51		*Petaloproctus sp.*	1	_	_	_	_	_	_	_	_	_	1
52	Chaetopteridae	*Phyllochaetopterus sp.*	1	_	_	_	_	_	_	_	_	1	2
53	Cirratulidae	*Cirriformia sp.*	_	_	_	_	_	2	32	18	11	_	63
54		*Cirratulus sp.*	4	_	_	_	_	_	2	27	_	_	33
55		*Tharyx sp.*	2	_	_	_	7	3	11	11	_	_	34
56		*Audouinia sp.*	2	_	_	_	_	_	_	5	_	_	7
57	Cossuridae	*Cossura sp.*	12	_	_	_	2	7	8	_	_	_	29
58	Eunicidae	*Marphysa sp.* 1	_	_	_	_	_	_	_	2	_	_	2
59		*Marphysa sp.* 2	12	_	_	_	_	_	_	24	67	34	137
60		*Marphysa sp.* 3	__-	-_	-_	-_	_-	_-	15	-_	-_	-_	15

Table 2 Continued

Sr.no	Family	Species	Stations										Total
			Light-house	Daung-Kumaw	Kyauk-Phya	Pahtaw-west	Masanpa	Maing	Panataung	Done-pale-aw	Sha-aw	Kyun-mweyar	
61	Eunicidae	*Eunice sp.*	_	_	_	_	_	_	_	_	_	2	2
62	Arabellidae	*Arabella sp.*	_	_	_	_	_	_	_	4	_	_	4
63	Lumbrineridae	*Lumbrinereis sp.*1	15	11	25	_	18	9	15	24	42	6	165
64		*Lumbrinereis sp.*2	6	_	_	3	2	_	10	37	19	13	90
65		*Lumbrinereis sp.*3	_	_	_	8	_	_	_	_	_	_	8
66		*Lumbrinereis sp.*4	7	5	9	_	5	4	9	_	_	_	39
67	Onuphidae	*Diopatra sp.*	45	_	_	_	8	51	23	5	6	_	138
68		*Onuphis sp.*	10	_	_	_	6	11	43	9	3	_	82
69	Flabelligeridae	*Pherusa sp.*	_	_	_	_	_	_	3	_	_	_	3
70		*Stylarioides sp.*	_	_	_	_	_	_	_	1	_	_	1
71	Magelonidae	*Magelona sp.*	9	_	_	_	_	3	_	_	_	_	12
72	Opheliidae	*Armandia sp.*	5	1	4	_	4	21	3	_	6	_	44
73		*Ophelina sp.*	12	_	2	_	7	8	4	_	_	_	33
74	Orbiniidae	*Orbina sp.*	4	_	_	3	_	_	2	9	_	_	18
75		*Haploscoloplos sp.*	1	_	_	_	_	_	18	_	_	_	19
76		*Scoloplos marsupialis*	56	_	_	_	3	33	_	21	7	_	120
77		*Scoloplos johnstonei*	13	_	_	_	_	_	1	_	_	_	14
78		*Scoloplos madagascariensis*	_	_	_	_	_	_	_	23	14	_	37
79		*Scoloplos armiger*	_	_	_	1	47	11	7	62	7	15	150
80		*Scoloplos sp.*	4	_	34	2	_	_	20	32	26	8	126

Table 2 Continued

Sr.no	Family	Species	stations										
			Light-house	Daung-Kumaw	Kyauk-Phya	Pahtaw-west	Masanpa	Maing	Panataung	Done-pale-aw	Sha-aw	Kyun-mweyar	Total
81		*Phylo sp.*	_	_	_	_	_	_	4	2	_	_	6
82	Paraonidae	*Aricidea sp.*	19	_	_	5	6	11	6	_	_	_	47
83		*Paraonis sp.*	11	_	_	3	6	4	2	3	_	2	31
84	Oweniidae	*Owenia sp.*	3	_	_	_	_	_	_	_	_	_	3
85	Sabellidae	*Potamilla sp.*	_	_	_	_	_	_	2	_	_	_	2
86		*Branchiomma sp.*	_	_	_	_	_	_	_	_	2	_	2
87		*Megalomma sp.*	_	_	_	_	_	_	1	_	_	_	1
88	Serpulidae	*Serpula sp.*	2	_	_	_	_	_	_	_	2	_	4
89		*Pomatoceros sp.*	_	_	_	_	_	_	_	_	_	2	2
90		*Hydroides sp.*	_	_	_	_	_	_	_	_	1	_	1
91		*Vermiliopsis sp.*	_	_	_	_	_	_	_	_	2	3	5
92	Spionidae	*Dispio sp.*	_	_	_	_	_	_	1	_	_	_	1
93		*Malacoceros sp.*	36	_	_	_	7	13	3	_	3	_	62
94		*Nerinides sp.*	18	5	1	_	10	1	2	3	1	_	41
95		*Orthoprionospio sp.*	_	_	_	_	_	_	1	_	_	_	1
96		*Spio sp.*	4	_	_	_	_	_	_	_	_	_	4
97		*Prionospio pinnata*	10	_	_	_	_	_	_	_	_	_	10
98		*P. malmgreni*	16	_	_	_	_	_	2	_	4	_	22
99		*P. sexoculata*	3	_	_	_	_	_	5	1	_	2	11
100		*P. steensterpuri*	1	-	-_	_-	-_	_-	_-	-_	-_	_-	1

Table 2 Continued

Sr.no	Family	Species	Stations										Total
			Light-house	Daung-Kumaw	Kyauk-Phya	Pahtaw-west	Masanpa	Maing	Panataung	Done-pale-aw	Sha-aw	Kyun-mweyar	
101	Spionidae	*Polydora caeca*	19	_	_	_	3	_	_	_	_	_	22
102		*P. flava*	_	_	_	_	_	_	_	1	_	_	1
103		*Pseudopolydora sp.*	4	_	_	_	_	_	_	_	5	_	9
104	Poecilochaetidae	*Poecilochaetus sp.*	10	_	_	_	_	5	29	_	_	_	44
105	Sternaspidae	*Sternaspis sp.*	2	_	_	_	2	3	_	_	_	_	7
106	Pectinariidae	*Pectinaria sp.*	_	_	_	_	_	_	3	_	_	_	3
107	Terebellidae	*Amaeana sp.*	_	_	_	_	_	_	19	_	4	_	23
108		*Lysilla sp.*	_	_	_	_	_	_	1	5	1	_	7
109		*Polycirrus sp.*1	_	_	_	_	_	_	2	_	_	_	2
110		*Polycirrus sp.*2	_	_	_	_	_	_	_	_	5	1	6
111		*Lanice sp.*	2	_	_	_	_	_	3	_	_	5	10
112		*Liomia sp.*	_	_	_	_	_	_	_	3	2	_	5
113		*Polymnia sp.*	_	_	_	_	_	_	_	_	_	6	6
114	Trichobranchidae	*Terebellides stroemi*	4	_	_	_	1	_	8	_	3	_	16
115		*Schistocomus sp.*	_	_	_	_	_	_	_	2	_	_	2
116	Ampharetidae	*Amphicteis sp.*	1	_	_	_	_	_	_	_	1	_	2
117	Terebellidae	Terebellid polychaete	_	_	_	_	_	_	1	_	_	_	1
118	Hesionidae	Hesionid polychaete	_	_	_	_	_	_	_	1	_	_	1
119		Sedentaria polychaete	_	_	_	_	_	_	_	_	1	_	1
120		Serpulid polychaete	_	_	_	_	_	_	_	_	_	3	3
	Total		1003	236	444	66	303	412	628	486	726	191	4495

Table 3 Total abundance and three diversity indices of intertidal polychaetes in Myeik Coastal Areas.

Sr. No.	Station	Number of Species	Abundance	Polychaete species		
				H´ (Diversity)	J´ (Evenness)	D (Richness)
1	Light-house	72	1003	3.59	0.84	10.27
2	Daung-kumaw	16	236	2.26	0.81	2.75
3	Kyauk-phya	17	444	2.49	0.88	2.62
4	Pahtaw-west	15	66	2.28	0.84	3.34
5	Masanpa	35	303	3.02	0.85	5.95
6	Maing	35	412	3.08	0.87	5.65
7	Panataung	69	628	3.62	0.86	10.55
8	Done-pale-aw	45	486	3.22	0.85	7.11
9	Sha-aw	40	726	2.73	0.74	5.92
10	Kyun-mweyar	27	191	2.82	0.86	4.95

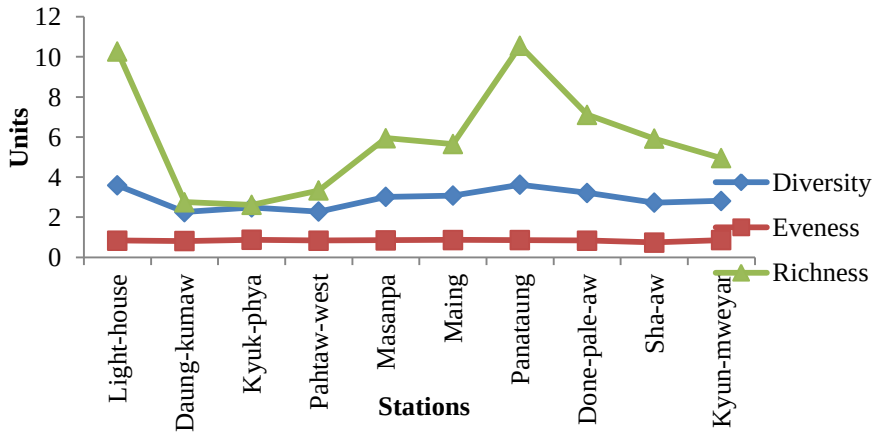

Figure 2 Three diversity indices of polychaetes from different stations of Myeik Coastal Areas.

Discussion

Polychaetes are the most important and abundant in the intertidal area. In the present study, 4495 individuals belonging to 120 species of polychaetes were collected from ten stations of Myeik Coastal Area. At near mainland, the highest abundance was recorded for Light-house Station (1003) followed by Panataung Station (628). The lowest abundance was recorded for Pahtaw-west (66). At away from mainland the highest abundance was recorded for Sha-aw Station (726) and the lowest abundance was recorded for Kyun-mweyar (191). While most of the species had limited distribution, some species showed wide distribution. Barrio Frojan *et al.* (2006) indicated that the variation in abundance of polychaete was probably caused by seasonal shift and environmental factors.

Three different terms of diversity indices (species richness, species evenness and species diversity) were used. The species richness (Margalef's index) is used to estimate the total number of species in a given area. The more the number of species, in a sample or the more species present in a species list of a given environment, the greater will be the species richness. High evenness occurs, when the species present are virtually in equal abundance, which, is conventionally equated with high diversity. The term species diversity is used for the number of species per number of individuals. The highest species diversity is possible when only one individual represents every species and the lowest diversity possible is when community consists of only one species (Soetaert and Heip 1990).

The highest number of species (72) was recorded at the Light-house sampling site. High values for species richness which were above 10 were recorded for Light-house and Panataung. The lowest values for species richness, which were less than 5, were recorded at Daung-kumaw, Kyauk-phya and Pahtaw-west. The high values for species diversity which were above 3 were recorded at Light-house, Masanpa, Maing , Panataung and Done-pale aw. The low values for species diversity (greater than 2) were recorded at Daung-kumaw, Kyauk-phya and Pahtaw-west, Kyun-mweyar and Sha-aw Stations.

The lowest species diversity and species richness were recorded at Daung-kunaw where the salinity was low due to freshwater inflow. The result of the present study indicated that at the sample site (Daung-kumaw Station) closer to the waste discharge point of the crab farm, the species richness as well as abundance of polychaete was low. At the sample site that was close to the human dwelling and fresh water inflow (Kyauk-phya Station), the species was less abundant. Besides, freshwater inflow might have contributed to low species diversity and species richness. At these two sites, Pilargids and Capitellids were widely distributed. Dahana Y Aka and Wijey Ara Tne (2006) described that Pilargids were widely distributed in the estuary. Low species composition and abundance at Pahtaw-west Station indicate the prevalence of stress condition due to dredging operations. The high species richness and species diversity of polychaetes were observed at Panataung and Light-house Stations. The high species richness and high diversity attributed to the more stable physical condition (Joydas and Damodaran 2001). Evenness values ranged from 0.74 to 0.88 during the two year's sampling. Evenness varied between 0.81 and 0.88 at near stations with mainland and indicating that species composition approached an equitable distribution.

Three indices at most stations were in the same parallel trend when the species diversity was high; the other two indices also positively followed. Highest species richness and diversity values were obtained from Light-house and Panataung attributing to the more stable physical conditions. Lowest species diversity and richness values were obtained from Daung-kumaw, Pahtaw-west and Kyauk-phya, suggesting poor environmental health due to anthropogenic activities.

Dean (2008) and Sukumuran and Devi (2009) revealed that decrease in species diversity led to increase in species dominance, because of effect of pollutants on the benthic environment. Low diversity and higher population density of a few organisms denote some major stress condition, which eliminated many species but promoted survival of a few. Lower H' value indicated poor environmental health. Sukumuran and Devi (2009) reported that in the good healthy environment Shannon diversity were higher than 2. And Barrio Frojan *et al.* (2006) revealed that H' values greater than 4 was considered as good as clean environment. In this study,

Light-house, Panataung and Done-pale aw Stations had diversity of > 3 and the remaining other stations had diversity of > 2. So it can be stated that the status of Myeik Coastal Areas indicate good healthy environment.

Conclusion

Near stations with Myeik city mainland were different the stations away from mainland in terms of species composition and abundance. A remarkable reduction in polychaete abundance and diversity was noticed at near three stations (Daung-kumaw, Pahtaw-west and Kyauk-phya) with Myeik city mainland due to waste discharge, fresh water inflow and anthropogenic activities. It is strongly believed that the present work will be valuable to be used as base line data to gauge any further change of polychaetes in Myeik Coastal Areas in some year to come.

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ISOLATION OF PIGMENTED FUNGUS FROM *LOBOPHORA VARIEGATA* (J.V.LAMOUROUX) AND EXTRACTION OF ANTIBACTERIAL ACTIVITY AGAINST ON *ESCHERICHIA COLI* AHU5436

Khin Thandar Linn[1]

Abstract

In this investigation, the isolation of pigmented fungus has been conducted from brown seaweed. The samples of brown seaweed species, *Lobophora variegata* (J.V.Lamouroux) was collected the natural beds from the rocky shore of Minn- Land coastal area, Ngwe- Saung region, Pathein township. The isolation of pigmented fungus was carried out by Washing method. During the preliminary study of antibacterial activity on *Escherichia coli* AHU5436, the paper disc diffusion assay method was used to check the activity of the fermented broth. The filter paper and four solvents such as 20% NH_4CL, water saturated n-butanol, n-butanol- acetic- water (3:1:1) and water saturated ethyl acetate were used for Paper chromatography according the method of Tomita,1988. Antibacterial metabolite was extracted with ethyl acetate from the fermented broth based on the results of (R_f value) paper chromatography.

Keywords: antibacterial activity, *Escherichia coli*, extracted, fermented broth, *Lobophora variegata*, pigmented fungus.

Introduction

Marine ecosystems cover about 70% of the planet surface and are still an under exploited source of useful metabolites. Among marine organisms, marine algae and seaweeds are rich sources of bioactive and their value as a source of novel substances has grown rapidly in recent years. Seaweeds are one of the major producers of marine ecosystem, found almost in all part of the coastal regions around the globe. They are defined as evolutionarily diverse assemblages of marine photosynthetic, non-vascular macro-algal forms, inhabiting the littoral zone in sea, which vary in their color, shape and size. The size of marine algal forms may be very small (few mm) or up to several centimeters. Seaweeds fall under three different categories including green (chlorophyceae), red (rhodophyceae), and brown (phaeophyceae). The characteristic colors of seaweeds are due to different pigmentation. Seaweeds are macroscopic benthic algal forms, different from microscopic algae and constitute one of the highest productive ecosystems. Macroalgae, also known as seaweeds, are conspicuous and dominant features in marine ecosystems. They differ from other plants, in that algae lack roots, leafy shoots, flowers, and vascular tissues (Suryanarayanan, T.S, 2012).

Seaweeds are the most primitive group of vegetation and they have gained great importance as a promising source of bioactive compounds that can be used for health-promoting substances such as polysaccharides, proteins, peptides and polyphenols, pigments, dietary fiber, omega-3 fatty acids, and vitamins with antibacterial, antiviral, and antifungal properties. (Hamed et. *al*., 2015). Bioactive compound in the seaweeds, algae, and fungi can be procured by employing many different processes and methods (Belattmania et. *al*., 2016). In this study period, marine algae especially brown algae *Lobophora variegata* (J.V.Lamourous) has been used for the isolation of marine pigmented fungus and its antibacterial activity on test organism *Escheria coli* AHU 5436. The present study was also focused on the investigation of fermentation period and the extractions of antibacterial activity were also studied.

[1] Dr, Assistant Lecturer, Marine Science Department, Pathein University

Materials and Methods

Sample Collection

Plants of the species *Lobophora variegata* (J.V.Lamourous) was collected in the form of live materials growing the natural beds from the rocky shore of Minn Land coastal area (Lat 16˚ 54.7564″ N ,Long 094˚ 89.754″ E), Ngwe - Saung, Pathein Township, Ayeyarwady Region. The samples were carried by ice box with natural seawater of salinity30 **‰**.The samples were identified based on the external morphology, Herbarium specimens, literatures and other related voucher specimens. The collected samples were photographed by using digital camera and the fresh samples were used for the isolation.

Figure 1 Map showing the location of sample collecting area

Isolation of Pigmented Fungus

In this study, the isolation method was carried out with the following scheme (Inaba and Ando 2004). In the first step, prepare the GPY agar medium, glucose 1.0g, polypeptone 0.5g, yeast extract 0.5g and agar 2.0 g and seawater 1ml were placed in a conical flask. Then 100ml of sterile distilled water was added to the medium. The flask was plugged with cotton wool and sterilized by autoclaving. After this the medium was poured into the plate near the flame of a spirit burner in the culture chamber. In the second step, the plant samples were washed with tap water for removing sands and then cut into smaller pieces. After cutting pieces, the samples were washed again with sterile distilled water and dipped into 70% methanol in 1minute for removing external dusts including unwanted microbes. After 1 minute, the pieces were rinsed with sterile distilled water and dry on tissues paper. After drying, they are plated on agar medium and incubated for 7 days. The isolated fungus was stored in PDA medium (Potato 20g, Dextrose 1.5g, Agar 1.8g and DW 100mL).

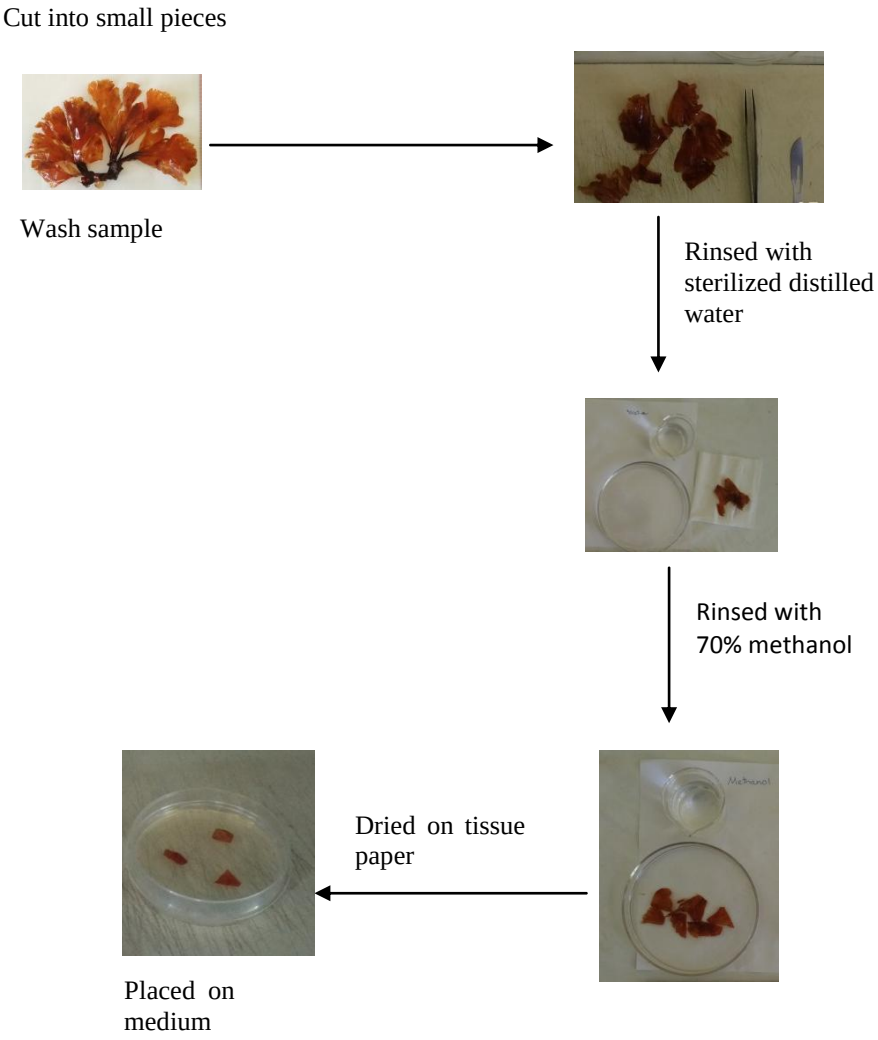


Figure 2 Preparation of isolation for pigmented fungus.

Preparation of fermented broth cultures for screening of antibacterial activity

The seed medium was prepared by mixing glucose 1.0g, polypeptone 0.3g, KNO_3 0.1g and K_2HPO_4 0.01g in a conical flask. Then 100 ml of sterile distilled water was added. After this, every 25 ml of the medium was poured into the 50ml conical flask. Then the flask was plugged with cotton wool and sterilized by autoclaving. The isolated fungus was grown on GSY medium in 7 days for sporulation. Then isolated fungus was inoculated on seed medium in the culture chamber for 3 days. After incubation for 3 days, the seed culture was transferred into the conical flask containing the fermentation media glucose 1.0g, yeast extract 0.5g ,$CaCo_3$ 0.1g, K_2 HPO_4 0.001g and $MgSO_4$ 0.001 were put in a conical flask with 100 ml distilled water . The fermentation period took 10 days. During the fermentation, the fermented broth was used for the paper disc diffusion assay.

Screening the antibacterial activity of the fermented broth by paper disc diffusion assay

The paper disc diffusion assay method (Tomitta, 1988) was used to check the activity of the obtained fermented broth. Test organisms *Escheria coli* AHU 5436 were used to test the activities. These test organisms were inoculated in 20ml of assay broth in conical flasks and incubated overnight. To get assay media, glucose1.0g, yeast extract 0.3and agar 2.0g were placed

in a conical flask and then 100 ml distilled water was added to obtain complete assay medium. The flask was plugged with cotton wool and sterilized by autoclaving. After this the assay medium was cooled. The one percent of test organisms was added to the assay medium, and then poured into plates in the culture chamber. After solidification, paper disc impregnated with samples were applied on the agar plates and the plates were incubated at room temperature for 24hours. Clear zones (inhibitory zones) around the test discs indicate the presence of antibiotic (bioactive compounds) which inhibits the growth of the test organisms selectively.

