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FOREWORD

On 26 February 2026, the Myanmar Academy of Arts and Science (MAAS) was reconstituted to accelerate the implementation of four different strategies:

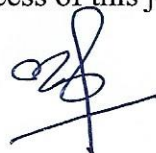
- (a) encouraging universities and colleges to engage in more research activities
- (b) organizing domestic and international research conferences
- (c) publishing research journals and proceedings, and
- (d) fostering research collaboration among local and international universities

Since the year 2001, the MAAS has been making every effort to fulfil these important functions in collaboration with universities and colleges across the country. As a result, the MAAS has published research papers in 23 volumes on diverse academic disciplines in the Journal of the Myanmar Academy of Arts and Science. It is a great honor to say that the 24th research conference was successfully held at the University of Yangon on 9–11 March 2024. The theme of this conference is '*Research and Innovation for Peace, Prosperity and Sustainability*'. A total of 369 research papers presented by both faculty and postgraduates at the conference have been published in Volume XXIII as follows:

Vol. XXIII, No. 1A	Chemistry
Vol. XXIII, No. 1B	Chemistry, Industrial Chemistry
Vol. XXIII, No. 2	Physics, Mathematics and Computer Studies
Vol. XXIII, No. 3	Zoology, Marine Science, Environment and Water Studies
Vol. XXIII, No. 4	Botany, Geology
Vol. XXIII, No. 5	English, History, Philosophy, Geography, Psychology, International Relations, Oriental Studies
Vol. XXIII, No. 6	Myanmar
Vol. XXIII, No. 7	Law, Library and Information Studies, Archaeology, Anthropology, Language and Linguistics
Vol. XXIII, No. 8	Applied Statistics, Management Studies, Statistics, Commerce, Economics, Journalism and Tourism
Vol. XXIII, No. 9A	Educational Theory and Management, Curriculum and Methodology
Vol. XXIII, No. 9B	Educational Psychology

I firmly believe that the empirical research findings from these papers will provide practical assistance for research candidates and those who are interested in research work. I am also hopeful that these findings will significantly contribute to the well-being of the community at large as well as the State's socio-economic development.

On behalf of the members of the MAAS, I would like to offer my special gratitude and thanks to those who have contributed their research papers to this journal. I also do appreciate the editing work done by senior professors and scholars of high standing as well as the members of the MAAS Editorial Board. Finally, my heartfelt thanks go to the members of the Publication Committee and the Department of Higher Education for their great dedication to the success of this journal.



Dr. Soe Win
President

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Journal of the Myanmar Academy of Arts and Science

Vol. XXIII. No.4

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EVOLUTIONARY TRENDS OF SOME WILD CEREAL FROM IN -DAW -GYI LAKE, MOHNYIN TOWNSHIP, KACHIN STATE*

Lwin Mar Saing¹

Abstract

All grasses belong to the angiosperm's family Poaceae under order Poales. There are more than 13000 species of grasses on the planet, and grass dominated ecosystems. In this research, 16 species of wild cereal grasses collected from In - daw- gyi Lake, Mohnyin Township, Kachin state. The survey area is the largest lake in South East Asia and richness of primitive plant diversity region. The collected species were classified and identified by Halfliger and Scholz's classification (1981) was based upon the detail morphological characters. This research presents the evolutionary affinities of wild cereal grasses that based on grass 's detail taxonomic morphological characters in principles of author' citations and highlights the primitive simplicity to advanced modified complexity of wild cereal grasses changed by their environmental adaptation status in survey region. This result found that the integrations status of primitive and advanced modified characters in individual species of grass's phylogeny. The aim and objectives of this research are supporting the better understanding of wild cereal grass's taxonomy and evolutionary trends in survey area for professional specialists, scientists, all academicians and researchers in family Poaceae.

Keywords: Grass's morphology, taxonomy, evolutionary trends

Introduction

Family Poaceae (Gramineae) is generally found in tropical areas to temperate and sufficient rainfall to form grassland. It is important role as a barrier to erosion of soil stabilization. Grass's taxonomy and systematic hierarchy is critical key for affinities among grasses diversity between their relationship evolutionary processes. The evidence from grass's microfossils with their reproductive organs and roots traced with dentition of fossil vertebrate indicated the grasses had stated to form a significance of global vegetation (Phillips.,1995).

Archaeological evidence suggests that grasses in the form of wild cereals were some of the earliest crop to be cultivated and it's estimated that at present they provide up to 52% of the carbohydrates in human diets worldwide (Stebbins,1956). The English word grass probably comes from the Old High German word "gras", generally used to describe any herbage suitable for livestock grazing. All global economic cereal derived from wild cereal grasses according to the adaptation with evolutionary status of environment and human civilization. The habit of grasses is usually herbaceous, reed liked or arborescent culms(stem) in monocotyledons flowering plants. Grass's morphological characters based on units of spikelet's (reproductive organs), aerial and underground parts (vegetative organs) with wind - pollinated. They rank among the top five families of flowering plants and most abundant and in terms of number of species important family of the Earth's flora. In this research, 16 species; 16 genera; 8 tribes in 5 subfamilies of wild cereal grasses from In - daw - gyi Lake, Mohnyin Township, Kachin state were collected during flowering periods. The collected species' identification, classification and evolutionary trends based on classification of (Hafliger and Scholz 1981) and many principles of author' citations. The finding outcomes of results are contributing the science knowledge of difficult and unique modified reproductive and vegetative characters of

* First Prize (2024)

¹ Department of Botany, Mohnyin University

grasses with their evolutionary trends of various environmental adaptation in survey area. According to Hundley and Chit Ko Ko (1987), 129 genera and 428 species of grasses reported 134 genera and 577 species of grasses were recorded in Myanmar. The future outcome of this research informs the science record of taxonomy and evolutionary unique characters of grasses for all academician and researchers.

Materials and Methods

Collection Procedure, Classification and Identification

Specimens were collected from In - daw - gyi Lake, Mohnyin Township, Kachin State during from November, 2023 to August, 2024 within the flowering periods. The study area lies between 24° 29' 09" and 25° 25' 35" North latitude and between 95° 48' 22" and 96° 48' 11" East longitude and the elevation nearly about 190 to 289 meters (about 948.16 fts). The collected specimens were pressed, dried and prepared for herbarium specimens. The herbarium technique is provided by short Guide to Preparing an Herbarium Specimens by (Nyo MOUNG, 2003, Plant Collection Techniques and Herbarium Management). Map of collection areas was shown in figures. The classification was used according to Halfliger and Scholz's Classification (1981). The identification and classification collected specimens were made by the using of the vegetative and reproductive characters. The identification was done by using of keys given by Hutchinson (1934); Rhind, (1945); Stebbins, 1956; Bor, (1960); Backer, (1965); Dissanayake, et al (1982). Halfliger, and Scholz (1981); Jacob, (1945); Nasir, and Ali, (1982); Hundley and Chit Ko Ko, (1987); Willis, and Macelusair, (2002); Simpson, (2006); and Byng *et al.* (APG IV 2016).

The Phylogenetic Position between the 16 Genera of Family Poaceae

The Phylogeny of family Poaceae is accomplished by principles of grass author' citations. In the present study, 13 different characters were chosen which show differences between the genera and based on the morphological specialization of evolutionary trends to establish an evolutionary diagram of genera within the general level of family Poaceae. The nearly related genera are collected in irregular figures. Which represent the relative position of the groups. The distance from a central point is the conception of the distance they have progressed from their hypothetical ancestor for each character studies the value 0, 0.5 and 1.0 has been assigned to the specialized advance conditions respectively. Small letters represent the value of 0.5 for and capital letters represent the value of 1.0 for each character. The open cycle (○) and close cycle (●) indicate the continuous divergence values and the end point.



Figure 1. Collection procedure in study area

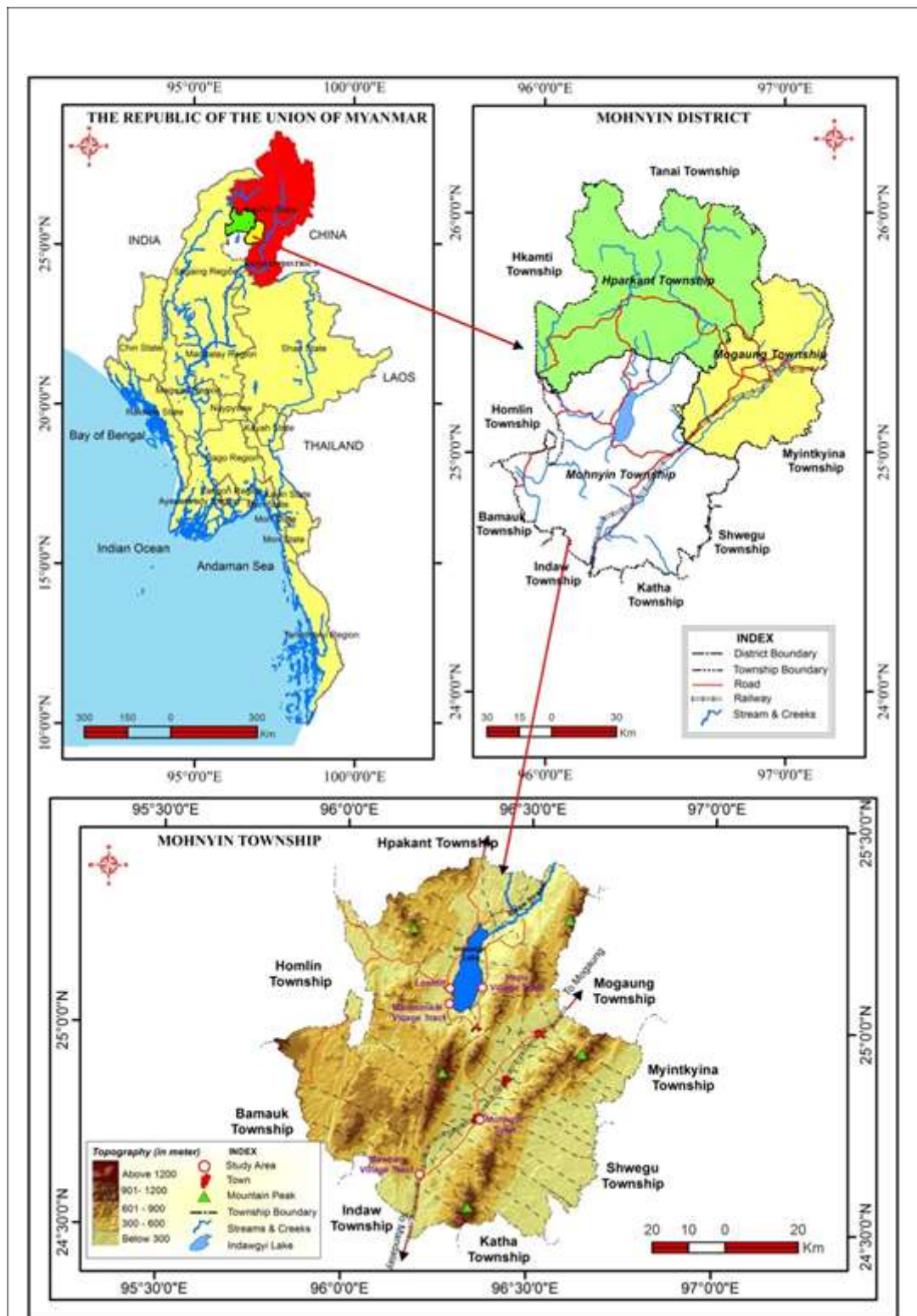


Figure 2 Location map of the study area

Source: Department of Geology, Mohnyin University, Kachin State

Results

Basic unit and key characters for identification, classification and evolutionary structure of grass's spikelet

Grass's morphological characters based on units of spikelet (reproductive organs), with modified aerial and underground parts (vegetative organs). The various features of grasses morphological units are unique and critical important for grasses identification, classification and evolution trends. spikelet is the unit of inflorescences. It can be differentiated into 1 to 2 flowered or 1 to many flowered spikelet's with articulation(joint). The spikelet may be terete, flattened, gibbus and rounded shape. Spikelet comprises glumes. The basally outer 2 glumes are lower empty glume and upper empty glumes. The flowering glumes are outer lemma and inner palea arranged above of empty glumes and this joint portion of empty glumes and flowering glumes called rachilla. All vegetative and reproductive glumes characters may be various modified characters etc. perfect, texture, silky hairs, bristles and awns.

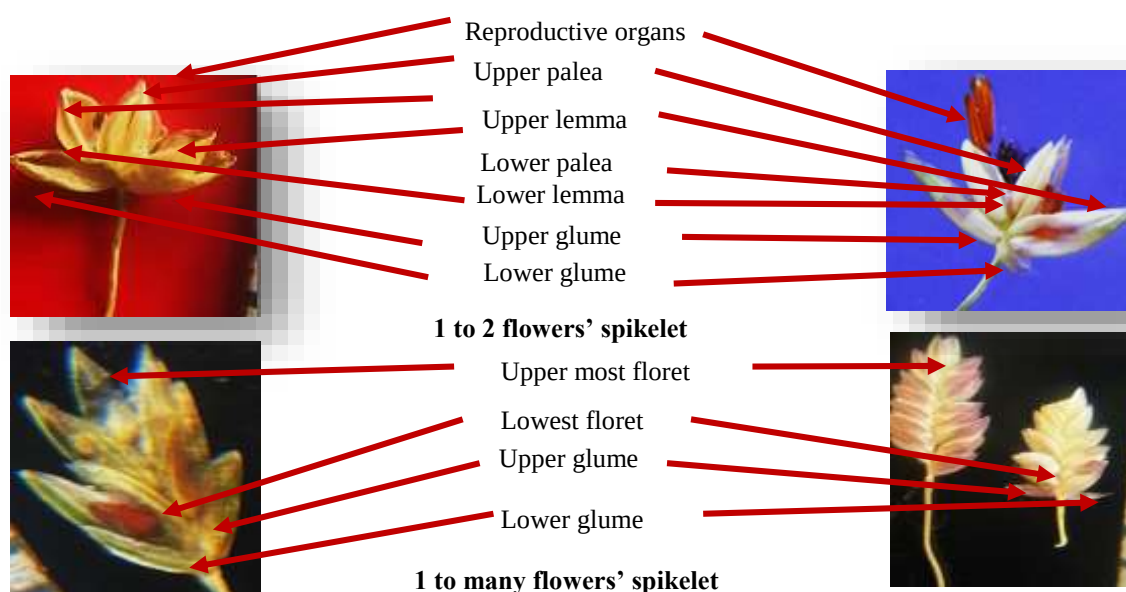


Figure 3 Structure of spikelet

I. Subfamily I. Bambusoideae Luer. (Tribe. Bambusee Nees) (1893).

1. *Cephalostachyum pergracile* Munro.(Myanmar name - Tin - wa)

Perennial, culms up to 120 - meter high, scandent. 4 -10 branching system. The dropping culm possess strongly underground sympodial rhizomes systems, the persistent and strongly thick texture of culm sheath and culm blade with falcate shape auricle which curved deeply folded dense curved hairs. The outer surface of culm sheath and blade with densely scabrous persistent hairs, culm blade reflexed and arranged many equally horizontal branchlets.

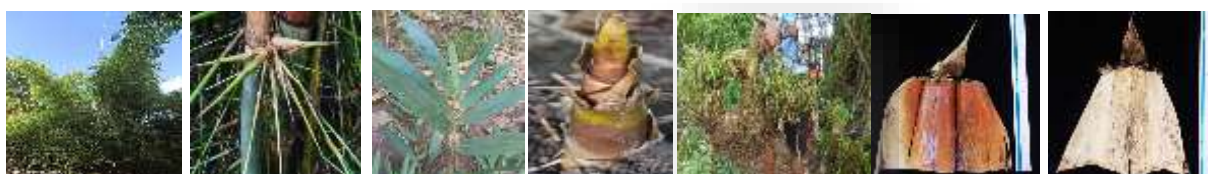


Figure 4 Habit, Branching, Leaves, Young shoot, Rhizome, (Dorsal and ventral Culm blade, sheath)

2. *Chimonobambusa communis* (Hsueh & T.P.Yi) T.H.Wen & Ohnrb.(1999).

(Myanmar name - Thike - wa)

Perennials, tufted, shrubby to arborescent, sympodial rhizomes, distinctly rooting system. Culm up to 60 - meter or more with underground sympodial rhizome. The culm sheath and blade deciduous and thin texture. 1 - 3 nearly equally branching systems. The deciduous culm sheath and blade with caducous softly hairs. The falcate auricle with softly ciliate and usually persistent on culm blade.



Figure 5 Habit, Branching, Leaves, Rhizome, Young shoot, (Dorsal and Ventral view) Culm blade and sheath

II. Subfamily Oryzoideae Kunth ex Beilschm (Tribe. Oryzeae. Dumort.(1824).

3. *Oryza rufipogon* Griffith, Not.Pl. Asiat.3:5.1851. (Myanmar name-Nat-sa-ba)

Annuals, erect, aquatic, tufted, rooting to stoloniferous rhizome. Culm 60 - meter high. It possesses distinct empty glumes. Flowering glume thick with crustaceous texture and six stamens with 1 fertile floret. The ligule very long and up to 10.0 -15.0 cm. Awn of lemma up to 30 – 45 cm long. Lemma and palea with pectinate scabrous hairs.



Figure 6 Habit, Leaves, Inflorescences, Versatile anthers, Spikelet, Awned floret, Ligule, Rhizomes

4. *Leersia hexandra* SW., Prod.Veg.Ind.Occ.21.1788. (Myanmar name -Thaman – myet)

Perennials, aquatic, erect, strongly rooting to stoloniferous rhizome, creeping rhizomes. Culm 20.0- 35.0 meter high, slender. Inflorescence raceme; spikelet without empty glume and 1 fertile floret perfect with pectinate lemma and palea. Fertile stamens six.



Figure 7 Habit t, Leaves, Inflorescences, Ligule, Spikelet, Spike, Floret, Rhizomes

III.Subfamily Pooideae Benth. (Tribe. Arundinellae Stapf in Benth.)

5. *Arundinella nepalensis* Trin. (Myanmar name - Yaman – myet)

Perennial or annual, culm up to 50 - meter high and strongly scaly rhizomatous system. Inflorescences simple raceme liked primary branches with modified raceme to advanced open panicle and densely silky hair spikelet. Spikelet possess 1 - 2 florets, lower floret staminate and upper floret bisexual, densely silkily hair, long awned with upper lemma; awn 1.5 - 3.5 mm long, awned scabrous and based on hairy. The lowest floret bisexual and uppermost floret reduced sterile structure.

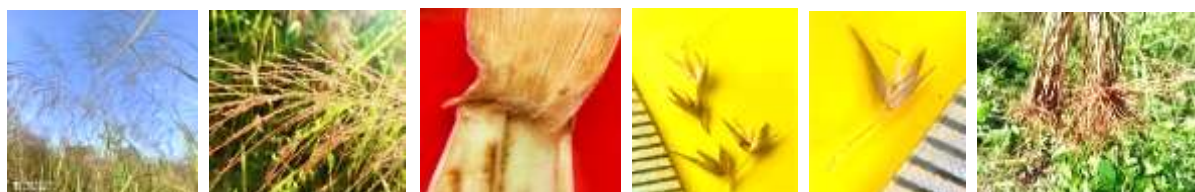


Figure 8 Habit, Leaves, Inflorescences with loosely panicles, Ligule, Spikelet, Floret, scaly

6. Subfamily Pooideae Benth. (Tribe. Arundineae Dumort.)

Phragmites karka (Retz.) Steud. (Myanmar name - Kaing)

Annuals or perennials, erect, strongly rhizomatous, reed liked arborescent, tufted. Culm 160 - meter high. The inflorescence dense open panicle, very densely persistent silkily hairs until at flower maturity. Spikelet pedicellate and sub-sessile; florets 5 - 6, the lowest floret sterile; the second to the fourth florets are neuter. The fifth lowest floret bisexual.

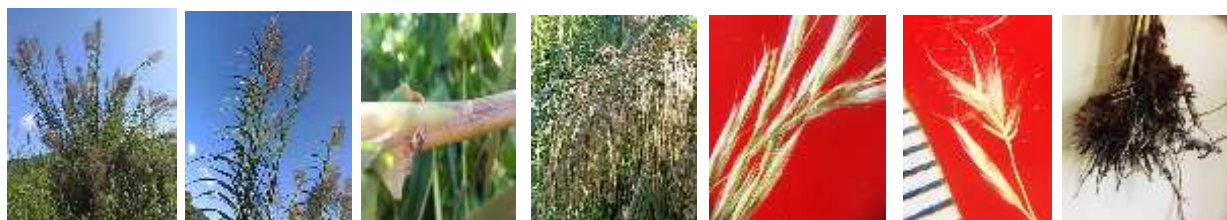


Figure 9 Habit, Leaves-Leaf sheath and blade, Inflorescences, Ligule, Spikelet, Floret, Rhizomes

IV. Subfamily Chloridoideae Duvall *et al.*, 1994. (Tribe. Eragrosteae Stapf.)

7. *Dactyloctenium aegyptium* (L.) Willd. (Myanmar name - Myet - lay - gwa)

Annuals or perennials, tufted, rhizomatous. Culm up to 1.5- meter high. Inflorescences is 4 - fork digitately arranged spike with 3 - 4 florets. the lowest bisexual perfect floret and the middle and upper most florets are staminate and middle nerve of lemmas with modified awned



Figure 10 Habit, Leaf, Tubercle based hairs leaf blade, Inflorescences, Ligule, Spikelet, Rhizome

8. *Eleusine indica* (L.) Gaertn. Fruct. Sem. Pl. 1: 8. 1788. (Myanmar name - Sinnago - myet)

Perennial, erect, tufted, flattened. Culm up to 1.0 - meter high and very strongly rooting system. Inflorescences 5 - 6 digitately arranged racemes. Spikelet possess 4 -5 florets with the lowest floret bisexual and the upper most florets reduced.



Figure 11 Habit, Leaves, Inflorescences, Ligule, Spikelet, Floret, Roots

9. *Eragrostis atrovirens* (Desf.) Trin. ex Steud., Nom. Bot. ed. 2.1: 562. 1840. (Myanmar name-unknown)

Annual, erect, tufted, rhizomatous. Culm up to 1.5- meter high and possess racemose arranged panicle. Spikelet comprises 14 - 34 advanced fertile florets number and all of florets fertile structures which fall down above the empty glumes at maturity.



Figure 12 Habit, Leaves, Ligule, Inflorescences with open panicles, Spikelet, Floret, Roots

Subfamily Chloridoideae Duvall *et al.*, 1994 (Tribe. Cynodoneae Dumort.)

10. *Cynodon dactylon* (L.) Pers., Syn.Pl.1:85.1805. (Myanmar name - Myesa - myet)

Perennials, tufted, rhizomatous to stoloniferous. Culm up to 1.0 - meter high, inflorescences 3 - 4 digitately arranged raceme and flattened spikelet with only 1 fertile floret.



Figure 13 Habit, Leaves, Inflorescences with digitate raceme, Ligule, Spikelet, Floret, Rhizomes

V. Subfamily Panicoideae Bouchena; Khelladi *et al.* (2008). (Tribe. Andropogoneae Dumort.)

11. *Themeda villosa* (Poir.) A. Camus (Myanmar name - Thekke - min)

Annuals or perennials, erect, strongly rhizomatous, reed liked arborescent, tufted. Culm up to 125.0 - meter high with basal portion flattened. Inflorescence provide branching racemes embraced by sheathing spatheoles, single or little branches on flexuous peduncles, gathered into a leafy false panicle; raceme compressing 2 homogamous pairs of spikelets forming a sort involucre bract, 1 - 4 sessile spikelet surrounded by 8 - 9 pedicellate spikelet. The bract with strongly scabrous to long bristles and coriaceous texture of upper lemma.

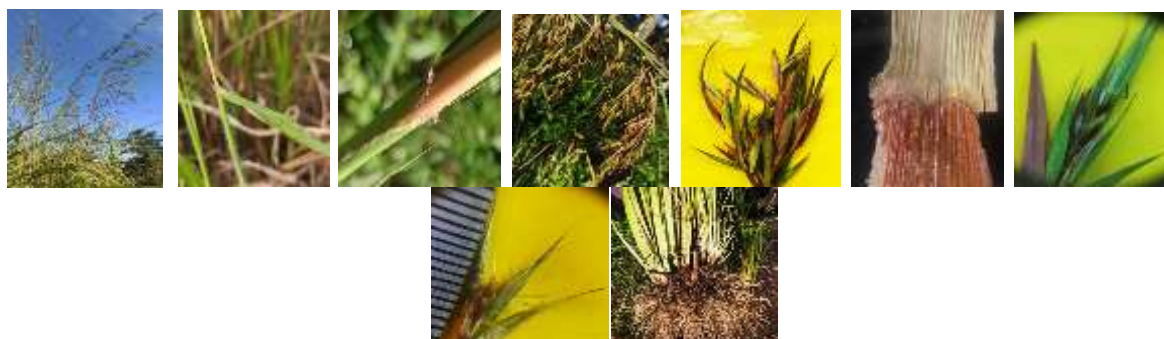


Figure 14 Habit, Leaves, Inflorescences, raceme with involucre, Ligule, Spikelet, rhizome

12. *Ischaemum timorense* Kunth.

Annual, creeping or erect, stoloniferous, reddish - brown color, glabrous. Culm up to 2. 0 -meter high and strongly stoloniferous. Inflorescences is 2 - 3 digitately arranged spike-like raceme with paired similar spikelet. Both of sessile and pedicellate spikelet perfect; lower floret staminate and upper bisexual. The cleft awn of upper lemma occurs in both spikelets. The upper floret in sessile spikelet and lower glume in pedicellate spikelet boat shape.



Figure 15 Habit, Leaves, Inflorescences, Node with ciliate, Ligule, Spike, Spikelet, Stolon

Subfamily Panicoideae Bouchenak - Khelladi *et al.* (2008). (Tribe. Paniceae R. Br.)

13. *Oplismenus undulatifolius* (Ard.) P. Beauv.

Perennials, erect, tufted, strongly the strongly rosette liked stoloniferous. Culm up to 0.5 m high and culm. The lower glume with modified awned up to 7.0 mm long. The awn of upper glume up to 5.0 mm long. lower floret neuter and upper floret bisexual and lower lemma provided to scabrous awned.



Figure 16 Habit, Leaves, Inflorescences, Ligule, Spike, Spikelet, floret, Stolon

14. *Panicum atosanguineum* Hochst. ex A. Rich.

Annual, aquatic, tufted, strongly rhizomatous to stoloniferous, reddish colored. culm up to 1.5 - meter high and. Inflorescences possess racemose to loosely arranged open panicle and leave blade distinctly narrowly linear shape. Spikelet composed 2 - flowered; persistent at maturity, the lower reduced or staminate florets and upper bisexual perfect floret. The texture of flowering glumes chartaceous.

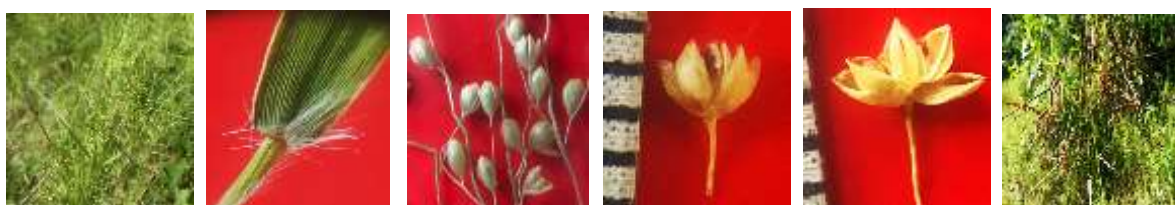


Figure 17 Habit, Leaves, Ligule, Inflorescences, Spikelet, Floret, Stolon

15. *Setaria pumila* (Poir.) Roem. & Schult.

Annuals, erect, tufted, fibrous roots. Culm 1.5 - meter high, flattened. Inflorescence narrowly cylindrical spike - liked racemes. Spikelet paired. Lower floret staminate and upper bisexual. All of flowering glumes crustaceous and upper lemma strongly rugose with densely involucre bristles.

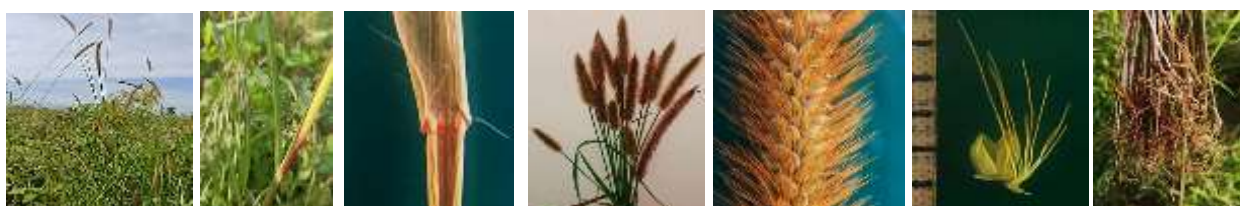


Figure 18 Habit, Leaves, Ligule, Inflorescences with mature densely bristles, Spikelet, Roots

16. *Axonopus compressus* (Sw.) P.Beauv., Ess.Agrostogr.12,154.1812. Myanmar nam: Hngetdaw - mi

Perennial, erect to prostrate, strongly stoloniferous, tufted, slender. Culm up to 1.0 meter high. Inflorescences 2-3 digitately arranged raceme, rachis wavy, spikelet secund and imbricate arranged on wavy rachis, digitately arranged raceme Spikelet borne single or pairs in rows on a narrowly flattened winged rachis winged, dorsally compressed to flat. The lower floret neuter and upper floret bisexual.



Figure 19 Habit, Leaves, Ligule, Digitate inflorescences, Spike, Spikelet, Floret, Rhizome

The Phylogenetic Position between the 16 genera of family Poaceae

The diagram is made by circular form with sections corresponding to the 16 genera of grasses. Within these sections are placed the genera at the distance from the central point indicating their relative evolutionary specialization. In this diagram, the concerning characters are given for each genus. Total divergence values of each genus are determined by each character. The relative characteristics between the genera are plotted on a concentric chart. A common hypothetical ancestor group is plotted at a point where divergence in character combination occurs. The diagram shown that a probable evolutionary trend for the genera of the Poaceae family. 13 morphological characters were used for the primitive and advanced characters of grass's evolutionary trends. The following morphological features were used for phylogeny. (A- Root and Rhizome System, B- Leaf sheath, C- Inflorescences, D- Rachilla, E- Glumes, F- Glumes with awned, G- Lemma, H- Palea, I- Lodicule, J- Stamens, K- Seed, L- Dispersal by Special devices (bristles, ciliate ,silky hairs),M- Members of fertile florets.). (a,c,h) are used as the common based ancestral character in this evolutionary trends of species.

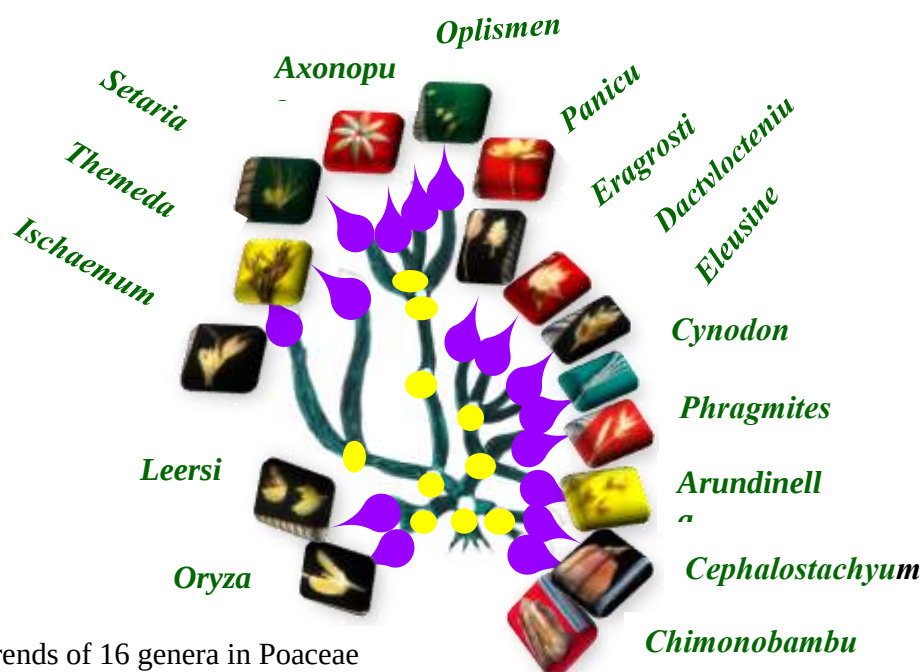


Figure 20 Evolutionary Trends of 16 genera in Poaceae

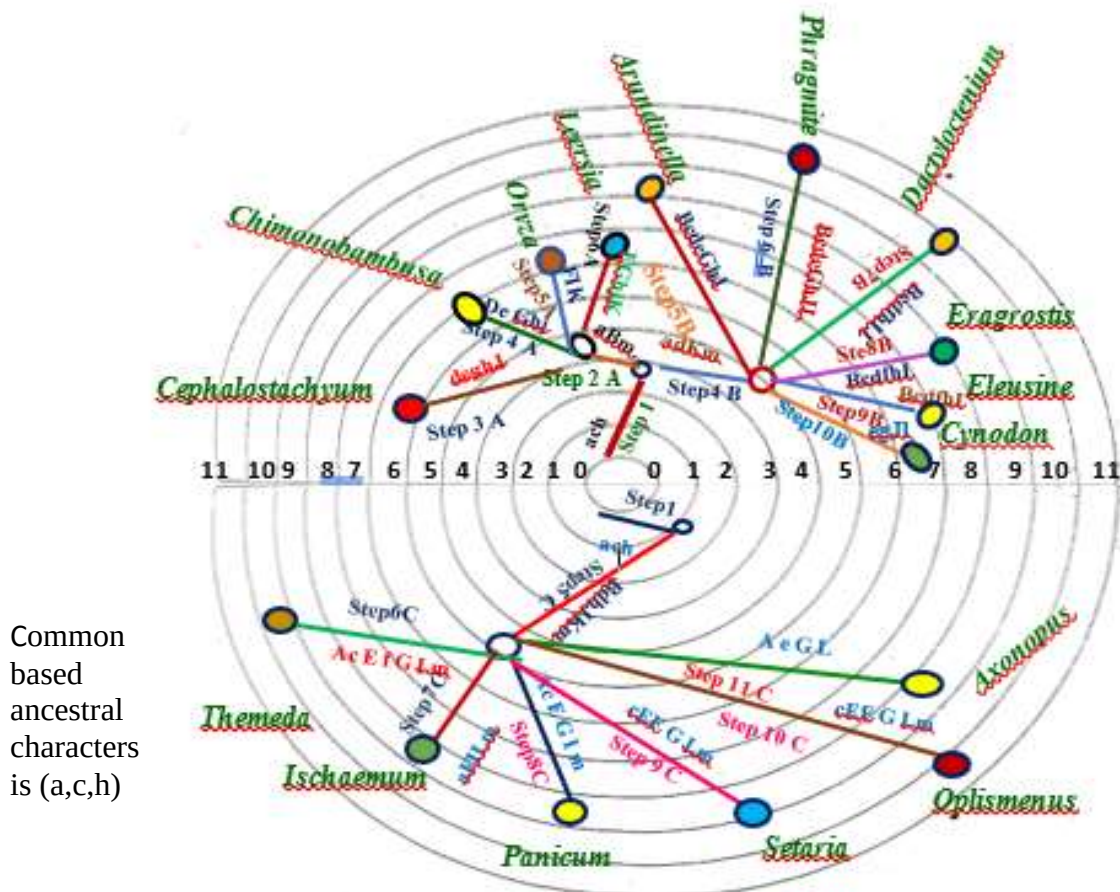


Figure 21 Cladistics Diagram of 16 Genera' Evolutionary Trends in Family Poaceae

Discussion and Conclusion

Grass belongs to graminoid clade of monocotyledonous and closely relationship with commelinid's clade. The traditional name of Gramineae is permitted by the International Code of Botanical Nomenclature, which also provided for use as family Poaceae, based upon the type of genus *Poa* L. under order Poales (Cope,1982)). In this research, 16 species, 16 genera, 8 tribes and 5 subfamilies of family Poaceae were recorded in In- daw -gyi Lake, Mohnyin Township, Kachin State, during from November ,2023, to August, 2024, within flowering time.

In this research, Poaceae was classified into 5 subfamilies according to Halfliger and Scholz's classification (1981). Namely of 5 subfamilies are Bambusoideae, Oryzoideae, Pooideae, Chloridoideae and Panicoideae

. Bamboo is the giant grass and the flowering time of subfamily Bambusoideae are not usually annual and after it's flower opening; the plant died. Thus, bamboo's identification is usually based on deeply used by modified vegetative features. Bamboo florets number are many and fertile florets with six stamen numbers. These stamens characters are primitive features of flower sexual status in grass's phylogeny, but they possess modified vegetative culm, underground rhizomes, node, branching system and various characters of culm sheath and culm blade. Subfamily Bambusoideae is the most primitive family by their arborescent vegetative habit characters in grasses taxonomy and evolution than those of the followed by 4 subfamilies.

In tribe Bambuseae of subfamily Bambusoideae; genus *Chimonobambusa communis* (Hsueh & T. P. Yi) T.H.Wen & Ohnrb possess distinctly rooting system of lower nodes of culm deciduous and thin texture of culm sheath and blade with curved auricle with softly ciliate hairs and loosely arranged 1-3 nearly equally branching systems. Genus *Cephalostachyum pergracile* Munro. K.N comprises modified advance features of strongly underground rhizomes systems, the persistent and thick texture and dense scabrous persistent hairs on the outer surface of culm sheath and culm blade with curved strongly folded densely curved hairs auricle and many equally horizontal branching systems. These features of *Cephalostachyum pergracile* Munro. K. N is more advanced modified vegetative structure than genus *Chimonobambusa communis* (Hsueh & T.P.Yi) T.H.Wen & Ohnrb in bamboo's evolution. These characters agree with Tzvelev, (1989).

Subfamily Oryzoideae is closely relative to subfamily Bambusoideae by similar six stamen numbers. This point shown that primitive structures of many stamens number characters in subfamily Oryzoideae. Thus, subfamily Oryzoideae is the second primitive evolutionary status than other than the rest of 3 subfamilies. The genus *Oryza rufipogon* Griffith. possess empty glumes. Genus *Leersia hexandra* Sw. stands without these features but strongly grow adaptation on survey area. This characters of *Leersia* (Soland.) Sw. are more advanced modified characters than genus of *Oryza* L. in tribe Oryzeae of subfamily Oryzoideae. These features agree with Moore, A.C. (1960).

In subfamily Pooideae are provided by rhizomatous underground systems, inflorescences with modified raceme to advanced open panicle and densely silky hairs spikelet. These characters are great advanced distribution mechanisms for grasses evolution. But the only the lowest floret bisexual and uppermost floret reduced sterile structure are primitive features. So, subfamily Pooideae is the third most primitive evolutionary stands other than the rest of 2 subfamilies. Genus *Arundinella nepalensis* Trin. in tribe Arundinelleae possess 1 - 2 florets, the upper floret bisexuals with long awned modified advanced characters. Genus *Phragmites karka* (Retz) Stead.in tribe Arundineae of subfamily Pooideae are advanced distinctly dense persistent silky hairs of distribution mechanisms structures than genus *Arundinella* of tribe Arundinellae Stapf in Benth. of subfamily Pooideae. This phylogeny point of view agreements with Bor, N.L, (1960).

Subfamily Chloridoideae possess inflorescences racemose features and digitately modified inflorescences characters with 2-34 fertile florets. Their many fertile florets structure with completely covered by flowering glumes. This character is more advanced morphological status than the previous recorded 3 subfamilies. In tribe Eragrosteae; genus *Dactyloctenium aegyptium* (Desf.) Beauv. is digitately spike with 3-4 florets. The lowest bisexual perfect floret, above florets are usually reduced and the uppermost florets are staminate and middle nerve of lemmas with modified awned. Genus *Elusine indica* (L.) Gaertn. possess 4 -5 florets with the lowest florets' bisexual and the upper most florets reduced. The reproductive and vegetative characters of genus *Elusine indica* (L.) Gaertn.is the same evolutionary stand with genus *Dactyloctenium aegyptium*(L.) Willd. Genus *Eragrostis* Wolf. possess racemose arranged panicle inflorescences and usually all of florets with 14 -34 advanced fertile florets number. Many fertile florets number of genus *Eargrostis* Wolf. is the most advanced evolutionary florets structure than genera of *Dactyloctenium aegyptium*(L.) Willd and *Elusine indica* (L.) Gaertn.in tribe Eragrosteae. Genus *Cynodon dactylon* (L.) Pres. in tribe Cynodoneae is rhizomatous to stoloniferous culm and only possess 1 fertile floret structure. Therefore, genus *Cynodon* Rich. are

the most primitive florets feature characters than all genera from tribe Eragrosteae in subfamily Chloridoideae. These evolutionary trends characters agreements with Moore, A.C. (1960).

Subfamily Panicoideae comprises tribe Andropogoneae and tribe Paniceae. Tribe Andropogoneae are usually provided by fragile paired sessile and pedicellate spikelets. Both of spikelets are usually awned or awn less; similar or dissimilar in texture and structure. Tribe Andropogoneae included 2 genera; Genus *Themeda villosa* (Poir.) A. Camus. is composed of strongly rhizomatous culm, branching racemose inflorescence which embraced by sheathing spathae's modified involucre bract and flexuous peduncles. Its sessile spikelet possesses 1 - 4 fertile florets were surrounded by 8-9 sterile pedicellate spikelet. The bract with strongly scabrous with long bristles. Their coriaceous texture of upper lemma is advanced grass's taxonomy in phylogeny. Genus *Ischaemum timorense* Kunth. is 2 - 3 digitately arranged spike-like raceme with paired similar spikelet. Both of sessile and pedicellate spikelet with perfect of lower floret staminate and upper bisexual. The cleft awn of upper lemma in both spikelet and boat shape of upper floret in sessile spikelet and lower glume in pedicellate spikelet features are modified adaptation dispersal mechanisms of grass's taxonomy. Genus *Themeda villosa* (Poir.) A. Camus comprises more modified advanced evolutionary features than genus *Ischaemum timorense* Kunth. by their reproductive and vegetative characters. Moreover, these 2 genera of tribe Andropogoneae in subfamily Panicoideae possess respectively individual modified advanced characters.

4 genera in tribe Paniceae of subfamily Panicoideae possess usually stoloniferous, rhizomatous, pedicellate or subsessile spikelet's awned or awn less, dorsally compress or terete spikelet. The strongly rosette like stoloniferous culm of genus *Oplismenus undulatifolius* (Ard.) P. Beauv. is up to 20.5 cm high and lower glume with modified awned up to 7.00 mm long. The awn of upper glume up to 5.0 mm long. Its lower lemma provided with scabrous awned. These awn characters are advanced complexity adaptation for species distribution for grass's phylogeny. Genus *Setaria pumila* (Poir.) Roem. & Schult is cylindrical panicle inflorescence, modified advanced densely persistent bristles and lower floret staminate and upper bisexual. The upper lemma rugose structure is advanced texture of covering for sexual organs. Genus *Panicum atrosanguineum* Hochst. ex A. Rich. possesses racemose to loosely arranged open panicle possess strongly rhizomatous to stoloniferous culm. It possesses lower reduced or staminate florets and upper bisexual provide the adaptation for distribution mechanisms of modified advanced reproductive and vegetative features. Genus *Axonopus compressus* (Sw.) P. Beauv., is stoloniferous culm, 2-3 digitately arranged raceme, spikelet flattened and borne singly or pairs in rows on a narrowly flattened winged rachis. Genera of *Panicum* L. and *Oplismenus* P. Beauv. are the most advanced modified characters than the 2 genera in tribe Paniceae of subfamily Panicoideae.

As the conclusion, tribe Paniceae is modified complexity advanced morphological characters than tribe Andropogoneae of subfamily Panicoideae in grass's evolution. These among 5 subfamilies; the subfamily Panicoideae possess the most advance modified vegetative and reproductive characters than the rest of previous 4 subfamilies. These evolutionary status agreements with the Nasir, E. and S. I. Ali (1982). The cladistic diagram with open cycle (○) (continuous divergence) and close cycle (●) (end point). The primitive, intermediate and advance level of evolutionary values are (0, 0.5, 1.0) respectively. The features of collected species found that the most primitive simplicity characteristics leading to the advanced complexities modified

character in collected species from survey area. Moreover, the primitive and advanced modified characters are integrations status occur in individual species of grass's phylogeny. This research highlights the modified taxonomy and evolutionary trends and informs the better understanding of wild cereal data for professional specialists, scientists, all academician and researchers in family Poaceae from In -daw -gyi Lake, Mohnyin Township, Kachin State, Myanmar.

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SCREENING OF PLANT GROWTH PROMOTING BACTERIA (PGPB) FROM THE SOIL AND THEIR EFFECTS ON THE GROWTH OF SELECTED PLANTS*

Phoo Wint Yee Thaw¹, Cho Cho², Hnin Win Yee³, Kay Thwe Lwin⁴

Abstract

Microorganisms in soil play a crucial role in crop production and sustainable development of agriculture. This study was intended to isolate the PGPB strains from different soil sample. The experiment was performed at the Molecular Research Laboratory, Department of Botany, Center for Research and Innovation, University of Yangon, in 2024. In this study, five bacterial strains have been isolated and given named as SP1 to SP5. In morphological characterization, all strains showed the circular shape, entire margin, raised elevation, smooth texture and transparent opacity except the opaque opacity in the strain SP1. Microscopical observations revealed that strains SP1, SP2, SP4 and S5 are bacilli shape, Gram-positive bacteria and only SP3 are Gram-negative bacteria with the form of bacilli. The different biochemical tests and nitrogen fixation test of each isolated strains were performed. The DNA extraction and purification from each strain were conducted and all extracted bacterial DNA produce relatively high purity of DNA, ranging between 1.8 and 2.0 in A_{260}/A_{280} ratio with less impurities in A_{260}/A_{230} ratio. Among five strains, strain SP2 and SP5 indicated the best DNA purity result. Moreover, the improvement of plant growth promotion performance indicated the positive effects on the selected plants at 15 days post inoculation, especially strains SP2 and SP5 showed the maximum results on plant growth parameters.

Keywords: soil bacteria, plant growth promoting bacteria (PGPB), DNA extraction, growth parameters

Introduction

Sustainable agricultural development is particularly important for world's population estimated to reach 9.1 billion by the year 2050 due to the necessity of improvement and enhancement of productivity of agricultural products (FAO, 2009). To meet the improvement of crop production, modern agriculture production strongly relies on synthetic fertilizers and pesticides to achieve high productivity levels. However, it can impact the decreasing organic matter content, soil degradation, pollution in groundwater system, and causing severe diseases (Adesemoye *et al.*, 2009, Bourguet and Guillemaud, 2016). Therefore, it is important to find eco-friendly and sustainable alternative from natural resources and microbiological approaches have become more popular for crop improvement. Nowadays, plant growth promoting bacteria (PGPB) are considered as environmental friendly and easily biodegradable for the enhancement of plant productivity (Jat Baloch *et al.*, 2023).

PGPB belongs to the free-living group of soil bacteria which improve both plant growth and crop yield when they are applied in different ways (Kumar *et al.*, 2014). PGPBs, initially described by Kloepper and Schroth in 1978, are mainly defined as a heterogeneous group of bacteria that promote the growth of plants by affecting the soil environment through direct or indirect mechanisms (Goswami *et al.*, 2013 and Wang *et al.*, 2014). PGPB belong to a vast variety of genera such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Di Benedetto *et al.*, 2017) which are applied to the production of various crops such as cereals and

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vegetables (Basu *et al.*, 2021). PGPB stimulate plant growth by various mechanisms including improvement of plant nutrition, unique enzymes secretion, phytohormones regulation, and suppression of pathogenic organisms. The exploitation of PGPB could reduce the application of synthetic fertilizers and help maintain soil health (Benizri, *et al.*, 2001).

Many recent studies have expanded the term PGPR to plant growth promoting bacteria (PGPB) by including various bacterial strains from a diverse range of sources, rather than rhizosphere (Goswami *et al.*, 2013). Among diverse PGPB groups, *Bacillus* spp. is the safe microorganisms that hold remarkable abilities for synthesizing a vast array of beneficial substances and it possess the potential plant growth promoting traits (Stein, 2005). In addition, the process of isolating bacterial DNA can be crucial step in identifying and studying beneficial bacteria (PGPB) when applied to plants, which can significantly enhance their plant growth by mechanisms like nutrients solubilization, hormone production and pathogen suppression. Purified DNA from soil bacteria can be used to study the role of soil microorganisms in plant growth (Parthasarathy, *et al.*, 2025).

Therefore, the isolation of indigenous PGPB strains with plant growth promoting potential is necessary for sustainable agricultural development. For this reason, the present study was proposed to isolate and characterize the PGPB strains from different soil samples, to conduct the morphological, microscopical and biochemical characterization, to extract and purify the genomic DNA from bacterial isolates, and to evaluate the effect of plant growth promotion on selected plants.

Materials and Methods

1. Sample Collection

The soil samples were collected from different soil in San Phu village, Meneitaung area, Hopong Township, Southern Shan State at the GPS location 20°39'51"N 97°18'3"E, 20°40'52"N 97°18'8"E and 20°40'42"N 97°17'57"E. The collected samples were placed in sealed plastic bags.