Preliminary Characterization of Metaboliteas by Using Paper Chromatography

Paper chromatography was carried out by the method of Tomita, 1988. The filter paper (Toyo Advantec, Japan) and four solvents such as 20%NH_4Cl, water saturated n-butanol, n-butanol- acetic acid- water (3:1:1) and water saturated ethyl acetate were used for preliminary characterization of metabolites. The fermented broth samples (100µl) were applied on the papers 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 hours. According to the inhibitory zone, R_f value was calculated for the corresponding metabolite.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

The Effects of pH and the Extraction of Antibacterial Metabolite

Fermented broth (each 10mL) was adjusted at pH (5,6,7 and 8) using sodium hydroxide (NaOH) and dilute Hydrochloric acid (HCl). By using paper disc diffusion assay method, the inhibitory zones were determined to obtain the effects of pH for the extraction. Antibacterial metabolite was extracted from the fermented broth based on the results (R_f value) of paper chromatography -bioautography.

Figure 3 Extraction of antibacterial compound

Results

Scientific classification

Phylum Phaeophyta
Class Phaeophyceae
Order Dictyotales
Family Dictyotaceae
Genus *Lobophora*
Species *Lobophora variegata* (J.V. lamouroux,1817)

Figure 4 External morphology of *Lobophora variegata*

Description- Plants orangy to dark brown colour, 7-10 cm broad, prostrate, overlapping in clusters, upper potions entire, lobate with variegated markings in concentric zones, thalli circular to fan shaped, attached by rhizoids arising from the basal parts of the fronds, grows intertidally or in shallow water in tropical and warm temperate seas.

Isolation of Marine Pigmented fungus

The morphological characteristic and photomicrograph of pigmented fungus isolated from *L.variegata* was shown in figures. The front view of isolated fungus is white color and the back view is red.

front view back view

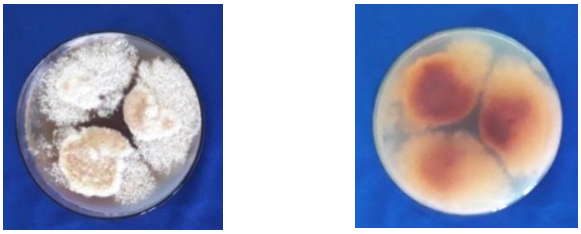


Figure 5 Morphological characteristic of front view and back view of isolated fungus from *L. variegata*

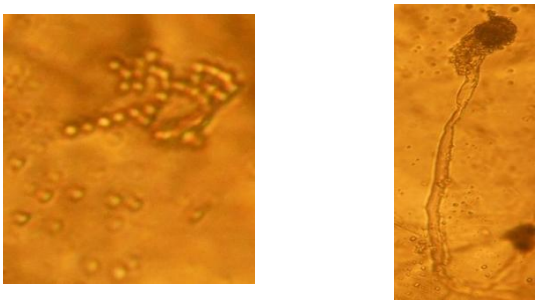


Figure 6 Photomicrograph of isolated fungus from *L. variegata*

Antibacterial Activity Against on *E.Coli* AHU5436

In the investigation of antibacterial activity, fermentation period 5, 6, 7 and 8days showed the activity against on test organism *E.coli* AHU5436. The 6 days of fermentation period showed the best activity on *E.coli* AHU 5436. The detail results were shown in Table 1 and Figure 7.

Table 1 Antibacterial activity of ten days fermentation period

Fermentation period	Clear zone(mm)
1-4 days	no activity
5day	18.42
6day	**20.06**
7day	15.48
8day	13.74
9-10days	no activity

Test organism----- *E.coli* AHU5436.

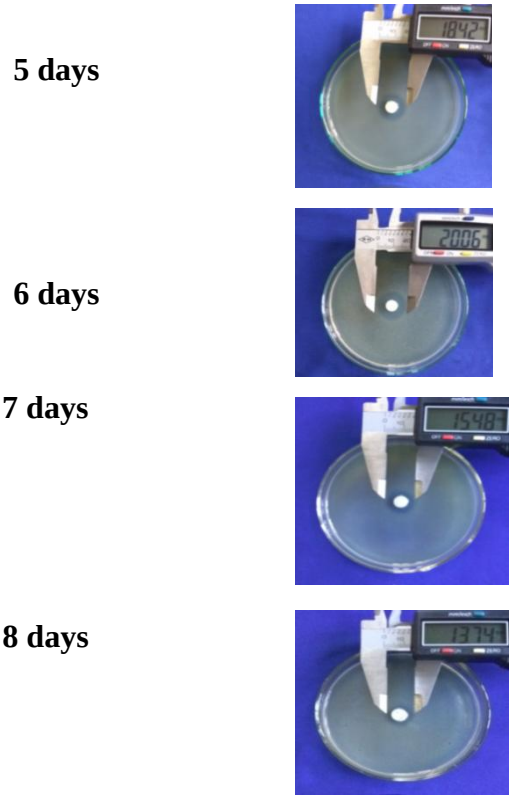


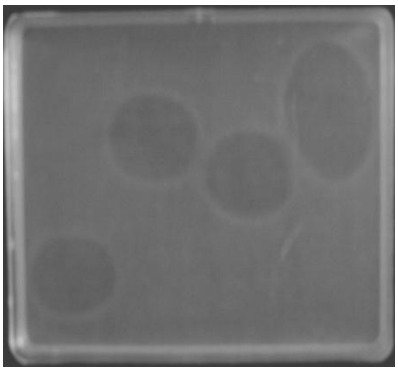
Figure 7 Fermentation period of antibacterial activity on *E.coli* AHU5436

Preliminary Characterization of Metabolites by using Paper Chromatography

According to the R_f values, it was considered that antibacterial metabolite is soluble in solvent No.4, ethyl acetate. Therefore, solvent No.4, ethyl acetate is suitable for the extraction of antibacterial metabolite. The results were showed in Figure 7.

Table 2 Solvent system and the result of R_f value

No	Solvent	R_f value
1	20% NH_4Cl	0.16
2	Water saturated *n*-BuOH,	0.6
3	n-BuOH-acetic acid- water	0.5
4	Water saturated ethyl acetate	0.8

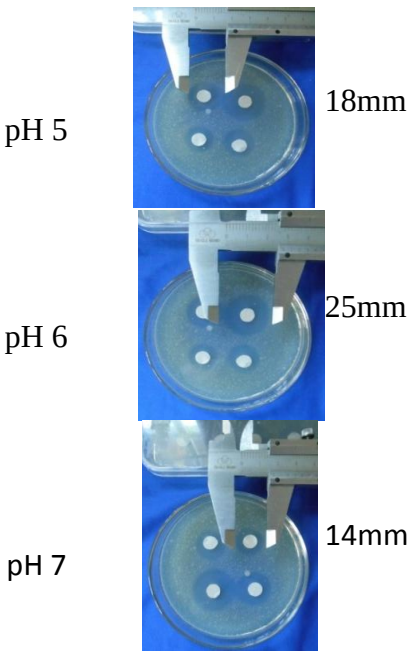


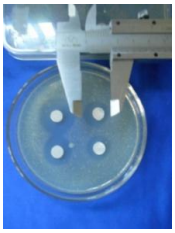
1 2 3 4

Figure 8 Bioautographic overlay assay

The Effects of pH and the Extraction of antibacterial metabolite

In the study of the effects of pH for the extraction with ethyl acetate, it was found that the fermented broths with adjusted pH exhibited the activities. However, pH 6.0 condition showed the best activity. Therefore, it was determined that pH 6.0 was suitable for the extraction of metabolite from the fermented broths with ethyl acetate. Five liters fermented brotwas used for the extraction of antibacterial metabolite from the isolated fungus.



pH 7  15mm

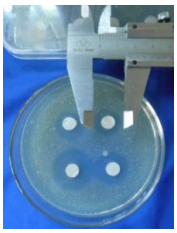
pH 8  14mm

Figure 9 The effects of pH for the extraction with ethyl acetate

Discussion

Marine fungi are potential producers of bioactive compounds that may have pharmacological and medicinal applications. The marine environment is extremely complex and contains a broad spectrum of fungal diversity. Fouillaud, M. et.*al*, 2016 state that the variety of new marine-derived fungal genera have been identified and evaluated, numerous marine fungi are identical to terrestrial fungi, e.g., *Aspergillus* spp., *Cephalosporium* spp, *Fusarium* spp, or *Penicillium* spp. Mayer et.*al*, 2013, report that the significant involvement of fungi in the industry, through the production of various useful substances, such as antibiotics, immune suppressants, anti-cancer drugs, plant hormones, enzymes, acids and also natural pigments. Zhang et.*al*, 2012 mentioned that marine derived fungi are regarded as a potential bright sources of new molecules with likely application in pigment production. Many genera producing pigments have been isolated from water, sediments, and decaying organic residues, or from living organisms such as invertebrates, plants or algae. This study initiated the search for pigmented fungi from marine brown algae *L.variegata* from Minn- Land coastal area, Ngwe-Saung region, Pathein township. The studied of antibacterial activity against on *E.coli* 5436 and the extraction of antibacterial metabolites with ethyl acetate for the results of Paper Chromatography bioautographic overlay assay.

Conclusion

In the screening of pigmented fungi, only one species was isolated from marine brown algae *L. variegata.* During the study of preliminary antibacterial activity, fermentation period was carried out 10 days by using on test organism *E.coli* 5436. The starting of fermentation period 1, 2, 3 days and the ending of fermentation period 9, 10 days do not showed the activity. The fermentation period 5, 6, 7 and 8 days showed the activity against on *E.coli* 5436. The fermentation period 6days activity showed the best in this study. In the using of four solvents for Paper chromatography method, solvent No.4 ethyl acetate showed the best R_f value. Therefore this solvent was used for the extraction. In the extraction of antibacterial metabolite, the effects of pH were checked by using sodium hydroxide (NaOH) and dilute hydrochloric acid (HCL). According to the pH results, pH 6.0 was more suitable for the extraction of antibacterial metabolites with ethyl acetate.

Acknowledgements

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DETERMINATION OF NITROGEN FIXING RATE AT THE RHIZOSPHERIC SEDIMENT OF MANGROVE FOREST IN SHWE THAUNG YAN MANGROVE FOREST, MYANMAR

Myat Thwe Myint Aung[1], Htay Aung[2], Motoo UTSUMI[3]

Abstract

Mangrove forests dominate the world's tropical and subtropical coastlines, and provide a unique ecosystem. Mangrove sediments play a critical role in biogeochemical processes by behaving as both source and sink for nutrients and other materials. The interactions of mangrove plant's growth rate and the sediment nutrients are complex and dynamic. Nitrogen fixation is a process that converted the atmospheric nitrogen gas to ammonium and it is an important source of new nutrient in the mangrove ecosystem. However, the study of nitrogen fixation rate in rhizospheric sediment of mangrove forest is rare, and the factors that regulate N fixation within the biome remain largely unknown. In this study, the rate of nitrogen fixation in mangrove sediment was investigated using the acetylene reduction technique. Sediment samples were collected from Shwe Thaung Yan mangrove forest and were calculated the nitrogen fixation rate. The differences between sediment depths and nitrogen fixation rate, and nitrogen fixation ability at the mangrove forest were described.

Keywords: Mangrove, Nitrogen, Nitrogen (N) fixation, ARA assay, Shwe Thaung Yan

Introduction

Mangrove represents unique and ecologically important coastal habitats throughout the tropical and subtropical which is occupying around 180,000 km^2 around the world. Chapman (1940), Davis (1940) and MacNae (1969) defined mangrove as a general term applied to plants which live in muddy, loose, wet soils in tropical tidal waters. They are circum-tropical on sheltered shores and often grow along the banks of rivers as far inland as the tide penetrates. Mangroves are recognized as highly productive ecosystems that provide organic matter and shelters to adjacent coastal ecosystems. Despite their high production rates, mangrove habitats are often nutrient limited, particularly for combined nitrogen (Ryther and Dunstan 1971).

Many previous researches showed the mangrove habitats were often nutrient limited, particularly for combined nitrogen, while nitrogen fixation may be an important nitrogen input term to these ecosystems because of its potential to provide nitrogen in usable form to plants (Zuberer and Silver 1978, 1979). High rates of nitrogen fixation are associated with mangrove bark, decaying mangrove leaves, pneumatophores roots, and mangrove rhizosphere soil (Zuberer and Silver 1978, 1979). In Raymond *et al.* (2004) research, for organisms to fix nitrogen, either in association with plants or not, the presence and activity of the nitrogenase enzyme complex is required. In this study, we describe details of physicochemical properties in mangrove sediments and estimation the rate of nitrogen fixation that exist within different sites of the mangrove forest. These results may be helpful for guidance to determine the nitrogen fixation rate of interest within these distinct sites.

[1] Dr, Assistant Lecturer, Department of Marine Science, Pathein University
[2] Dr, Professor, Department of Marine Science, Pathein University
[3] Dr, Faculty of Life and Environmental Sciences, University of Tsukuba, Japan

Materials and Methods

Study sites

Mangroves sediment samples were collected from Magyi channel of Shwe Thaung Yan coastal, Ayeyarwady region. Site 1 (17º 04.222' N 94º 28.913' E) is located by the mouth of Magyi tidal creek and dominance mangrove specie by *Bugueria gymnorrhiza* spp. Site 2 (17º 04.324'N 94º 27.917'E) is located by the middle of tidal creek and dominance mangrove specie by *Ceriops tagal* spp. Site 3 (17º 04.701'N 94º 28.141'E) is located in the marine park (Mangrove conservation area of Shwe Thaung Yan coastal) and dominance mangrove specie by *Rhizophora mucronata* spp. All study sites are along the Magyi tidal creek (Figure 1).

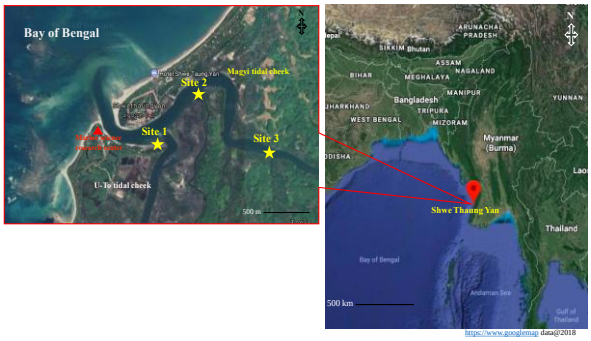


Figure 1 Study sites in Shwe Thaung Yan mangrove forest along the Magyi tidal cheek, the Republic of the Union of Myanmar.

Sampling

For physicochemical and microbial analysis, approximately 200 g of soil samples were collected from each of the three locations at low tide. Samples were collected at the rhizosphere region using by spade and triplicate from four different depth i.e, 1) 0 - 5 cm; 2) 5 - 10 cm; 3) 10 - 15 cm; 4) 15 - 20 cm of each sites. Soil samples were always taken from near parts of the roots (Figure 2). All collected samples were put into a plastic zipper bag and immediately transferred into an icebox transported to Japan for analyses.

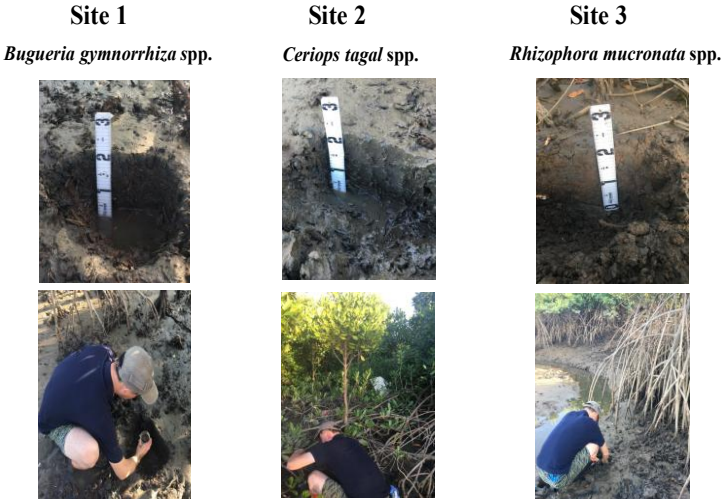


Figure 2 Study three sites information in Shwe Thaung Yan mangrove forest along the Magyi tidal cheek, Myanmar.

In situ measurement of physicochemical parameters in mangrove forest

Temperature, redox potential, and pH of the sediment samples were measured *in situ* using the thermometer, electric digital pH and conductivity meter (Horiba). And also, the soil samples were analyzed for their relative sediment structure because of microbe and vegetation types of mangrove are dependent on the soil types (e.g. muddy or sandy loam or silt-mud type).

Procedure for measuring soil nitrogenase activity (Acetylene Reduction Assay)

Nitrogen fixation rates were measured by the acetylene reduction technique (Capone and Montoya 2001) based on nitrogenase enzyme activity. Acetylene reduction assay (ARA) is a commonly used assay and is widely accepted as an effective and reproducible, instantaneous nitrogen fixation rate measurement. Before the incubation, 100 mL glass vials purge with Argon gas put into the flushing headspace vials (to create an anaerobic environment).

About 10 mL wet sediment sample was extruded from each layer sediment sample bag using 5 mL sterile syringes (with the top end of the syringe removed) put into the vials and sealed with an open cap over a Teflon - silicone septa. 10 % of the atmosphere of each vial containing 10 mL soil was substituted with gaseous acetylene (10 %) from a commercial cylinder inject to each vial (0.1 atm, 10 % C_2H_2 by volume) through the Teflon-silicone septa. The vials were shaken by hand in order to avoid heterogeneities within the gas phase. Samples were incubated in the dark at ambient water temperature (24^0C) for 24 h. From each incubation assay, 1 mL gas sample was taken from the vials at every 6 hours interval (specifically 0, 6, 12,18 and 24) and measured by gas chromatograph. The ethylene concentration was determined by a GC-14B gas chromatograph with FID detector (Shimadzu, Japan). Representative retention times in minutes are methane, 0.8; acetylene, 5.6; and ethylene, 7.8. 1 mL of gas sample from each vial was injected into GC-14B and Sincarbon ST (50/80 mesh) packed column with 3 m and 3 mm a stainless-steel column (column temperature 60^0C). Temperature at both the injector and detector was 210^0C while oven temperature was kept at 160ºC. The carrier gases flow rates were 60, 50 and 50 mL/min of helium (He), hydrogen (H_2) and oxygen (O_2) respectively (Table 1). The chromatograms were used to integrate the areas of the curves of acetylene (C_2H_2) and ethylene (C_2H_4) to estimate C_2H_4 production (Holguin *et al.* 1992, 2001). The acetylene reduction rates were calculated based on dry weight of sediments (samples were oven-dried at 60^0C for 48 h). Ethylene peak heights were measured and related to calibrations made with standard C_2H_2 (0.976%) and C_2H_4 (0.965%) concentration. The acetylene reduction rates were converted to rates of nitrogen fixation using the theoretical factor of three acetylene molecules equivalent to one nitrogen molecule (Turner and Gibson, 1980).

Table 1 Measurement condition of gas chromatography.

GC-14B	
Detector	FID detector (hydrogen flame ionization detector)
Column	Sincarbon ST (50/80 mesh), packed, length 3 m × inner diameter 3 mm stainless-steel
Sample injection volume	1 mL
Column temperature.	60°C
Injector temperature	210°C
Detector temperature	210°C
Oven	160°C
Carrier gas	He (60 mL/min), H_2 (50 mL/min), O_2 (50 mL/min)

Results

In situ measurement of physicochemical parameters in mangrove forest

The physicochemical properties of three sites of sediment samples were shown in Table 2. The pH of all sites was less than 7.0, indicating that the soil samples were slightly acidic. Electrical conductivity (EC) contents were 1750 µS/cm (Site 1), 1725 µS/cm (Site 2) and 1675 µS/cm (Site 3), respectively. Seawater and sediment porewater salinity at Site 1 (*B. gymnorrhiza* spp.) was much higher than Site 2 (*R. mucronata* spp.) and Site 3 (*C. tagal* spp.). Seawater and soil salinity ranged from 31 ‰ to 35 ‰. The sediment temperature measured at three depths (5 cm, 10 cm, and 15 cm) ranged from 27.9 ˚C to 29.2 ˚C. Three distinct sites, comprising three bulk sediment types, namely clay - loam (Site 1), mud - clay (Site 2), and mud - clay - loam (Site 3) rhizosphere soils. This indicated that the rhizosphere soil samples were substantially different from each other.

Table 2 Physico-chemical environmental parameters of sampling three sites at Shwe Thaung Yan mangrove forest, the Republic of the Union of Myanmar

Site	Dominant mangrove Species	Depth (cm)	Temp (℃)	pH	EC (µS/cm)	Sediment Texture	Salinity Sediment	Salinity Seawater
1	*Bugueria gymnorrhiza*	5	28.5	5.9	1.750	Clay loam	35.0	32.0
		10	28.4					
		15	28.1					
2	*Ceriops tagal*	5	28.5	6.0	1.725	Mud-clay	34.5	31.0
		10	28.5					
		15	28.3					
3	*Rhizophora mucronata*	5	29.2	6.3	1.675	Mud- Clay- loam	33.5	32.0
		10	28.5					
		15	27.9					

Nitrogenase activity in mangrove sediments

Reduction of C_2H_2 to C_2H_4 by nitrogenase of mangrove sediment samples were examined with respect to a wide variety of characteristics, and the striking similarities between nitrogen fixation and C_2H_2 reduction were reported in these experiments. It follows that, for calibration of the relationship between acetylene reduction and nitrogen fixation, the acetylene concentration should be adjusted to give equimolar concentrations of acetylene and nitrogen in solution. To confirm the occurrence of nitrogen fixation in the samples of mangrove rhizosphere sediments with high nitrogen intake, additionally examined the acetylene-reducing activities in four-layer samples from each site. In this assay, each layer sample was divided into two: one was analyzed aerobic condition and the other was an anaerobic condition. By comparing these two types of sample, also evaluated the effect of both conditions on the nitrogen-fixing activity. In general, the anaerobic condition of C_2H_2 reduction activities was low and aerobic condition sediments generally exhibited high. All the samples of aerobic condition were showed acetylene-reducing activities (ranging from 0.14 to 2.36 nmol of C_2H_4/g (dry sediment)/h), but under the anaerobic condition, it was 0.05 to 0.88 (nmol of C_2H_4/g (dry sediment)/h). Aerobic activities showed 2.8 to 2.7 times higher than the values of the corresponding anaerobic condition (Tables 3, 4, 5). Rates of ethylene production showed a similar pattern for two sites (1 and 2) with relatively from 0 to

24 h during incubation. The highest rates observed were 2.36 and 1.10 (nmol of C_2H_4/g (dry sediment)/h) for surface layer (0 - 5 cm) and the second layer (5 - 10 cm) in Site 3. There was no statistically significant difference in nitrogenase activity between all sediment samples during the incubation (R_2= 0.878 (Adjusted R_2= 0.857). The highest nitrogen fixation rate was occurred in Site 3 under the two conditions (aerobic and anaerobic) (Figure 5; A and B), followed by Site 1 (Figure 3; A and B), and Site 2 (Figure 4; A and B). In contrast, the activities in the deepest sediment layer (15 - 20 cm) of all sites were very low. The differences of nitrogen fixation rates in aerobic and anaerobic conditions were statistically significant (Multiple Regression: $p < 0.001$, $n = 4$). Aerobic and anaerobic rates were highly significantly different, and the acetylene-reduction rates were correlated positively with the time during the incubation periods. The nitrogen fixation rates were estimated the theoretical reduction ratio C_2H_2:N_2 = 3:1 was used.