Figure. 1. Location map of Sample Collection

2. Isolation of Bacteria from Soil Samples

The soil microorganisms were isolated by serial dilution technique (Phay and Ando 2019). In this method, 2 g of soil samples was suspended in 18 ml of distilled water. Then, the suspension was shake for 30 minutes and transferred 2 mL into new tube containing 18 ml of distilled water. Mixed them very well and transferred 1 mL into new tube with 9 mL DW (1/1000). From the total 10mL tube, transferred 2 mL to new tube containing 8 mL of DW

(1/5000) and 1 mL to 9 mL tube (1/10000). The dilution series were prepared between 10^{-1} to 10^{-5} . These dilutions were spread on nutrient agar medium (NAM) and incubated for 24 hours at room temperature. After incubation, pure culture maintained at 4 °C for further studies.

3. Morphological and Microscopical Characterization

The morphological characteristics of isolates such as colony color, shape, elevation, margin, optical density and size were determined by observing the colonies on the plates. Microscopical characterization was examined by Gram staining (Bergey, *et al.*, 1994).

4. Biochemical Characterization

Biochemical tests such as (1) Catalase test, (2) Citrate utilization test, (3) Gelatinase hydrolysis test, (4) Indole test, (5) Methyl red (MR) test, (6) Motility test, (7) Vogas Proskauer (VP) test, (8) Triple sugar iron (TSI) test, and (9) Urease test, were performed (MacFaddin 2000). Similarly, the estimation of N-fixation was checked using N-free Jensen's agar medium (Kayasth *et al.*, 2014).

5. Extraction and Purification of Genomic DNA

The genomic DNA extraction from bacterial isolates was conducted by using the Wizard® Genomic DNA Purification Kit (Promega Corp. 2005). First, 1ml of overnight culture was centrifuged for 3 minutes at 14,000 rpm to get the pellet cells and discard the supernatant. For Gram positive bacteria, suspend cells in 480µl 50mM EDTA and add 120µl of lysozyme. Then, incubate at 37°C for 1 hour and centrifuge at 14,000 rpm. After, remove the supernatant and add 600µl of Nuclei Lysis Solution. Again, incubate for 5 minutes at 80°C, then cool to room temperature and add 3µl of RNase Solution. The cell incubates again at 37°C for 1 hour, then cool to room temperature. For Protein Precipitation, 200µl of Protein Precipitation Solution add to the tube and incubate on ice for 5 minutes. Then, centrifuged at for 3 minutes and the supernatant transferred to a clean new tube containing 600µl of isopropanol. Then, centrifuged again and decant the supernatant. The 600µl of 70% ethanol added to the tube and centrifuged again. The DNA pellet was air-dried for 10-15 minutes and rehydrated with 100µl of Rehydration Solution. Then, incubated for 1 hour at 65°C or overnight at 4°C for further studies. After the DNA isolation studies, checking the DNA concentration and purity of bacterial strains were performed by using NanoDrop NanoPhotometer® N60 based on the absorbance ratio at 260 and 280 nm and other contamination at 260 and 230 nm (Desjardin & Conklin, 2010). To check the DNA quality, 1 µL sample was pipetted onto the lower pedestal of NanoDrop apparatus. The ratio of A_{260}/A_{280} values between 1.7 and 2.0 indicate DNA samples with good quality. The A_{260}/A_{230} ratio should be greater than 1.5, ideally close to 1.8 (Brescia, 2012 and Vesty *et al.*, 2017).

6. Evaluating the Effect of Bacterial isolates on Plant Growth Promotion

The plant inoculation experiment, including all the process (bacterial inoculum and seed preparation, and experimental design for plant assay) were performed. In the current study, black gram, red flat bean and corn were selected for plant inoculation test. Firstly, fresh bacterial culture was prepared 1 day before seeds treatment. The isolates were grown in falcon tube containing 10 mL of Nutrient broth (NB) and incubated in a shaking condition at 150 rpm for 48 hours at room temperature. Then, seeds were washed thoroughly with distilled water for 10 times. Again, the seeds were sterilized with 70% (v/v) ethanol for 3 min and properly washed again with distilled water. Then, placed on the sterilized paper for 2-3 days and germination

percentage were analyzed. The soil was sterilized by autoclaving at 121°C for 15 minutes. Then, good, germinated seedlings were selected and transferred to the plastic cup (top) containing autoclaved soil and each cup containing 1 seed, and 1 ml of bacterial inoculum (1×10^9 CFU/ mL) was added to each cavity of seedling. The cup (bottom) was filled with sterilized distilled water. Non-inoculated seedlings were used as a control. These cups were kept in a place full of sun light for proper growth. Plant weight, plant height, root length and root weight of selected plants were evaluated after 15 days of post-inoculation.

Results

1. Morphological and Microscopical characterization of isolated bacteria

The five bacterial strains have been isolated from different soil samples. Among them, strains (SP1 to SP5) showed the circular shape, entire margin, raised elevation, smooth texture, and transparent opacity except with the strain SP1 showed opaque opacity in morphological characterization. Microscopical observations showed all bacterial strains are bacilli shape, Gram-positive bacteria and SP3 was Gram-negative bacteria with bacilli form.

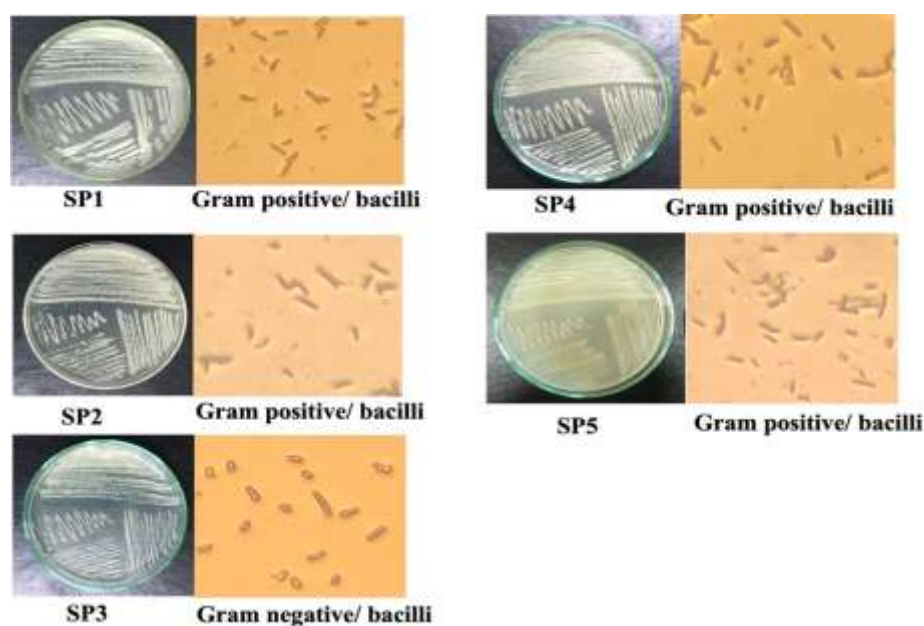


Figure.2. Morphological and Microscopical Characters of isolated strains (X 400)

Table. 1. Morphological and Microscopical Characterization of Five Bacterial Strains

No	Shape	Margin	Elevation	Texture	Opacity	Colony Color		Gram Staining
						Surface color	Reverse color	
SP1	circular	entire	raised	smooth	opaque	white	white	+
SP2	circular	entire	raised	smooth	transparent	white	white	+
SP3	circular	entire	raised	smooth	transparent	white	cream	-
S4P	circular	entire	raised	smooth	transparent	yellow	yellow	+
SP5	circular	entire	raised	smooth	transparent	yellowish cream	yellowish cream	+

2. Biochemical Characterization

Table. 2. Biochemical Characterization of Five Bacterial Strains

No.	Catalase test	Citrate test	Gelatin hydrolysis test	Indole test	(MR) test	Motility test	(TSI) test	Urea Test	(VP) test	N-fixation
SP1	+	-	+	-	-	+	A/A	+	-	+
SP2	+	+	+	-	-	+	K/A (H ₂ S)	+	-	+
SP3	+	-	-	-	-	+	A/A	-	-	+
SP4	+	-	-	-	+	+	A/A	-	-	+
SP5	+	+	-	-	+	+	K/K (H ₂ S)	-	-	+

+ = positive, - = negative, K/A= al/ac - dextrose only fermented, A/A= ac/ac - dextrose, lactose, sucrose fermented, K/K= al/al no sugar fermented, Black = H₂S production



Figure. 3. Biochemical Characters of isolated bacterial strains

3. Extraction and Purification of Genomic DNA

The concentration and purity of bacterial DNA were analysed by using NanoDrop spectrophotometer which measure A_{260}/A_{280} and A_{260}/A_{230} ratio. The DNA ratio between A_{260}/A_{280} indicated 1.8 to 2.0 which means all strains indicated the best DNA quality. The strain SP5 showed the best DNA concentration. In A_{260}/A_{230} ratio, the value showed less than 2.0 indicates the presence of contamination. However, strains SP2 and SP5 showed the less contaminations.

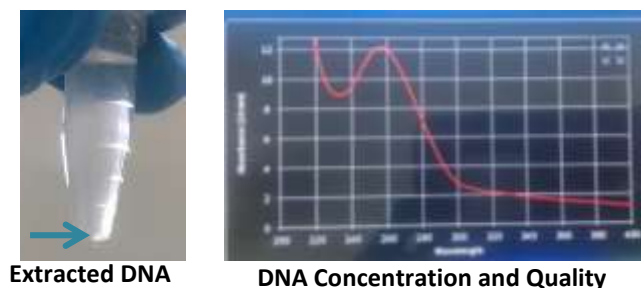


Figure. 4. Extracted DNA and DNA Concentration and Quality Using NanoDrop Spectrophotometer

Table. 3. Genomic DNA Concentration and Quality Using NanoDrop Spectrophotometer

Strains	DNA Concentration (ng/μL)	Indication Ratio for DNA purity ($A_{260}/A_{280} = \sim 1.8 - 2.0$)	Indication Ratio for Impurities Contamination ($A_{260}/A_{230} = 2.0 - 2.2$)
SP1	125.65	1.878	1.166
SP 2	69.250	2.031	1.345
SP 3	160.10	1.911	1.192
SP 4	59.750	2.012	1.150
SP 5	494.35	2.021	1.445

4. Evaluating the Effect of Bacterial isolates on Plant Growth Promotion

The effect of five bacterial isolates on the plant growth of black gram, red flat bean and corn was evaluated at 15 days post inoculation. Among five strains, four strains except SP3 indicated the significantly increase in plant height compared with the uninoculated treatment (control). Strain SP2 and SP5 showed the maximum results on plant height, plant weight, root length and root weight in all selected plants.

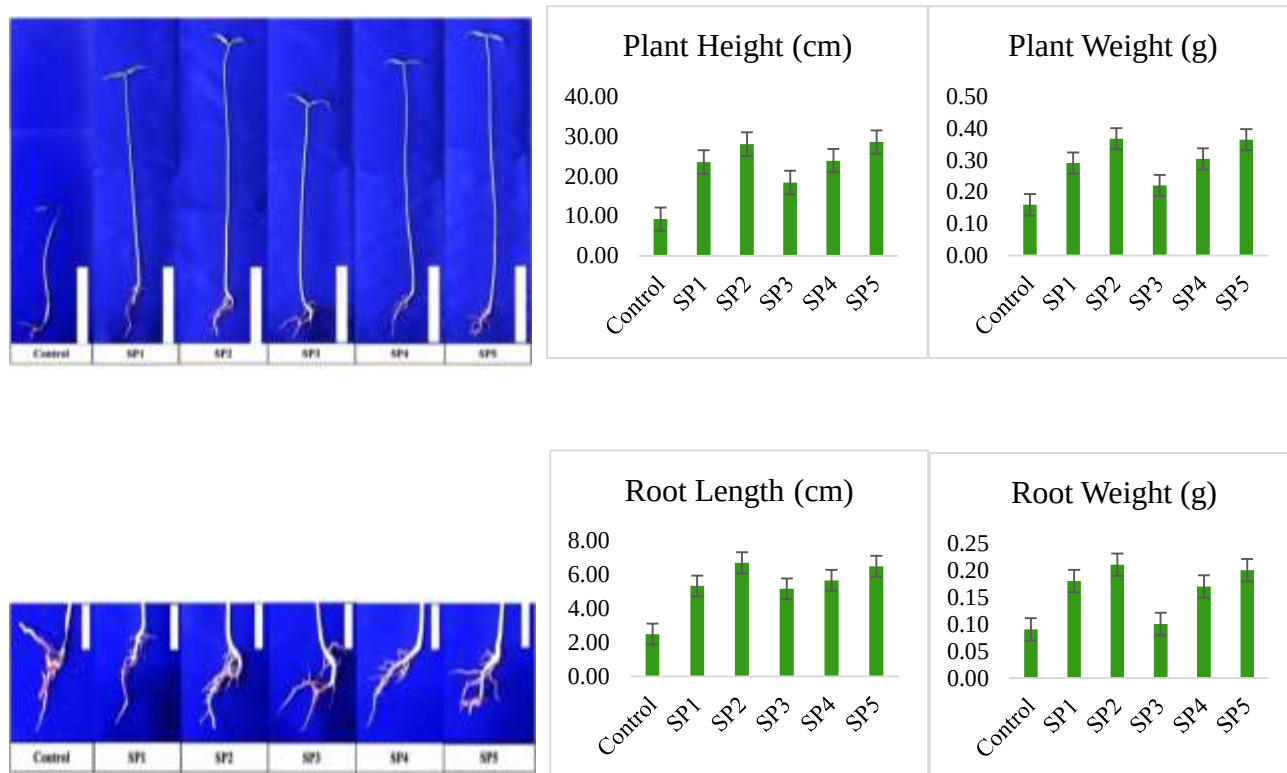


Figure. 5. Comparison of plant growth parameters (plant height, plant weight, root length and root weight) of five strains on black gram, inoculated with no-inoculation (control) at 15 days after sowing. The histograms at each treatment are significantly different at 15 days, $P > 0.05$ (t-test).

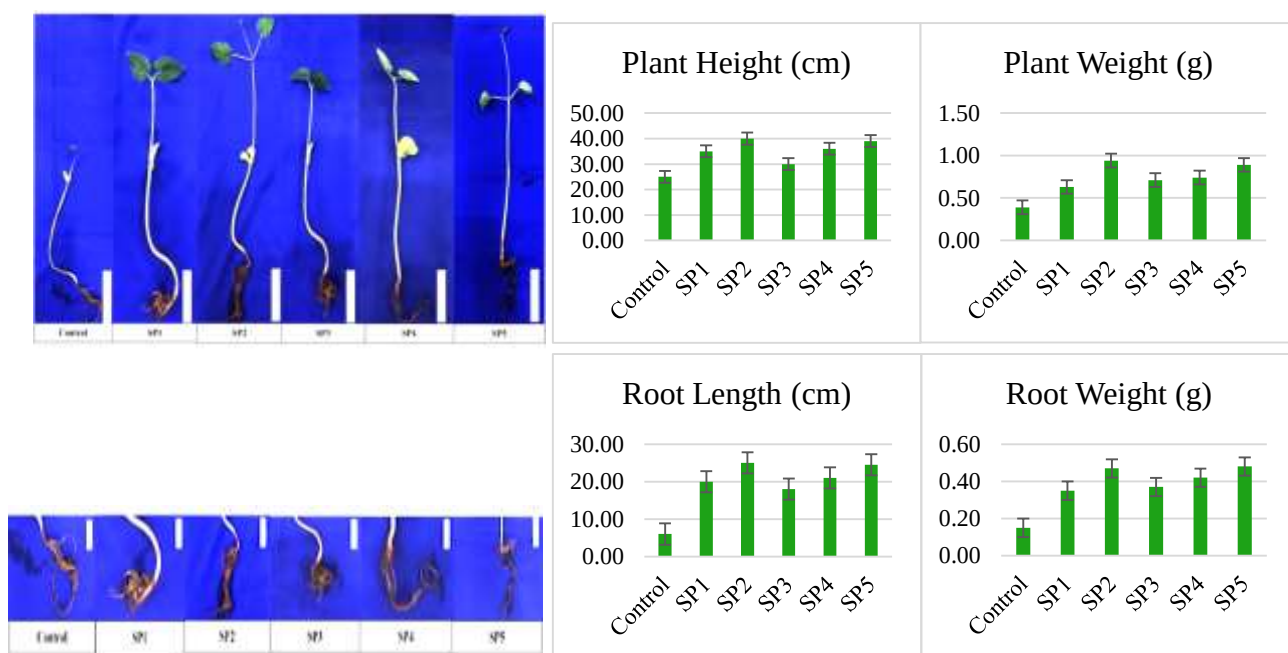


Figure. 6. Comparison of plant growth parameters (plant height, plant weight, root length and root weight) of five strains on red flat bean, inoculated with no-inoculation (control), at 15 days after sowing. The histograms at each treatment are significantly different at 15 days, $P > 0.05$ (t-test).

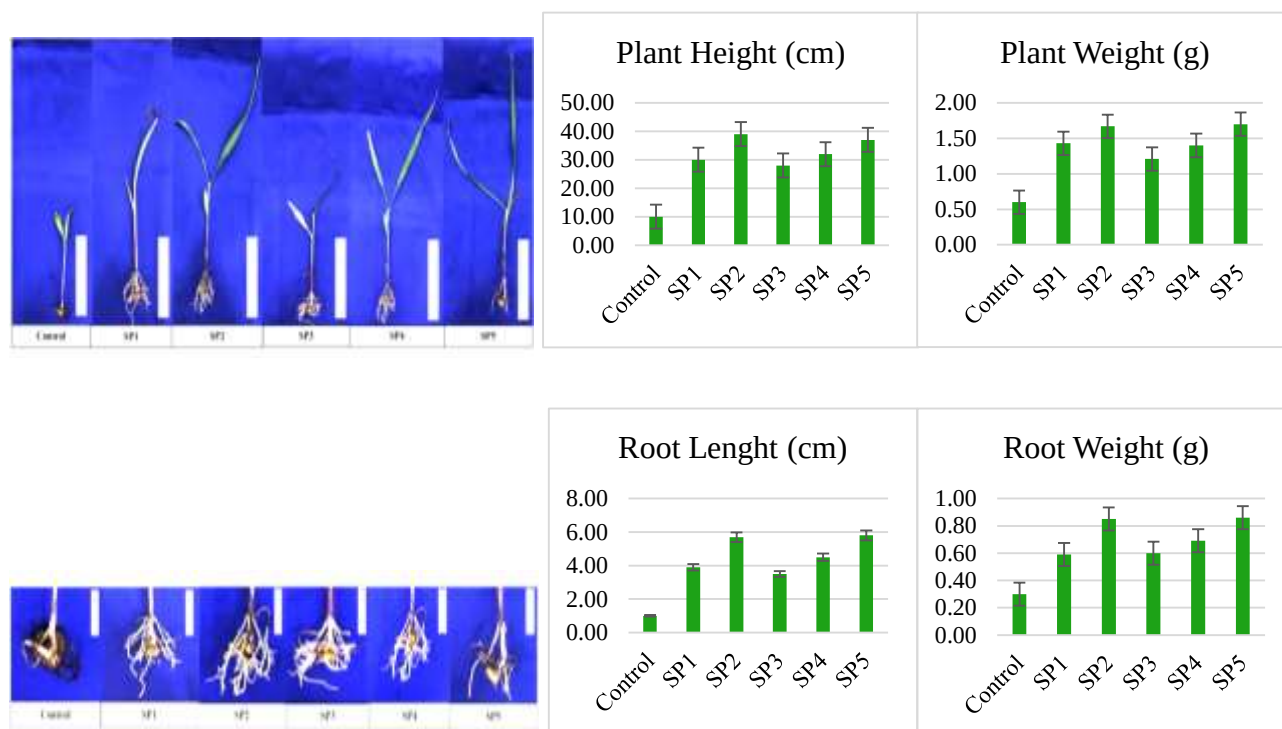


Figure. 7. Comparison of plant growth parameters (plant height, plant weight, root length and root weight) of five strains on corn, inoculated with no-inoculation (control), at 15 days after sowing. The histograms at each treatment are significantly different at 15 days, $P > 0.05$ (t-test).

Discussion and Conclusion

Plant growth-promoting bacteria (PGPB) are one of the most generalized findings of plant probiotic microbes (PPM) in soil, having *Bacilli* and *Pseudomonas* being the most common genera reported (Glick *et al.*, 1997 and Hashem *et al.*, 2019). In this study, five bacterial strains have been isolated from different soil samples and given named as SP1 to SP5. In the morphological characterization, all strains (SP1 to SP5) showed the circular shape, entire margin, raised elevation, smooth texture, and transparent opacity with the exception of opaque opacity in strain SP1. Microscopic observations demonstrated that strains SP1, SP2, SP4 and SP5 were Gram-positive with bacilli form and SP3 were observed as Gram-negative bacteria in the bacilli form.

In biochemical characterization, all isolates showed the positive results in catalase test, motility test, and TSI test. In contrast, the negative results of all bacterial strains were observed in indole test and VP test. The three isolates SP1, SP3 and SP4 indicated the negative result in citrate test and positive result were obtained in SP2 and SP5 which similar to the findings of Neihaya and Zaki (2018). For the MR test, SP1, SP2, and SP3 showed the negative results and SP4, and SP5 showed the positive results, which similar to study of Ambeng *et al.*, (2019). In urea test, SP1 and SP2 showed the positive result, and the negative result were observed in S3, S4 and S5. These are close agreement with the studies of (Anand *et al.*, 2006 and Mandragutti *et al.*, 2021). In Triple Sugar Iron Agar test, strains SP1, SP3 and SP4 indicated that the acid fermentation of dextrose, lactose, and sucrose. Strains SP2 taken place only dextrose fermentation and SP5 showed the absence of carbohydrates fermentation but the production of

H₂S gas was observed in both strains. Hiranmayee *et al.*, (2023) described that TSI test provide carbohydrate fermentation and H₂S production, it can impact the plant growth. In the current study, the strains SP2 and SP5 showed the H₂S production which means it might be effectively promote the plant growth.

Demirkan *et al.* 2014 reported the positive results in motility, catalase and negative results in indole production from the *Bacillus* sp. strains isolated from soil samples. Similarly, many studies reported that the white colony color, smooth texture, Gram positive with rod shape in morphological characters as well as the positive results in catalase, and H₂S gas production and the negative results in MR, VP and citrate utilization test were identified as *Bacillus* spp. (Younes *et al.*, 2012 and Neihaya and Zaki, 2018). The current study is closely related with their findings. For this reason, the current findings were assumed that isolated bacteria SP1, SP2, SP4, and SP5 might be *Bacillus* species except strain SP3. In addition to improvement of plant growth, nitrogen-fixing potential is an important attribute for plant growth promoting bacteria. In the observation of nitrogen fixing ability on a selective media, all isolated strains showed the growth of bacterial colonies after incubation at room temperature for 5-7 days which indicated the positive results of nitrogen fixation ability. Lihan *et al.*, (2021) reported that all isolated PGPB strains, Gram-positive *Bacillus* sp. bacteria showed the positive reaction of growth on nitrogen free media and these results are the same with the current observation.

Moreover, high quality DNA from bacteria can promote plant growth by improving the plants resistance to stress, increasing soil fertility, and enhancing the fitness of plants (Parthasarathy, 2025). In the experiment of DNA extraction and determination of DNA concentration and purity by using Promega kit, all extracted bacterial DNA in the current study produce relatively high purity of DNA, the value of A₂₆₀/A₂₈₀ indicated between 1.8 and 2.0 and the value of A₂₆₀/A₂₃₀ showed the range under 2.0, which indicates the presence of contamination such as RNA, salt, and protein. However, strains SP2 and SP5 indicated the less contaminations in A₂₆₀/A₂₃₀ ratio due to the results of nearly ~1.5. Similar observations were reported in the studies of Wilson, (1997), Courtois, *et al* (2001), and Tiago *et al.*, (2015). Dwiyitno *et al.*, (2018) reported that commercial kit/ Promega produced relatively high DNA quality (A_{260/280} = 1.79-2.12) which indicated the similar results of present findings because strain SP5 showed the best DNA purity result.

The plant growth parameters including plant height, plant weight, root length and root weight of bacterial isolates indicated the positive effects on the selected plants (black gram, red flat bean and corn) at 15 days post inoculation. Among five bacterial strains, strains SP2 and SP5 indicated the maximum plant height, plant weight, root length and root weight in all selected plants, it might be SP2 and SP5 promote plant growth due to the H₂S production. Tozlu *et al.*, (2012) described the application of *Bacillus megaterium* strain M-3 as biofertilizer improved the bean production. Similarly, the inoculation of corn with various *Bacillus* sp. significantly increases the shoot and root weight (Efthimiadou *et al.*, 2020). In 2022, AlAli *et al.*, stated that *Bacillus baekryungensis* DPM17 isolated from a non-agricultural area, and it improved the growth parameters such as root length, dry weight, and branching. Therefore, *Bacillus* spp. isolated form current study is closely agreement with their findings due to the achievement of highest effects on the growth of selected plants (bean and corn). Interestingly, strain SP3 was not identified as *Bacillus* although it showed the positive results on plant growth compared with control (uninoculated) plants. Moreover, the results of DNA indicated the best DNA purity ratio

with less impurities in SP2, and SP5 showed highest plant growth while other strains with some impurities in DNA result indicated moderate plant growth.

In conclusion, this study explores the importance of beneficial soil microorganisms which have the unique ability to promote the plant growth for agricultural productivity might be *Bacillus* species except one strain and preliminary molecular approaches such as DNA extraction and purification of all bacterial isolates indicated the high purified DNA with less impurities. These findings collectively point out that inoculation of plants with *Bacillus* spp. could be the excellent manner for speedy enhancement of plant morphological characteristics and might be play important role for sustainable agricultural development by reducing the use of chemical fertilizers. In future, it is remained to study the different PGPB traits of isolated bacterial strains and more efficiency test under greenhouse and field conditions as well as to clarify the role of PGPR as biofertilizers that exert useful consequences on plant growth and development.

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EFFECT OF INOCULATING ENDOPHYTIC FUNGI ON AGARWOOD FORMATION IN *AQUILARIA MALACCENSIS* LAM.*

Cherry Mon¹, Zaw Lwin Oo² and Kalaya Lu³

Abstract

The fourteen endophytic fungi were isolated from the healthy plant parts such as leaves, bark, wood and fruit of *Aquilaria malaccensis* Lam. The fourteen isolated fungal strains were tested for enzymatic activities such as cellulase, laccase, lipase, protease and pectinase. *Aspergillus* sp. (CM 2 and CM 11) showed cellulase, lipase and pectinase activities. *Curvularia* sp. (CM 6) showed laccase. *Aspergillus* sp. (CM 2 and CM 11) and *Curvularia* sp. (CM 6) were selected as the potential endophytic fungi for inducing the agarwood formation. The three strains of endophytic fungi such as *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6) and *Aspergillus* sp. (CM 11) were used as inoculation materials. The fungal inoculation was done in the stem of *Aquilaria malaccensis* Lam. using the syringe method. The induced agarwood was harvested after 3 months of inoculation. The inoculation of *Aspergillus* sp. (CM 11) showed better results in average agarwood yield (3.5 %) per tree. The average agarwood yield per tree treated with *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6) and Potato dextrose broth (Control) was showed 1.9 %, 0.7 % and 0.2 % respectively. The 2.53 % of agarospirol production was induced successfully using the fungi *Aspergillus* sp. (CM 11) within 3 months only, after inducing the infection. Therefore, *Aspergillus* sp. (CM 11) is an efficient and promising fungal strain for artificial agarwood production during commercial-scale cultivation of *Aquilaria malaccensis* Lam. which can lead to the ecology and ecological stabilities for communities in near future.

Keywords: isolation, endophytic fungi, enzymatic activity, inoculation, agarwood

Introduction

Agarwood is a resinous portion of *Aquilaria* trees, a genus belonging to the family Thymelaeaceae (Gao *et al.* 2019). Agarwood-producing trees are widely distributed in Asia, including India, Sri Lanka, Bangladesh, Bhutan, Myanmar, Laos, Vietnam, Cambodia, China, Brunei, Malaysia, Indonesia, Philippines, Thailand, and Papua New Guinea (Turjaman *et al.* 2016). The healthy wood of *Aquilaria* Lam. trees is white, soft, and without scented resins. The dark resinous material of *Aquilaria* is created as a response to natural injury by lightning strike, animal grazing, insect attack, and microbial invasion (Blanchette & Heuveling 2009).

The natural production of agarwood is rare and slow, as only 7-10 % of old trees can produce agarwood, resulting in high agarwood prices on the global market. Agarwood can fetch as much as US\$ 100,000 per kilogram for superior pure wood material or US\$ 100 per kilogram for low quality. The high economic and medicinal value of agarwood has increased market demand, leading to the overharvesting of agarwood resources and destruction of natural *Aquilaria* forests (Naef, 2011).

Agarwood oleoresin composed of sesquiterpene and chromone derivatives and their composition determines the quality or grade of agarwood in the market (Chhipa & Kaushik 2017). The oleoresin produced by the tree is a highly valuable component of incense, perfumes, drugs, stimulants, cardiac tonics, and carminatives (Mengling *et al.*, 2005). The over-use of

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agarwood has seriously affected the natural resources of all *Aquilaria* species capable of producing agarwood, thus these endangered species listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 2004 (Chen *et al.* 2011).

To protect the wild plant resources and to meet the demand for sustainable agarwood production, *Aquilaria* trees are now being planted in large area by using a variety of artificial cultivation mode (Lei *et al.* 2019). The three common induction techniques are physical, chemical, and biological (Arzen *et al.* 2019). Biological induction techniques involve the stimulation of the plant's immune response through artificial infection of "pure" or "mixed" fungal strains. The majority of fungi used for the inoculation are endophytes isolated from healthy plants or the infected tissue of agarwood-producing trees (Rasool & Mohamed 2016).

Along with the continuously decreasing supply of wild agarwood and in order to meet market demand, much effort will make to preserve natural *Aqualaria* species populations and to increase agarwood supply. Therefore, the study on the effect of inoculating the endophytic fungi to induce the agarwood formation is important to improve cultivation of *Aqualaria* species.

The general aim and objectives of this research is to earn income generation through sustainable and artificial induction technique. The specific aim and objectives of this research are to isolate and identify the endophytic fungi from healthy plant parts of *Aquilaria malaccensis* Lam., to study the enzymatic activities of endophytic fungi, to induce agarwood formation by inoculating with endophytic fungi, to compare the quantity of agarwood formation and to test the quality of agarwood formed through fungal infection.

Materials and Methods

Collection of Plant Samples

The plant parts of 10-year-old *Aquilaria malaccensis* Lam. were collected from agarwood plantation situated at Sin Gaung Lay village, Yat Kwet Gyi 13, Pyin Oo Lwin Township, Mandalay Region.

Isolation of Endophytic Fungi

The healthy plant parts such as leaves, bark, wood and fruit of *Aquilaria malaccensis* Lam. were used for the isolation of endophytic fungi. The isolation of fungi was done by the methods of Cui *et al.* (2011). The potato dextrose agar (PDA) medium and water glucose agar (WGA) medium were used for the macroscopical and microscopical characters of the fungi. The isolated fungi were identified by referring to Gilman (1957).

Analysis of Enzymatic Activities of Endophytic Fungi

The isolated fungal strains were screened for enzymatic activities such as cellulase, laccase, lipase, protease and pectinase by the methods of Sunitha *et al.* (2013), Bisht *et al.* (2017), Sharma *et al.* (2015) and Elsabayt *et al.* (2015).

Inoculation of Endophytic Fungi into *Aquilaria malaccensis* Lam.

The three strains of endophytic fungi such as *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6) and *Aspergillus* sp. (CM 11) were used as inoculation materials to inoculate *Aquilaria malaccensis* Lam. *Aspergillus* sp. (CM 2) were cultured in Czapek dextrose broth (CZB) and *Curvularia* sp. (CM 6), *Aspergillus* sp. (CM 11) were cultured in Potato dextrose broth (PDB). These fungal strains were incubated at 25°C for 7 days at Microbiology Laboratory of Botany Department, University of Mandalay. Inoculation of endophytic fungi was carried out in an agarwood plantation situated at Sin Gaung Lay village, Yat Kwet Gyi 13, Pyin Oo Lwin Township, Mandalay Region from March 2024 to October 2024. The 13-years old *Aquilaria malaccensis* Lam. trees used in this study were randomly selected based on diameter at breast height (DBH) between 30 cm and 50 cm with height range between 5 m and 6 m. The fungal inoculation was done in *Aquilaria malaccensis* Lam. using syringe method. The holes were made along the trunks with an electric drill. The hole diameter was 0.5 cm and the inward depth was one-third of the stem diameter. The initial hole was made 30 cm above the ground. The vertical space between holes was 20 cm and in each horizontal line distributed three holes (Wahyuni & Prihantini 2020). 30 ml of homogenized fungal cultures was inoculated in each hole using a sterile syringe. A sterilized PDB medium was used as a control (Figure 1). The inoculation holes were subsequently covered with silicon to avoid contamination from insects and water (rain). Each treatment was taken three replications on different trees. The second times of fungal inoculation was carried out after 2 months of the first inoculation times.



Figure 1 Inoculation process of selected endophytic fungi into the stem of *Aquilaria malaccensis* Lam.

Collection of Induced Agarwood by Inoculating Endophytic Fungi

The induced agarwood was collected when the fungus inoculated on the stem of *Aquilaria malaccensis* Lam. trees, had grown for 3 months. The inoculation site changed in color from white to brown and was dug up by a chisel. The inoculated trees were cut down and inoculated trunks were weighed. The inoculated trunks were then carved to obtain the agarwood. The produced agarwood was collected and weighed (Wahyuni & Prihantini 2020). The yield of agarwood production was calculated using the following formula:

$$Y = \frac{\text{Output}}{\text{Input}} \times 100 \%$$

Y = yield of agarwood production (%)

Input = log of wood before carving (kg)

Output = agarwood production after carving (kg)

Analysis of the Chemical Constituents in Essential Oils Agarwood (CM 11) of *Aquilaria malaccensis* Lam.

The agarwood induced by *Aspergillus* sp. (CM 11) was selected to extract the essential oil. The 50 g of agarwood powder was soaked in distilled water (800 ml) for 24 h. Hydrodistillation process was taken at 100 °C for 4 h to extract the oil (Liu *et al.* 2013). The chemical constituents in essential oils were analyzed by gas chromatography-mass spectrometry, GC-MS-QP2010-Ultra Shimadzu, at the Department of Chemistry, University of Mandalay.

Results

Morphological Characters of Endophytic Fungi

The macroscopical and microscopical characters of endophytic fungi isolated from the healthy plant parts of *Aquilaria malaccensis* Lam. were studied. The fourteen strains of endophytic fungi were tentatively designated as CM 1 to CM 14. The characteristics of selected endophytic fungi such as *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6) and *Aspergillus* sp. (CM 11) were described in Table 1 and Figure 2 - 4.

Enzymatic Activities of Endophytic Fungi

The enzymatic activities such as cellulase, laccase, lipase, protease and pectinase of the fourteen isolated fungal strains were tested. Among the fourteen fungal strains, the three fungal strains such as *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6) and *Aspergillus* sp. (CM 11) were showed the enzymatic activities (Table 2 and Figure 5 – 8).

Effect of Endophytic Fungi on Agarwood Production

In the present study, the stem of *Aquilaria malaccensis* Lam. was inoculated with selected endophytic fungi such as *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6), *Aspergillus* sp. (CM 11)

and Potato dextrose broth (Control) for inducing agarwood formation. The induced agarwood was harvested after 3 months of inoculation. The cross sections of the trees treated with selected endophytic fungi were showed in Figure 9. The average percentage of agarwood yield per tree treated with different fungal strains was estimated. The inoculation of *Aspergillus* sp. (CM 11) showed better results in average agarwood yield (3.5 %) per tree. The average agarwood yield per tree treated with *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6) and Potato dextrose broth (Control) was showed 1.9 %, 0.7 % and 0.2 % respectively (Table 3 and Figure 10). The chemical constituents in essential oils were analyzed by gas chromatography-mass spectrometry (Figure 11). The total 30 chemical compounds were identified, in which agarospirol (2.53 %), 2-Butanone, 4-phenyl- also known as benzylacetone (3.89 %), 2-Butanone, 4-(4-methoxyphenyl)- also known as anisylacetone (2.13 %), guaicol (2.74 %) and (-)-aristolene (2.41 %) are in the group of sesquiterpenes which is responsible for fragrance in agarwood (Table 4).

Table 1 Characteristics of Isolated Endophytic Fungi

Endophytic Fungi	Source	Macroscopical Characters		Microscopical Characters
		Surface	Reverse	
<i>Aspergillus</i> sp. (CM 2)	Leaf	White to black	White to cream	Conidiophores nonseptate, vesicle globose, phialide biserial, conidia globose, brown
<i>Curvularia</i> sp. (CM 6)	Bark	Grey	Pale brown to black	Conidiophores brown, septate, conidia pale brown, ellipsoidal, 3-septate
<i>Aspergillus</i> sp. (CM 11)	Fruit	Olivaceous to brown	Pale yellow to olivaceous buff	Conidiophores nonseptate, vesicle globose, phialides uniseriate, conidia globose, hyaline

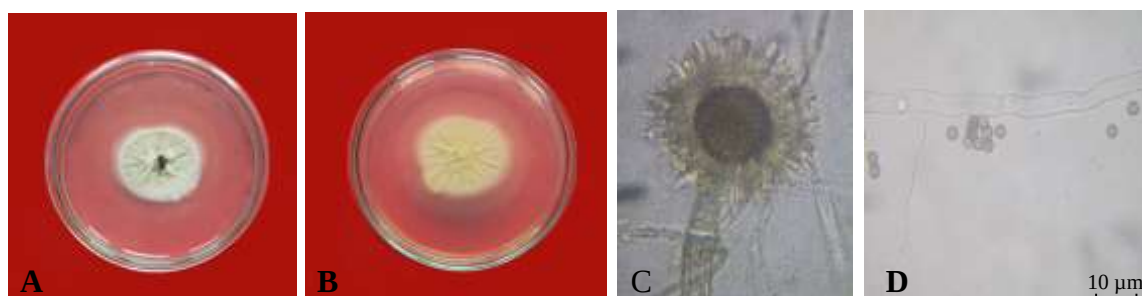
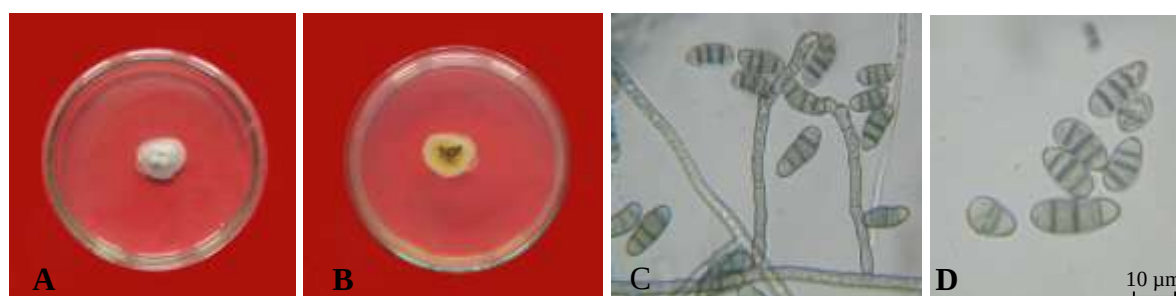
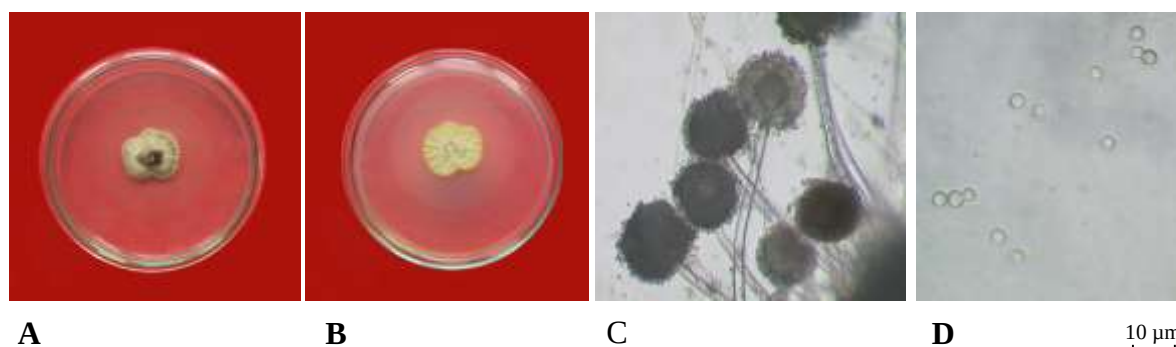
Table 2 Enzymatic Activity of Endophytic Fungi

Endophytic fungi	Cellulase	Laccase	Lipase	Protease	Pectinase
<i>Aspergillus</i> sp. (CM 2)	+	-	+	-	+
<i>Curvularia</i> sp. (CM 6)	-	+	-	-	-
<i>Aspergillus</i> sp. (CM 11)	+	-	+	-	+

+ = Positive reaction, - = Negative reaction

Table 3 The Average Yields of Agarwood Production after 3 months

Treatment	Wood (kg)	Agarwood (kg)	Yield (%)
Potato Dextrose Broth (Control)	19.21	0.043	0.2
<i>Aspergillus</i> sp. (CM 2)	11.16	0.21	1.9
<i>Curvularia</i> sp. (CM 6)	21.54	0.16	0.7
<i>Aspergillus</i> sp. (CM 11)	17.00	0.60	3.5

**Figure 2** Macroscopical and microscopical characters of isolated *Aspergillus* sp. (CM 2) from leaves of *Aquilaria malaccensis* Lam. A. Surface colony (3 days), B. Reverse colony (3 days), C. Photomicrograph of a conidial head, D. Photomicrograph of conidia**Figure 3** Macroscopical and microscopical characters of isolated *Curvularia* sp. (CM 6) from bark of *Aquilaria malaccensis* Lam. A. Surface colony (3 days), B. Reverse colony (3 days), C. Photomicrograph of conidiophore and conidia, D. Photomicrograph of conidia**Figure 4** Macroscopical and microscopical characters of isolated *Aspergillus* sp. (CM 11) from fruit of *Aquilaria malaccensis* Lam. A. Surface colony (3 days), B. Reverse colony (3 days), C. Photomicrograph of conidial heads, D. Photomicrograph of conidia

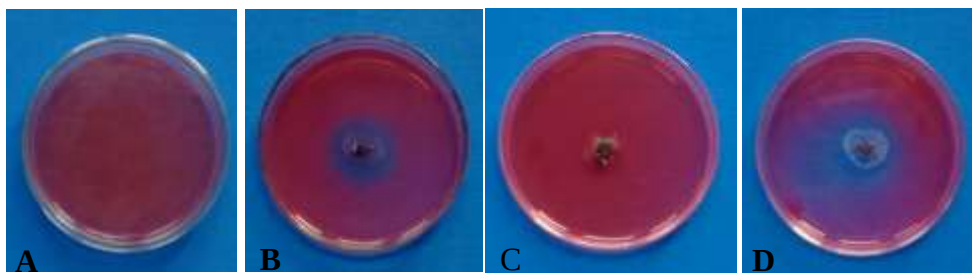


Figure 5 Cellulase Activities of Isolated Endophytic Fungi A. Control B. *Aspergillus* sp. (Positive) C. *Curvularia* sp. (Negative) D. *Aspergillus* sp. (Positive)

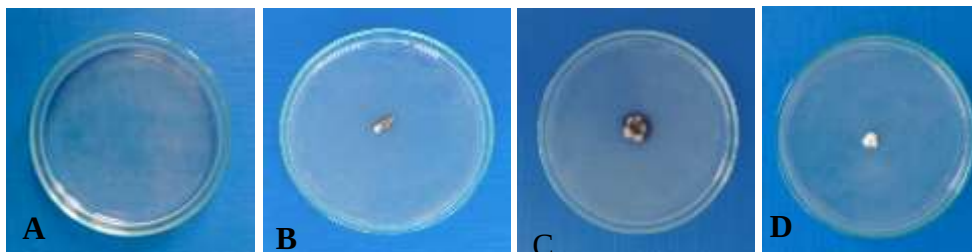


Figure 6 Laccase Activities of Isolated Endophytic Fungi A. Control, B. *Aspergillus* sp. (Negative), C. *Curvularia* sp. (Positive), D. *Aspergillus* sp. (Negative)

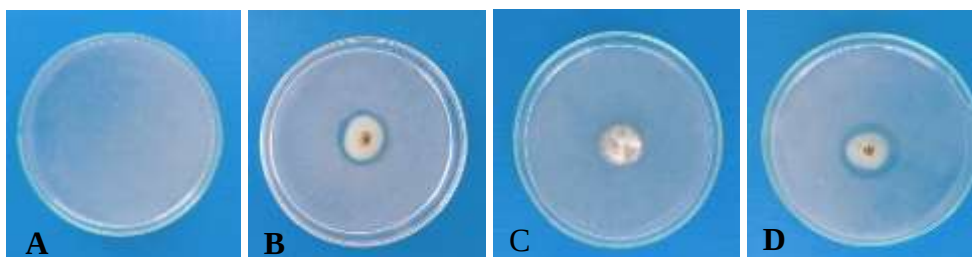


Figure 7 Lipase Activities of Isolated Endophytic Fungi A. Control, B. *Aspergillus* sp. (Positive), C. *Curvularia* sp. (Negative), D. *Aspergillus* sp. (Positive)

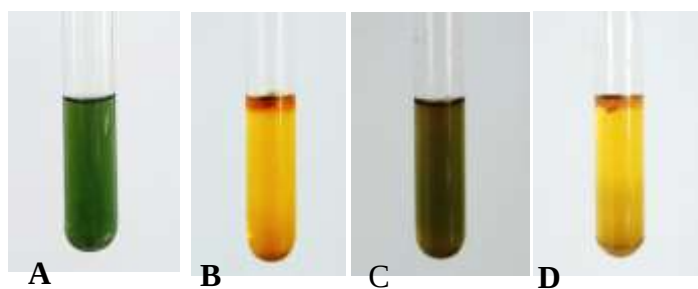


Figure 8 Pectinase Activities of Isolated Endophytic Fungi A. Control, B. *Aspergillus* sp. (Positive), C. *Curvularia* sp. (Negative), D. *Aspergillus* sp. (Positive)

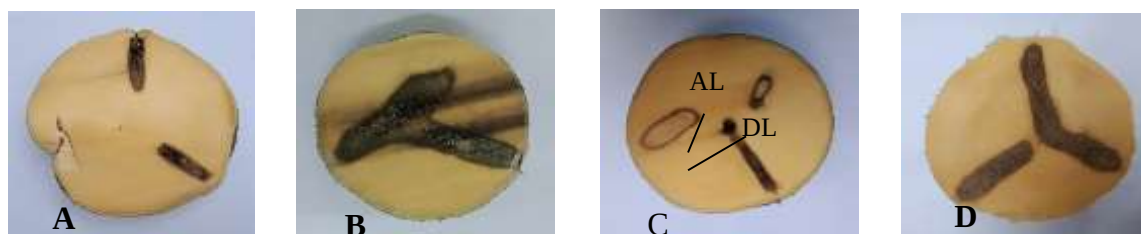


Figure 9 Agarwood formation in the stem of *Aquilaria malaccensis* Lam. after 3-month treatment with selected endophytic fungi A. Potato Dextrose Broth (control) B. *Aspergillus* sp. (CM 2) C. *Curvularia* sp. (CM 6) D. *Aspergillus* sp. (CM 11)

DL = Decaying layer, AL = Agarwood layer



Figure 10 Agarwood formation after 3-month treatment with selected endophytic fungi

A. Potato Dextrose Broth (control) B. *Aspergillus* sp. (CM 2)

C. *Curvularia* sp. (CM 6) D. *Aspergillus* sp. (CM 11)

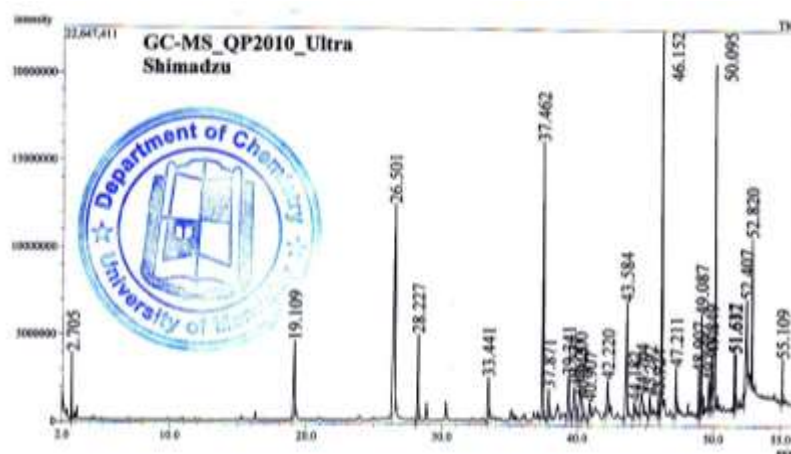


Figure 11 Chromatogram for GC-MS analysis of essential oils from agarwood (CM 11) of *Aquilaria malaccensis* Lam.