Table 3 Rates of ethylene production and nitrogen fixation in Site 1 sediments incubated with 0.1 atm acetylene under (a) aerobic condition, (b) anaerobic condition

(a) Aerobic condition

Depth (cm)	C_2H_4 (nmole/g(dry weight)/h)	N_2 – fixation rate (nmole/g(dry weight)/h)	Molar ratio	
0-5	2.03±0.31	0.68	3:1	(n=4)
5-10	2.38±0.48	0.80	3:1	(n=4)
10-15	0.52±0.13	0.17	3:1	(n=4)
15-20	0.73±0.13	0.24	3:1	(n=4)

(b) Anaerobic condition

Depth (cm)	C_2H_4 (nmole/g(dry weight)/h)	N_2 – fixation rate (nmole/g(dry weight)/h)	Molar ratio	
0-5	0.66±0.09	0.22	3:1	(n=4)
5-10	0.72±0.11	0.24	3:1	(n=4)
10-15	0.35±0.05	0.12	3:1	(n=4)
15-20	0.33±0.05	0.11	3:1	(n=4)

Table 4 Rates of ethylene production and nitrogen fixation in Site 2 sediments incubated with 0.1 atm acetylene under (a) aerobic condition, (b) anaerobic condition.

(a) Aerobic condition

Depth (cm)	C_2H_4 (nmole/g(dry weight)/h)	N_2 – fixation rate (nmole/g(dry weight)/h)	Molar ratio	
0-5	3.53±0.77	1.18	3:1	(n=4)
5-10	0.80±0.14	0.27	3:1	(n=4)
10-15	0.85±0.14	0.28	3:1	(n=4)
15-20	0.43±0.08	0.14	3:1	(n=4)

(b) Anaerobic condition

Depth (cm)	C_2H_4 (nmole/g(dry weight)/h)	N_2 – fixation rate (nmole/g(dry weight)/h)	Molar ratio	
0-5	0.53±0.77	0.18	3:1	(n=4)
5-10	0.64±0.10	0.21	3:1	(n=4)
10-15	0.40±0.06	0.13	3:1	(n=4)
15-20	0.15±0.01	0.05	3:1	(n=4)

Table 5 Rates of ethylene production and nitrogen fixation in Site 3 sediments incubated with 0.1 atm acetylene under (a) aerobic condition, (b) anaerobic condition.

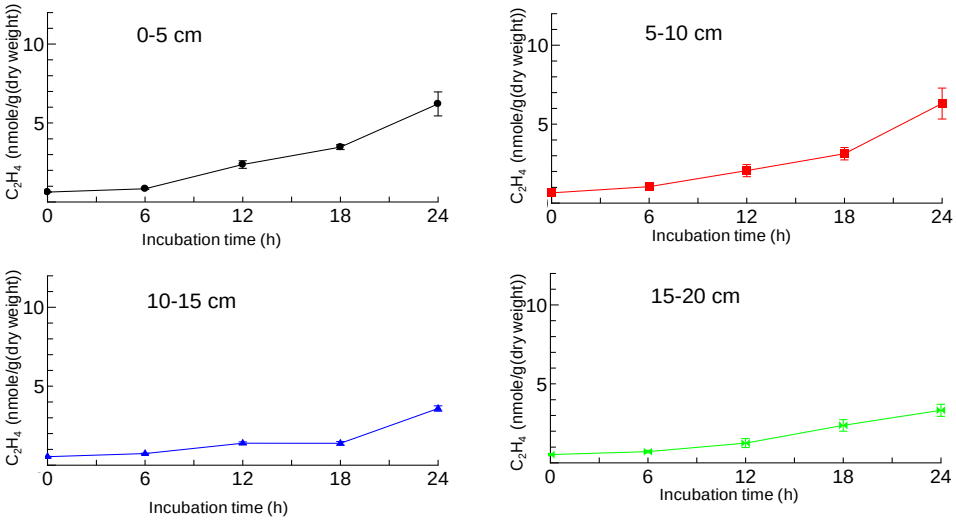
(a) Aerobic condition

Depth (cm)	C_2H_4 (nmole/g(dry weight)/h)	N_2 – fixation rate (nmole/g(dry weight)/h)	Molar ratio	
0-5	7.08±1.38	2.36	3:1	(n=4)
5-10	3.31±0.69	1.10	3:1	(n=4)
10-15	1.84±0.33	0.61	3:1	(n=4)
15-20	2.32±0.45	0.77	3:1	(n=4)

(b) Anaerobic condition

Depth (cm)	C_2H_4 (nmole/g(dry weight)/h)	N_2 – fixation rate (nmole/g(dry weight)/h)	Molar ratio	
0-5	2.65±0.44	0.88	3:1	(n=4)
5-10	1.45±0.25	0.48	3:1	(n=4)
10-15	1.59±0.27	0.53	3:1	(n=4)
15-20	0.58±0.09	0.19	3:1	(n=4)

(A) Aerobic condition



(B) Anaerobic condition

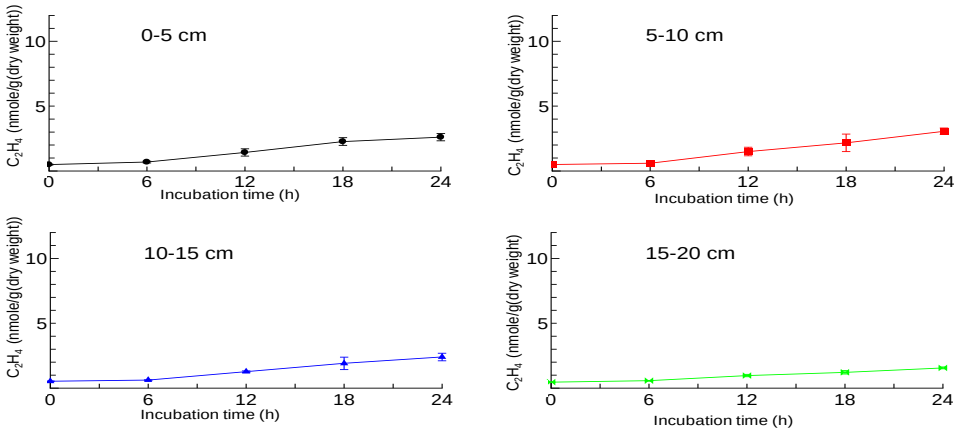
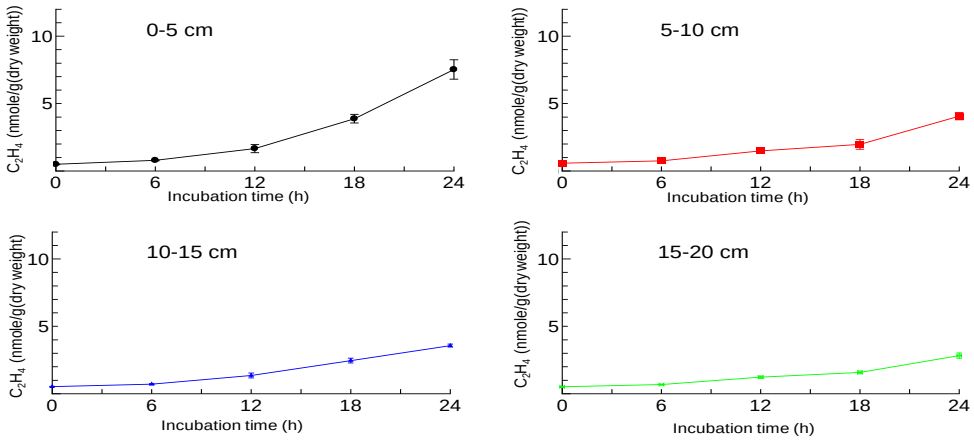


Figure 3 Acetylene reduction assay under aerobic condition (A), anaerobic condition (B) using sediment samples of Site 1

(A) Aerobic condition



(B) Anaerobic condition

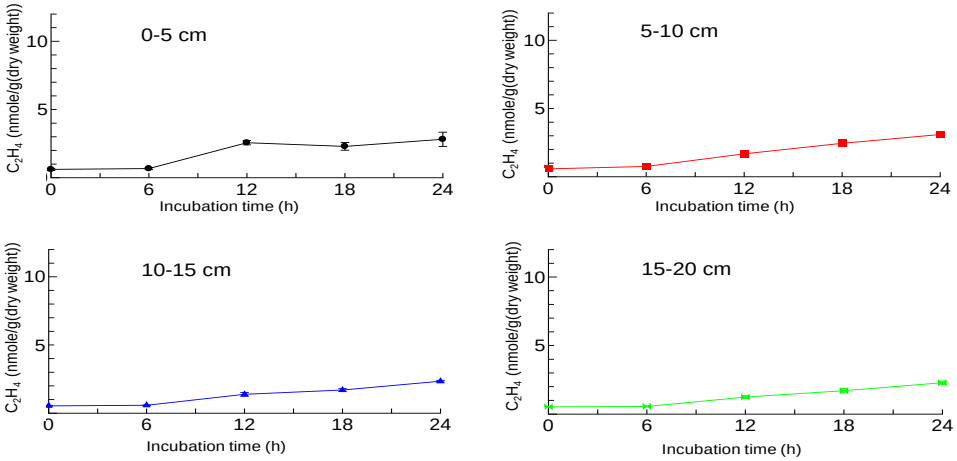
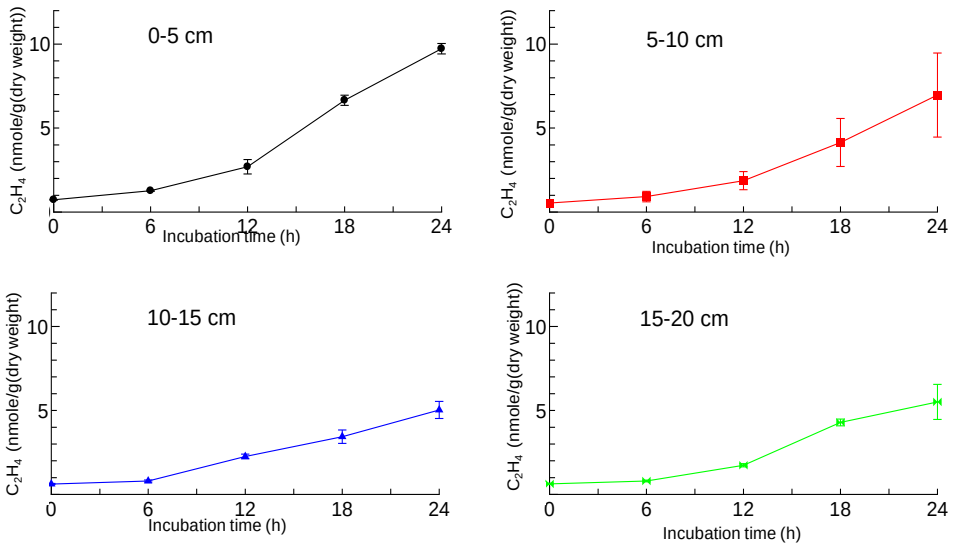


Figure 4 Acetylene reduction assay under aerobic condition (A), anaerobic condition (B) using sediment samples of Site 2

(A) Aerobic condition



(B) Anaerobic condition

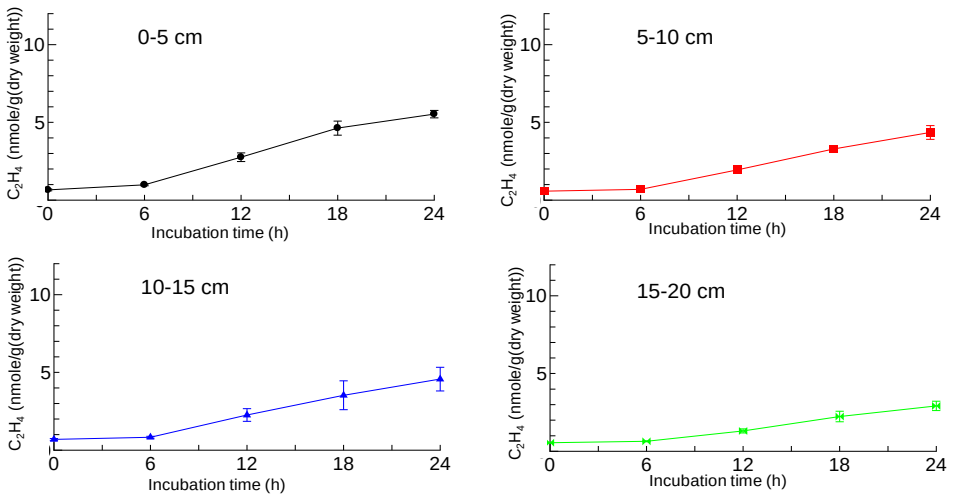


Figure 5 Acetylene reduction assay under aerobic condition (A), anaerobic condition (B) using sediment samples of Site 3

Discussion and Conclusion

The physicochemical indicators in seawater and sediment (pH, EC, and salinity) were similar to those of each site, with the exception of the extreme salinity at Site 1 (mouth of the channel). And then, the relatively high salinity was found at Site 3. Possibly, circulation of water is poor, and high air temperature (29.2˚C) leading to higher salinity at Site 3. However, acetylene reduction assay (ARA) showed that nitrogen fixation was taking place at all sites. Site 1 had low N_2-fixing activity, probably a consequence of extremely high salinity in the sediment, and the sediment structure was clay loam type. So far, Site 1 and Site 2 showed the similar pattern of N_2-fixing activity and these sites sediment also appeared similar physicochemical characteristics. The highest nitrogen-fixing activity was detected at Site 3, perhaps because of the relatively high concentration of mud loam soil and sediment salinity was lower than other two sites. Although,

the best predictor for the activity of nitrogen fixation in all sites sediment pH was around 6.1. On the other hand, one of the strong effects is pollution. Site 1 and 2 were located on the tidal cheek and it is very near to the local village. And Site 3 was located in the park and it is far from local village. Some reports (Dias *et al.* 2010; Holguin *et al*. 2001) were suggested a correlation between nitrogen pollution and low nitrogen fixation activities in mangrove forests. Also, Mohammadi *et al.* (2012) reported that the environment factors such as temperature, pH, nutrient availability and soil condition had a significant difference in nitrogen fixation activities. Zuberer and Silver (1978) recommenced, nitrogenase activity was associated with many different components of the mangrove ecosystem. These included sediments, mangrove root systems, mangrove leaf litter, and litter from macro-algae and seagrasses, as well as low activity were found in fresh and healthy mangrove leave. Evidence for nitrogen fixation has been confined chiefly to the sediments, with highest rates found within the surface layer (0-5 cm) and mostly in aerobic condition. Expressed in terms of ammonia nitrogen fixed, rates in this layer ranged from 0.22 to 1.18 nitrogen fixation rate (nM/g (dry weight)/h). The mangrove sediment habitat can occur nitrogen fixation because the main reason is the oxygen gradient, thus including the anaerobic conditions, the presence of carbonaceous and other nutrients (Andreote *et al.* 2012; Li *et al.* 2011).

This research implied its effectiveness for measurement of nitrogen fixation rate in Shwe Thaung Yan mangrove forest. The higher nitrogen fixation rate was occurred in mangrove park (Site 3) than other two sites. In this study, Site 1 was more frequently suffers waste pollution from the village, that would be likely to increase exogenous nitrogen inputs (Zedler *et al.* 2008) and inhibit nitrogen fixation rates (Shi *et al.* 2006). However, these phenomenon is probably not important as a nitrogen source in the mangrove sediments because of low fixation rates found at deep layers. The results of these studies indicated that N_2 fixation in mangrove communities is quite widespread, and this study indicates that the level of activity is of significance to the well-being of the community, especially in areas subject to nitrogen limitation. All of these results suggested that nitrogen fixation in sediments would be used as an indicator of the health of a mangrove forests ecosystem in the tropical environment.

Acknowledgements

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ISOLATION AND SCREENING ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC FUNGI DERIVED FROM TWO MARINE SPONGES AGAINST *ESCHERICHIA COLI*

S Aung Myo Htay[1]

Abstract

Escherichia coli is known to cause several infectious diseases such as urinary tract infection and gastrointestinal disease. Marine endophytic fungi from marine sponges have potential as source of new compound against *Escherichia coli*. In the present study, two marine sponges were collected in Shwe-Thaung-Yan coastal area for the isolation of marine fungi on August, 2018. The isolation was undertaken by surface sterilization method. In this study, seven endophytic fungi were isolated from two marine sponges. Among these isolated fungi, two isolates, SF-04 and SF-05 showed antibacterial activity against *Escherichia coli*. According to fungi identification results, fungus SF-04 was identified as *Trichoderma* sp. and fungus SF-05 was identified as *Aspergillus* sp..

Keywords: antibacterial activity, *Aspergillus* sp, endophytic fungi, *Escherichia coli*, marine sponge, *Trichoderma* sp.

Introduction

Microorganisms are a rich source of structurally unique bioactive substances. Since the 1940s, over 30,000 natural products have been discovered from microorganisms, more than 10,000 of which are biologically active (Fenical, 1993). Several characteristics of microorganisms make them important sources of bioactive natural products. Many bioactive compounds, especially antibiotics, have been isolated from microbiological sources. Of the natural products that have been developed into drugs, many come from plant sources, but there have been a considerable number of important drugs harvested from microorganisms and marine sources (da Rocha, 2001). Marine derived fungi are a rich source of structurally new natural products with a wide range of biological activities (Somei and Yamada, 2005, Blunt et *al.*, 2006).

Sponges are a part of the benthic fauna and live in all areas of the marine world, from the shallow coastal seas to the deepest oceans (Levi, 1998). Most of them occur in the marine environment and only about 1% inhibits freshwater (Belarbi et *al.*, 2003). Sponges are well known to be hosts for a large community of microorganisms. Some bioactive compounds isolated from marine organisms have been shown to exhibit anticancer, antimicrobial, antifungal and other pharmacological activities (Natori et *al.*, 1994). Majority of the marine natural products have been isolated from the wide variety of marine microorganisms living in sponges' tissues (Williams et *al.*, 2007).

Escherichia coli are a common inhabitant of the gastrointestinal tract of humans and animals. They are often the most abundant facultative anaerobes in this environment. There are *E.coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of humans and animals. The pathogenic *E. coli* are divided into those strains causing disease inside the intestinal tract and others capable of infection at extra-intestinal sites (Ketia et *al.*, 2012). Therefore, isolation and screening antibacterial activity of endophytic fungi from marine sponges were carried out in this research work.

[1] Dr, Lecturer, Marine Science Department, Pathien University

Materials and Methods

Sampling Site and Sponge Collection

Two marine sponge samples, *Cloina* sp. (Duchassaing and Michelotti, 1864) and *Amphimedon* sp. (Duchassaing and Michelotti, 1864) were collected at Shwe-Thaung-Yan coastal area (Lat. 17 °04' N, Long. 94° 26') nine miles up away from Chaungtha Beach of Ayeyarwady Region. Specimen collection was carried out on August, 2018. All samples of each species were collected at the depths of 0.5-3 m in the intertidal zone and gloves were worn during collection. Specimens were transferred directly to sterile plastic bags containing seawater to prevent contact of sponge tissue from air. The specimens were transported to the laboratory and processed immediately for the isolation and cultivation of fungi. Alternatively, sponge tissues were stored in refrigerator at - 80˚C for identification and future studies.

Figure 1 Map showing the location of sample collected area

Isolation of endophytic fungi

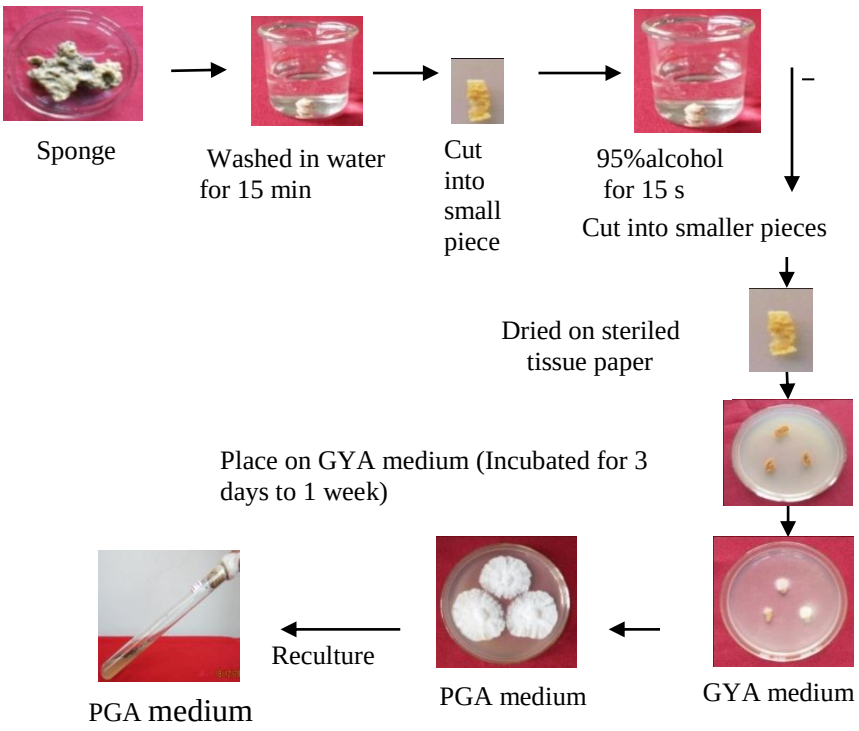
Isolation of fungi was undertaken by Surface Sterilization Method (NITE, 2004) (Figure. 2). The live sponge sample was washed with water for 15 minutes to get rid of nonspecific fungal species from seawater column on sponge surface and cut into small pieces. The cutting samples were disinfected with 95 % alcohol for 15 second and then, cut into the smaller pieces at each end and dried on the sterile tissue paper. After drying, sponge samples were plated on glucose yeast extract agar (GYA) medium containing chloramphenicol. The plates were incubated at 25˚C for 3 days to 1 week until the morphology of fungi could be distinguished. Each isolate was picked up and transferred onto the plate of potato glucose agar medium containing chloramphenicol. The resulting plates were also incubated at room

temperature for pure culture and then, maintained in the slants containing PGA medium for further studies.

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
DW	80 ml
Seawater	20 ml
pH	6.5
(after autoclaving chloramphenicol was added to the medium.)	

PGA medium (Potato Glucose Agar Medium)	
Glucose	2.0 g
Potato	20g
Agar	1.8 g
DW	80 ml
Seawater	20 ml
pH	6.5
(after autoclaving chloramphenicol was added to the medium.)	



GYA medium = Glucose Yeast extract Agar medium

PGA medium= Potato Glucose Agar medium

Figure 2 Surface Sterilization Method for the isolation of endophytic fungi from marine sponge

Study on the Antibacterial Activities

Preliminary study for antibacterial activity against *Escherichia coli* was carried out by the paper disc diffusion assay method (NITE, 2004).

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 50 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 and a non-impregnated sterile paper disc was also placed on the assay plate as a control. 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.

Media used for antimicrobial activity test

Seed Medium

Glucose	2.0 g
Yeast extract	1.0 g
NaCl	0.1 g
K_2HPO_4	0.001 g
DW	80 ml
Seawater	20 ml (28 %)
pH	6.5

Fermentation Medium

Glucose	3.0 g
Glycerol	0.3 ml
Yeast extract	0.3g
Polypeptone	1.2 g
K_2HPO_4	0.001 g
$MgSO_4$	0.001 g
$CaCO_3$	0.1 g
DW	80 ml (28 %)
Seawater	20 ml
pH	6.5

Assay Medium

Glucose	1.0g
Peptone	0.3g
Agar	1.8 g
DW	80ml
Seawater	20 ml (28 %)
pH	6.5

Identification of endophytic fungi

Identification was achieved by taxonomic process set up direct comparison of specimen and by the use of keys, description and illustration. The microscopic examinations of cultures were done on PGA medium under microscope (400X) and identified according to Ando and Inaba (2004).

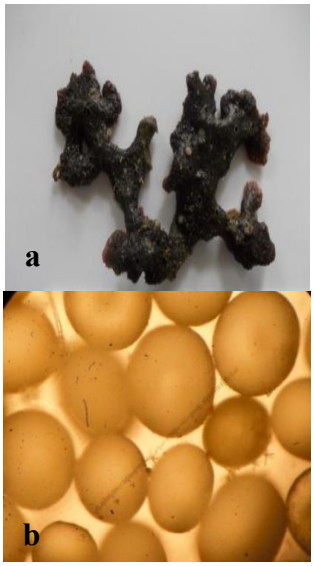
Selection of test organism

The test organism, *Escherichia coli* was obtained from the laboratory of BRBDC of Pathien University.

Results

Classified lists of sponge species collected from study area

Two species of marine sponges were collected from Shwe- Thaung- Yan coastal area. The classification system of the recorded species was followed after Khin Zar Nyo (2004).



Phylum- Porifera
Class- Demospongiae
Sub Class- Monaxonida
Order- Haplosclerida
Family- Niphatidae
Species- *Amphimedon* sp.
(Duchassaing and Michelotti, 1864)

Description - massive to repent branches (1-3cm in diameter); contains volcano-shaped oscules (2-5mm); light gray in color; soft and compressible in consistency; found on shallow reefs and seagrass beds.

Figure 3 (a) Morphology of *Amphimedon* sp. (Duchassaing and Michelotti, 1864)
(b) Micrograph of spicules (400X)

Phylum - Porifera
Class - Demospongiae
Sub-Class- Monaxonida
Order- Hadromerida
Family- Clionidae
Species - *Cliona* sp. (Duchassaing and Michelotti, 1864)

Description- thin to thick encrusting (0.2-5cm in thickness), or massive lobate; smooth; velvety surface with oscules (0.2-3cm in diameter) bearing cream colored membranes; firm and rubbery; found on the reef and seagrass environments.

Figure 4 (a) Morphology of *Cliona* sp. (Duchassaing and Michelotti, 1864)
(b) Micrograph of spicules (400X)

Isolation of Endophytic Fungi from Marine Sponges

In this study, seven endophytic fungi were isolated from two marine sponges by Surface Sterilization Method (Table 2).

In the prescent study, four fungi were isolated from marine sponge *Amphimedon* sp. and three fungI from *Cliona* sp. (Table 1).

Table 1 Isolated fungi from two sponges' species by surface sterilization method

Sample	Total Isolated Fungi	
	Total Isolated Fungi	Fungi No
Amphimedon sp.	4	**SF-01,02,03,04**
Cliona sp.	3	**SF-05,06,07**
Total Isolated Fungi	**7**	**7**

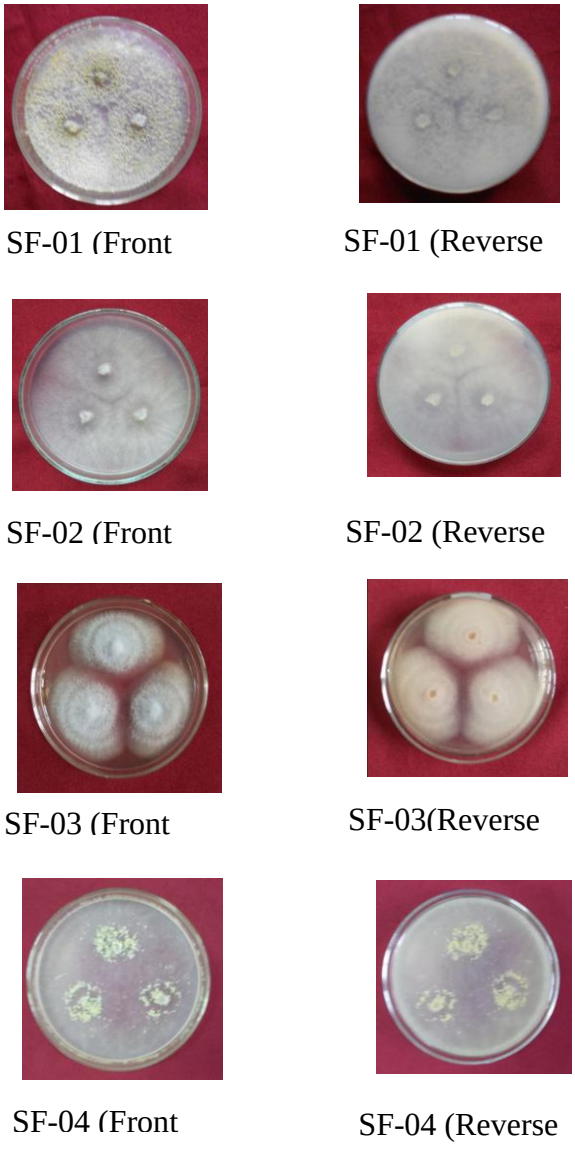
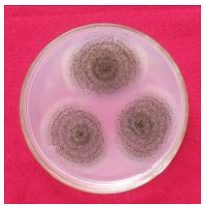


Figure 5 Morphologies of endophytic fungi isolated from *Amphimedon* sp.

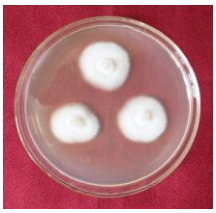


SF-05 (Front

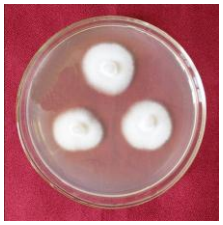
SF-05 (Reverse side)

SF-06 (Front

SF-06 (Reverse side)



SF-07 (Front



SF-07(Reverse

Figure 6 Morphologies of endophytic fungi isolated from *Cliona* sp.

Antibacterial Activity of Isolated Fungi against *Escherichia coli* by Paper Disc Diffusion Assay

Antibacterial activity was carried out by the paper disc diffusion assay method. In the present study, the fungus SF - 04 isolated from *Amphimedon* sp. and the fungus SF – 05 isolated from *Cliona* sp. were shown distinct clear zone against *Escherichia coli* and the other fungi were not shown any inhabitation zone (Table 2).

Table 2 Antibacterial Activity of Isolated Fungi against *Escherichia coli* by Paper Disc Diffusion Assay (7 days fermentation)

Fungi No.	**Inhibitory zone (mm)**
SF-01	no activity
SF-02	no activity
SF-03	no activity
SF-04	29.84
SF-05	35.48
SF-06	no activity
SF-07	no activity

Figure 7 Antibacterial Activity of Isolated Fungus SF - 04 against *Escherichia coli*

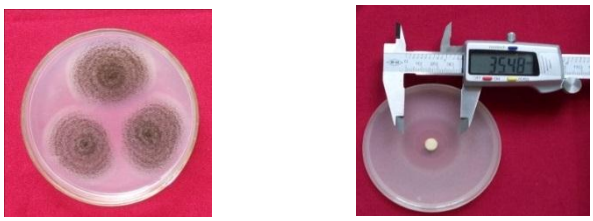


Figure 8 Antibacterial Activity of Isolated Fungus SF - 05 against *Escherichia coli*

Identification of endophytic fungus SF - 04 and SF - 05

Identification of endophytic fungui which showed the clear zone of inhabitation against *Escherichia coli* was done according to Ando and Enaba (2004). According to fungi identification results, fungus SF-4 was identified as *Trichoderma* sp. (Figure 9) and fungus SF-5 was identified as *Aspergillus* sp. (Figure 10).

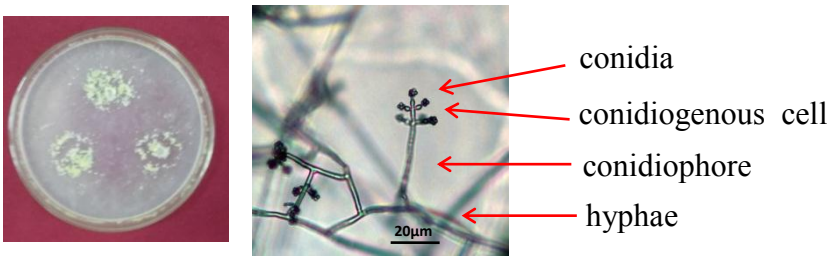


Figure 9 Morphology and photomicrograph (400 X) of fungus SF-04 (7 days old culture on PGA medium)

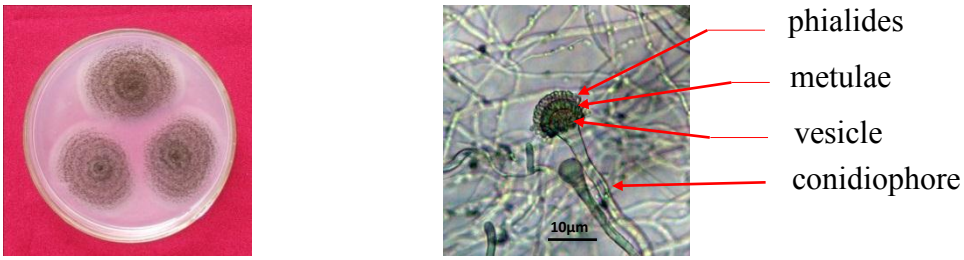


Figure 10 Morphology and photomicrograph (400 X) of fungus SF-05 (7 days old culture on PGA medium)

Identification Keys of Mitosporic Fungi (Ando and Enaba, 2004)

1. Synnemata form ---------- Synnematous fungi

1. Synnemata not form ---------- 2

2. Conidium axis curved through more than 180 ---------- Helicoconidium

2. Conidium axis not curved through more than 180 ---------- 3

3. Conidium with more than one axis; protuberances(s), other than setulae, present and more than ¼ the length of the spore body -- Stauroconidium

3. Conidium with only one axis; any protuberances, other than setuale, if present, not more than ¼ the length of the spore body ---------- 4

3. Conidial shape not above ---------- Miscellaneous fungi

4 Length/ width ratio of conidium body exceeding 15:1---------- Scolecoconidium

4. Length/ width ratio of conidium body less than 15:1---------- 5

5. Conidium with 1septa ---------- Didymoconidium

5. Conidium lacking septa (Ameroconidium) ---------- 6

6. Conidiophores not produced or not clear ---------- *Arthrinium* sp.

6. Conidiophores with or without septa, developed single and enteroblastic conidia ---------- 7

7. Conidiophores without septa, not branched and multi phialides with parallel arrangement and straight ending in a large vesicle---------- *Aspergillus* sp.

7. Conidiophores with septa, developed single and branched cluster into fascicles---------- *Trichoderma* sp.

Discussion

Marine fungi have been recognized as an important and untapped resource for novel bioactive compounds. The chemical compounds of marine microorganisms are not well known as terrestrial counterparts. However, in the last decade, several bioactive compounds have been isolated from marine fungi and bacteria. These natural compounds are new resources for the development of medically useful compounds (Donia and Haman, 2003, Anand et *al.*, 2006). Antibacterial activities among marine fungi and bacteria were a well-known phenomenon and had been demonstrated in a number of studies (Isnansetyo and Kamei, 2003, Uzair et *al.*, 2006).

In the present study, two marine sponges collected from Shwe Thaung Yan coastal area were employed for the isolation of marine endophytic fungi. Endophytic fungi were isolated by the method of Surface sterilization Method (Figure 2). In this study, seven endophytic fungi were isolated from two marine sponges (Table 1). Four fungi were isolated from *Amphimedon* sp. and three fungi were isolated from *Cliona* sp.. Easson et *al.,* 2014 mentioned the microbial diversity of *Cliona varians* to find the relationships between the microbial community patterns and sponge population structure. Antibacterial activity of all isolates against *Escherichia coli* was carried out

by paper disc diffusion assay method. In the present investigation, endophytic fungus SF – 04 inhibited on *Escherichia coli* (29.84 mm) and SF – 05 inhibited on *Escherichia coli* (35.48 mm) (Figure 7 and Figure 8). By the microscopic observations, fungus SF-04 was identified as *Trichoderma* sp. and fungus SF-05 was identified as *Aspergillus* sp. (Figure 9 and Figure 10). According to Ghisalberti and Sivasithaamparam (1991), *Trichoderma* species were well-known as antifungal producers and they have been used as biological control agent for various phytopathogenic fungi.

Conclusion

Although marine sponges have been shown the bioactive potential for various natural products, the microbial study on marine sponges are very rare. For this reason, the present study was carried out on the antibacterial activity of marine microbes associated with marine sponges 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.

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BRACHIONID ROTIFER OF YE RIVER MOUTH IN SOUTHERN MON COASTAL WATER

Naung Naung Oo[1]

Abstract

Studies on diversity of brachionid rotifers were conducted at Ye River Mouth (Lat. 15° 04′ N and Lat. 15° 12' N, Long. 97° 46' E and Long. 97° 48' E) in southern Mon coastal water from January to December 2018. Mon coastal water rotifers are least studied and authenticated records are not available for sufficing the data on their biodiversity. Rotifers were collected from brackish water to know their species diversity. A total of 12 species of branchionid rotifers identified during this study are *Brachionus angularis* Gosse, 1851, *B. calyciflorus* (Pallas, 1766), *B. caudatus* Barrois & Daday, 1894, *B. diversicornis* (Daday, 1883), *B. donneri* Brehm, 1851, *B. falcatus* (Zacharias, 1898), *B. forficula* Wierzejski, 1891, *B. murrayi* (Fadeew, 1925), *B. plicatilis* (Müller, 1786), *B. quadridentatus* Hermann, 1783, *B. rotundiformis* (Tschugunoff, 1921) and *B. urceolaris* (Müller, 1773). This is a primary record on brachionids of euryhaline water of Ye River Mouth. The detailed description of the rotifers recorded during the study in southern Mon coastal water is presented for substantiating the taxonomic relevance of the study.

Keywords: Brachionid, rotifer, brackish, Ye River Mouth, southern Mon coastal water.

Introduction

Rotifers make up a phylum of microscopic and near-microscopic pseudocoelomate animals. There are some pioneering efforts towards the end of eighteenth century to provide a systematics for rotifers based on morphological details (Hudson and Gosse, 1889). Rotifers may be free swimming or truly planktonic, others move by inch worming along the substrate whilst some are sessile, living inside tubes or gelatinous holdfasts. Most species of rotifers are about 200 to 500 μm long. However a few species, such as *Rotaria neptunia* may be longer than a millimeter.

Rotifers are microscopic animals, their diet consist of matter small enough to fit through their tiny mouths during filter feeding. Rotifers are primarily omnivorous, but some species have been known to be cannibalistic. The diet of rotifers most commonly consists of dead or decomposing organic materials, as well as phytoplankton that are primary producers in aquatic communities. Such feeding habits make some rotifers primary consumers. There are about 2000 species of rotifers, divided into two classes, Monogononta and Bdelloidea. Monogononta is the largest group with around 1500 different species. Order Bdelloida is of particular note because of the absence of males and the ability of cryptobiosis.

In Myanmar context the study about rotifers are few when compared to the global research. The history of Myanmar rotifers dates back to the initial period of rotifer systematics started with a brief note on rotifers in some Myanmar waters. The available literature shows that the earlier works on Myanmar rotifers were limited to certain regional water bodies. Over the last three decades there were a few research priorities to address these lacunae on observing and classifying rotifer fauna in Myanmar waters. Hitherto, the study about the rotifers present in the brackish waters is meagre. Since coastal ecosystems like mangroves and coral reefs harbour a variety of fishes, it is imperative to study the rotifers in the ecosystem which are the driving force behind the survival of most of them. The objective of this study is to observe how many species of brachionid rotifers in brackish water of Ye River Mouth in southern Mon coastal water.

[1] Dr, Assistant Lecturer, Department of Marine Science, Mawlamyine University

Materials and Methods

Rotifer samples were collected from the brackish waters of Ye River Mouth in southern Mon coastal water (Fig. 1). Since the density of rotifer present in pure saline waters of Mon coastal water are very less, the present study is restricted to the brackish water areas only. Samples were collected from January to December 2018. Rotifer samples were collected by filtering 5 litres of water from a particular site. Water samples at a site were collected from various depths instead of a particular point to avoid sampling errors.

In the present study, the samplings were done in the evening time between 3 to 5 p.m., as rotifers have the tendency to migrate vertically for grazing on nutrient rich phytoplankton in the upper water column. During the filtering process, water was collected from various depths and different points using a small bucket of 5 litres and was filtered through the net of mesh size 40 µm. Collected samples were transferred to a 100 ml sampling bottle and fixed with 5% formaldehyde immediately to avoid clumping of rotifers. Fixed samples were carried to the laboratory for further analysis.

Samples were kept in dark and cool area. Primarily all the samples were analysed for the density of the rotifers present in the samples. The density was counted using sedge wick-rafter plankton counting cell. Further analysis for the species identification was done with binocular microscope. The samples were thoroughly analysed for the presence of various rotifer species and photographs of the desired species were taken using digital camera. The identification of rotifer samples were followed by Battish (1992), Fontaneto, De Smet and Melone (2008), and Phan and Le (2012).

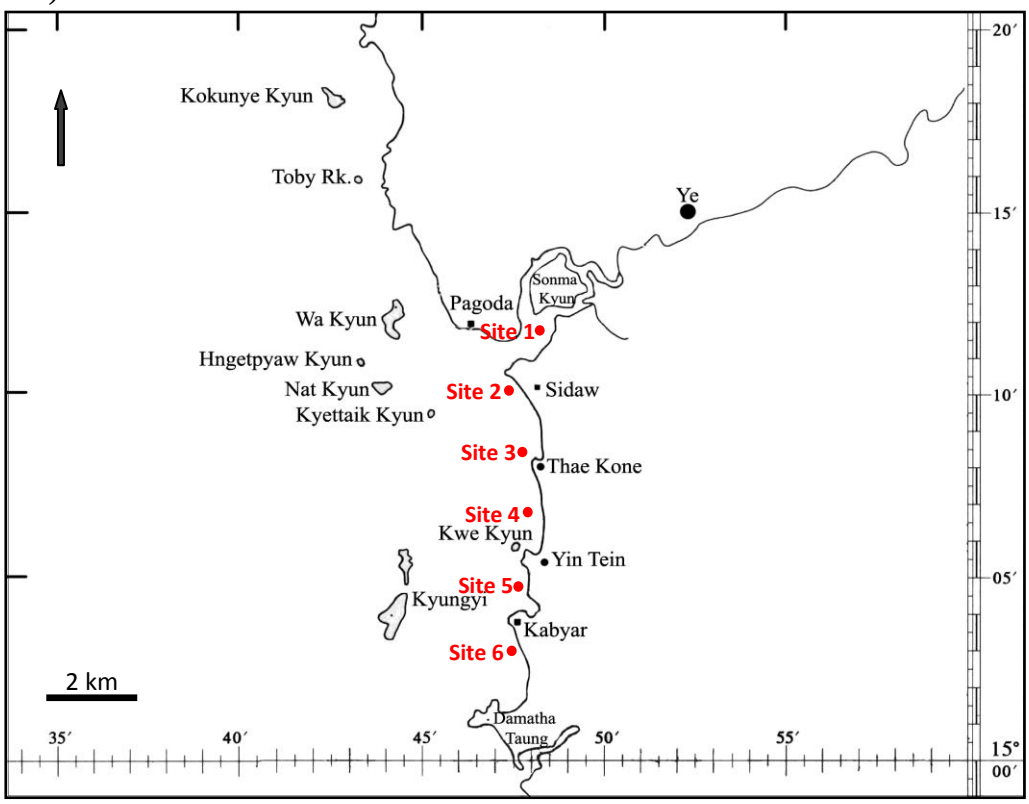


Figure 1 Map showing the sampling sites of brachionid rotifers in Ye River Mouth, southern Mon coastal area

Results and Discussion

Rotifer fauna of southern Mon coastal waters is rich in *Brachionus* species. The presence of *Brachionus plicatilis* species complex is a notable character of the rotifers present in Ye River Mouth waters. Backwaters of Sitaw describe the *Brachionus* species complex as a combination of *B. plicatilis*, *B. rotundiformis* and *B. murrayi*. But there is a slight variation in the description of this complex as *B. murrayi* is less envisaged in the complex with a majority of them being a combination of *B. plicatilis* and *B. rotundiformis*. Earlier study describes the *Brachionus* species complex in Andaman waters as *Brachionus* 'S' and 'L' and 'SS' forms as smaller *Brachionus*, larger *Brachionus* and tiny *Brachionus* (Madhu, Rema and Soundrarajan 2004). But the present study reveals that they are *Brachionus rotundiformis, B. murrayi* and *B. plicatilis* respectively in occurrence with the reports from Cochin backwaters (Anitha and Rani 2006). But the difference observed among southern Mon coastal water species is with respect to the lesser availability of *B. murrayi* among the various samples of the complex collected from southern Mon coastal waters. Other species of rotifers are present in very less number. The representatives of other species are specific for certain areas but *Brachionus* species complex consisting of *Brachionus rotundiformis* and *B. plicatilis* is common for most of the areas (Table 2). The rotifers identified during this study are *Brachionus angularis*, *B. calyciflorus*, *B. caudatus*, *B. diversicornis*, *B. donneri*, *B. falcatus*, *B. forficula*, *B. murrayi*, *B. plicatilis*, *B. quadridentatus*, *B. rotundiformis* and *B. urceolaris* (Table 1 and Fig. 2). The maximum density of rotifers was noticed from the sample collected from Kamawkin water (Site. 1), near Sonma Kyun (Table 2). The sample from that area consisted of *Brachionus rotundiformis* only. Various other rotifers are mainly identified from the samples collected from mangrove areas.