Table 4 Chemical composition of essential oils from agarwood (CM 11) of *Aquilaria malaccensis* Lam.

Peak#	R.Time	Area %	Name
2	19.109	3.89	2-Butanone, 4-phenyl-
5	33.441	2.13	2-Butanone, 4-(4-methoxyphenyl)-
8	39.341	2.53	Agarospinol
9	39.771	2.41	(-)-Aristolene
10	40.200	2.74	Guaiol

Discussion

In the present study, the fourteen endophytic fungi were isolated from healthy plant parts such as leaves, bark, wood and fruit of *Aquilaria malaccensis* Lam. The microscopical characters of CM 2 showed that conidiophores were nonseptate, vesicle globose, phialides biserial, conidia globose, brown, one-celled. The microscopical characters of CM 11 showed that conidiophores were nonseptate, vesicle globose, phialides uniserial, conidia globose, hyaline, one-celled. Gilman (1957) stated that the conidiophores of genus *Aspergillus* were nonseptate or septate, usually enlarging upward and broadening into elliptical, hemispherical or globose vesicles bearing phialides; phialides uniserial or biserial; conidia spherical or elliptical, one celled. These characters of CM 2 and CM 11 were in agreement with the statements of Gilman (1957). Therefore, CM 2 and CM 11 were confirmed as *Aspergillus* sp.

The macroscopical characters of CM 6 colony were grey. Reverse colony was pale brown to black in color. Conidiophores were brown, septate. Conidia were pale brown, ellipsoidal, 3-septate. Gilman (1957) stated that conidiophores of genus *Curvularia* were brown, septate, unbranched, conidia long-ellipsoidal, weakly curved, very pale brown, with three cross walls. These characters of strain CM 6 were in agreement with the statements of Gilman (1957). Therefore, CM 6 fungal strain was confirmed as *Curvularia* sp.

Abe *et al.* (2015) reported that the best genera for cellulase production were *Cladosporium*, *Aspergillus* and *Penicillium*. Sunitha *et al.* (2013) reported that the endophytic fungi *Aspergillus* sp. (Ci 10) and *Pestalotiopsis* sp. (Ci 4) isolated from *Calophyllum inophyllum* produced extracellular amylase, pectinase and proteinase. Fareed *et al.* (2017) reported that *Aspergillus calidoustus* fungi show the highest amount of lipase enzyme and *Penicillium marneffe* and *Aspergillus fumigatus* have no production of enzyme. Patel & Bhaskaran (2016) revealed that *Curvularia lunata* showed highest laccase activities (166.2 U/ml) on 3rd day of incubation in submerged medium.

In the present study, the fourteen isolated fungal strains were tested the enzymatic activities such as cellulase, laccase, lipase, protease and pectinase. *Aspergillus* sp. (CM 2 and CM 11) showed positive for cellulase, lipase and pectinase activities. These findings were agreed with Sunitha *et al.* (2013), Abe *et al.* (2015) and Fareed *et al.* (2017).

Curvularia sp. (CM 6) showed laccase activity. These findings were agreed with the statement of Patel & Bhaskaran (2016). All of the isolated endophytic fungi in this study did not show protease activity.

Wahyuni & Prihantini (2020) reported that the averages yield of production agarwood by simpori and implant techniques after six months of inoculation were 0.66 % and 0.64 % respectively. Subasinghe *et al.* (2022) studied agarwood resin inducement method using mycotoxins extracts of selected fungal species such as *Aspergillus niger* and *Fusarium solani* in *Aquilaria crassna*. The average resin contents of the individual trees inoculated with mycotoxins of the *Aspergillus niger* after seven months varied from 0.89 ± 0.35 % to 3.24 ± 0.86 % for a volume of 25 ml, from 0.89 ± 0.36 % to 4.44 ± 1.50 % for 50 ml and from 1.91 ± 0.10 % to 2.20 ± 0.46 % for 100 ml.

Among the fourteen fungal strains, the three strains of endophytic fungi isolated from healthy plant parts were used to assess their potential for induction of artificial infection in *Aquilaria malaccensis* Lam. *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6), *Aspergillus* sp. (CM 11) were isolated from healthy plant parts such as leaves, bark, fruit of *Aquilaria malaccensis* Lam.

The stem of *Aquilaria malaccensis* Lam. was inoculated with selected endophytic fungi and Potato dextrose broth (Control) for inducing agarwood formation. *Aspergillus* sp. (CM 11) after 3 months of inoculation showed better results in average agarwood yield (3.5 %) per tree. The average agarwood yield per tree treated with *Aspergillus* sp. (CM 2), *Curvularia* sp. (CM 6) and Potato dextrose broth (Control) was showed 1.9 %, 0.7 % and 0.2 % respectively. These results were agreed with the statements of Wahyuni & Prihantini (2020) and Subasinghe *et al.* (2022).

Agarospinol is one of the major responsible compounds for fragrance in agarwood. The presence of agarospinol compound in the agarwood confirmed the initiation of infection in the tree. The highest agarospinol content was measured in wood infected by fungi *Penicillium polonicum* (3.33 %) within 3 months (Chhipa & Kaushik 2017). Ahmaed & Kulkarni (2017) stated that agarospinol, benzylacetone, anisylacetone, guaiol and aristolene included in essential oil of *Aquilaria malaccensis* Lam.

In the present study, 2.53 % of agarospinol production was induced successfully using the fungi *Aspergillus* sp. (CM 11) within 3 months only, after inducing the infection. The other chemical constituents in essential oil included 2-Butanone, 4-phenyl- also known as benzylacetone (3.89 %), 2-Butanone, 4-(4-methoxyphenyl)- also known as anisylacetone (2.13 %), guaiol (2.74 %) and (-)-aristolene (2.41 %). These are in the group of sesquiterpenes which is responsible for fragrance in agarwood. These results were agreed with the statements of Chhipa & Kaushik (2017) and Ahmaed & Kulkarni (2017).

Endophyte infection may change the plant hormones, which can induce the plant defensive enzymes and stimulate production of plant secondary metabolites or even new compounds (Yang *et al.* 2022). The series of enzymes produced by endophytic fungi could play a major role in defense mechanism which ultimately becomes responsible for agarwood production.

In the present study, the agarwood resin formation can be induced in *Aquilaria malaccensis* Lam. by using selected endophytic fungi. Among the three fungal strains, *Aspergillus* sp. (CM 2 and CM 11) was indicated promising results (210 g and 600 g) in agarwood production within 3 months of infection. *Aspergillus* genus was confirmed to be the high potential agent for artificial production of agarwood.

Conclusion

The artificial induction of agarwood by means of fungal inoculation in this study has been proven to be a promising technique for agarwood formation. Therefore, *Aspergillus* sp. (CM 11) is an efficient and promising fungal strain for artificial agarwood producing during commercial-scale cultivation of *Aquilaria malaccensis* Lam. which can lead to the ecology and ecological stabilities for communities in near future.

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PALYNOLOGICAL STUDY ON SOME MANGROVE PLANTS FOUND IN SITTWAY COASTAL AREA*

Kyi Kyi Iwin¹, Oo Soe Than², Aye Myat San³

Abstract

Pollen morphology of 14 mangrove species belonging to 10 genera of 9 families were studied, classified and identified by available literatures. Pollen of 14 mangrove species (*Nypa fruticans*, *Ticantio crista*, *Kandelia obovate*, *Excoecaria agallocha*, *Sonneratia apetala*, *S. caseolaris*, *Sesuvium portulacastrum*, *Aegiceras corniculatum*, *A. floridum*, *Vincetoxicum flexuosum* var. *tenuis*, *Acanthus ilicifolius*, *Avicennia alba*, *A. marina*, *A. officinalis*) were observed with a binocular electric microscope. Pollen study area is in Yae- chan- pyin village, Kyauk- tan village, Aung- mingalar village, Nar- yae- kan village and along the Sittway costal, Rakhine State. The pollen key to the species has been constructed based on the acquired palynological assessment. Pollen of all the species have been recorded with aperture number, position, shape, size, and sculpture of the exine; investigated species were pollinia and monads; varying form porate, colpate, colporate to sulcate. The equatorial and polar views of each taxon were recorded by photomicrographs. The present study revealed that the pollen morphology of some mangrove species in study area. Mangrove pollen is crucial for the reproduction of mangrove trees, which are key components of coastal ecosystems. These trees provide habitats for numerous species, contributing to biodiversity. Therefore, the pollen morphology of mangrove plants needs to be protected and regularly assessed.

Keywords: Mangrove; micromorphology; pollen; Sittway costal area

Introduction

The mangrove of the world grows in the tropical and temperate zone of the 123 countries. There are about 73 kinds of trees and in 152,000 square meter area. About 94 species belonging to 67 genera of 41 families in Myanmar. Draft (2005) stated that, 29 species mangroves plants belong to 15 genera were presented in Myanmar. and Cherry Aung (2016) reported that 44 species of true mangroves and associated plants belonging to 22 genera of 14 families were presented. And then, 40 species, true mangroves and associated plants belonging to 28 genera of 21 families were found in Yae-Chan-Pyin and Aung-Mingalar village, Rakhine State (Saw Saw Khine, 2015).

In this study, 14 mangrove species belonging to 10 genera of 9 families of pollen morphology were investigated. Pollen morphology, such as grain size, the number of pores and exine sculpturing are the most distinctive features for species delimitation. Pollen morphology, including its shape and structure, can provide valuable information for identifying and classifying various taxa and solving taxonomic issues within a particular group of plants (Britannica, 2023). This study aimed to determine the pollen morphology of various mangrove species in study area. The study is expected to add value to the taxonomic study of mangrove species and to provide the valuable pollen characters that can be used in plant classification and identification.

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Materials and Methods

The specimens were collected from some Sittway coastal area in January to September 2022. The collected plants were photographed to record their habits, inflorescences, and flowers. Identification of specimens was accomplished in accordance with the taxonomic procedures. By using floristic literature of Gilbert (1994), Dassanayake (1980-2000) and HU Qi-ming and WU Delin (SCGB) (2007-2009). Myanmar names were referred to Hundley and Chit Ko Ko (1987). Pollen samples of specimens were freshly collected from the anther in blooming flowers. According to Erdtman method (1952), the pollen grains were observed with acetolysis method. The terminology used in the identification of pollens was according to Erdtman, (1952), Punt et al. (2007), Hesse et al. (2009) and Halbritter et al. (2018).

Results

In pollen morphology of mangrove plants, 14 species belonging to 10 genera of 9 families have been studied. They were monocotyledonous and eudicotyledonous plants. The families were listed according to APG IV of Byng et al. (2016) systems as follows in Table 1 and the species according to alphabetical order.

Table 1. List of mangrove species studied in Sittway coastal area

Family	Scientific name	Myanmar name	Rakhine name
Arecaceae	1. <i>Nypa fruticans</i> Wurm	Dah-ni	Ye-ohn
Fabaceae	2. <i>Ticanto crista</i> L.	Sukyanbo	Kaung-chit-sue
Rhizophoraceae	3. <i>Kandelia obovata</i> Sheue.	Byu	Byu
Euphorbiaceae	4. <i>Excoecaria agallocha</i> L.	Tayaw	Jakant-pin
Lythraceae	5. <i>Sonneratia apetala</i> Banks,	Kambala	Pyar-zee
	6. <i>Sonneratia caseolaris</i> (L.) Engl	Lamu	Lamu
Aizoaceae	7. <i>Sesuvium portulacastrum</i> (L.) L.	Pinle-teji	Pinle-teji
Primulaceae	8. <i>Aegiceras corniculatum</i> (L.) Blanco	Ye-khaya	Jetrome
	9. <i>Aegiceras floridum</i> Roem. & Schult.	Pya-pin	Sapaung
Apocynaceae	10. <i>Vincetoxicum flexuosum</i> var. <i>tenuis</i> (Blume) Schneidt,	Not known	Ma-line-twat
Acanthaceae	11. <i>Acanthus ilicifolius</i> L.	Kaya	Phad-yan sue
	12. <i>Avicennia alba</i> Blume	La-me	Pya-chun
	13. <i>Avicennia marina</i> (Forssk.) Vierh.	Tha-mai phyu	Pyar-phyu
	14. <i>Avicennia officinalis</i> L.	Thame	Pyar-wine

1. *Nypa fruticans* Wurmb

Family : Arecaceae

Myanmar Name : Dah-ni

Rakhine Name : Ye-ohn

Pollen morphology

Disulcate, zonaulcate, spheroidal, medium, 32.5-38.7 μm in diameter; amb ribbon shaped; sulcus long and narrow, about 0.7x25.0 μm in length and breadth; exine 5.0-6.2 μm thick, sexine slightly thinner than nexine; sculpturing echinate, spines 2.5-5.0 μm in length, straight, pointed, basal cushion 2.0-2.5 μm in width, the interspinal spaces 2.5-3.7 μm in width, the interspinal areas reticulate.

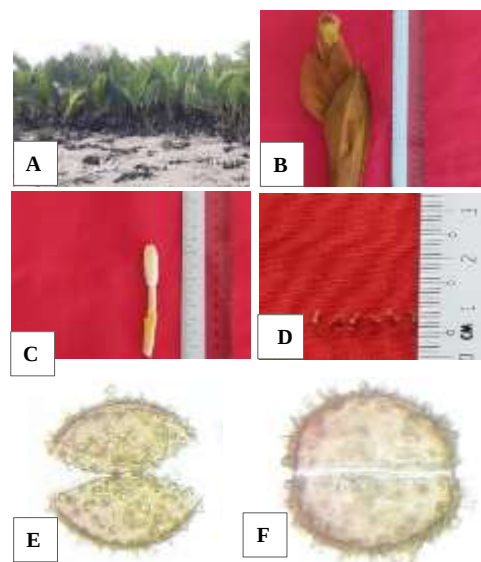


Figure.1 *Nypa fruticans* Wurmb
(A)Habit (B)Inflorescences (C)Flowers
(D)Androecium (E)Polar view (F)Equatorial view

Specimen examined: Sittway area, Kyauk-tan village & Sittway coastal; January 2022.

2. *Ticanto crista* L.

Family : Fabaceae

Myanmar name : Sukyanbo

Rakhine name :Kaung-chit- sue

Pollen morphology

Tricolporate, zonocolporate, subspheroidal, medium, 31.2-38.7 x 38.7-37.5 μm in length and breadth; amb rounded; colpi longicolpate, 25.0-27.5 x 7.5 μm in length and breadth; pori lolongate, about 5.0x2.5 μm in length and breadth; exine 2.0-2.5 μm thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 2.0- 3.7 μm in width, the muri simplibaculate, about 1.2 μm in width

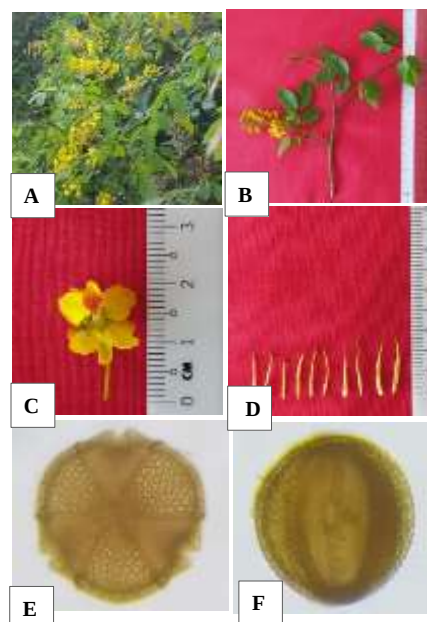


Figure.2 *Caesalpinia crista* L.
(A)Habit (B)Inflorescences (C)Flower
(D)Androecium (E)Polar view (F)Equatorial view
Specimen examined: Sittway area; Aung-mingalar village; June 2022.

3. *Kandelia obovata* Shue.

Family : Rhizophoraceae

Myanmar name : Byu

Rakhine Name : Byu

Pollen morphology

Tricolporate, zonocolporate, subprolate, small, 17.5-18.7 x 14.5-15.0 μm in length and breadth; amb rounded triangular; colpi longicollate, 12.5-15.0 x 2.0-2.5 μm in length and breadth; pori lalongate, 3.0-5.0 x 2.2-2.5 μm in length and breadth; exine 2.0-2.5 μm thick, sexine thinner than nexine; sculpturing striate.

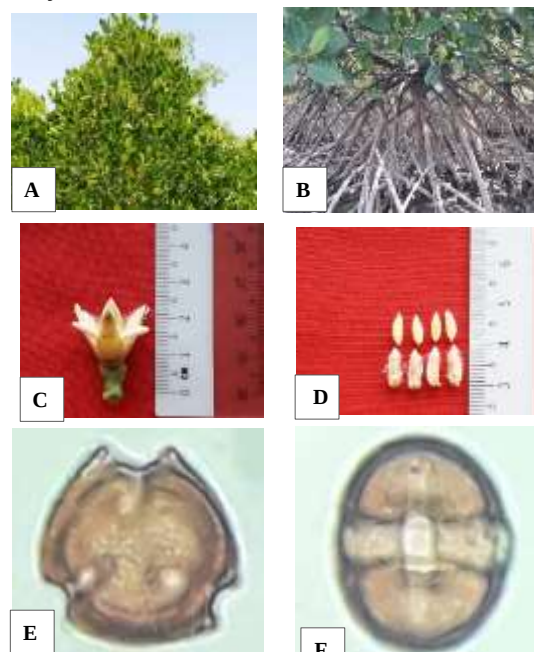


Figure.3. *Kandelia obovata* Shue. (A)Habit (B)Inflorescences (C)Flower (D)Androecium (E)Polar view (F)Equatorial view Specimen examined: Sittway area, Nar-ya-kan village, April 2022.

4. *Excoecaria agallocha* L.

Family : Euphorbiaceae

Myanmar name : Tayaw

Rakhine name : Jakant-pin

Pollen morphology

Tricolporate, zonocolporate, oblate spheroidal, small, 20.0-22.5 x 22.5-25.0 μm in length and breadth; amb rounded; colpi longicollate, 13.7-16.2 x 1.2 μm in length and breadth; pori lalongate, 2.5 x 3.7-5.0 μm in length and breadth; exine 2.0-2.5 μm thick, sexine as thick as nexine; sculpturing striate.

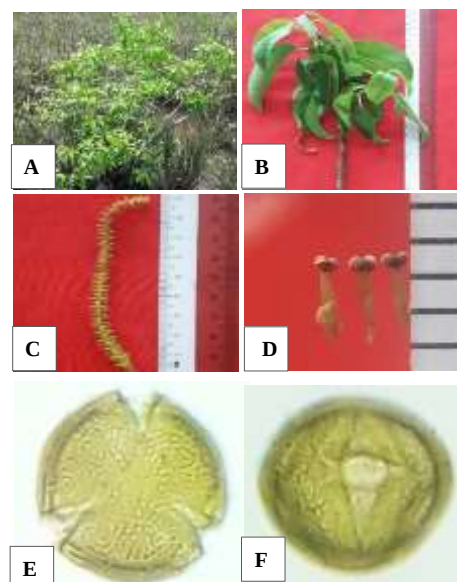


Figure.4. *Excoecaria agallocha* L. (A)Habit (B)Inflorescences (C) Flower (D)Androecium (E) Polar view (F) Equatorial view **Specimen examined:** Sittway area, Yae-chan-pyin, September 2022

5. *Sonneratia apetala* Banks,

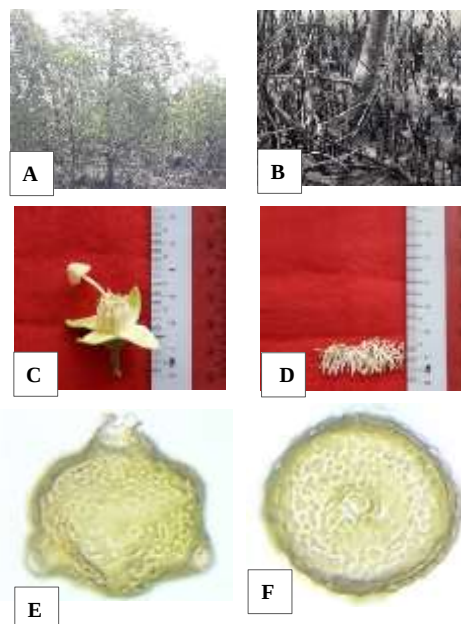
Family : Lythraceae

Myanmar name : Kambala

Rakhine name : Pyar-zee

Pollen morphology

Triporate, zonoporate, oblate spheroidal, small, 19.5-23.7 x 20.0-25.0 μm in length and breadth; amb rounded hexagonal; 3 pseudocolpi present; pori circular (crassi marginate), about 3.0 μm in diameter; exine 2.5-3.7 μm thick, sexine thicker than nexine; sculpturing gemmate.

**Figure.5 *Sonneratia apetala* Banks**

(A)Habit (B)Roots (C)Flower (D)Androecium

(E)Polar view (F)Equatorial view

Specimen examined: Sittway area, Nar-yae-kan, September 2022.

6. *Sonneratia caseolaris* (L.)**Engl.**

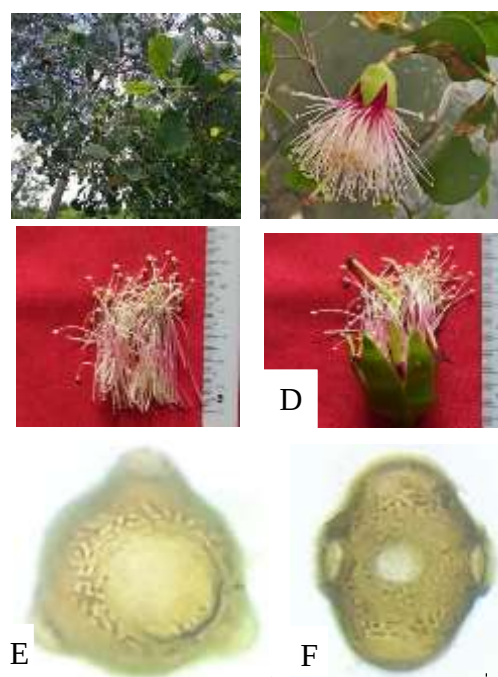
Family : Lythraceae

Myanmar name : Lamu

Rakhine Name : Lamu

Pollen morphology

Triporate, zonoporate, prolate, medium, 27.5-30.0 x 21.7-22.5 μm in length and breadth; amb rounded hexagonal; 3 pseudocolpoidorate; pori circular, surrounded by distinctly thin collar, 4.7-5.0 μm in diameter; exine 2.5-3.5 μm thick, sexine thicker than nexine; sculpturing gemmate.

**Figure.6 *Sonneratia caseolaris* (L.) Engl.**

(A)Habit (B)Inflorescences (C) Flower

(D)Androecium(E)Polar view (F) Equatorial view

Specimen examined: Sittway area; Nar-yae-kan; January 2022

7. *Sesuvium portulacastrum* (L.)

Family : Aizoaceae

Myanmar Name : Pinle-teji

Rakhine Name : Pinle-teji

Pollen morphology

Tricolpate, zonocolpate, oblate spheroidal, small, 13.7-15.0 x 15.0-17.0 μm in length and breadth; amb rounded; colpi longicollpate, 11.2-13.7 x 5.0-7.5 μm in length and breadth; exine 1.2-2.0 μm thick, sexine as thick as nexine; sculpturing striate.

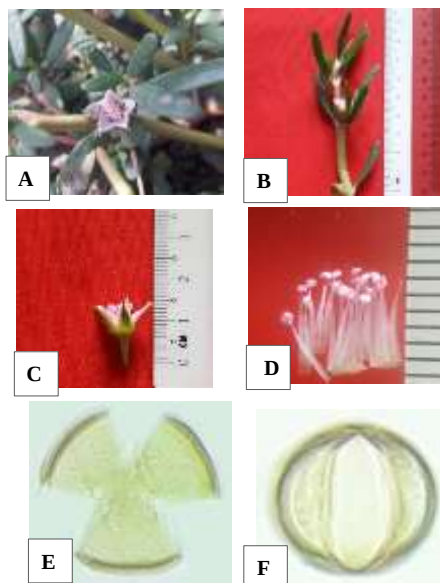


Figure.7 *Sesuvium portulacastrum* (L.)

(A)Habit (B)Inflorescences (C)Flower

(D) Androecium (E)Polar view (F) Equatorial view

Specimen examined: Sittway area; Yae-chan-pyin; May 2022

8. *Aegiceras corniculatum* (L.) Blanco

Family : Primulaceae

Myanmar Name : Ye-khaya

Rakhine Name : Jetrome

Pollen morphology

Tricolporate, zonocolporate, prolate spheroidal, small, 12.7-15.5 x 12.5-15.0 μm in length and breadth; amb rounded; colpi longicollpate, 10.0-11.2 x 0.7 μm in length and breadth; pori lalongate, 2.5 x 4.5-5.0 μm in length and breadth; exine 2.0-2.5 μm thick, sexine as thick as nexine; sculpturing reticulate, the lumina heterobrochate 1.2-2.5 μm in width, the muri simplibaculate about 0.5 μm in width.

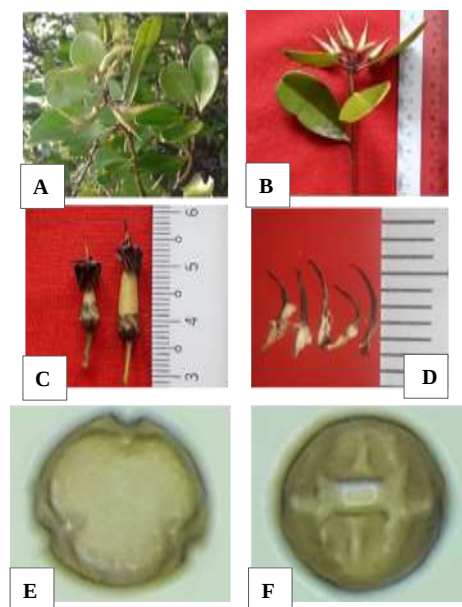


Figure 8. *Aegiceras corniculatum* (L.) Blanco

(A)Habit (B)Inflorescences (C) Flower

(D)Androecium (E)Polar view (F) Equatorial view

Specimen examined: Sittway area; Yae-chan-pyin; May 2022

9. *Aegiceras floridum* Roem. & Schult.

Family : Primulaceae

Myanmar Name : Pya-pin

Rakhine Name : Sapaung

Pollen morphology

Tricolpate, zonocolpate, oblate, large, 37.5-50.0 x 62.5-72.5 μm in length and breadth; amb rounded triangular; colpi longicolpate, 27.5-35.0 x 6.2-17.5 μm in length and breadth; exine 3.7-5.0 μm thick, sexine thicker than nexine; sculpturing retipilate.



Figure 9. *Aegiceras floridum* L.
(A)Habit (B)Inflorescences (C) Flower
(D)Androecium (E)Polar view
(F) Equatorial view **Specimen examined:**
Sittway area; Yae-chan-pyin; May 2022

10. *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt,

Family : Apocynaceae

Myanmar Name : Notknown

Rakhine Name : Ma-line-twat

Pollen morphology

Pollinia (a coherent mass of pollen grains), pollinial sac 51.2-73.7 x 27.5-50.0 μm in length and breadth, obovate shaped with rounded at the apex, golden yellow, orientation of pollinum pendulus; corpusculum 67.5-71.2 x 16.2-18.7 μm in length and breadth, linear oblong shaped of head, dark brown; translator arms 57.5-75.0 x 15.0-16.2 μm in length and breadth, cylindrical in shape, creamy yellow, the translator attachment to the pollinial terminal.

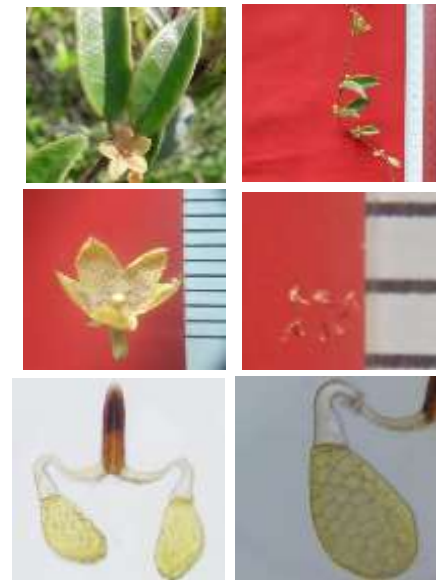


Figure 10. *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt,
(A)Habit (B)Inflorescences (C)Flower
(D)Androecium (E) Pollinia (F)Pollinium
Specimen examined: Sittway area; Nar-yae-kan; June 2022

11. *Acanthces ilicifolius* L.

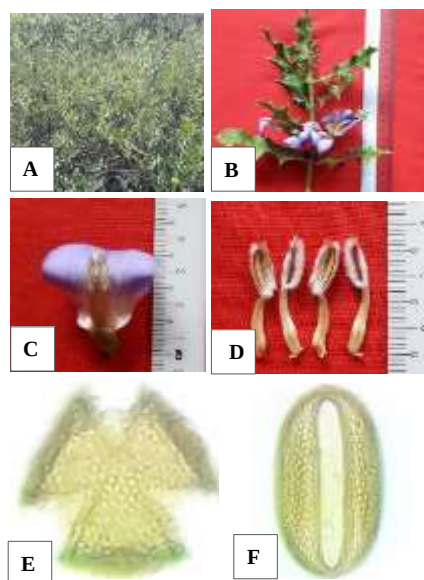
Family : Acanthaceae

Myanmar Name : Kaya

Rakhine Name : Phet-yan-sue

Pollen morphology

Tricolpate, zonocolpate, perprolate, small, 32.5-37.5 x 17.5-22.5 μm in length and breadth; amb triangular; colpi longicolpate, 37.5-38.7 x 4.5-5.0 μm in length and breadth; exine 2.0-2.5 μm thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 1.2-2.0 μm in width, the muri simplibaculate, about 0.7 μm in width.

**Figure 11. *Acanthus ilicifolius* L.**

(A)Habit (B)Inflorescences

(C)Flower(D)Androecium (E)Polar view

(F) Equatorial view **Specimen examined:**

Sittway area; Yae-chan-pyin; May 2022

12. *Avicennia alba* Blume.

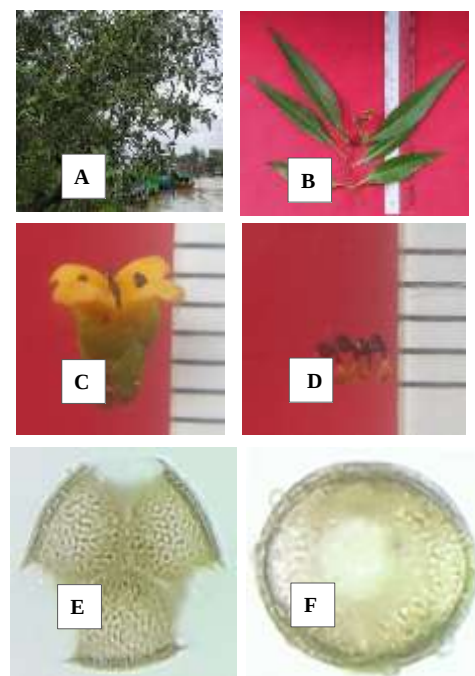
Family : Acanthaceae

Myanmar Name : La-me

Rakhine Name : Pyae shun

Pollen morphology

Tricolporate, zonocolporate, suboblate, small, 16.2-18.7 x 20.0-22.5 μm in length and breadth; amb rounded triangular; colpi syncolpate, 13.0-15.0 x 4.7-5.0 μm in length and breadth; pori lolongate, 7.5-8.7 x 6.2-7.5 μm in length and breadth; exine 2.0 μm thick, sexine thicker than nexine; sculpturing striato-reticulate, the lumina heterobrochate, 1.2-2.0 μm in width, the muri simplibaculate, about 0.7 μm in width.

**Figure 12. *Avicennia alba* Blume.**

(A)Habit (B)Inflorescences (C)

Flower(D)Androecium (E)Polar view

(F) Equatorial view **Specimen examined:**

Sittway area; Yae-chan-pyin; April 2022

13. *Avicennia marina* (Forssk) Vierth.

Family : Acanthaceae

Myanmar Name : Tha-mai-phyu

Rakhine Name : Pyar-phyu

Pollen morphology

Tricolporate, zonocolporate, oblate spheroidal, small, 19.7-20.2 x 20.0-22.5 μm in length and breadth; amb rounded triangular; colpi longicollate, 14.7-15.0 x 3.7-5.0 μm in length and breadth; pori lolongate, 7.5-8.7 x 5.0-6.5 μm in length and breadth; exine about 2.5 μm thick, sexine as thick as nexine; sculpturing striate.

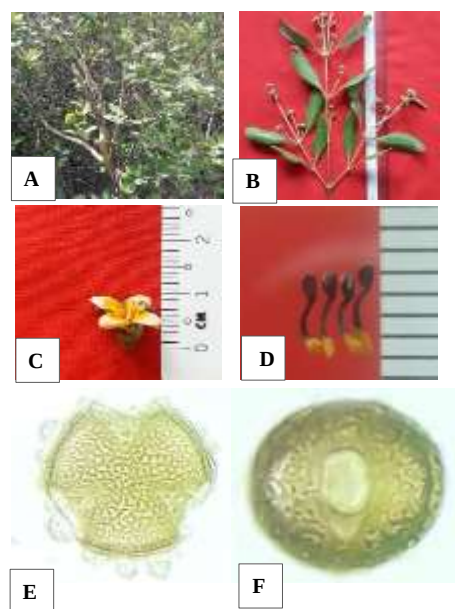


Figure 13. *Avicennia marina* (Forssk) Vierth (A) Habit (B) Inflorescences (C) Flower (D) Androecium (E) Polar view (F) Equatorial view **Specimen examined:** Sittway area; Yae-chan-pyin; May 2022

14. *Avicennia officinalis* L.

Family : Acanthaceae

Myanmar Name : Thame

Rakhine Name : Pyar wine

Pollen morphology

Tricolporate, zonocolporate, oblate spheroidal, small, 20.5-22.5 x 23.0-25.0 μm in length and breadth; amb rounded triangular; colpi longicollate, 15.0-17.2 x 6.2-7.5 μm in length and breadth; pori lolongate, 7.5 x 5.7-5.0 μm in length and breadth; exine 2.0-2.5 μm thick, sexine thinner than nexine; sculpturing striato-reticulate, the lumina heterobrochate, 0.7-1.2 μm in width, the muri simplibaculate, about 0.2 μm in width.

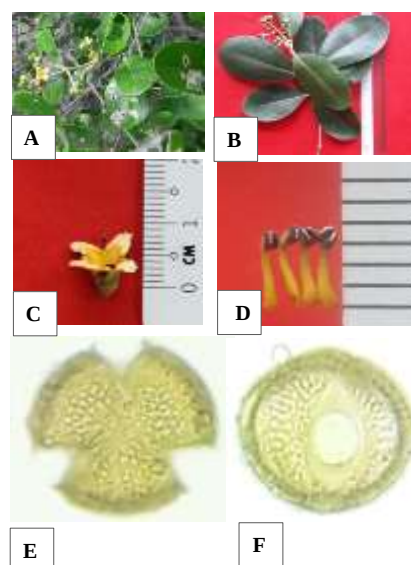


Figure 14. *Avicennia officinalis* L. (A) Habit (B) Inflorescences (C) Flower (D) Androecium (E) Polar view (F) Equatorial view **Specimen examined:** Sittway area; Yae-chan-pyin; May 2022

Artificial key to the species by using pollen morphology

1. Pollen grains pollinia ----- 10. *Vincetoxicum flexuosum*
1. Pollen grains free from each other -----2
 2. Aperture sulcate or colpate -----3
 2. Aperture porate or colpate -----6
3. Aperture disulcate ----- 1. *Nypa fruticans*
3. Aperture tricolpate ----- 4
 4. Grains large; exine more than 3.5 μm thick ----- 9. *Aegiceras floridum*
 4. Grains small; exine less than 2.5 μm thick-----5
5. Shape perprolate; sculpturing distinctly reticulate-----11. *Acathus ilicifolius*
5. Shape oblate spheroidal; sculpturing striate----- 7. *Sesuvium portulacastrum*
 6. Aperture porate-----7
 6. Aperture colpate-----8
7. Grains small; shape oblate spheroidal ----- 5. *Sonneratia apetala*
7. Grains medium; shape prolate ----- 6. *Sonneratia caseolaris*
 8. Pori lalongate -----9
 8. Pori lolongate -----10
9. Sculpturing striate ----- 4. *Excoecaria agallocha*
9. Sculpturing reticulate ----- 8. *Aegiceras corniculatum*
 10. Sexine thicker than nexine ----- 11
 10. Sexine as thick as or thinner than nexine -----12
11. Grains small; colpi syncolpate -----12. *Avicennia alba*
11. Grains medium; colpi longicolpate ----- 2. *Ticanto crista*
 12. Sexine as thick as nexine -----13. *Avicennia marina*
 12. Sexine thinner than nexine -----13
13. Shape subprolate; grains size 14.5-15.0 μm in breadth -----3. *Kandelia obovata*
13. Shape oblate spheroidal; grains size 23.0-25.0 μm in breadth -----14. *Avicennia officinalis*

Discussion and Conclusion

Pollen of 14 mangrove species in Sittway coastal area were observed with a binocular electric microscope. All the species are classified as true mangrove species. Most species belong to the Acanthaceae (4 species) followed by Primulaceae (2 species), Lythraceae (2 species), Arecaceae (1 species), Fabaceae (1 species), Rhizophoraceae (1 species), Euphorbiaceae (1 species), Aizoaceae (1 species) and Apocynaceae (1 species).

Pollen characters of mangrove species were studied, namely the ratio of polar/ equatorial (P/E), shape, size, outline, polar view, equatorial view, aperture and ornamentation.

The types of pollen grains found in the study were pollinia and monads; pollinia in only one species (*Vincetoxicum flexuosum* var. *tenuis*) and the rest species were monads.

With respect to the number of apertures, pollen may be classified as inaperturate and aperturate. In aperturate, the investigated pollens were sulcate, colpate, colpoate and porate. Disulcate aperture was found in *Nypa fruticans*; tricolpate was found in *Sesuvium portulacastrum*, *Aegiceras floridum* and *Acathus ilicifolius*; triporate was found in *Sonneratia apetala* and *Sonneratia caseolaris*; tricolporate was found in the rest species.

Based on the shape of equatorial view, angiosperms pollen can be grouped into 9 classes such as peroblate, oblate, subspheroidal, suboblate, oblate spheroidal, spheroidal, subprolate, prolate and perprolate (Erdtman, 1946 & Walker and Doyle, 1975). In this research, pollen shape was described by the polar axis and equatorial diameter ratio: spheroidal, subspheroidal, oblate spheroidal, oblate, suboblate, subprolate, prolate and perprolate were found.

Schoch-Bodmer (1940) has demonstrated that the size of pollen grains depends on the state of nutrition of the plant. Heese et al. (2009) and Halbritter et al. (2018) stated that pollen size varies less than 10 µm to more than 100 µm. In the present study, the large pollen size was found in *Aegiceras floridum*; medium was found in *Nypa fruticans*, *Ticanto crista*, *Sonneratia caseolaris* and *Vincetoxicum flexuosum* var. *tenuis* while the rest species were small grains.

In this study, variation in pollen grains outline was discovered, specifically obovate, rounded, rounded triangular, rounded hexagonal, ribbon shaped, triangular.

In palynology, ornamentation is applied to study the surface feature of pollen. Walker and Doyle (1975) stated that the sculpture patterns' appearance also varies significantly from one species to another. In this research, the sculpture patterns of the pollens were observed echinate, striate, gemmate, and various reticulate patterns.

In 2016, Cherry Aung conducted a research work on the mangrove of Myanmar and collected 44 species altogether; the study of mangrove Myanmar by draft, 2005 and he accomplished recordings 29 species. But this research is done on palynological study of some mangrove plants found in Sittway coastal areas. No one has yet worked on the pollen morphology of mangrove plants from Sittway coastal areas. The benefit of mangroves is improving the water quality flowing from rivers and streams into the estuaries and ocean environment. Therefore, mangrove scientists around the world are involved in studying pollen from different points of view and from different points of interest and practical uses.

The aim of the current study is to provide knowledge on pollen morphology of mangrove plants. The importance of pollen in environmental studies, ancient geography of the continents,

climate in certain places were also informed in this paper. It is sincerely hoped that the present study will support as a useful information for further studies and identification of families distributed in Myanmar.

Acknowledgements

We are greatly indebted to Dr Thet Thet Mar Win, Professor and Head, Department of Botany, University of Yangon for her kind permission to apply this paper.

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COMPARATIVE STUDY ON HISTOLOGICAL CHARACTERS, PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *ANDROGRAPHIS PANICULATA* (BURM.F.) WALL. EX NEES AND *ANDROGRAPHIS ECHIOIDES* (L.) NEES

Moe Moe¹, Ei Ei Phyu², Than Than Soe³, Thet Thet Zin⁴

Abstract

Andrographis paniculata (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees belong to the family Acanthaceae. These species were collected from Patheingyi Township, Mandalay Region, during August 2022 to March 2023. They are known as traditional medicinal plants not only in Myanmar but also in Asia. The present studies were undertaken to establish a comparative study of morphological, histological characters, phytochemicals analysis, antioxidant and antimicrobial activities of two species. The morphological studies of two species have annual erect herbs. The leaves of *A. paniculata* (Burm. f.) Wall. ex Nees was lanceolate, glabrous on both surfaces. *A. echioides* (L.) Nees was oblong, pubescent on both surfaces. In histological characters, diacytic types of stomata found only on abaxial surface and stomata index were 13.3-21.43 in *A. paniculata* (Burm. f.) Wall. ex Nees. *A. echioides* (L.) Nees observed on both surfaces and stomata index were 14.28-25.0, 20.0-33.33. The phytochemical analysis of aqueous leaves extract showed the presence of high number phytochemicals when compared with ethanol and methanol. It showed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, proteins and reducing sugar in both species. The free radicals scavenging of leaves of two species have excellent antioxidant activity showing IC₅₀ value 5.98 and 3.44 mg/l. Antimicrobial activities of the different solvent extracts of leaves were tested on five pathogenic microorganisms. The antibacterial activity of the chloroform extracts of *A. paniculata* (Burm.f.) Wall. ex Nees were tested against *Micrococcus luteus*. In *A. echioides* (L.) Nees, ethyl-acetate extract was found active against the strains of *Pseudomonas albicans*. The results of present studies can serve as a useful tool in the identification, authentication, and efficacy of the plants, which leads to pharmaceutical application.

Keywords: Morphology, histology, phytochemical, antioxidant, pathogenic microorganisms

Introduction

Acanthaceae represent about 229 genera and 3450 species, distributed in the tropical regions, Central America, Brazil and Africa. Plants are herbs, shrubs, small trees with cystoliths. Leaves are simple, opposite, and exstipulate. Flowers bisexual, hypogynous with bracts and bracteoles. Fruit elastically dehiscent bivalve capsule. Seeds are often on hook like outgrowths the so called retinacula or jaculators (Ankush and Sicjandra. 2023).

The genus *Andrographis* comprising of about 40 species, is essentially distributed in southern India and in tropical Asia. *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) Nees plants have mostly in dry places, such as India, Sri Lanka and South Asian countries. In Myanmar, *A. paniculata* (Burm.f.) all. ex Nees is commonly known as Se ga gyi or Shan Se ga gyi. It has various pharmacological effects and used as medicinal herb

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for treating upper gastrointestinal tract, upper respiratory infections, herpes, other chronic diseases and its constituents relating to Covid-19 (Nyeem *et al.* 2017, Hossain *et al.* 2021).

A. echinoides (L.) Nees is commonly known as Se ga gyi hmwe htu. It is highly medicinal important and used in liver diseases, bacterial malaria, fungal disease and antioxidant, and anti-inflammatory (Pande *et al.* 2018). The leaf juice of this plant is used to treating fever (Elaiyaraja *et al.* 2015). Both species are widely distributed in Mandalay Region. Medicinal plants have been a long history of usage in traditional medicine. The authentic knowledge of the usage of medicinal plants passed from one generation to another, after refining and addition (Haq *et al.* 2011).

Medicinal plants are an important source of valuable therapeutic agents, both in modern and in traditional medicine. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid and phenolic compounds. Plants act as antimicrobial compounds due to the presence of secondary metabolites. The medicinal properties and capabilities of the plant, the chemical analysis of phytochemical content and antioxidant activity assay. Antioxidants help in preventing degenerative diseases such as cancer, heart disease and even altitude sickness (Keita *et al.* 2022). Both species are widely used as traditional medicine. In Mandalay Region, almost every household cultivated *A. paniculata* (Burm.f.) Wall. ex Nees in the style of homegardens.

In this study, general aim and objectives to promote green economy and green growth through economic scale cultivation of these species. Specific aim and objectives to use as tool in the identification, authentication and standardization of the plant material in pharmaceutical application because of having potential for manufacturing pallet for traditional medicine.

Materials and Methods

Plant Sample collection

Andrographis paniculata (Burm.f.) Wall. ex Nees and *Andrographis echinoides* (L.) Nees were collected from Patheingyi Township, Mandalay Region, during August 2022 to March 2023. The collected specimens were studied at the Department of Botany, University of Mandalay. The morphological studies of the plants were identified with the help of literatures; Backer *et al.* (1965), Hooker (1885), Dassanayake (1998) and Kress *et al.* (2003).

Histological Studies

According to Johnsen's method (1940), killing and fixation, dehydration, infiltration, embedding, staining and mounting were made. Free hand section was made by using chloral hydrate solution and a new razor blade and stomatal index was calculated according to Kokate (2000), Metcalfe & Chalk (1957). Histological studies were carried out Department of Botany, University of Mandalay.

Phytochemical Screening Analysis

Phytochemical screening analysis of aqueous, ethanol and methanol leaves extract of two species were carried out using standard procedures to identify the presence of secondary metabolites as described by Harbone (1998), Sofowora (1993). Phytochemical analysis were

examined at Department of Botany, University of Mandalay, University Research Center (URC), University of Mandalay.

Determination of Antioxidant Activity Analysis by DPPH Method

The antioxidant activity of leaves extracts of two species were assessed by the ability of the extract to scavenge DPPH free radicals. Ascorbic acid was used as the standard compound. The antioxidant activity of samples were shown as % inhibition of DPPH absorbance reported by Sharma & Bhat (2009) at the U Kar Ka Food Testing Laboratory, Mandalay, Myanmar.

Antimicrobial Activity Analysis

The antimicrobial activity analysis of two species leaves were performed by agar well diffusion method reported by Balouiri *et al.* (2016) at the Microbiology Laboratory in Department of Botany, University of Mandalay. Antimicrobial activities of the different solvents extract of leaves were tested on five pathogenic microorganisms.