Table 1 Systematic of brachionid rotifer of Ye River Mouth in southern Mon coastal water

Phylum	**:**	Rotifera Cuvier, 1817
Class	**:**	Monogononta Plate, 1889
Order	**:**	Ploima Hudson & Gosse, 1886
Family	**:**	Brachionidae Ehrenberg, 1838
Genus	**:**	*Brachionus* Pallas, 1766
Species	1	*B. angularis* Gosse, 1851
	2	*B. calyciflorus* (Pallas, 1766)
	3	*B. caudatus* Barrois & Daday, 1894
	4	*B. diversicornis* (Daday, 1883)
	5	*B. donneri* Brehm, 1851
	6	*B. falcatus* (Zacharias, 1898)
	7	*B. forficula* Wierzejski, 1891
	8	*B. murrayi* (Fadeew, 1925)
	9	*B. plicatilis* (Müller, 1786)
	10	*B. quadridentatus* Hermann, 1783
	11	*B. rotundiformis* (Tschugunoff, 1921)
	12	*B. urceolaris* (Müller, 1773)

Artificial key to the species of *Brachionus* recorded from study areas

1.a. Two small anterior median spines …….. **2**

1.b. Four or six well developed anterior spines ……. **3**

2.a. Two divergent posterior spines present ……***Brachionus caudatus***

2.b. Posterior spines absent ……..***Brachionus angularis***

3.a. Anterior lorica margin with four spines ……. **4**

3.b. Anterior lorica margin with six spines ……... **6**

4.a. Lorica smooth, transparent, posterior lorica circular ……***Brachionus calyciflorus***

4.b. Posterior lorica with two spines …….. **5**

5.a. Length of posterior right spine longer than left spine ……***Brachionus diversicornis***

5.b. Length of posterior spines stout and inward curved ……..***Brachionus forficula***

6.a. Anterior spines length less equal …… **7**

6.b. Anterior spines length unequal ……... **8**

7.a. Posterior lorica without spines ……...***Brachionus plicatilis***

7.b. Posterior lorica with two large spines …….***Brachionus donneri***

8.a. Antero-intermediate spines longer antero-median and lateral ….***Brachionus falcatus***

8.b. Antero-intermediate spines shorter antero-median and lateral ……... **9**

9.a. Posterior lateral without spines ……***Brachionus urceolaris***

9.b. Posterior lateral with spines ……. **10**

10.a. Lorica barrel-shaped ……..***Brachionus quadridentatus***

10.a. Lorica vase shaped ……..... **11**

11.a. Lorica miniature more rounded and not sharply …….***Brachionus rotundiformis***

11.b. Lorica small ovoid to elliptical and not sharply ……...***Brachionus murrayi***

This work is an earnest attempt to add to the knowledge of the occurrence of rotifers in brackish water systems in Mon coastal water. Since, the identified rotifers show some new records for the locality, description of identified rotifers in southern Mon coastal water are given below in concurrence with the similar descriptions and their distribution and ecology.

Brachionus angularis Gosse, 1851

Ahlstrom, 1932. p 234; Ahlstrom, 1940. p 154-155, pl V: figs 1-13; Osorio Tafall, 1942. p 42, pl I, II: figs 1-19; Wang, 1961. p 70-71, pl V: figs 46a-c; Edmondson, 1966. p 451, fig 18.29c; Koste, 1978. p 91-92, pl 13: figs 1-7, 11-14, 16; Dang et al., 1980. p 56-57, fig 36; Battish, 1992. p 87-88, fig 72; Chung et al., 1992. p 45-46, pl II: fig 3; De Manuel, 2000. p 97, fig 3o; Dhanapathi, 2000. p 37, pl II: fig 2.

Synonym: *B. daitojimensis* Sudzuki, 1992; *B. donghuensis* Sudzuki & Huang, 1997; *B. lyratus yonaguniensis* Sudzuki, 1992; *B. minimus* Bartsch, 1877; *B. morondavaensis* Sudzuki, 1998; *B.*

papuanus Daday, 1897; *B. pseudokeikoa* Sudzuki, 1992; *B. pyriformis* Sudzuki & Huang, 1997; *B. syennensis* Schmarda, 1859; *B. testudo* Herberg, 1853.

Description: Lorica ovate, posterior rounded, surface smooth, compressed dorsal-ventral. Anterior dorsal margin with a pair of small spines in the centre of occipital, forming a deep broad U-shaped sinus. Posterior without spines. Foot opening rather large, U-shaped aperture, with a pair of protuberances in the ventral plate.

Measurements: Total length 110 μm, Lorica length 100 μm.

Distribution and Ecology: This species is a small rotifer, and is widely distributed in freshwater and estuarine water. Common in the Mekong River (Phan Doan Dang *et.al* 2015).

Brachionus calyciflorus (Pallas, 1766)

Hada, 1938. p173, fig I; Ahlstrom, 1940. p 150-152, pl III: figs 1-9, pl XX: figs 7-8; Osorio Tafall, 1942. p 45-46, pl VIII: figs 83, 86-90; Wang, 1961. p 71-72, pl V: figs 47a-c; Edmondson, 1966. p 451, fig 18.29b; Shirota, 1966. p 45, fig 637; Koste, 1978. p 86-87, fig 33b, pl 12: figs 1a-e; Dang et al., 1980. p 58-59, fig 38; Battish, 1992. p 79-80, figs 64 (1-3); Chung et al., 1992. p 44, pl III: fig 1; De Manuel, 2000. p 98, figs 3d, g; Dhanapathi, 2000. p 39, pl III: fig 1.

Synonym: *B. amphiceros* Ehrenberg, 1838; *B. dorcas* Gosse, 1851; *B. gillardi* Hauer, 1966; *B. pala* Ehrenberg, 1838; *B. pala anuraeiformis* Brehm, 1909.

Description: Lorica oval, flexible, smooth, slightly compressed dorsal-ventral, anterior dorsal margin with four broad based spines of similar size, triangular, median longer than lateral. Lateral posterior with a pair of large spines or absent. Anterior ventral margin slightly elevated, with a shallow V or U-shaped notches. Foot opening usually with two broad-based and stretched-like spines.

Measurements: Total length 300 μm; length of lorica 210 μm.

Distribution and Ecology: Estuarine water species, body rather large, distributed widely in ponds, lakes and rivers (De Manuel 2000). This species is widely used in ecological studies, eco-toxicological and aquaculture and is found in the Mekong River Basin.

Brachionus caudatus Barrois & Daday, 1894

Ahlstrom, 1940. p 155-156, pl VI: figs 1-11; Osorio Tafall, 1942. p 46-47, pl III, IV: figs 20-35, 39, 44-45; De Ridder, 1966. p 24, figs 4-5; Koste, 1978. p 94-95, pl 13, 14; Dang et al., 1980. p 60, fig 40; Battish, 1992. p 82, figs 67 (1-5); Dhanapathi, 2000. p 38, pl II: fig 4.

Synonym: *B. caudatus singapurensis* Sudzuki, 1989

Description: Lorica ovate, transparent, medial slightly wide, posterior narrowing. Lorica surface ornamentation, with four occipital spines, the lateral slightly longer than the medians, two median spines separated by a U-shaped sinus, posterior with a pair spines long, stout, slightly curved. Ventral plate with slightly convex between the base of posterior spine.

Measurements: Total length 150 μm; lorica length 113 μm.

Distribution and Ecology: This species is distributed in tropical and subtropical regions and is found in the Mekong River Basin (Phan Doan Dang *et.al* 2015).

Brachionus diversicornis (Daday, 1883)

Ahlstrom, 1940. p 161-162, pl IX: figs 6-7, pl XX: figs 3-5; Wang, 1961. p 77-78, pl VI: fig 54; Koste, 1978. p 68, pl 15: fig 5; Dang et al., 1980. p 59, fig 39; Battish, 1992. p 88, fig 73; Chung et al., 1992. p 48, pl III: fig 3; Dhanapathi, 2000. p 38, pl II: fig 7.

Synonym: *Schizocerca diversicornis* Daday, 1883

Description: Lorica tumbler-shaped, firm, elongate, half of anterior wider than posterior, slight compressed dorsal-ventral, with four occipital spines, laterals longer than medians. Posterior lorica with two spines unequal, right posterior spine longer than left spine.

Measurements: Total length 230 μm; lorica length 160 μm.

Distribution and Ecology: Estuarine water species, distributed widely in ponds, lakes and rivers, found in the Mekong River Basin (Phan Doan Dang *et.al* 2015).

Brachionus donneri Brehm, 1851

Voigt, 1956. pl 106, fig 12; Bērziņš, 1973. p 458, figs 12-14; Koste, 1978. p 70, fig 24.11, pl 14: fig 6; Sharma, 1983. figs 1-2; Phan et al., 2012. p 16, fig 3.

Synonym: Non.

Description: Lorica lateral view ovate or rounded, transparent, compressed dorsal-ventral. Anterior dorsal margin with six spines, equal, blunted. Anterior ventral margin with four spines, blunted, laterals longer medians spines, two medians spines separated by a U-shaped sinus. Lateral dorsal margin with two pairs spines, pair of anterior spines small, blunted, pairs of posterior large, pointed. Posterior margin with two large spines, mace-shaped, ending of rounded, two spines separated by a deep V-shaped, broad.

Measurements: Total length 110 μm - 150 μm.

Distribution and Ecology: Estuarine and freshwater species was found in the lower of the Mekong River (Vietnam), Tonle Sap (Cambodia). This species is also found in Sri Lanka, Panama, India (Sharma 1983 and Dhanapathy 2000).

Brachionus falcatus (Zacharias, 1898)

Ahlstrom, 1940. p 164-165, pl X: figs 1-3; Wang, 1961. p 77, pl VI: fig 53; Shirota, 1966. p 45, fig 638; Koste, 1978. p 83, pl 14: figs 2a-b; Dang et al., 1980. p 62-63, fig 43; Battish, 1992. p 84 – 85, figs 68 (1-2); Chung et al., 1992. p 49, p I, fig 7; De Manuel, 2000. p 98, fig 3i; Dhanapathi, 2000. p 41, pl VI: figs 1-3.

Synonym: *B. falcatus reductus* Koste & Shiel, 1987

Description: Lorica ovate, surface with stippled, compressed dorsal-ventral. Anterior dorsal margin with six spines, unequal, intermediates spines considerably longer than other spines, curved ventrally at the end. Lateral and median spines short, subequal. Posterior lorica margin with two spines very long, slightly curved inward. Foot opening between bases of posterior spines.

Measurements: Total length 170 μm; Lorica length 80 μm.

Distribution and Ecology: Estuarine and freshwater species, distributed in tropical and subtropical areas, found in the Mekong River (Phan Doan Dang *et.al* 2015).

Brachionus forficula Wierzejski, 1891

Ahlstrom, 1940. p 162-163, pl VII: fig 8, pl XX: figs 1-2; Wang, 1961. p 72-73, pl V: figs 48a-b; Koste, 1978. p 95-96, pl 14: fig 7; Dang et al., 1980. p 61-62, fig 42; Battish, 1992. p 85, figs 69 (1-3); Chung et al., 1992. p 47, pl II: fig 5; Altindağ et al., 2005. p 101, fig 2b.

Synonym: Non.

Description: Lorica firm, stippled, anterior dorsal margin with four occipital spines, lateral spines slightly longer than median spines, Posterior of lorica with a pair of spines stout, long, subequal, curved inward, base of spines wide, near their bases are knee-like swellings, tapering to points. Foot opening between the bases of posterior spines.

Measurements: Total length 180 μm, Lorica length 100 μm.

Distribution and Ecology: Estuarine and freshwater species, common in ponds, lakes, rivers, found in the Mekong River (Phan Doan Dang *et.al* 2015).

Brachionus murrayi (Fadeew, 1925)

Ahlstrom, 1940. p 175-176, pl XIX: figs 1-4; Osorio Tafall, 1942. p 59-60, pl XI, XII: figs 109, 110, 135; Wang, 1961. p 81-82, pl VI: fig 57; Edmondson, 1966. p 451, figs 18.30b, d; Shirota, 1966. p 45, fig 663; Koste, 1978. pl 8: figs 1, 2a, 3, 6; Dang et al., 1980. p 65-66, fig 47; Fernando et al., 1981. p 209, figs 4-5; Battish, 1992. p 88-89, fig 74; Chung et al., 1992. p 41, pl I: fig 2; Dhanapathi, 2000. p 43-44, pl IV: fig 5; Fontaneto et al., 2008. p 88, fig 55.

Synonym: Non.

Description: Lorica small ovoid to elliptical and not sharply separated into dorsal and ventral plates; occipital spines six in number which are narrow markedly above the broad, inflated base and end in thin acutely pointed tips or small based equilateral, equidistant triangular spines; the pectoral margin rigid and scalloped, shows considerable variations, irregularity of the four rounded projections; the occipital spines also show considerable variations especially in the relative length of intermediate spines; posterior spines absent; foot opening with a small sub square aperture ventrally.

Measurements: Total length 190 μm; Lorica width 120 μm.

Distribution and Ecology: This species is widespread in estuarine and freshwater and is found in the Mekong River (Wallace and Snell 2001, Wallace *et.al* 2006, Wallace and Smith 2013).

Brachionus plicatilis (Müller, 1786)

Ahlstrom, 1940. p 149-150, pl II: figs 1-9; Osorio Tafall, 1942. p 55-56, pl IV, VII: figs 38, 75-79; Edmondson, 1966. p 451, fig 18.29a; Shirota, 1966. p 45, figs 640-641; Koste, 1978. p 77, pl 9, fig 1a-e, pl 12, fig 7; Dang et al., 1980. p 60-61, fig 41; De Maeseneer, 1980. p 117, pl 1: fig 3; Battish, 1992. p 89-90, figs 75 (1-2); De Manuel, 2000. p 100, fig 3n; Dhanapathi, 2000. p 42, pl IV: fig 3; Fontaneto et al., 2008. p 88, fig 52.

Synonym: *B. hepatotomeus* Gosse, 1851; *B. mulleri* Ehrenberg, 1834

Description: Lorica oval, elongate, relatively soft, slightly compressed dorsal-ventral. Anterior dorsal margin with six spines, base of spines broad, saw-tooth spines, nearly equal. Anterior

ventral margin with four spines, very broad and blunted. Posterior lorica without spine. Foot opening posterior, aperture clearly U-shaped.

Measurements: Total length 160 μm; Lorica width 110 μm.

Distribution and Ecology: This species widely distributed in brackish or salt water (Rao and Chandra 1984). Only found in the Mekong River in Vietnam.

Brachionus quadridentatus Hermann, 1783

Ahlstrom, 1940. p 165-166, pl XI: fig 9, pl XII: figs 1-9; Osorio Tafall, 1942. p 57-58, pl IX: figs 91-94, 96-97; Edmondson, 1966. p 451, fig 18.29e; Shirota, 1966. p 45, fig 644; Koste, 1978. p 72-73, fig 32a, pl 11: figs 4a-b; Dang et al., 1980. p 64, fig 45; Battish, 1992. p 80-81, figs 65 (1-8); Chung et al., 1992. p 42, pl I: fig 5; De Manuel, 2000. p 101, figs 3a, c; Dhanapathi, 2000. p 42, pl V: figs 1-2.

Synonym: *B. ancylognathus* Schmarda, 1859; *B. brevispinus* Ehrenberg, 1832; *B. capsuliflorus* Pallas, 1766; *B. cluniorbicularis* Skorikov, 1894; *B. cluniorbicularis isigakiensis* Sudzuki, 1992; *B. rhenanus* Lauterborn, 1893

Description: Lorica barrel-shaped, width broader than length, surface stippled or pustulate. Anterior dorsal margin with six spines, medians a pair, spines longer than laterals and intermediates spines, curved outward. Anterior ventral margin undulate, somewhat elevated toward the centre, with a median sinus. Lateral of posterior lorica margin with two spines, somewhat unequal. Foot opening tubular shaped, with two sides stretched like spines.

Measurements: Total length 160 μm; Lorica width 95 μm.

Distribution and Ecology: Fresh and brackish water species, found in both northern and southern hemispheres, common in tropical and subtropical regions (Murray 1913, Segers *et.al* 1992 and Segers 2007). This species is the most common of genus *Brachionus* in the Mekong River Basin (Phan Doan Dang *et.al* 2015).

Brachionus rotundiformis (Tschugunoff, 1921)

Ahlstrom, 1932. p 248; Ahlstrom, 1940. p 174-175, pl XVIII: fig 6-9; Osorio Tafall, 1942. p 60-61, pl XI, XII: figs 111, 113, 134; Wang, 1961. p 80-81, pl VI: figs 56a-b; Shirota, 1966. p 45, fig 664; Koste, 1978. p 63-64, pl 6: figs 1-2, pl 7: figs 1-2;Dang et al., 1980. p 65, fig 46; Battish, 1992. p 90-91, figs 76 (1-2); Chung et al., 1992. p 49, pl III: fig 4; De Manuel, 2000. p 112; Fontaneto et al., 2008. p 88, fig 54.

Synonym: Non.

Description: Lorica miniature more rounded and not sharply split into dorsal and ventral plates; occipital margin with small based acutely sharp spines; pectoral margin four-lobed, lateral ones roughly triangular; foot opening with sub square aperture ventrally and fairly ovoid aperture dorsally.

Measurements: Total length 180 μm; Lorica width 100 μm.

Distribution and Ecology: Estuarine and freshwater species, common in ponds, lakes, rivers. Found in both northern and southern hemispheres, common in tropical and subtropical regions. It found in the Mekong Basin (Wallace and Snell 2001, Wallace *et.al* 2006, Wallace and Smith 2013).

Brachionus urceolaris (Müller, 1773)

Ahlstrom, 1940. p 171-172, pl XVI: figs 1-11; Osorio Tafall, 1942. p 59, pl IV: figs 36-37; Wang, 1961. p 75-76, pl V: fig 51; Koste, 1978. p 78-79, figs 30a, 31, pl 9: figs 3a-e; Dang et al., 1980. p 41, fig 44; De Manuel, 2000. p 101, fig 3m; Dhanapathi, 2000. p 43, pl. IV: fig 4.

Synonym: *Tubipora urceus* Linnaeus, 1758

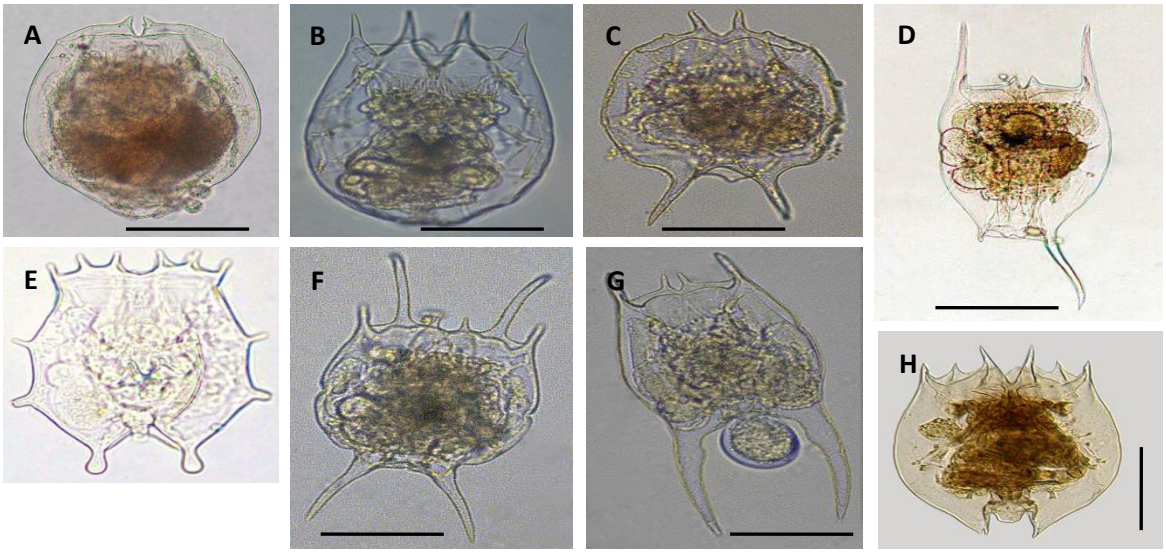
Description: Lorica ovate, wide in the middle lower part of lorica, posterior rounded, surface smooth. Anterior dorsal margin with six spines, medians longer than other spines, laterals longer than intermediates, two median spines separated by a V-shaped sinus. Anterior ventral margin undulate. Foot opening rather large, somewhat deflected to dorsal plate, aperture dorsally U-shaped and large oval aperture ventral plate.

Measurements: Total length 115 µm; Lorica width 75 µm.

Distribution and Ecology: Estuarine and freshwater, common in ponds, lakes and rivers with eutrophication. Widespread throughout Asia, found in the Mekong Basin (Wang 1961).

Table 2 Density and location specific availability of rotifers (+ indicates presence of the species)

Collection site	Mean Density (No/100 ml)	Brachionid rotifer											
		B. angularis	*B. calyciflorus*	*B. caudatus*	*B. diversicornis*	*B. donneri*	*B. falcatus*	*B. forficula*	*B. murrayi*	*B. plicatilis*	*B. quadridentatus*	*B. rotundiformis*	*B. urceolaris*
Site 1 (Kawmawkin, mangrove area)	98											+	
Site 2 (Sitaw, mangrove area)	46	+		+	+			+		+	+	+	
Site 3 (Thaekone, mangrove area)	32		+			+	+		+	+		+	+
Site 4 (Yintein, mangrove area)	44	+		+	+			+	+	+	+	+	+
Site 5 (Kabyarwa, mangrove area)	40	+	+			+	+		+	+	+	+	
Site 6 (Kabyarwa, mangrove area)	12			+	+		+	+	+	+	+	+	



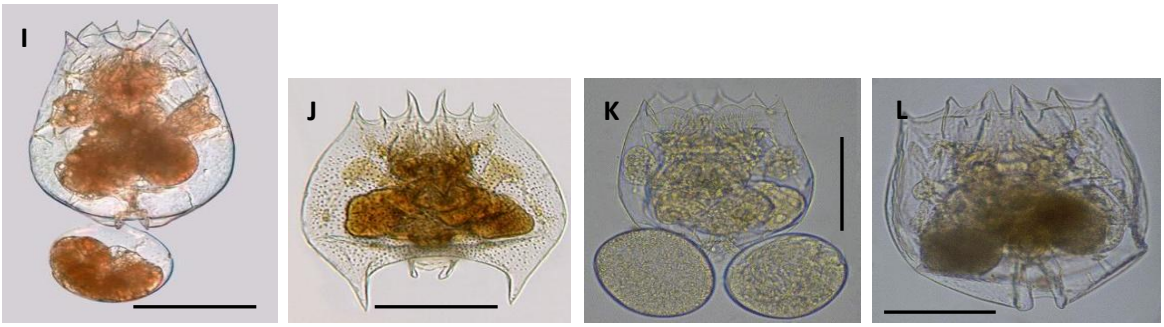


Figure 2 (A-L): Brachionid rotifer of Ye River Mouth in southern Mon coastal water. A) *Brachionus angularis* Gosse, 1851; B) *B. calyciflorus* (Pallas, 1766); C) *B. caudatus* Barrois & Daday, 1894; D) *B. diversicornis* (Daday, 1883); E) *B. donneri* Brehm, 1851; F) *B. falcatus* (Zacharias, 1898); G) *B. forficula* Wierzejski, 1891; H) *B. murrayi* (Fadeew, 1925); I) *B. plicatilis* (Müller, 1786); J) *B. quadridentatus* Hermann, 1783; K) *B. rotundiformis* (Tschugunoff, 1921); L) *B. urceolaris* (Müller, 1773). Scale bars = 100 μm.

Conclusion

Apart from the basic taxonomy interest in this effort, the notable absence of *Brachionus murrayi* among the *Brachionus* species complex in Mon coastal waters is a cue for further studies. It is imperative to investigate the potential of these complexes and other rotifers in determining the diversity and abundance of aquatic organisms in Mon coastal waters. Further, the taxonomic cues provided by the study calls upon intensive exploratory works to strengthen the database on rotifers of Mon coastal waters to promote the various ongoing captive seed production programmes of marine ornamental and food fishes.

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LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF SOME COMMERCIAL FISH SPECIES FROM KYAIKKHAMI FISH LANDING CENTER, MON COASTAL AREA

Zarni Ko Ko[1]

Abstract

The study was conducted to analyze the length-weight relationship and condition factor of some commercial fish species from Kyaikkhami Fish Landing Center, Mon Coastal Area during June, 2018 to March, 2019. In present study period, a total of 873 fish species in size ranging from 20.2cm to 49cm for *Tenualosa ilisha*, from 20.6cm to 49.3cm for *Tenualosa toli*, from 24.8cm to 45.9cm for *Scomberomorus guttatus* and from 16.5cm to 30cm for *Setipinna tenuifilis* was recorded. In present findings, the exponential forms of equations derived between total length and total weight for *T. toli* was $W=0.1223TL^{2.2272}$ (r=0.9262), for *T. ilisha* was $W=0.0094TL^{3.001}$ (r=0.9582), for *S. guttatus* was $W=0.138TL^{2.1396}$ (r=0.9161) and for *S. tenuifilis* was $W=0.138TL^{2.1396}$ (r=0.9161). The 'b' values of length-weight relationship of *T. toli*, *S. guttatus* and *S. tenuifilis* were observed to be 2.2272, 2.1396 and 1.1262 indicating negative allometric on species. Otherwise *T. ilisha* was 3 which indicated isometric in nature. Moreover, the mean condition factor of all of fish species was 0.95±0.16, 0.95±0.12, 0.69±0.13 and 1.09±0.43, indicating that its growth has been more or less normal (k<1or k>1).

Keywords: Length-weight relationship, condition factor, some commercial fish species, fish landing center, Mon Coast.

Introduction

The length-weight relationship (LWR) is a very important parameter to understand the growth dynamics of the fish population. Length and weight data are useful standard results of any fish sampling program. LWR of fishes are important in fisheries biology because they allow the estimation of the average weight of fish of a given length group by establishing a mathematical relation between the two parameters. The LWR is particularly important in parameterizing yield equations and in estimations of stock size. The exact relationship between length and weight differs among species of fish according to their inherited body shape, and within a species according to the condition (robustness) of individual fish. The study of morphometric characters in fishes is important because they can be used for the differentiation of taxonomic units. In fisheries science, the condition factor is used in order to compare the "condition", "fatness" or wellbeing of fish. The condition factor usually increases with sexual maturation (Dutta *et al*, 2012).

Length-weight relationship has been studied for many fish species in Myanmar Coastal Waters; these included Zin Zin Zaw (2010) on *Scomberomous guttatus*, Zaw Zaw Aung (2010) on *Pampus argenteus*, Min Ye Lwin Oo (2013) on Chirocentridae, Thazin Aye (2013) on Anchovy fishes, Tint Swe (2007) on some fish of stationary bag-nets fishery, Khaing Myat Myat Htwe (2008) on two species of herring fishes, Ohmar Min (2009) on Sciaenid fishes, Mi Mi Mya Thet (2009) on polynemid fishes, Nyo Nyo Tun (2009) on Sardinella species, Nang Mya Han (2010) on Mugilidae, Khin May Chit Maung (2012) on Leiognathidae, Su Su Hlaing (2012) on Family-Engralidae, Thu Thu Min (2017) on Caragidae, Zin Mar Aye (2019) on Scombrid fishes.

The objectives of this study are 1) to estimate length-weight relationship and 2) to investigate the condition factor of some commercial fish species from Kyaikkhami Fish Landing Centre.

[1]Assistant Lecturer, Department of Marine Science, Mawlamyine University

Materials and Methods

Length-weight relationship of four commercial marine fish species viz. *Tenualosa ilisha* (Nga-tha-lauk)**,** *Tenualosa toli* (Nga-tha-lauk-yout-pha), *Scomberomorus guttatus* (Nga-kon-shat) and *Setipinna tenuifilis* (Nga-byar) were measured. A total of 873 individual four fish species were measured from June 2018 and March 2019 from Kyaikkhami Fish Landing Center (16°05'N and 97°34'E) (Figure 1).

Determination of Length-weight relationship

Data on length frequency distribution of fishes were collected from Kyaikkhami fish landing center was undertaken. The total length (TL) was measured in centimeters from the snout to the end of caudal fin by using measuring board. The total weight was measured in grams (g) by using digital balance.

The length-weight relationship was determined by the methods of least square using the formula, which was followed after Pauly (1984) as.

$$W = a\ L^b$$

Where, W= weight of fish (g)

L= total length (TL) of fish in (cm)

a= constant (intercept)

b= length exponent (slope)

The "a" and "b" values were obtained from a linear regression of the length and weight of fish. The logarithmic form of the equation is given as; Log W = log a + b log L. The coefficient of correlation (r) was calculated by standard statistical formula.

Determination of Fulton's Condition factor (K)

The mean weight and length of the experimental fish were used to estimate condition factor using equation followed by Fulton, 1904 (as cited in Froese, 2006):

$$K = (W \times 100)/L^3$$

Where, W= Weight in grammes (g), L= Total length of fish in centimeters (cm)

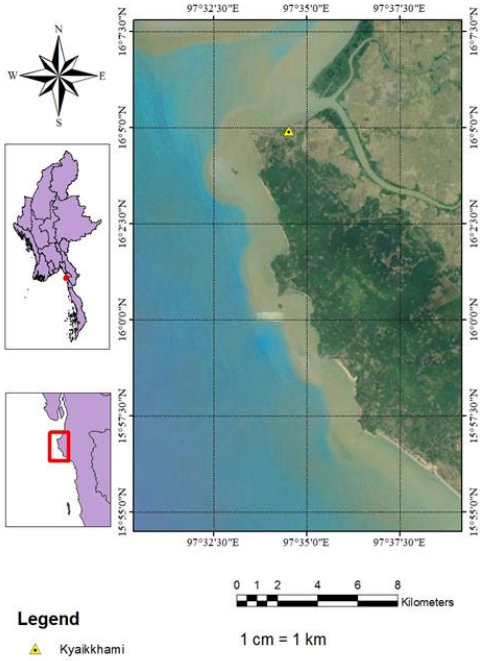

Figure 1 Map showing the fish landing centre during study period

Results and Discussion

Length-weight relationship: The length weight relationships of 873 individual fishes of four different species have been detailed on table 1.

The study of length - weight relationship of fish is of paramount importance in studying the growth, gonadal development and general wellbeing of fish population and for comparing life history of fish from different habitats stressed upon the importance of length weight relationship in modeling aquatic ecosystems (Mushtaq *et al*., 2016). For a fish having an unchanged body form and specific gravity, the value of 'b' is 3 which describe isometric growth. The value of 'b' can also fluctuate between 2.5 and 4 (Hile, 1936) and 2.2 - 4.5 (Carlander, 1969) which describe allometric growth (as cited in Balli, 2005). Allometric coefficient "b" larger or smaller than 3.0 shows an allometric growth, value b>3 shows a positive allometric growth, while value b<3 indicates a negative allometric growth. It is isometric growth when value b is equal to 3.0 (Bangenal and Tesch, 1978) (as cited in Ahmed *et al*, 2014). The value of r > 0.8 regardless of sex and season represent a strong relationship between length and weight and indicate whether the relationship was significant or not, indicated that if r > 0. 9 and weight increases in length, then it is clear that the fish maintains its shape throughout its life (Rahman *et al*., 2004).

A total of 235 individual of *Tenualosa ilisha* (Nga-tha-lauk) were measured during this study period. The length distribution of *T. ilisha* ranging in size from 20.2-49cm and the body weight varied between 92g and 1200g. The 'b' value of *T. ilisha* was 3 while the 'r' value was 0.9582. The length-weight relationship of *T. ilisha* was found to be $W=0.0094*L^{3.001}$. So, *T. ilisha* was observed to be isometric growth in nature in present study area (Figure 2). However, Khine Myat Myat Htwe (2012) reported positive allometric in nature (b=3.027) in Kyaikkhami fish landing centre and negative allometric in nature (b=2.423) in Mawlamyine fish landing centre. Mohamed Abdul-Razak and Qusim Audai (2004) also reported the positive allometric in

Iraqui Marine Waters, northwest Arabian Gulf. From Bangladesh water, the exponential form of equation ($W = 0.00305\ TL^{3.381}$) of *T. ilisha* (*Hilsa Shad),* which indicated that the growth relationship between length and weight of the fish was positive allometric in nature (b=3.381, b >3) (Nurul Amin, 2005). Moreover, positive allometric in Northern Bay of Bengal was reported by Dutta, *et al*, 2012. However, Reuben et al (1997) was established that the negative growth of *T.ilisha* from northeast coast of India (b<3) (as cited in Dutta, et al, 2012). And the relationship between length and weight of *T. ilisha* was significant and the negative allometric was found in Chilika Lake, Odisha was described by Mohanty and Nayak, 2017.

In present study, a total of 200 *Tenualosa toli* (Nga-tha-lauk-yout-pha) of various size groups were measured every month. Based on data measurement, the total length ranged and total weight ranged of *T. toli* was 20.6cm and 49.3cm and 126g and 960g. The LWR of *T. toli* was $W=0.1223*L^{2.2272}$ where the correlation coefficient value (r) was 0.9262. The negative allometric growth of *T. toli* was found so that the 'b' value is less than 3 (b<3) (Figure 3). Similarity, Khin Myat Myat Htwe (2012) reported that the growth of *T. toli* was negative allometric in nature while the length weight relationship was $W=0.012*L^{2.904}$ and the "b" value was less than 3 in Kyaikkhami fish landing centre. And then the LWR of *T. toli* was found as $W=0.012*L^{2.822}$ showing negative allometric growth where the "b" value was 2.822 (Dar Shabir, Thomas Saly, Chakraborty, and Jaiswar, 2014).

Scomberomorus guttatus (Nga-kon-shat), a total of 220 individual of fish species were measured in present study. The ranges of length and weight of were between 24.8cm and 45.9cm and between 115g and 530g. The length-weight relationship of *S. guttatus* was $W=0.1396*L^{2.1396}$, where "r" value was 0.9161. From this relationship, the 'b' value was 2.1396, it can be inferred that their growth is negative allometric in nature (Figure 4). Similarity, Zin Zin Zaw (2010), reported the LWR of *S. guttatus* was $W=0.0203*L^{2.687}$ and the regression coefficient r= 0.9894 so the growth indicated negative allometric in nature (b<3) in Mawlamyine fish landing centre. Moreover, Rashid, Mustafa and Dewan (2010) indicated growth of *S. guttatus* was also negative allometric as the value of allometric coefficient "b" was less than 3. Moreover, the LWR of *S. guttatus* was $W = 0.00001\ L^{2.894}$, where the r^2 value was 0.9915, from this relationship it can be inferred that their growth is negative (b<3) at different fish landing centers of West Bengal (Dutta, *et al*, 2012).

A total of 218 *Setipinna tenuifilis* (Nga-byar) were measured during this study period. The length distribution of *S. tenuifilis* ranging in size from 16.5cm-30cmcm and the body weight varied between 65g and 230g. The growth of *S. tenuifilis* is negative allometric as its 'b' value was found to be 1.1262 and the LWR was $W=3.6842*L^{1.1262}$. The correlation coefficient 'r' was 0.3585 in present study (Figure 5). In Su Hlaing Hlaing (2016), the negative allometric growth of *S. tenuifilis* was observed in Mon Coastal Water beacause the mean 'b' was lower than 3.

Condition factor: Fulton's condition factor (K) represents health condition or well-being of fish (Nash, Valencia and Geffen, 2006). The value of K > 1 indicates the well-being of the fish to be good. The higher values of 'K' in a particular period seem to be the preparation for the reproductive activities (Telvekar, Chakraborty and Janissary, 2006). The mean K values of commercial fish species in current study fluctuated between 0.56 and 0.95 as shown in table 1. From present study period, the condition factor (K) value for *Tenualosa ilisha* ranged from 0.49 to 1.46 and mean value was 0.95 ±0.12, for *Tenualosa toli* ranged from 0.40 to 1.46 and mean value was 0.95 ±0.16, for *Scomberomorus guttatus* ranged from 0.35 to 1.33 and mean

value was 0.69 ±0.13 for *Setipinna tenuifilis* ranged from 0.56 to 2.89 and mean value was 1.09 ±0.43. So, the mean value of K of some commercial fish species was lower than 1 which indicates the well-being of the fish to be poor but *Setipinna tenuifilis* was good health in nature (Table 2 and Figure 6).

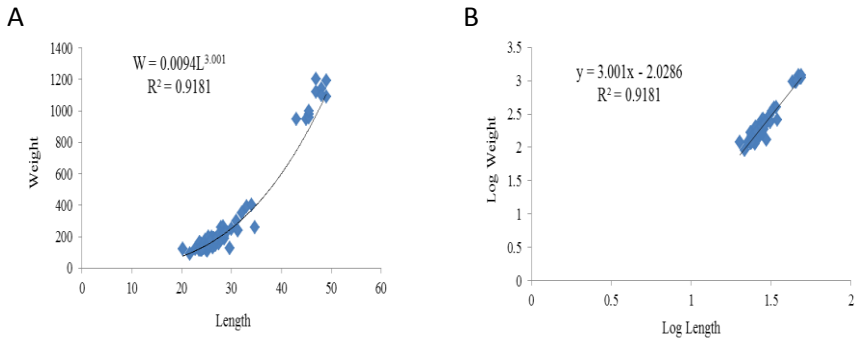

Figure 2 Length-weight relationship of *Tenualosa ilisha*; A) Non Linear relationship and B) Linear relationship

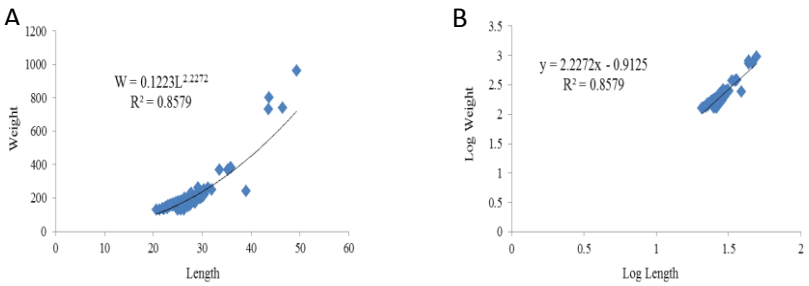

Figure 3 Length-weight relationship of *Tenualosa toli*; A) Non Linear relationship and B) Linear relationship

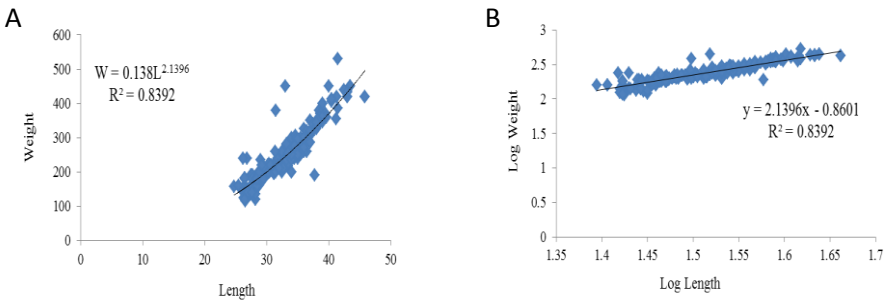

Figure 4 Length-weight relationship of *Scomberomorus guttatus*; A) Non Linear relationship and B) Linear relationship

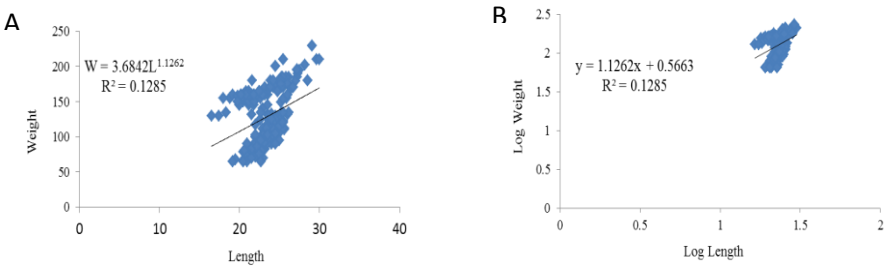

Figure 5 Length-weight relationship of *Setipinna tenuifilis*; A) Non Linear relationship and B) Linear relationship

Table 1 Summary of length-weight relationship parameters of some commercial fish species from Kyaikkhami Fish Landing Center in present study.

Species	N	Characteristics (TL)		Characteristics (TW)		L W R			Exponential Equation
		L_{min}-L_{max}	$\bar{X}\pm SD$	W_{min}-W_{max}	$\bar{X}\pm SD$	a	b	r	
Tenualosa ilisha	235	20.2-49	25.51±3.07	92-1200	210.89±194.66	-2.02	3	0.958	$W= 0.0094*L^{3.001}$
Tenualosa toli	200	20.6-49.3	24.37±1.51	128-960	218.59±131.60	-0.91	2.23	0.926	$W= 0.1223*L^{2.2272}$
Scomberomorus guttatus	220	24.8-45.9	15.01±1.73	115-530	259.79±84.47	-0.86	2.14	0.916	$W= 0.138*L^{2.1396}$
Setipinna tenuifilis	218	16.5-30	30.54±2.93	65-230	132.54±39.02	0.57	1.13	0.358	$W= 3.6842*L^{1.1262}$

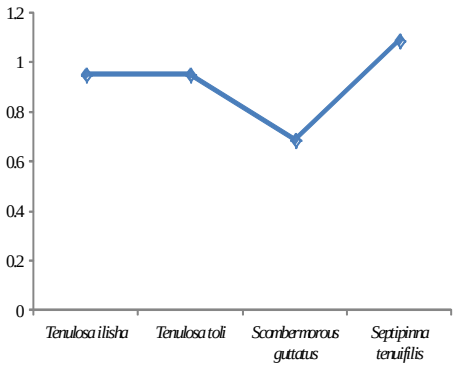

Figure 6 Mean Condition factor (K) of some commercial fish species from Kyaikkhami fish landing centre

Table 2 Mean Condition factor (K) of some commercial fish species from Kyaikkhami fish landing centre in present study.

Mean Condition Factor (K)	***Tenualosa ilisha***	***Tenualosa toli***	***Scomberomorus guttatus***	***Setipinna tenuifilis***
	0.95 ±0.12	0.95 ±0.16	0.69 ±0.13	1.09 ±0.43

Conclusion

The LWR of these commercially important marine fishes helps to manage their stock in the present study area. From the length weight relationship study, it is clear that the growth of *Tenulosa ilisha* was isometric in nature but *Tenulosa toli, Scomberomous guttatus* and *Setipinna tenuifilis* were negative allometric in nature and the length and the weight of fish species are significantly correlated (r<1, nearly 1) except *Setipinna tenuifilis* (r=0.358). Present studies indicated negative allometric and poor growth of the fish. So the coefficient "b" of *T. toli, S.*

guttatus and *S. tenuifilis* became to be less rotund when the length of fish increases. So the mean condition factor value (K) of all fish species was lower than 1 except *Setipinna tenuifilis*, the condition of all species was poor health of fishes in present study. The condition factor of *Setipinna tenuifilis* was good in present study. It is concluded that the result of the study will be useful to the researchers and policy planners in this study area.

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I am indebted to Dr Aung Myat Kyaw Sein, Rector of Mawlamyine University and Dr Mie Mie Sein, Pro-Rector and Dr San San Aye, Pro-Rector, 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. Finally, my infinite thanks are to my beloved family for their kind and financial support throughout this study period.

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STUDY ON THE FISHERY STATUS OF COACH WHIPRAY, *HIMANTURA UARNAK* AT TWO FISH LANDING SITES OF MON STATE

Myo Min Tun[1]

Abstract

The coach whipray *Himantura uarnak* (Forsskal, 1775) was collected from two fish landing sites namely Zeephyuthaung and Ahlayt coastal areas, Mon State during April 2018 to March 2019. Average highest and lowest landings were 85.5 kg and 11.3 kg in the months of July 2019 and in December 2018 at Zeephyuthaung and the highest volume was 102 kg in April and the lowest was 14.3 in June at Ahlayt respectively. During the study period, *Himantura uarnak* contributed only 338.3 kg in April at Ahlayt followed by 307.6 kg in February at Ahlayt and 307.5 kg in April and 307 kg in July at Zeephyuthaung, and the least is 31.5 kg in June at Ahlayt of the year wise total landing volume of sharks and rays respectively. *H. uarnak* was found in throughout of the year in study period. There is no comprehensive data of fish landing in Mon State.

Keywords: Abundance, coach whipray, CPUE, fish landing sites, *Himantura uarnak*, Mon State

Introduction

In Myanmar, the fishery sector is the second most commercially vital sector after the agriculture sector to fulfill the protein requirements of the people of Myanmar and to provide the food security as well as employment opportunity to a large number of fishery communities and rural dwellers. The total fish stocks of Myanmar are about 1.75 million tones of which 1.05 million tones can be harvested annually. Along the Myanmar coastline, there are 139 fishing grounds. Among them, Mon State has 14 fishing grounds.

The ray fishes spend their entire lives in close contact with the bottom but many others spend most of their life in the water column. They are located on top of the head and can be used to draw water into the gill chambers while the fish is lying motionless on the bottom, waiting to ambush prey or avoiding predators. Their scales are typically found only as a few rows of large denticles on the back, which are sometimes modified into spines. The rays are characterized by ventral gill openings, enlarged pectoral fins that attach to the side of the head, lack of an anal fin, eyes and spiracles located on top of the head and pavement-like teeth. Of the global current chondrichthyan fauna (more than 1200 species), at least 315 species recorded in Southeast Asian Region, which including 174 species of sharks from 8 orders (29 families) and 141 rays from 5 orders (19 families). As for rays, Indonesia also recorded the highest number with 101 species and 17 families followed by Malaysia (82 species; 14 families), Philippines (66 species; 18 families), Thailand (55 species; 12 families), Cambodia (54 species; 14 families), Myanmar (46 species; 11 families), Vietnam (39 species; 12 families) and Brunei Darussalam (35 species; 11 families) (Ahmad et al. 2013).

In the present study, an attempt has been made on the study of distribution of the ray, *Himantura uarnak* in two fish landing sites along the Mon coastal areas included Ahlayt, and Zeephyuthaung. The objectives of the present study are to know the monthly catch rates of the ray, *H. uarnak* populations along the coastline of Mon and to investigate the abundance of the ray, *H. uarnak* in Mon coastal waters.