Results

Morphological Studies

Table 1. Comparative morphological characters of *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Andrographis echiodides* (L.) Nees

Scientific Name	<i>A. paniculata</i> (Burm.f.) Wall. ex Nees	<i>A. echiodides</i> (L.) Nees
Myanmar Name	Se ga gyi	Se ga gyi hmwe tu
English Name	King of bitter	False water willow
Habit	Annual herb	Annual herb
Stem shaped	Quadrangular stem, glabrous	Quadrangular stem with hairy
Leaves	Simple, opposite and decussate, glabrous on both surfaces	Simple, opposite and decussate, pubescent on both surfaces
Leaf base	Cuneate	Cuneate
Leaf apex	Acute	Obtuse
Inflorescence	Terminal or axially paniculate racemes	Terminal or axially paniculate racemes, divericate
Flowers	White with purple spots, glabrous, bisexual, zygomorphic, hypogynous	White or pink with dark purple spots, pubescent, bisexual, zygomorphic, hypogynous
Androecium	Stamens 2, epipetalous, exserted, white, anther ditheous, basifixed	Stamens 2, epipetalous, exserted, white, anther ditheous, basifixed

Scientific Name	<i>A. paniculata</i> (Burm.f.) Wall. ex Nees	<i>A. echioides</i> (L.) Nees
Gynoecium	Carpel 2, fused, Ovary superior, ovoid. many ovule in each locule on the axile placenta, stigma bifid	Carpel 2, fused, Ovary superior, ovoid. many ovule in each locule on the axile placenta, stigma bifid
Fruit type	Loculicidal capsule, linear-oblong acute at both ends, glabrous	Loculicidal capsule, linear-oblong acute at both ends, pubescent
Seed	12 seeded, ovate, nonendospermic	4 seeded, oblongoid, nonendospermic

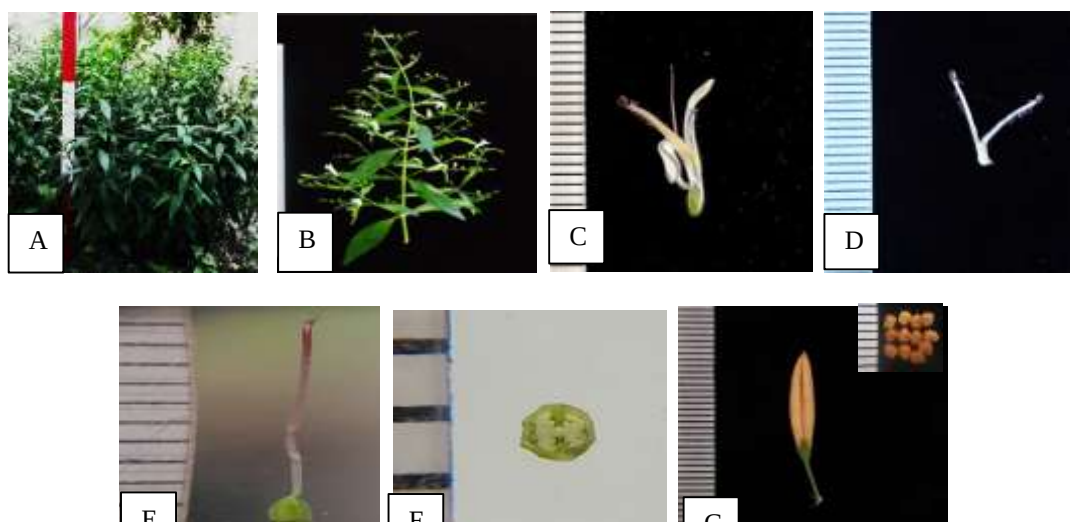


Figure 1. Morphological characters of *Andrographis paniculata* (Burm.f.) Wall. ex Nees

A. Habit B. Inflorescence C. L.S of flower D. Androecium
E. Gynoecium F. T.S of ovary G. Fruit and seeds

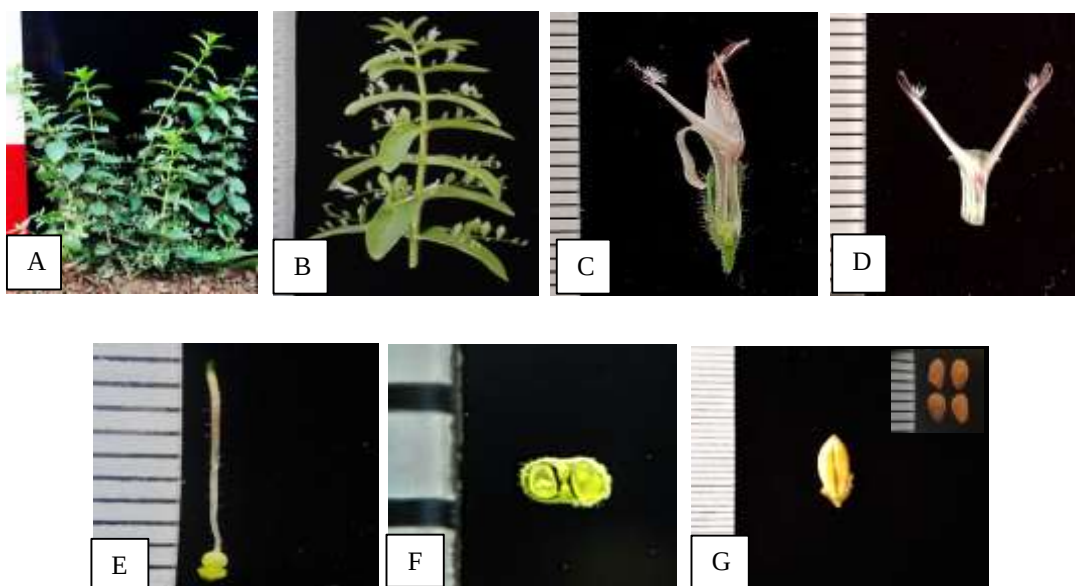


Figure 2. Morphological characters of *Andrographis echioides* (L.) Nees

A. Habit B. Inflorescence C. L.S of flower D. Androecium
E. Gynoecium F. T.S of ovary G. Fruit and seeds

Histological Studies

Table 2. Comparative histological characters of *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Andrographis echiodides* (L.) Nees

Plant part	<i>A. paniculata</i> (Burm.f.) Wall. ex Nees	<i>A. echiodides</i> (L.) Nees
Shape of petiole	Rectangular shape	Crescent shape
Shape of midrib	Triangular	Rectangular
Stomatal type & Stomatal Index	Diacytic stomata only in lower surface and 13.3 - 21.43	Diacytic stomata on both surfaces and 14.28 - 25.0, 20.0 - 33.33
Vascular bundle	Crescent shape and collateral	Crescent shape and collateral
Cystoliths	Round	Elongated
Trichomes	Uniseriate unicellular, sessile glandular trichomes	Uniseriate multicellular, non-glandular trichomes
Stem shape	Quadrangular with distinct wing	Quadrangular with slightly distinct wing

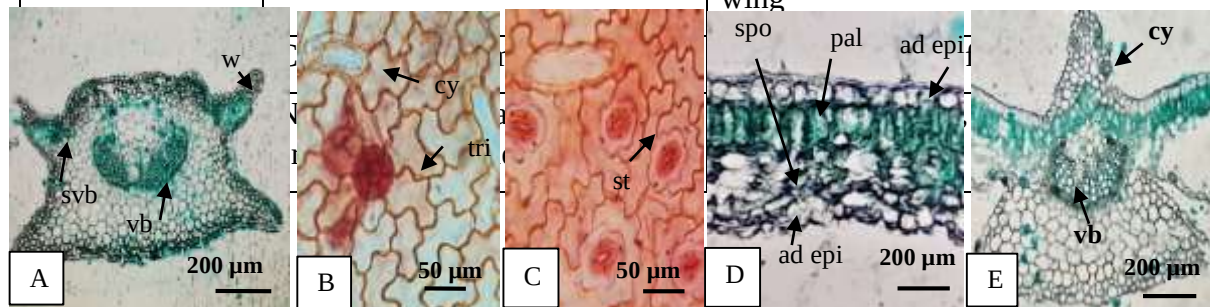


Figure 3. Internal structure of leaf of *Andrographis paniculata* (Burm.f.)Wall. ex Nees

A. T.S of petiole B. Adaxial surface view of lamina C. Abaxial surface view of lamina showing diacytic types of stomata D. T.S of lamina E. T.S of midrib

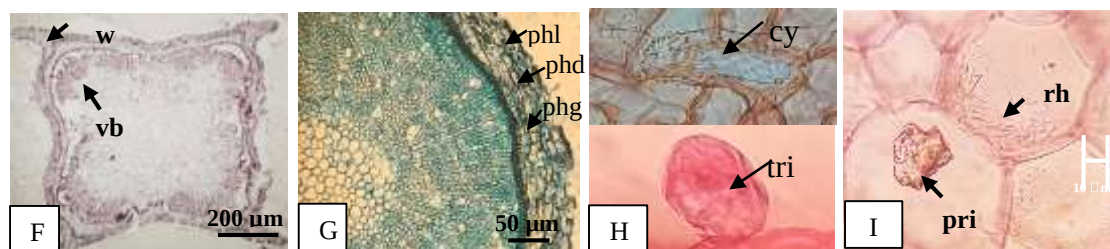


Figure 4. Internal structure of stem & root of *Andrographis paniculata* (Burm.f.) Wall. ex Nees

F. T.S of stem G. T.S of root H. Trichome & cystolith I. Prismatic & needle shaped

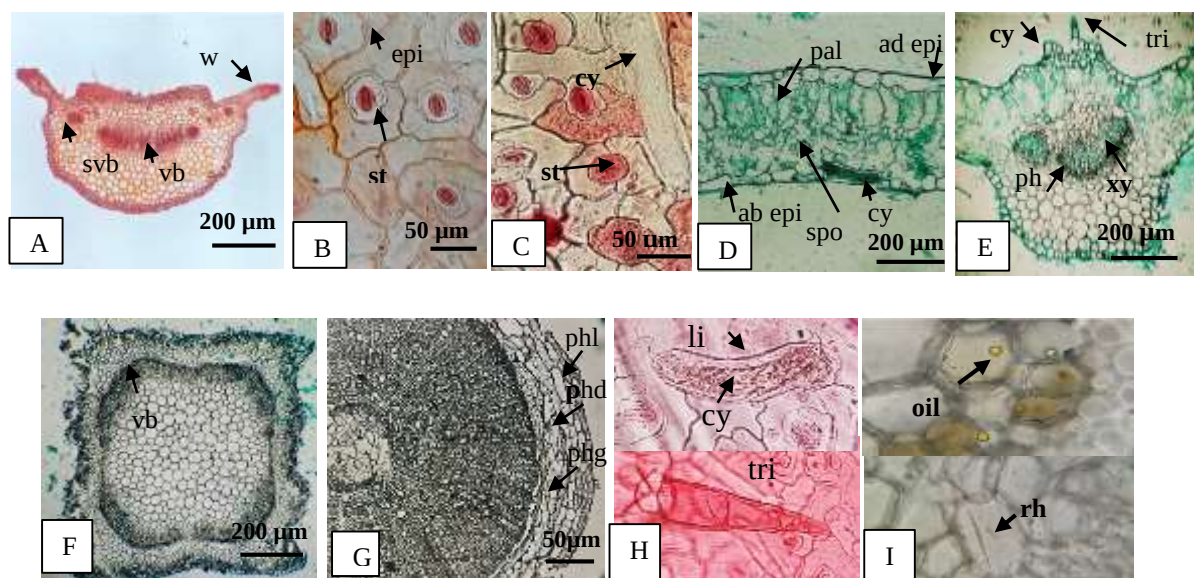


Figure 5. Internal structure of leaf, stem & root of *Andrographis echioides* (L.) Nees

A. T.S of petiole B. Adaxial surface view of lamina C. Abaxial surface view of lamina showing diacytic types of stomata D. T.S of lamina E. T.S of midrib showing a vascular bundle F. T.S of stem G. T.S of root H. Trichome & cystolith I. Needle shaped & oil globule

ad epi= adaxial epidermal cell, ab epi= abaxial epidermal cell, svb= small vascular bundle, pal= palisade parenchyma cell, spo= spongy parenchyma cell, tri= trichome, vb= vascular bundle, ph= phloem xy= xylem, epi= epidermis, w= wing, st= stomata, li= lithocyst, pri=prismatic crystal, rh= single raphide

Preliminary phytochemical Analysis

Table 3. Phytochemical analysis of powder leaves extract were examined by using various chemical reagents for the presence or absence of chemical constituents

No.	Phytochemical	<i>A. paniculata</i> (Burm.f.) Wall. ex Nees				<i>A. echioides</i> (L.) Nees			
		H ₂ O	EtOH	MeOH	Observation	H ₂ O	EtOH	MeOH	Observation
1.	Alkaloids (Mayer's reagent)	+	+	+	Cream ppt.	+	+	+	Cream ppt
	Alkaloids (Wagner's reagent)	+	+	+	Reddish brown ppt.	+	+	+	Reddish brown ppt
	Alkaloids (Hager's reagent's)	+	+	+	Yellow ppt.	+	+	+	Yellow ppt.
2.	Steroids	+	+	-	Upper reddish	+	-	-	Upper reddish
3.	Terpenoids	-	+	-	Lower greenish yellow	-	-	-	No Lower greenish yellow

No.	Phytochemical	<i>A. paniculata</i> (Burm.f.)Wall. ex Nees				<i>A. echiodides</i> (L.) Nees			
		H ₂ O	EtOH	MeOH	Observation	H ₂ O	EtOH	MeOH	Observation
4.	Glycosides	+	-	+	Yellow	+	-	+	Yellow
5.	Phenolic compounds	+	+	+	Dark green	+	+	+	Dark green
6.	Flavonoids	+	+	+	Pale green	+	+	+	Yellow
7.	Tannins	+	+	+	Black	+	+	+	Black
8.	Saponins	+	+	-	Frothing	+	-	+	Frothing
9.	α - amino acids	+	+	+	Purple spot	+	+	+	Purple spot
10.	Proteins	+	+	+	Yellow ppt	+	+	+	Yellow ppt
11.	Reducing sugars	+	+	+	Green	+	-	+	Green
12.	Starch	+	+	+	Black	-	+	+	Black
13.	Carbohydrates	+	-	-	Purple ring	+	-	-	Purple ring
14.	Cyanogenic glycosides	-	-	-	No brickish color	-	-	-	No brickish color

(+) = present, (-) = absent, ppt= precipitate, H₂O= Aqueous, EtOH= Ethanol, MeOH= Methanol

Antioxidant capacity determination by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay

Table 4. Analysis of DPPH radical scavenging of leaves extract of *Andrographis. paniculata* (Burm.f.) Wall. ex Nees and *Andrographis. echiodides* (L.) Nees

No.	Extract Name	Sample concentration	Absorbance	% inhibition	IC ₅₀ Value mg/l
1.	Ascorbic acid	50	0.264	89.41459503	6.75
		25	0.724	70.97032879	
		12.5	1.123	54.97193264	
		6.25	1.390	44.26623897	
		3.125	1.511	39.41459503	
1	Hot water extract of <i>A. paniculata</i> (Burm.f.) Wall. ex	100	1.169	53.12751	5.98
		50	1.210	51.48356	
		25	1.217	51.20289	
		12.5	1.231	50.64154	

No.	Extract Name	Sample concentration	Absorbance	% inhibition	IC ₅₀ Value mg/l
	Nees	6.25	1.240	50.28067	
2	Hot water extract of <i>A. echioides</i> (L.) Nees	100	1.203	51.95687	3.44
		50	1.217	51.39776	
		25	1.227	50.99840	
		12.5	1.230	50.87879	
		6.25	1.233	50.75879	

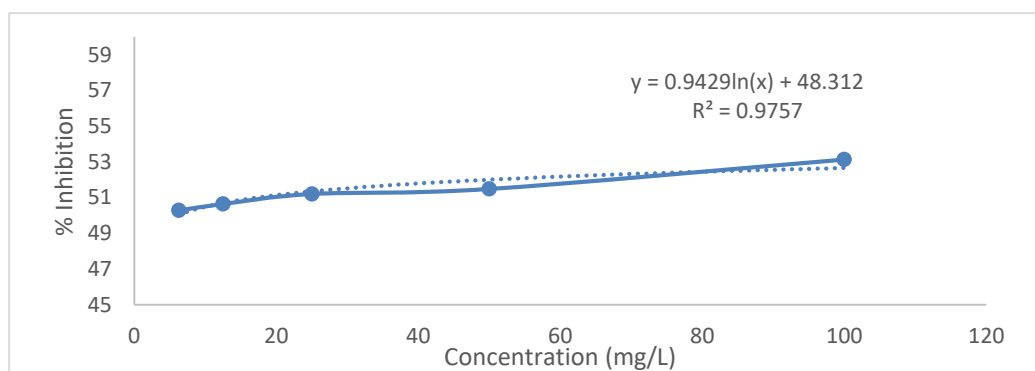


Figure 6. % inhibition & Different concentration of leaves of *A. paniculata* (Burm.f.) Wall. ex Nees

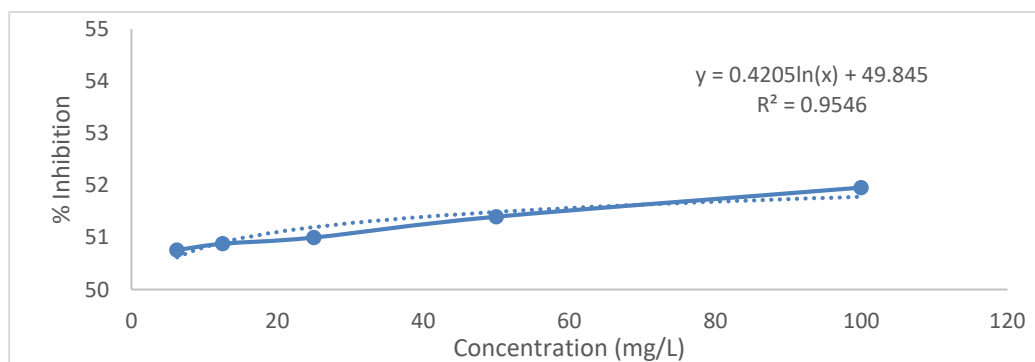


Figure 7. % inhibition & Different concentration of leaves of *A. echioides* (L.) Nees

Antimicrobial Activity Analysis

Table 5. Antimicrobial activity of various solvents from powdered leaves of *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Andrographis echioides* (L.) against five pathogenic microorganisms

Extract	Inhibition zone diameter(mm)									
	<i>A. paniculata</i> (Burm.f.) Wall. ex Nees					<i>A. echioides</i> (L.) Nees				
	<i>Escherichia coli</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas albicans</i>	<i>Candida albicans</i>	<i>Escherichia coli</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas albicans</i>	<i>Candida albicans</i>
Chloroform	17.8	21.9	20.0	18.7	18.3	-	10.8	10.8	12.8	13.0
Pet-ether	10.4	9.8	-	10.4	-	-	-	-	-	-
Ethyl-acetate	18.7	17.3	18.3	19.2	19.2	17.9	18.7	17.8	20.1	15.6
Ethanol	18.7	18.7	16.9	19.7	18.9	12.3	14.2	15.2	-	13.5
Methanol	18.2	16.9	15.9	16.9	17.0	9.3	11.7	11.9	11.2	12.1
Water	-	-	-	-	-	-	-	-	-	-

Agar well = 6mm

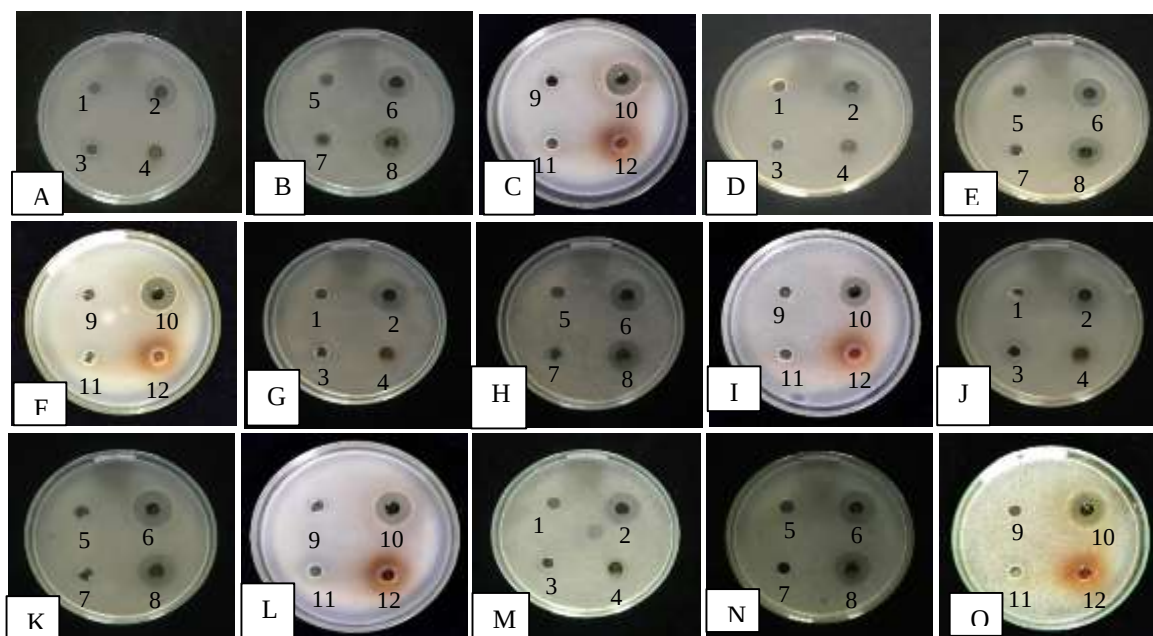


Figure 8. Antimicrobial Activity of different extracts from leaves of *A. paniculata* (Burm.f.) Wall. ex Nees

A, B, C = Test against *Escherichia coli* D, E, F =Test against *Micrococcus luteus*
 G,H,I. =Test against *Staphylococcus aureus* J, K, I.= Test against *Pseudomonas albicans*
 M, N, O = Test against *Candida albicans* 1, 3,5,7,9,11 = Control 2 = Test for chloroform
 4 = Test for pet-ether 6 = Test for ethyl-acetate 8= Test for ethanol
 10 =Test for methanol 12 = Test for aqueous

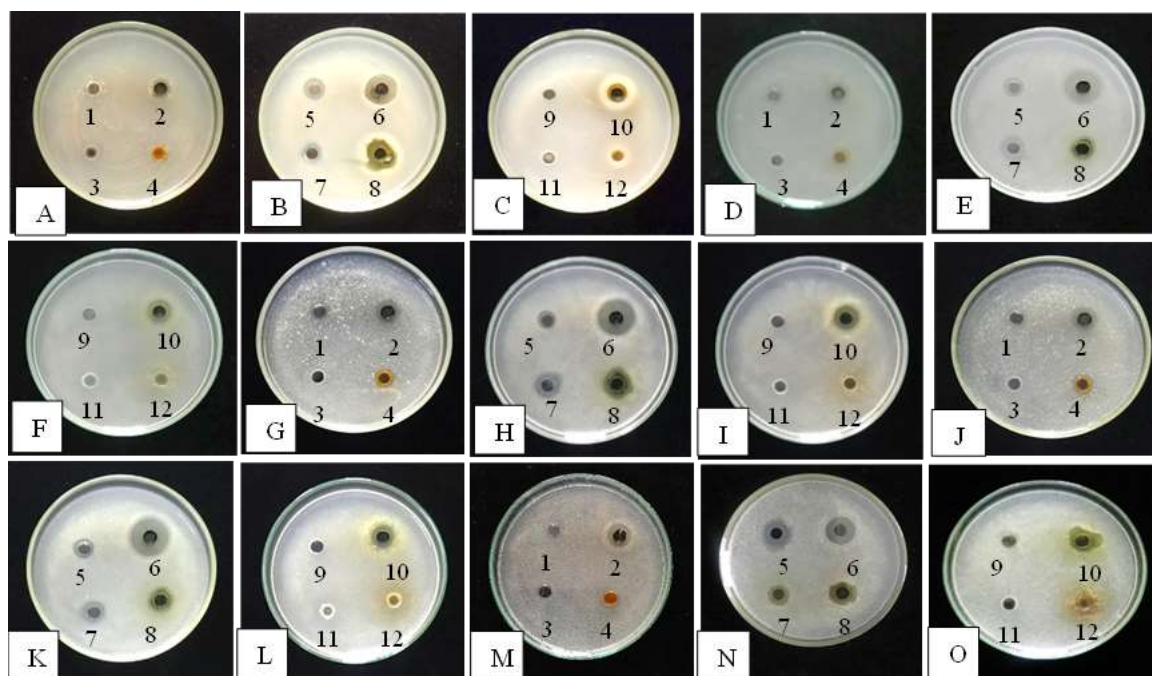


Figure 9. Antimicrobial Activity of different extracts from leaves of *A. echioides* (L.) Nees
 A,B,C = Test against *Escherichia coli* D,E,F =Test against *Micrococcus luteus* G,H,I =
 Test against *Staphylococcus aureus* J,K,I = Test against *Pseudomonas albicans* M,N,O
 = Test against *Candida albicans* 1,3,5,7,9,11 = Control 2 = Test for chloroform 4 = Test
 for pet-ether 6 = Test for ethyl-acetate 8= Test for ethanol 10 = Test for methanol 12 =
 Test for aqueous

Discussion

In this research, *A. paniculata* (Burm.f.) Wall. ex Nees and *A. echioides* (L.) Nees belong to the family Acanthaceae had been studied. The morphology characters of two species were annual herbs, quadrangular stems, leaves opposite and decussate. Inflorescences were axially or terminal spikes. Flowers were white with purple spots, bisexual, hypogynous and ovary superior. The loculicidal dehiscence fruits were found in two species. These characters were similar to Hooker (1885), Backer *et al* (1965), Dassanayake (1998).

In histology characters of leaves were diacytic types of stomata were found in two species. The stomata found only in abaxial surface and stoma index was 13.3-21.43 in *A. paniculata* (Burm.f.) Wall. ex Nees. *A. echioides* (L.) Nees observed on both surfaces and stoma index were 14.28-25.0, 20.0-33.33. The transverse section of *A. paniculata* (Burm.f.) Wall. ex Nees petiole was rectangular shaped and *A. echioides* (L.) Nees was crescent shaped. In transverse section of midrib, *A. paniculata* (Burm.f.) Wall. ex Nees was triangular and the other one rectangular. The vascular bundles of two species were crescent shaped and collateral types. These characters were agreement with Metcalf & Chalk (1957), Kokate (2000).

The transverse section of stem of two species were found in quadrangular shaped. Vascular bundles of stem were forming a circular ring and collateral type. One of the most outstanding histological features is the widespread occurrence of various shaped cystoliths located in both stem and leaf. The present data were similar to Metcalf & Chalk (1957).

The root of two species found circular in shaped. Periderm formation occurred at the outer part of the cortex. It consists of phellogen, phelloderm and phellem. These characters were in accordance with Metcalf & Chalk (1957).

The phytochemical analysis of the leaves of two species is summarized in Table 3. The aqueous extract of leaves shows the presence of high number of phytochemicals when compared with ethanol and methanol. It shows the presence of alkaloids, glycoside, tannins, phenolic compounds, flavonoids, saponins, α -amino acids, proteins, and reducing sugar whereas cyanogenic glycosides were not present in both species. Phytochemicals have highest therapeutic efficiency in pharmaceutical field. These results were similar to Ankush and Sicgandra (2023) Pande *et al.* (2018).