[1] Dr, Assistant Lecturer, Department of Marine Science, Mawlamyine University

Materials and Methods

Fish specimens in this study were collected from Mon coastal waters such as Zeephyuthaung (Lat. 15º 19' N, Long. 97º 78' E), and Ahlayt (Lat. 16º 33' N, Long. 97º 21' E). All specimens were collected from two fish landing sites of Mon coastal areas during April 2018 to March 2019. The map of the study areas were shown in Fig. 1. During the study period, monthly catch rates of this species was also conducted in the fish landing sites. The volumes of total catch, the catch of rays caught by stationary bag net at each sampling site were recorded. Based on each catch data, catch rate or catch per unit of effort (CPUE) was computed by dividing the total weight of this species by the number of fishing days (kg/day). Species composition was estimated from the total weight (kg) of this species sample and expressed in percentage. The catches of rays from inshore and offshore catches of longline and trawl fisheries were provided. In the field, the samples were washed with fresh water and then preserved in 10% formaldehyde solutions. Photographs of the external morphological structure of sharks and rays were taken with a FUJI digital camera. The samples were arranged for analyzing in fresh condition and measured the total length (TL) and disc length (DL) by measuring board in centimeter (cm). The body weight (BW) was measured by the KANEKO balance respectively. To continue other identification, all specimens were carried out to in the laboratory, Department of Marine Science, Mawlamyine University for future study. The classification and identification of collected specimens were adopted from Compagno (1984), Carpenter and Niem (1998) Vol. **2** and **3**, Yano *et al.*(2005), Ahmad *et al.* (2014), Ahmad *et al.* (2017) and Ahmad, Lim and U Saw Han Shein (2015).

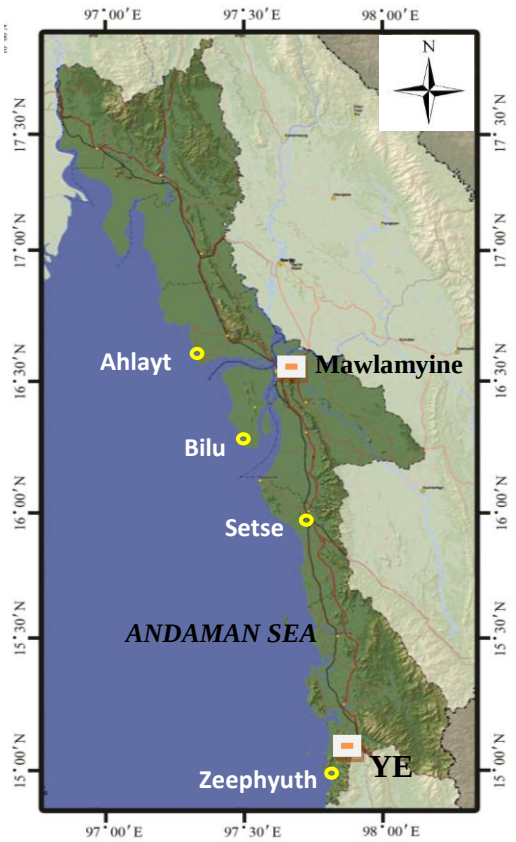

Figure 1 Map showing the sample collection sites of the study areas.

Results and Discussion

Classification of the coach whipray *Himantrura uarnak* from Mon Coastal Waters

Phylum Chordata

Class Chondrichthyes

Order Myliobatiformes

Family Dasyatidae

Genus *Himantura*

Species *Himantura uarnak* (Forsskal, 1775)

Family Dasyatidae: The family Dasyatidae includes stingrays, or whiprays and river stingrays, emcompassing nine genera and about 70 species. Like other rays, they have enlarged pectoral fins that form a disc. In this family, the disc stretches forward to include the head and ranges from less than 30 cm to over 2 m diameter. Stingrays can be found in all tropical and subtropical seas. River rays form a freshwater subfamily Dasyatidae and live only in freshwater in parts of South America and Africa. Most stingrays are benthic, burying themselves partially under sand or mud in relatively shallowwater. Members of Dasyatidae are viviparous and invest a lot of energy in relatively few young over a lifetime. This reproductive strategy renders them potentially vulnerable to human activity.

Description of the coach whipray *Himantrura uarnak* from Mon Coastal Waters

***Himantura uarnak* (Forsskal, 1775)**

(Figure 2 A-B)

Disc rhomboidal and flat; snout broadly triangular, tip distinctly pointed; pectoral fin apex narrowly rounded; anterior margin almost straight. Eyes are rather small and interorbital space broad. Mouth narrow, with 5 papillae on floor; labial furrows weakly developed; lower labial folds and papillae present. Internasal flap skirt-shaped; relatively short and broad, margin with fine fringe; nostrils long, narrow; lower jaw deeply concave near symphysis. Denticles low, flat, heart-shaped in a broad band from interorbit, extending along centre of disc and onto tail density increasing with size; 2 prominent pearl thorns in centre of disc but enlarged thorns absent from midline of tail and dorsal surface of disc largely smooth. The coloration of this species varies substantially with age and locality. Adults generally have a dorsal pattern of numerous closely spaced dark brown spots or reticulations on beige to yellow-brown background, which becomes blackish past the spine with lighter bands on the sides. The underside is pale, without markings.

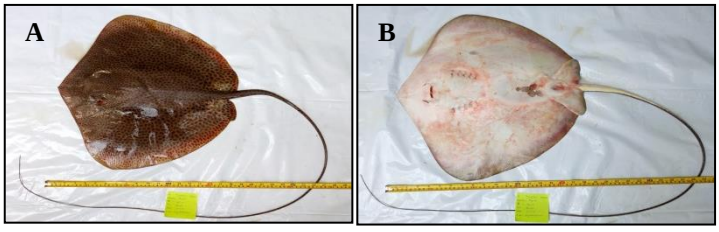

Figure 2 (A- B): The coach whipray *Himantura uarnak*: A) dorsal view and B) ventral view

Habitat and biology: They found in inshore and soft substrates. They found in coral reefs, brackish water and marine. During the day, this species generally in active and spends much time resting motionless on sea floor, sometimes buried in sand. They are viviparous and feed on benthic and neritic organisms, including crabs, shrimps, mantis shrimps, bivalves, gastropods, worms, jellyfish and bony fishes.

Geographical distribution: Malaysia, Myanmar, Indo-Pacific, west to South Africa and the Mediterranean Sea, north to Taiwan.

IUCN Red List Status: Vulnerable (VU) (assessed: 2015)

Abundance of coach whipray *Himantura uarnak* from Mon coastal waters

Table 1 and 2 described the monthly catch weight of ray *Himantura uarnak* in two fish landing sites of Mon State. Total catch (kg), catch of species (kg), catch per unit effort, CPUE (kg/day) and percentage composition of species in total catch (%) were presented in these tables. Catch per unit effort was calculated in terms of catch per days (kg/day).

Monthly catches of *Himantura uarnak* ranged from the minimum of 11.3 kg (December) to the maximum of 85.5 kg (July) in Zeephyuthaung fish landing site with an average catches of 39 kg (Table 1). The catches of this species increased from 43.9 kg in May to 85.5 kg in July. After that the catches decreased to 53.2 kg (August), 18.5 kg (September) and 15 kg (October). The catch rates of September to February are little differed (Figure 3). The range of monthly catches of Ahlayt was from 14.3 kg (June) to 102 kg (April) with an average catches of 29.4 kg (Table 2). The catch weight (102 kg) of April decreased to 14.3 kg in June and then increased to the catch weight of 67.8 kg in September. After that the catch weight decreased to 14.6 kg (November) and then increased to 75.6 kg (February). The catch weights of 102 kg (April), 67.8 kg (September), 75.6 kg (February) and 71.1 kg (March) were the highest catch rate months in Ahlayt as shown in Figure 4.

With regard to monthly catch per unit effort of this species, it ranged from 11.3 kg/day (December) to 85.5 kg/day (July) at Zeephyuthaung and 14.3 kg/day (June) to 102 kg/day (April) at Ahlayt. The average effort was found to be highest at Ahlayt (14.3 kg/day). During the study period, *H. uarnak* contributed only 338.3 kg in April at Ahlayt followed by 307.6 kg in February at Ahlayt and 307.5 kg in April and 307 kg in July at Zeephyuthaung, and the least is 31.5 kg in June at Ahlayt of the year wise total landing volume of sharks and rays respectively.

The percentage composition of *H. uarnak* varied from the minimum of 7.5% (October) to the maximum of 43.4% (August) of the total catch weight an average composition of 22.5% at Zeephyuthaung and from the minimum of 10% (July) to the maximum of 45.4% (June) of the total catch weight an average composition of 25.2% at Ahlayt landing site. *H. uarnak* was found in throughout of the year in study period.

The coach whipray, *Himantura uarnak* fights strongly on hook-and-line and is popular with recreational anglers, who usually release it alive. This species is caught by intensive artisanal and commercial fisheries operating in Southeast Asia and parts of the Indian Ocean, using bottom trawls, gillnets, beach seines and longlines. The meat, skin and cartilage are utilized, though this species is suitable price for human consumption.

Table 1 Monthly catch weight of the ray *Himantura uarnak* of Zeephyuthaung landing site.

Months	Total catch (kg)	Catch of species (kg)	Catch per unit effort (kg/day) of species	Percentage composition of species in total catch (%)
April (2018)	307.5	78.7	15.7	25.6
May	150.5	43.9	8.8	29.3
June	295.6	58.6	11.7	19.8
July	307.0	85.5	17.1	27.9
August	122.5	53.2	10.6	43.4
September	78.3	18.5	3.7	23.6
October	200.0	15.0	3.0	7.5
November	220.8	18.9	3.8	8.6
December	75.0	11.3	2.3	15.0
January (2019)	92.2	16.8	3.4	18.2
February	60.0	14.4	2.9	24.0
March	184.5	52.5	10.5	28.5
Average	174.5	39.0	7.8	22.5

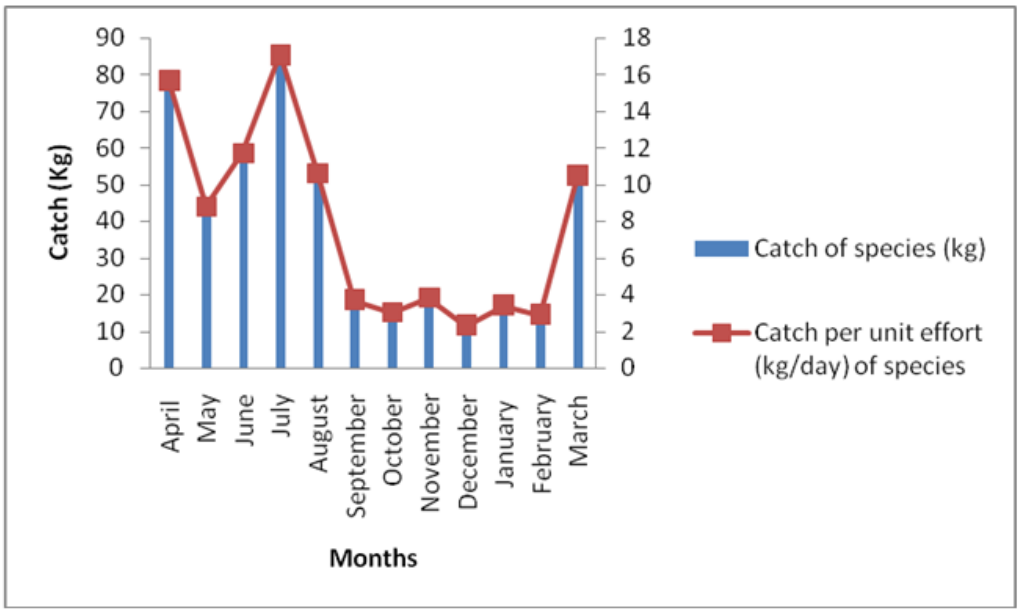

Figure 3 Monthly variations in catch and CPUE of *Himantura uarnak* at Zeephyuthaung fish landing site during April 2018 to March 2019.

Table 2 Monthly catch weight of the ray *Himantura uarnak* species of Ahlayt landing site.

Months	Total catch (kg)	Catch of species (kg)	Catch per unit effort (kg/day) of species	Percentage composition of species in total catch (%)
April (2018)	338.3	102.0	20.4	30.2
May	176.9	59.1	11.8	33.4
June	31.5	14.3	2.9	45.4
July	153.8	15.4	3.1	10.0
August	93.0	23.0	4.6	24.7
September	246.2	67.8	13.6	27.5
October	92.3	23.1	4.6	25.0
November	120.0	14.6	2.9	12.2
December	115.4	22.5	4.5	19.5
January (2019)	152.5	29.4	5.9	19.3
February	307.6	75.6	15.1	24.6
March	228.8	71.1	14.2	31.1
Average	171.3	43.2	8.6	25.2

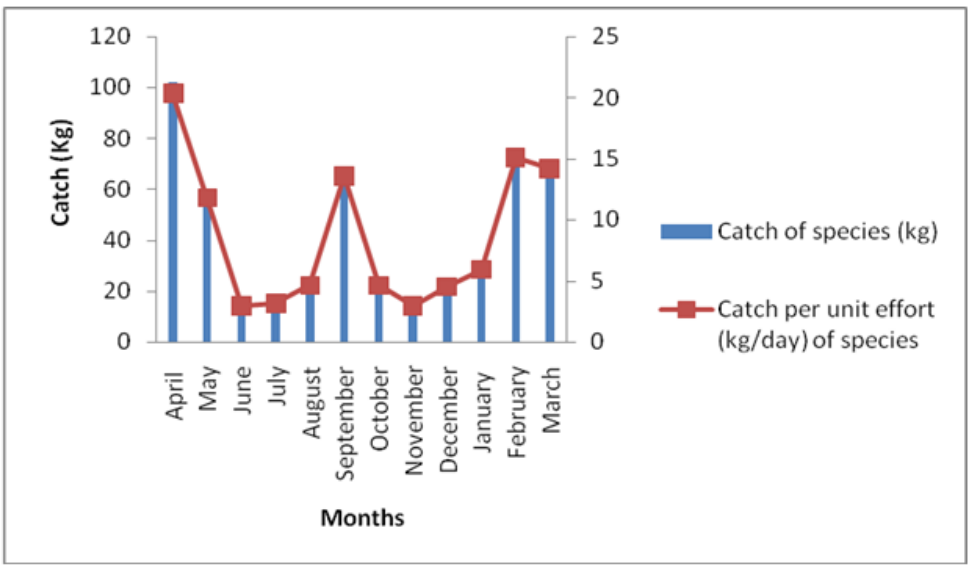

Figure 4 Monthly variations in catch and CPUE of *Himantura uarnak* at Ahlayt fish landing site during April 2018 to March 2019.

Conclusion

Mon State in Myanmar is one of the regions for its inland, inshore and offshore fisheries. The major account responsible for the fisheries is the Thanlwin River and its associated estuarines, river mouth and adjacent sea. A significant dilution of seawater by fresh water takes place forming an estuary. The vast stretch of estuary provides excellent condidions for concentration and development of a variety of fish species and their supportive ecosystems. These have been exploited by small-scale fishermen settling along the coastal fishing villages. Fish is an important part of the diet in Myanmar and the main role of the fishery sector which has been as a provider of food. During the study period, the Ahlayt station was more abundant species than Zeephyuthaung station. All species of rays are utilized for human consumption both fresh and salted. The skins of rays are used to produce leathers for purses and bags. There are strong consumers for marine fishes being mainly preferred in the coastal population and land territory. The marine fisheries sector has gradually developed during the late 1980.

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STUDY ON SOME PHYTOPLANKTON OF MAUNG MA KAN BEACH, LONGLONE TOWNSHIP, TANINTHARYI REGION, SOUTHERN MYANMAR

Yin Yin Htay[1], Moe Lwin Lwin[2], Tin Tin Kyu[2], Aung Aung Aye[2]

Abstract

Results indicated 47 genera consisted of 88 species of phytoplankton in the samples from Maung Ma Kan water at 2018. Among these, 41 genera was represented by 76 species were diatoms of Bacillariophyta and 6 genera with 12 species were dinoflagellates of Dinoflagellata. In Premonsoon, *Bellerochea horologicalis, Chaetoceros pseudocurvisetum, Proboscia alata, Rhizosolenia robusta* and *Syringidium americanum* dominantly occurred. Among these, the most abundant was *Proboscia alata,* 262 cell/L and it was 15 % of the total abundance of phytoplankton. In Monsoon, *Bacteriastrum varians, Bacteriastrum hyalinum, Lauderia annulata, Thalassionema frauenfeldii* and *Thalassionema nitzschioides* were abundantly recorded. Among these, the most maximum species was *Bacteriastrum varians,* 265 cell/L and it was 17 % of the total abundance of phytoplankton. In Postmonsoon, *Aulacodiscus argus, Bellerochea horologicalis, Palaria sulcata, Proboscia alata* and *Thalassionema nitzschioides* were dominant. Among these, the most dominant species was *Bellerochea horologicalis*, 261 cell/L and it was 43 % of the total abundance of phytoplankton. Moreover, the maximum total abundance was recorded as Premonsoon (1720 cell/L) followed by Monsoon (1518 cell/L) however Postmonsoon (604 cell/L) was observed.

Keywords: Abundance, Monsoon, phytoplankton, Postmonsoon and Premonsoon.

Introduction

Maung Ma Kan beach is Myanmar's second most culturally significant beach after Ngapali. Situated only 12 miles along a well-sealed road from Dawei City the beach is very under developed and primarily servicing the local Dawei residents. It is also situated in Southern Myanmar. And then it is located in front of Mocos Island that is situated in the Andaman Sea. Myanmar has tropical monsoon climate, Myanmar coastal area is influenced by strong monsoon regimes.

Phytoplankton always lives near the surface of the sea because, like all plants, they require light for photosynthesis. Phytoplankton is single-celled organisms, primary producers that serve as the base of the marine food chain. Phytoplankton, as the basis of trophic chain, constitutes the most important biological community in any aquatic system. The species composition and population density of phytoplankton are sensitive to environmental changes.

The objectives of this research: 1) to identify the diatoms and dinoflagellate species from Maung Ma Kan water; 2) to know the phytoplankton diversity in Maung Ma Kan water; 3) to recognize the seasonal variations of species composition and distribution; 4) to observe the seasonal abundance of phytoplankton from Maung Ma Kan water; 5) to understand the seasonal dominant species of phytoplankton community in Maung Ma Kan water during the study period.

[1] Dr , Lecturer, Department of Marine Science, Myeik University
[2] Assistant Lecturer, Department of Marine Science, Myeik University

Materials and Methods

The phytoplankton was collected from Maung Ma Kan water, Longlone Township, Taninthayi Region, Southern Myanmar (Lat. 14º 8.5' N and Long. 98º 4.5' E) at 2018. These samples were seasonal collected with a 10 µm mesh size standard plankton net (2 feet long and 8 inches wide) through 10m in length from a boat for 3 minutes to obtain a sufficient amount of sample for making the species identification. Premonsoon is from March to May; Monsoon is from June to October and Postmonsoon is November to February. The sample was preserved in 2 % formalin/sea water mixture and stored in the Department of Marine Science, Myeik University. And then 3 Liters of water sample were taken for the calculation of species abundance. Some environmental parameters were measured.

Results

A total of 88 species included 47 genera of phytoplankton were recorded. Among these, 76 species were diatoms and 12 species were dinoflagellates (Table. 2). In Premonsoon, *Bellerochea horologicalis* (139 cell/L), *Chaetoceros pseudocurvisetum* (183 cell/L), *Proboscia alata* (262 cell/L), *Rhizosolenia robusta* (151 cell/L) and *Syringidium americanum* (130 cell/L) dominantly occurred among 48 species (Table. 3 and Figure. 3). Therefore the most dominant species was *Proboscia alata* (262 cell/L) and 15 % of total abundance of phytoplankton (Figure. 6). The total abundance (1720 cell/L) was recorded during the study period. One dinoflagellate and forty seven diatoms were recorded during this season. The environmental factors, salinity 32-36‰, temperature 27ºC, pH 7.4, transparency 2.8 m, ammonia nitrogen 0.05 ppm, nitrite nitrogen 0.03 ppm and nitrate nitrogen 0.14 ppm were recorded during the study period (Table. 4).

Figure 1 Map showing the location of sample collection site, Maung Ma Kan water, Longlone Township, Tanintharyi Region, Southern Myanmar.

In Monsoon, *Bacteriastrum varians* (265 cell/L), *Bacteriastrum hyalinum* (147 cell/L), *Lauderia annulata* (146 cell/L), *Thalassionema frauenfeldii* (113 cell/L) and *Thalassionema nitzschioides* (95 cell/L) were abundantly recorded (Table. 3 and Figure. 4). Among these, the most maximum species was *Bacteriastrum varians*, 265 cell/L and 17 % of total abundance of phytoplankton (Figure. 7). The total abundance (1518 cell/L) was recorded during the study period (Table. 1). More dinoflagellate species was recorded in Monsoon than other season. The environmental factors of the Monsoon, salinity 20-30‰, temperature 26ºC, pH 7.2, transparency 2.7 m, ammonia nitrogen 0.07 ppm, nitrite nitrogen 0.04 ppm and nitrate nitrogen 0.15 ppm were

recorded during the study period (Table. 4). A total of 52 species, consisting of 43 diatoms and 9 dinoflagellate species occurred.

In Postmonsoon, *Bellerochea horologicalis* (261 cell/L), *Thalassionema nitzschioides* (47 cell/L), *Proboscia alata* (40 cell/L), *Aulacodiscus argus* and *Palaria sulcata* (25 cell/L) were dominant in the study area (Table. 3 and Figure. 5). Among these, the most dominant species was *Bellerochea horologicalis*, 261 cell/L and it was 43 % of the total abundance of phytoplankton (Figure. 8). Moreover, the maximum total abundance (604 cell/L) was recorded during the study period. Some environmental factors such as salinity 30-40‰, temperature 27 ºC, pH 7.5,

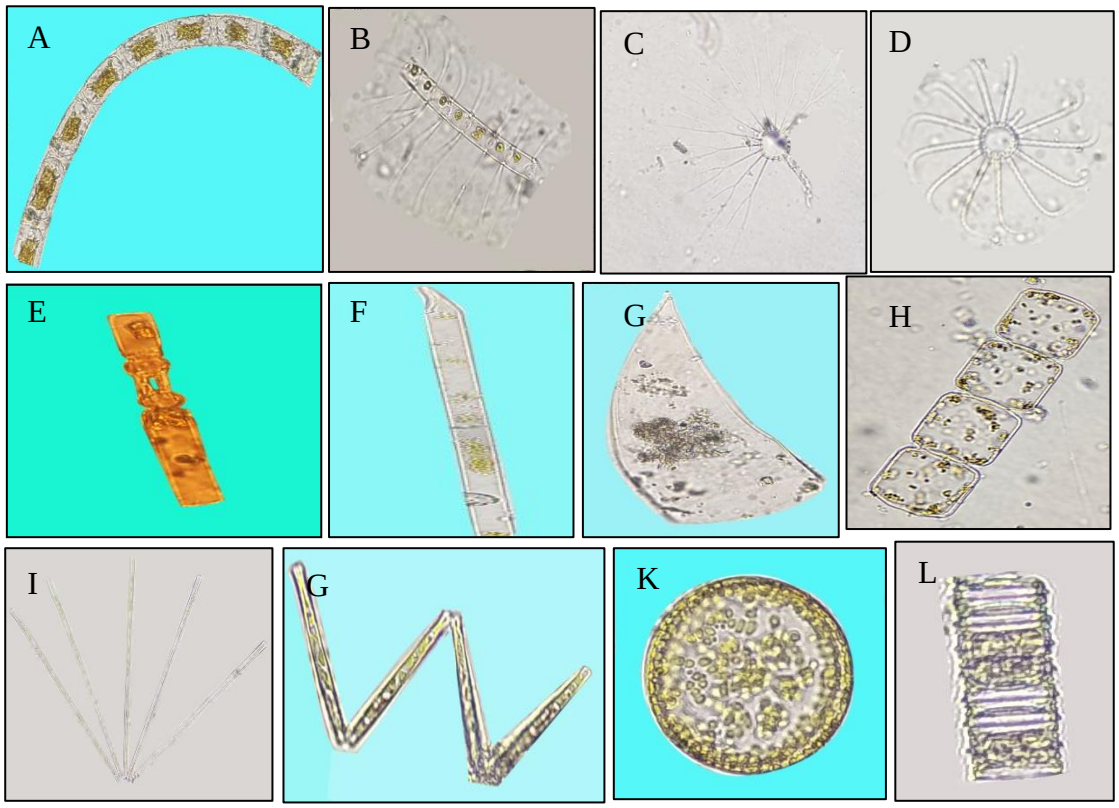

Figure 2 A-L) The dominant species of three seasons during the study period. A) *Bellerochea horologicalis*; B) *Chaetoceros pseudocurvisetum*; (C) *Bacteriastrum hyalinum* Lauder; D) *B. varians* Lauder; E) *Syringidium americanum* Bailey; F) *Proboscia alata* (Brightwell) Sundstrom; G) *Rhizosolenia robusta* Norman ex Pritchard; H) *Lauderia annulata* Cleve; I) *Thalassionema frauenfeldii* (Grumow) Hallegraeff; J) *T. nitzschioides* (Grunow) Mereschkowsky; K) *Aulacodiscus argus* (Ehrenberg) A.Schmidt; L) *Paralia sulcata* (Ehrenberg) Cleve.

transparency 3 m, ammonia nitrogen 0.06 ppm, nitrite nitrogen 0.05 ppm and nitrate nitrogen 0.17 ppm were recorded during the Postmonsoon (Table. 4). A total of 52 species containing 44 diatoms and 8 dinoflagellates were recorded in this season.

Discussion

The phytoplankton community of the study area's water inhabited 88 species consisted of 56 Bacillariophyceae, 20 species of Coscinodiscophyceae, 11 species of Dinophyceae and 1 species of Dictyochophyceae (Table. 2). In the present investigation, the following environmental factors were recorded. Salinity was more in Premonsoon and Postmonsoon than Monsoon. The

temperature changed a little in different three seasons. The most pH (7.5) was recorded in the Postmonsoon. More transparency (3 m) was recorded in Postmonsoon than in Premonsoon and Monsoon. The more ammonia nitrogen 0.07 ppm was recorded in Monsoon than other seasons however the nitrate nitrogen 0.17 ppm and nitrite nitrogen 0.05 ppm were higher in the Postmonsoon than other (Table. 4).

Table 1 Seasonal abundance (cell/L) of phytoplankton at Maung Ma Kan water during 2018

Sr. No	Species	Pre-Monsoon	Monsoon	Post Monsoon
1.	*Aulacoseira epidendron*	1	0	0
2.	*Asterionellopsis glacialis*	1	0	0
3.	*Asteromphalu sheptactis*	0	2	0
4.	*A. cleveanus*	0	2	0
5.	*Aulacodiscus argus*	0	1	25
6.	*Bacteriastrum elongatum*	3	0	0
7.	*B. hyalinum*	0	147	7
8.	*B. varians*	24	265	1
9.	*Bellerochea horologicalis*	139	0	261
10.	*B. reticulata*	0	0	2
11.	*Campylodiscus sp.*	0	0	1
12.	*Chaetoceros peruvianus*	45	6	0
13.	*C. curvisetus*	2	60	2
14.	*C. brevis*	2	3	0
15.	*C. pseudocurvisetum*	183	0	1
16.	*C. lorenzianum*	30	45	0
17.	*C. atlanticus*	1	10	0
18.	*C. subtilis*	0	60	0
19.	*C. pseudocrinitus*	3	0	0
20.	*C. coarctatus*	0	0	1
21.	*C. costatum*	15	0	0
22.	*C. dichaetus*	0	10	0
23.	*C. diversum*	0	33	0
24.	*C. compressus*	0	15	0
25.	*Coscinodiscus radiatus*	21	7	4
26.	*C. granii*	0	6	2
27.	*C. gigas*	0	1	0
28.	*Climacodium frauenfeldianum*	0	2	0
29.	*Ceratiumfusus*	0	0	4
30.	*C. macroceros*	0	1	3
31.	*C. furca*	0	25	2
32.	*Corethron criophilum var. inflatum*	0	13	0
33.	*Cyclotellastriata*	8	5	1
34.	*Cylindrotheca closterium*	0	0	1
35.	*Dinophysis caudata*	0	8	1
36.	*Ditylum sol*	11	85	10
37.	*D. brightwellii*	12	5	0
38.	*Dictyocha fibula*	21	0	0
39.	*Entomoneis alata*	1	1	1
40.	*Eucampia cornuta*	2	0	0

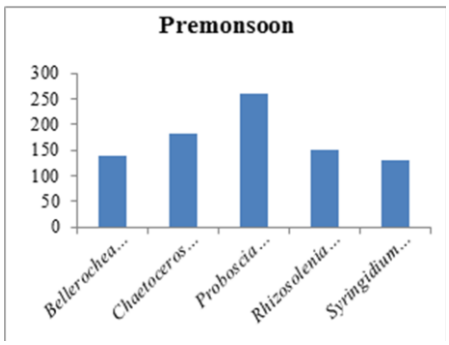

Figure 3 The abundance of dominant species of phytoplankton at Maung Ma Kan water during

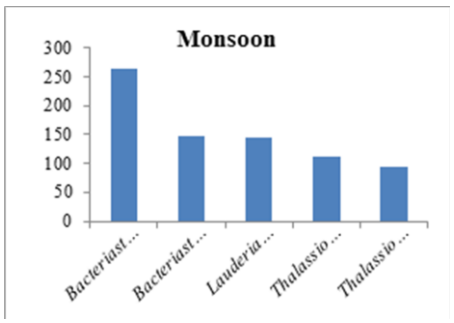

Figure 4 The abundance of dominant species of phytoplankton at Maung Ma Kan water during Monsoon, 2018.

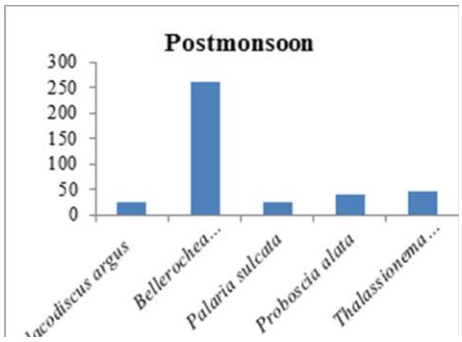

Figure 5 The abundance of dominant species of phytoplankton at Maung Ma Kan water during Postmonsoon,2018.

41.	*E. zodiacus*	0	0	9
42.	*Guinardia flaccida*	14	0	8
43.	*G. striata*	8	65	1
44.	*Gyrosigma balticum var. californicum*	11	0	0
45.	*Helicotheca tamensis*	0	1	7
46.	*Hemiaulus sinensis*	0	36	0
Sr. No	Species	Pre-Mon soon	Mon soon	Post Mon soon
47.	*H. indicus*	1	0	0
48.	*H. hauckii*	0	7	0
49.	*Hemidiscus cuneiformis*	0	12	5
50.	*Lauderia annulata*	47	146	6
51.	*Leptocylindrus danicus*	19	12	0
52.	*Lyrella granulata*	0	0	1
53.	*Meuniera membranacea*	9	0	4
54.	*Minidiscus trioculatus*	0	0	3
55.	*Neoceratium tripos*	0	1	0
56.	*N. breve*	0	0	2
57.	*Nitzschia sigma*	2	0	5
58.	*N. filiformis*	1	0	0
59.	*Odontella mobiliensis*	1	2	13
60.	*O. sinensis*	40	24	23
61.	*O. granulata*	0	0	5
62.	*Palariasulcata*	55	13	25
63.	*Peridinium quinquecorne*	0	1	0
64.	*Protoperidinium oceanicum*	0	37	2
65.	*P. conicum*	0	7	7
66.	*P. depressum*	0	1	0
67.	*Pleurosigma normanii*	40	3	4
68.	*P. elongatum*	5	0	2
69.	*Plantoniella sol*	1	0	0
70.	*Pseudonitzschia lineola*	0	0	2
71.	*P. seriata*	0	6	0
72.	*Prorocentrum micans*	0	4	2
73.	*Proboscia alata*	262	17	40
74.	*P. indica*	11	0	0
75.	*Rhizosolenia calcar. avis*	60	0	8
76.	*R. setigera*	36	16	3
77.	*R. imbricata*	67	10	15
78.	*R. robusta*	151	0	2
79.	*Schroderella delicatula*	70	20	8
80.	*Syringidium americanum*	130	0	0
81.	*Thalassionema frauenfeldii*	13	113	0
82.	*T. nitzschioides*	110	95	47
83.	*xcentricaThalassiosira e*	0	0	1
84.	*T. leptopus*	0	0	9
85.	*T. punctigera*	8	0	1
86.	*T. subtalis*	0	50	0
87.	*Triceratium favus*	12	1	3
88.	*Ulnaria ulna*	11	0	1
	Total	1720	1518	604

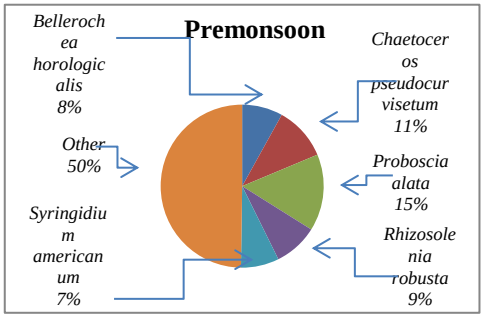

Figure 6 The composition (%) of dominant species at Maung Ma Kan water during Premonsoon, 2018.

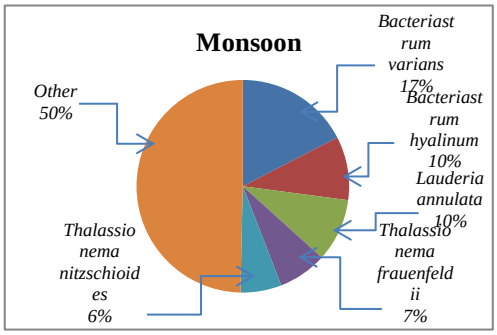

Figure 7 The composition (%) of dominant species at Maung Ma Kan water during Monsoon, 2018.

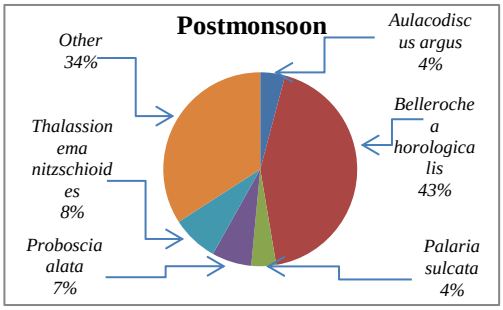

Figure8 The composition (%) of dominant species at Maung Ma Kan water during Postmonsoon, 2018.

Table 2 The presence-absence index of phytoplankton species by three seasons in Maung Ma Kan water during 2018.

Sr. No.	Species	Premonsoon	Monsoon	Postmonsoon
1.	*Aulacoseira epidendron*	+	-	-
2.	*Asterionellopsis glacialis*	+	-	-
3.	*Asteromphalus heptactis*	-	+	-
4.	*A. cleveanus*	-	+	-
5.	*Aulacodiscus argus*	-	+	+
6.	*Bacteriastrum elongatum*	+	-	-
7.	*B. hyalinum*	-	+	+
8.	*B. varians*	+	+	+
9.	*Bellerochea horologicalis*	+	-	+
10.	*B. reticulata*	-	-	+
11.	*Campylodiscus sp.*	-	-	+
12.	*Chaetoceros peruvianus*	+	+	-
13.	*C. curvisetus*	+	+	+
14.	*C. brevis*	+	+	-
15.	*C. pseudocurvisetum*	+	-	+
16.	*C. lorenzianum*	+	+	-
17.	*C. atlanticus*	+	+	-
18.	*C. subtilis*	-	+	-
19.	*C. pseudocrinitus*	+	-	-
20.	*C. coarctatus*	-	-	+
21.	*C. costatum*	+	-	-
22.	*C. dichaetus*	-	+	-
23.	*C.diversum*	-	+	-
24.	*C. compressus*	-	+	-
25.	*Coscinodiscus radiatus*	+	+	+
26.	*C. granii*	-	+	+
27.	*C. gigas*	-	+	-
28.	*Climacodium frauenfeldianum*	-	+	-
29.	*Ceratiumfusus*	-	-	+
30.	*C. macroceros*	-	+	+
31.	*C. furca*	-	+	+
32.	*Corethron criophilum var. inflatum*	-	+	-
33.	*Cyclotella striata*	+	+	+
34.	*Cylindrotheca closterium*	-	-	+
35.	*Dinophysis caudata*	-	+	+
36.	*Ditylum sol*	+	+	+
37.	*D. brightwellii*	+	+	-
38.	*Dictyocha fibula*	+	-	-
39.	*Entomoneis alata*	+	+	+
40.	*Eucampia cornuta*	+	-	-
41.	*E. zodiacus*	-	-	+
42.	*Guinardia flaccida*	+	-	+
43.	*G. striata*	+	+	+
44.	*Gyrosigma balticum var. californicum*	+	-	-
45.	*Helicotheca tamensis*	-	+	+
46.	*Hemiaulus sinensis*	-	+	-
47.	*H. indicus*	+	-	-
48.	*H. hauckii*	-	+	-
49.	*Hemidiscus cuneiformis*	-	+	+
50.	*Lauderia annulata*	+	+	+
51.	*Leptocylindrus danicus*	+	+	-
52.	*Lyrella fogedii*	-	-	+

Table. 2.Continued:

53	*Meuniera membranacea*	+	-	+
53.	*Minidiscus trioculatus*	-	-	+
54.	*Neoceratium tripos*	-	+	-
55.	*N. breve*	-	-	+
56.	*Nitzschia longissima*	+	-	+
57.	*N. angularis*	+	-	-
58.	*Odontella mobiliensis*	+	+	+
59.	*O. sinensis*	+	+	+
60.	*O. granulata*	-	-	+
61.	*Palaria sulcata*	+	+	+
62.	*Peridinium quinquecorne*	-	+	-
63.	*Protoperidinium oceanicum*	-	+	+
64.	*P. conicum*	-	+	+
65.	*P. depressum*	-	+	-
66.	*Pleurosigma normanii*	+	+	+
67.	*P. elongatum*	+	-	+
68.	*Plantoniella sol*	+	-	-
69.	*Pseudonitzschia lineola*	-	-	+
70.	*P. seriata*	-	+	-
71.	*Prorocentrum micans*	-	+	+
72.	*Probosciaalata*	+	+	+
73.	*P. indica*	+	-	-
74.	*Rhizosolenia calcar. avis*	+	-	+
75.	*R. setigera*	+	+	+
76.	*R. imbricata*	+	+	+
77.	*R. robusta*	+	-	+
78.	*Schroderella delicatula*	+	+	+
79.	*Syringidium americanum*	+	-	-
80.	*Thalassionema frauenfeldii*	+	+	-
81.	*T. nitzschioides*	+	+	+
82.	*Thalassiosira excentrica*	-	-	+
83.	*T. leptopus*	-	-	+
84.	*T. punctigera*	+	-	+
85.	*T. subtalis*	-	+	-
86.	*Triceratium favus*	+	+	+
87.	*Ulnaria ulna*	+	-	+
	No. of species	48	52	52

In the present study, the most abundance of phytoplankton 1720 cell/L was recorded in Premonsoon however the lowest one 604 cell/L was observed in Postmonsoon (Table. 1). Forty eight species of phytoplankton distributed in the Premonsoon however 52 species was recorded in the Monsoon and Postmonsoon (Table. 2). Boonyapiwat (1997) described that the greatest phytoplankton bloom occurred by the highest cell density of *Skeletonema costatum* in the Postmonsoon season near the end of Peninsular Malaysia. However *Bellerochea horologicalis* dominantly occurred during the study period.

Table 3 Abundance of dominant phytoplankton species surveyed from Maung Ma Kan water during the study period.

	Premonsoon		**Monsoon**		**Postmonsoon**	
Sr. No.	**Species**	**cell/L**	**Species**	**cell/L**	**Species**	**cell/L**
1	*Proboscia alata*	262	*Bacteriastrum varians*	265	*Bellerochea horologicalis*	261
2	*Chaetoceros pseudocurvisetum*	183	*Bacteriastrum hyalinum*	147	*Thalassionema nitzschioides*	47
3	*Rhizosolenia robusta*	151	*Lauderia annulata*	146	*Proboscia alata*	40
4	*Bellerochea horologicalis*	139	*Thalassionema frauenfeldii*	113	*Palaria sulcata*	25
5	*Syringidium americanum*	130	*Thalassionema nitzschioides*	95	*Aulacodiscus argus*	25

Table 4 The environmental parameters from Maung Ma Kan water during the study period.

Sr. No	**Parameters**	**Premonsoon**	**Monsoon**	**Postmonsoon**
1.	Salinity (‰)	32-36	20-30	30-40
2.	Temperature (ºC)	27	26	27
3.	pH	7.4	7.2	7.5
4.	Transparency (m)	2.8	2.7	3
5.	Ammonia nitrogen (ppm)	0.05	0.07	0.06
6.	Nitrite nitrogen (ppm)	0.03	0.04	0.05
7.	Nitrate nitrogen (ppm)	0.14	0.15	0.17

Rao and Al-Yamani (1998) described 18 species known to be harmful were present from the waters between Shatt Al-Arab and Straits of Hormuz, Arabian Gulf. However a few harmful species occurred in the present study. Ariyadej *et al*. (2004) described *Cyclotella meneghiniana* Kutzing and *Melosira varians* Agardh were indicators from the Banglang Reservoir, Yala Province. However *Cyclotella striata* normally occurred in the present study.

Boonyapiwat *et al*. (2007) recorded that *Proboscia alata* was dominant species in the Bay of Bengal. This result was near similar to the present study. Saravanakumar *et al*. (2008) reported the high densities of phytoplankton in the creek waters of western mangrove of Kachchh-Gujarat during monsoon and early winter season. This result was the same as the present observation. Si Thu Hein (2010) described that *Ceratium sp.* was dominantly found in Pahtaw water and *Ceratium furca* was dominantly observed in the present study.

Khin Yu Nwe (2011) reported *Thalassionema frauenfeldii, T. nitzschioides, Coscinodiscus sp.* and *Chaetoceros sp*. were dominant species in Myeik adjacent waters. These results were the same as the present study. Ogbuagu and Ayoade (2012) observed that seasonal peaking in abundance of phytoplankton could be attributed to periods of concentrations of nutrients from freshwater in Etche, Nigeria. This research was similar to the present study. Su Myat (2013) described that *Lauderia annulata, Bellerochea horologicalis* and *Thalassionema* spp. were commonly detected in Southern Myanmar. These results were similar to the present study.

Kocer and Sen **(**2014**)** *Melosira* sp. and *Meridion circulare* were the dominant taxa of the surface phytoplankton from Lake Hazar, Turkey in March and June, respectively. However *Bellerochea horologicalis, C. pseudocurvisetum, Proboscia alata, Rhizosolenia robusta* and *Syringidium americanum* were dominant in the present study during this period.

The dominant species was variable accordance to the season. In Premonsoon, *Bellerochea horologicalis, Chaetoceros pseudocurvisetum, Proboscia alata, Rhizosolenia robusta* and *Syringidium americanum* were dominantly occurred however in Monsoon, *Bacteriastrum varians, Bacteriastrum hyalinum, Lauderia annulata, Thalassionema frauenfeldii* and *Thalassionema nitzschioides* were abundantly recorded. In Postmonsoon, *Bellerochea horologicalis, Thalassionema nitzschioides, Proboscia alata, Aulacodiscus argus* and *Palaria sulcata* were dominant in the study area (Table. 3) (Figure. 2). Pandiyarajan *et al*. (2014) described that Bacillariophyceae was dominant than other from the inshore waters of Nizampatnam, South East coast of Indian. This result was the same to the present study.

Yin Yin Htay (2014) observed the most abundant species were *Chaetoceros curvisetus, Coscinodiscus radiatus, C. granii, Ditylum sol, Lauderia annulata, Melosira nummuloides, Odontella sinensis, Pleurosigma normanii, Proboscia alata, Pseudonitzschia seriata, Rhizosolenia setigera, Thalassiosira eccentrica, Thalassionema frauenfeldii* and *T. nitzschioides* in Myeik Archipelago. These results were a little similar to the present study however *Bellerochea horologicalis, Bacteriastrum varians* and *Rhizosolenia robusta* were dominantly observed in the present study.

Yin Yin Htay (2014) reported *Thalassionema nitzschioides, T. frauenfeldii* and one dinoflagellate species *Ceratium furca* were dominantly observed in Thal Chaung, Eastern part of Domel Island, Myeik Archipelago. This result was the same as the present study. Yin Yin Htay (2014) described that *Thalassionema nitzschioides, Chaetoceros curvisetus, Lauderia annulata, Bacteriastrum varians, Ditylum sol,* and one dinoflagellate species *Ceratium furca* were dominantly found in Tasaki Shin Kyun, Southern part of Domel Island, Myeik Archipelago. This observation was quite similar to the present study.

Zarni Ko Ko (2014) described *Lauderia annulata, Chaetoceros curvisetus* and *Thalassionema nitzschioides* were dominant species in Elphinstone Island waters, Myeik Archipelago. These results were similar to the present study. Yin Yin Htay (2016) described *Chaetoceros curvisetus* Cleve, *C. denticulatum* Lauder, *C. peruvianum* Brightwell, *C. lauderi* Ralfs and *C. lorenzianum* Grunow were more abundant in Myeik coastal waters. However *Chaetoceros pseudocurvisetum* was more abundant than other species during the present study. Yin Yin Htay (2018) described the phytoplankton abundance was the negative correlation with nitrate, in the Myeik coastal waters. This result was nearly similar to the present study.

Conclusions

The variation of phytoplankton distribution, abundance, the dominant species and species composition were mostly influenced by the seasonal changes in environmental parameters (salinity, temperature, pH, transparency, ammonia nitrogen, nitrite nitrogen, nitrate nitrogen). The more abundance of phytoplankton 1720 cell/L was recorded at the Premonsoon season during the low nutrient (ammonia nitrogen 0.05 ppm; nitrite nitrogen 0.03 ppm and nitrate nitrogen 0.14 ppm) than Monsoon and Postmonsoon seasons. Maung Ma Kan water is high productivity.

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