The free radicals scavenging activity of leaves of two species have been observed that the leaves extracts have excellent antioxidant activity showing IC₅₀ value of 5.98 and 3.44 mg/l compared to standard ascorbic acid 6.75 mg/l. The antioxidant activity of the leaves extracts are observed in lower IC₅₀. Antioxidants are natural substances that can help to delay cell damage and defend cells against. These resulting data were similar to those of Jeevanantham and Hussain (2021).

The antimicrobial activity of chloroform extract of *A. paniculata* (Burm.f.) Wall. ex Nees showed against maximum antimicrobial activity of *Micrococcus luteus* and aqueous extract did not show. In *A. echioides* (L.) Nees, ethyl-acetate of leaves extract were investigated for its antimicrobial properties against *Pseudomonas albicans* whereas aqueous and pet-ether extracts were not effective against. The antimicrobial activity of the medicinal plant extracts depends on the presence of different concentration of secondary metabolites. The present data similar to Pande *et al.* (2018), Keita *et al.* (2022).

Medicinal plants have been a long history of usage in traditional medicine. Plant leaves have been used as herbal medicine for their healing properties since ancient times. Plant natural products have been explored and used in the treatment of countless diseases.

Conclusion

The result of this study revealed the antibacterial and antifungal potential of the studied medicinal plants against drug resistant pathogens. The phytochemical constituents which are responsible for many pharmacological activities. Additionally, these medicinal plants could be used as a natural source of antioxidants. *A. paniculata* (Burm.f.) Wall. ex Nees is widely used as traditional medicinal soup. In Mandalay Region almost every household cultivated in the style of homegarden. Traditional medicines have been gaining momentum in popularity, to prevent Covid-19 sickness and demand has been increasing year by year. *A. echioides* (L.) Nees has potential source of medicine as anti-bacterial and antioxidant activity. Therefore, two species can be hoped that, homegarden scale cultivation can also be gained the medium scale and commercial cultivation, which would generate green economy in future.

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ACUTE ORAL TOXICITY AND ANTIOXIDANT ACTIVITY ON LEAVES OF *STROBILANTHES TONKINENSIS* LINDAU (LAT-PHAT-HMWE)

Aye Sandar Htay¹, Nwe Oo², Aye Minn Maw³, Ei Ei Phyu⁴

Abstract

Strobilanthes tonkinensis Lindau (lat-phat-hmwe) is a medicinal and aromatic plant native to Yunan Province, China. The specimen was collected from Sin Phyu Inn Village, Pyin Oo Lwin Township, Mandalay Region. In the present study, microscopical evaluation on the aqueous, 95% and 70% ethanolic extraction, acute oral toxicity test and antioxidant activity of leaves of *Strobilanthes tonkinensis* Lindau were investigated. In microscopical evaluation, the presence of glandular and non-glandular trichomes, cystoliths and diacytic stomata showed the distinct characters. The leaves were extracted with 70% and 95% ethanol and water using a reflux extraction method at Research Division, University of Traditional Medicine, Mandalay in 2023. In acute oral toxicity study, six female mice (25 ± 5 g) were used for each extract by using main test of Organization for Economic Co-operation and Development (OECD) 425 guidelines (2008) at Department of Medical Research, Pyin Oo Lwin Branch. In this study, there are no toxic signs and mortality in mice at the various doses of extract (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period. Therefore, the LD₅₀ values of *Strobilanthes tonkinensis* Lindau was found to be more than 5000 mg/kg. Evaluation of antioxidant activity of *Strobilanthes tonkinensis* Lindau were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assays method at Department of Chemistry, University of Research Centre, Mandalay. In the antioxidant activity, the IC₅₀ value of standard ascorbic acid is 4.271 µg/mL and the aqueous extracts, 70% and 95% ethanolic extracts are 63.618 µg/mL, 5.026 µg/mL and 2.925 µg/mL respectively. Thus, 95% ethanolic extract was the most effective antioxidant activity than the other extracts. Therefore, the leaves of *Strobilanthes tonkinensis* Lindau are non-toxic and a good source of phytoconstituents including with potential antioxidant and used safely in medicinal purposes.

Keywords: *Strobilanthes tonkinensis* Lindau, Microscopical characters, Extraction, Acute oral toxicity, Antioxidant activity

Introduction

The various types of bioactive compounds derived from medicinal plants serve as raw materials for new drug discovery. Therefore, plants are a source of medicinal compounds and play an important role in the maintenance of human health (WHO, 2013). *Strobilanthes tonkinensis* Lindau is a medicinal and aromatic plant belonging to the family Acanthaceae, native to Yunan Province, China and is now extensively distributed across Thailand and Vietnam including Myanmar. It has a unique aroma of glutinous rice when dried and is rich in biologically active compounds such as amino acids and flavonoids and aromatic components. According to the World Health Organization, plant extracts or their active constituents are used as folk medicine in traditional therapies by 80% of the world's population (WHO, 2011).

Extraction is a process whereby the constituents of a plant are removed using a solvent. Watery and ethanolic extracts have different properties. Ethanol dissolves alkaloids, flavonoids,

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glycosides, tannins and essential oils but does not dissolve proteins. Water dissolves flavonoids, glycosides, tannins, saponins, sugar, proteins and mineral salts but does not dissolve fats, resins and essential oils. Choice of solvent should be based on the polarity of the plant constituents of interest (Harborne, 1984).

The acute toxicity has been defined as the adverse effects occurring within a short time of administration of a single dose within 24 hours. The acute toxicity test was done to determine the symptoms consequent to administration of the test agent and the LD50 (medium lethal oral dose) of that agent. LD50 is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of animals when administered by the oral route.

In Myanmar, green tea leaves are mixed with the leaves of *Strobilanthes tonkinensis* Lindau also called the glutinous rice flavour leaves is now popular for the very noticeable and pleasant sticky rice aroma with green tea taste. Moreover, dried aromatic powders of *Strobilanthes tonkinensis* Lindau leaves is now used in the betel by mixing with other ingredients. It has been widely and commercially cultivated in Mohnyin Township, Kachin State neighboring with Chinese Province of Southern Yunnan, the place where the native and indigenous of this plant. It is also cultivated in Pyin Oo Lwin Township, Mandalay Region, Taunggyi Township and Nantsam Township, Shan State.

Strobilanthes tonkinensis Lindau is one of the edible Myanmar indigenous plants and popularly marketable for its characteristic scent. It is a natural source of locally available useful medicinal plants. However, there were only a few researches for this plant. Therefore, this present research focused on the evaluation of pharmacological activities of these medicinal and edible leaves. For safety use of this plant, the present study aimed to examine the acute oral toxicity of the leaves of this plant. Moreover, this investigation also intended to evaluate the antioxidant activity of the leaves of this plant.

Materials and Methods

Plant samples collection and preparation

The plant samples were collected from Sin Phyu Inn Village, Pyin Oo Lwin Township, Mandalay Region. The collected leaves samples were cleaned thoroughly to remove impurities. Then, the leaves were air-dried, grinded and kept in the air-tight containers for the study of diagnostic characteristics.

Extraction from leaves of *Strobilanthes tonkinensis* Lindau

In this study, the aqueous extract, 95% and 70% ethanolic extract were extracted by reflux extraction method at Research Division, University of Traditional Medicine, Mandalay in 2023.

Aqueous extraction

The aqueous extract will be done by 100 g of leaves of *Strobilanthes tonkinensis* Lindau with 500 ml of distilled water by using reflux apparatus for 3 hours at 60°C for 3 times. The extract will be filtered by using filter paper and filtration will be concentrated by using a vacuum rotary evaporator at 60°C. The concentrated extract will be made powder by freeze dryer to get dry powder extract.

95% and 70 % ethanolic extraction

The powder of leaves of *Strobilanthes tonkinensis* Lindau was successively extracted with 95% and 70 % ethanol and water by using Soxhlet apparatus 6 hrs. The ethanol-soluble fraction was dried by rotary evaporator under reduced pressure and concentrated on water bath at 60°C at Research Division, University of Traditional Medicine. (Alekhya *et al.*, 2013; Tailor & Goyal, 2014).



Figure 1 Extraction of leaves by Soxhlet



Figure 2 Removal of solvent by Rotary Evaporator



Figure 3 Drying of water extract by Freeze Dryer

Acute oral study from leaves of *Strobilanthes tonkinensis* Lindau

Acute oral toxicity study from aqueous and 70% ethanolic extract from leaves of *Strobilanthes tonkinensis* Lindau was performed at Department of Medical Research, Pyin Oo Lwin Branch. (OECD) 425 guidelines (2008).

Acute oral toxicity study from aqueous extract and 70% ethanolic extract from leaves of *Strobilanthes tonkinensis* Lindau

The two extracts of aqueous and 70 %ethanolic extract were tested on adult healthy 12 female albino mice of $25 \pm 5g$ for acute oral toxicity. It was carried out according to Organization for Economic Co-operation and Development (OECD) guideline 425. The main test was performed because there is no previous report about the toxicity and lethality of the leaves of *Strobilanthes tonkinensis* Lindau.

The mice were fasted food but not water for 4 hours prior to dosing. The fasted body weight of the first mice was determined and initiated at 175 mg/kg. This mouse was observed carefully each interval for 30 minutes, 1 hr, 4 hours and then 24 hrs. After 48 hours observation, the next mice were selected by using a factor of 3.2 times the original dose if the first mice survived. Therefore, the similar dose progression was done. The second mice were given 550 mg/kg, the third mice were 1750 mg/kg. the fourth mice were 5000 mg/kg and then the fifth and sixth mice were 5000 mg/kg. Body weight for each was determined for 7th day, 14th day.

Preparation of animals

For acute toxicity test, only female mice (body weight $25 \pm 5g$) were used. This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between sexes, but in those case where differences are observed, females are

generally slightly more sensitive. The animals were provided by Laboratory Animal Service Division, Department of Medical Research, POL Branch. The animals were kept in the cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals maintained in laboratory with unlimited supply of food and water (OECD, 2008).

Preparation and administration of doses

The 70% ethanolic extract of *Strobilanthes tonkinensis* Lindau was dissolved in distilled water for required concentration to be administered. Animals were fasted prior to dosing. Food but not water was withheld for 3-4 hours in mice. The fasted body weight of each animal was determined. The dose was calculated according to fasted body weight. By using cannula, doses of the test substances administered. Then, food was withheld for 1-2 hours (OECD, 2008). Body weights of mice were measured and recorded once a week. At the end of the test period, the mice were weighed again.



Figure 4 Preparation and administration of doses

Clinical observations

The mice were observed continuously during the first 30 minutes after dosing and observed periodically for the next 24 hours to monitor the systemic toxic effects and then daily thereafter, for 14 days. All observations were systematically recorded with individual worksheet being maintained for each animal. The observations of parameters were recorded skin and fur changes, eyes, mucous membrane, respiratory rate, motor activity and behavioral pattern were found to be normal. Salivation, convulsion, cyanosis, tremors, and diarrhoea did not occur in all animals for 14 days (OECD 2008).

Screening of Antioxidant Activity on leaves of *Strobilanthes tonkinensis* Lindau by DPPH free Radical Scavenging Assay Method

The antioxidant activity of aqueous extract and 70% and 95% ethanolic extract were measured by using DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging assay method at Department of Chemistry, University of Research Centre, Mandalay in 2023 (Neha & Mishra, 2011).

Preparation of Test Sample Solution for Crude Extract

About 0.01g of leaf extract was dissolved in 10 ml of 95 % ethanol under vigorous shaking. This solution was used as a stock solution (1 mg/ mL). This stock solution was diluted with ethanol to obtain desired concentrations of 25, 50, 100, 200, 400 µg/mL.

Preparation of 60 µM DPPH Solution

DPPH powder (0.0024 g) was thoroughly and gently dissolved in ethanol (100 mL). This solution was freshly prepared and stored in brown coloured reagent bottle. It was kept in the refrigerator for no longer than 24 hours.

Preparation of Standard Ascorbic Acid Solution

Ascorbic acid (0.01g) was dissolved in 100 mL of 95 % ethanol. It was diluted with ethanol to obtain the standard solution with the concentrations of 1, 2, 4, 8 and 16 µg/mL).

Measurement of DPPH Radical Scavenging Activity by Spectrophotometric Method

The control solution was prepared by mixing 2 mL of 60 µM DPPH solution and 2.0 mL of ethanol. Then, the test sample solutions were also prepared by mixing the test solution (2.0 mL) and 60 µM DPPH solution (2.0 mL) with various concentrations. The blank solution was also prepared by mixing the test sample solution (2.0 mL) and 95 % ethanol (2.0 mL). Furthermore, these solutions were allowed to stand at room temperature for 30 minutes. Moreover, the absorbance of each solution was measured at 519 nm by using UV-1800 spectrophotometer.

% inhibition value was calculated by using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{\text{Abs}_{\text{control}}} \times 100$$

$\text{Abs}_{\text{control}}$ = absorbance of control solution
 $\text{Abs}_{\text{sample}}$ = absorbance of test sample solution
 $\text{Abs}_{\text{blank}}$ = absorbance of blank solution

Results

Morphological and Microscopical characters of *Strobilanthes tonkinensis* Lindau

Aromatic herb; stem and branches quadrangular; inflorescence terminal and axillary spike, leaves simple, opposite; flowers white, bisexual, zygomorphic, hypogynous, bracteate, bracteoate, glandular trihomes present; calyx (5), lobes linear; corolla (5), tubular; stamens 4, didynamous, carpels (2), glandular stipitate in upper portion of ovary, yellow disc at the base of ovary. In microscopical evaluation the presence of glandular trichomes, cystoliths and diacytic stomata showed the distinct characters. SEM images also revealed the presence of cystoliths and glandular trichomes on the surfaces of leaves. The results were shown in Figure:5–18.



Figure 5 Habit



Figure 6 Inflorescence

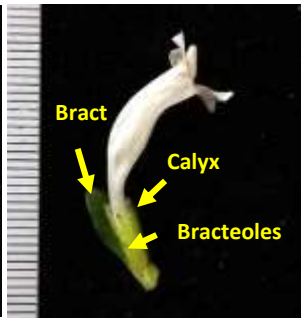


Figure 7 Flower

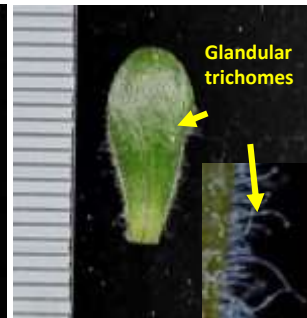


Figure 8 Bract

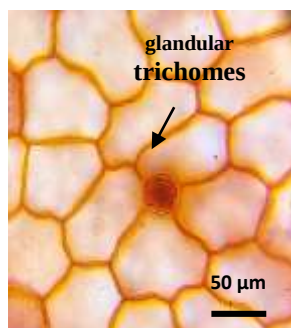


Figure 9 Adaxial surface view of lamina

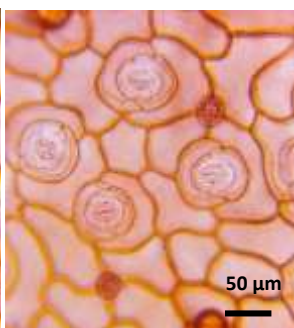


Figure 10 Abaxial surface view of lamina

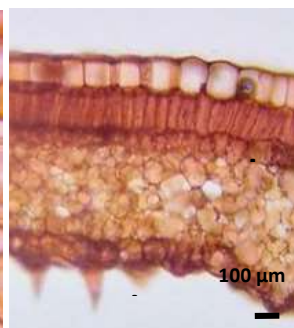


Figure 11 T.S. of lamina

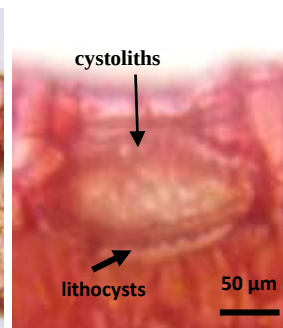


Figure 12 Cystolith within lithocyst in the epidermal layer of T.S. of lamina



Figure 13 Cystolith



Figure 14 Non-glandular trichome

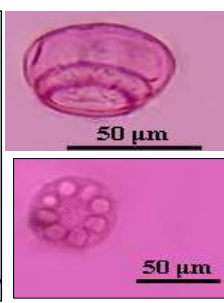


Figure 15 Glandular trichome

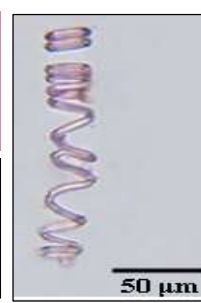


Figure 16 Spiral vessels

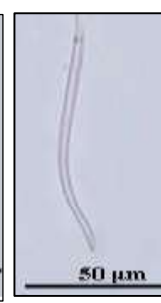


Figure 17 Aseptate fiber vessels

Scanning electron microscopy (SEM)

Figure 18 SEM images of leaves of *Strobilanthes tonkinensis* Lindau

Acute Toxicity Study from Aqueous Extract and 70% Ethanolic Extract of *Strobilanthes tonkinensis* Lindau

Acute toxicity test was done by using main test of OECD 425 guideline (2008). In the acute toxicity study, there were no toxic signs and mortality in mice at the various doses of 70 % ethanolic extract of *Strobilanthes tonkinensis* Lindau (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period.

Observations signs of toxicity and mortality of the mice were recorded. There were no toxic signs and mortality during the observation period of 14 days. Skin and fur changes, eyes, mucous membranes, motor activity and behavioral patterns were found to be normal. Salivation, convulsion, paralysis of limbs, tremors and diarrhea were not found in all mice and was shown in table (5). Therefore, the LD₅₀ value of 70% ethanolic extract of *Strobilanthes tonkinensis* Lindau was found to be more than 5000 mg/kg (raw powder 35.7 g/kg) body weight.

Acute toxicity test was done for aqueous extract by using main test according to OECD 425 guideline (2008). It showed that at the dose of 5000mg/kg of watery extract of *Strobilanthes tonkinensis* Lindau there was no lethality of the mice up to 14 days observation period. Therefore, median lethal dose (LD₅₀) of extract was supposed to be greater than 5000 mg/kg.

Clinical observations

For evaluation of toxicity, no significant changes were observed in toxic parameters. Skin and fur changes, eyes, mucous membrane, respiratory rate, motor activity and behavioral pattern were found to be normal. Salivation, convulsion, cyanosis, tremors, and diarrhoea did not occur in all animals. There were no abnormalities detected.

Body weight

Single oral doses of 175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg of *Strobilanthes tonkinensis* Lindau were administered to the mice. The animals were observed for 14 days. There were no significance changes in body weight of the mice before and after administration of the tested drug.

Table 1 Result of acute toxicity of aqueous extract of *Strobilanthes tonkinensis* Lindau on mice after single oral administration of the test drug according to main test in OECD 425 guideline

Different doses of test drug (mg/kg)	Number of tested mice	Observed period (Day)	Body weight (g)			Observed death
			0 week	1 st week	2 nd week	
175	1	14	24g	24g	24g	0
550	1	14	22g	22g	23g	0
1750	1	14	23g	22g	22g	0
5000	1	14	22g	21g	20g	0
5000	1	14	29g	25g	26g	0
5000	1	14	23g	22g	22g	0

Table 2 Result of acute toxicity of 70% ethanolic extract of *Strobilanthes tonkinensis* Lindau on mice after single oral administration of the test drug according to main test in OECD 425 guideline

Different doses of test drug (mg/kg)	Number of tested mice	Observed period (Day)	Body weight (g)			Observed death
			0 week	1 st week	2 nd week	
175	1	14	21g	21g	20g	0
550	1	14	22g	22g	21g	0
1750	1	14	23g	21g	21g	0
5000	1	14	24g	23g	23g	0
5000	1	14	26g	24g	25g	0
5000	1	14	26g	24g	24g	0

Antioxidant Activities of leaves of *Strobilanthes tonkinensis* Lindau

Antioxidant activities of aqueous extract and 70% ethanolic extract were determined by DPPH radical scavenging method and compared with standard ascorbic acid. DPPH scavenging activity of standard and sample solutions showed by reducing the purple coloured radical DPPH to the yellow coloured non-radical form DPPH. DPPH is a stable organic, free radical with an absorption band at 517 nm. The absorbance and % inhibition values at various concentrations of standard ascorbic acid and solution of two different extracts.

Antioxidant activities of standard and test sample solutions were expressed as percentage of DPPH radical inhibition and IC₅₀ values (µg/mL). The IC₅₀ value of standard ascorbic acid is 4.271 µg/mL and the aqueous extracts, 70% and 95% ethanolic extracts are 63.618 µg/mL, 5.026 µg/mL and 2.925 µg/mL respectively.

Table 3 Scavenging activity of standard Ascorbic acid at different concentrations

Sample	Concentration (µg/mL)	Absorbance	% Inhibition	IC ₅₀ (µg/mL)
Ascorbic acid	1	0.228	32.34	4.271
	2	0.166	43.91	
	4	0.129	50.74	
	8	0.109	67.65	
	16	0.086	90.91	

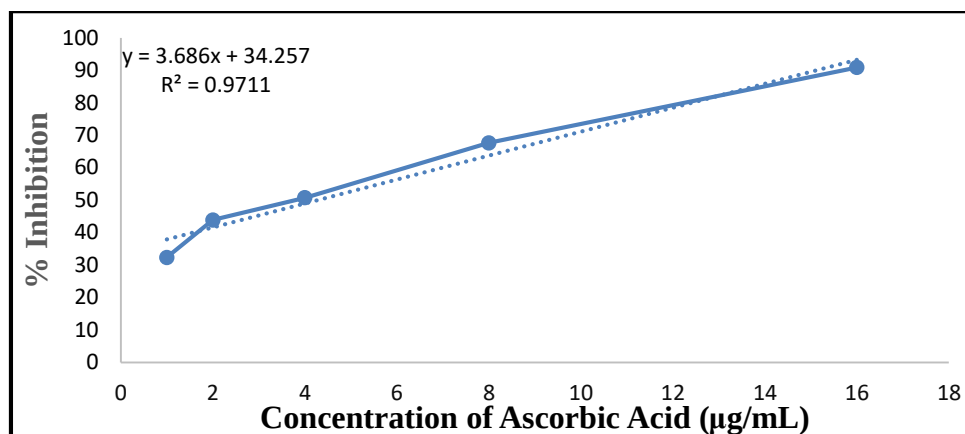


Figure: 19 Percent Inhibition at Different Concentration of Standard Ascorbic Acid

Table 4 Scavenging activity of aqueous extract on leaves of *Strobilanthes tonkinensis* Lindau at different concentrations

Extract	Concentration (µg/mL)	Absorbance	% Inhibition	IC ₅₀ (µg/mL)
Watery extract	25	0.801	41.125	63.618
	50	0.543	49.559	
	100	0.422	58.941	
	200	0.256	64.400	
	400	0.089	86.205	

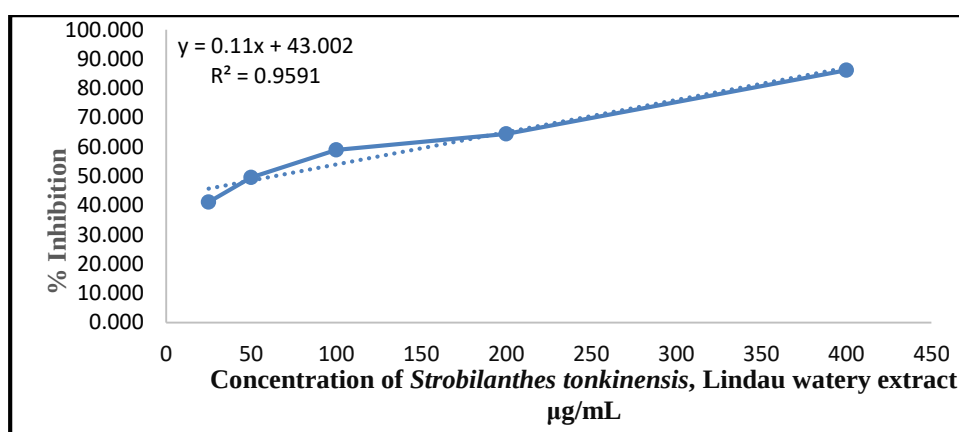


Figure 20 Percent Inhibition at Different Concentration of *Strobilanthes tonkinensis* Lindau aqueous extract µg/mL

Table 5. Scavenging activity of 70% ethanolic extract on leaves of *Strobilanthes tonkinensis* Lindau at different concentrations

Extract	Concentration	Absorbance	% Inhibition	IC ₅₀ (µg/mL)
	(µg/mL)			
70% Ethanol	2.5	0.192	42.169	5.026
	5	0.153	53.916	
	10	0.141	57.530	
	20	0.102	69.277	
	40	0.018	94.578	

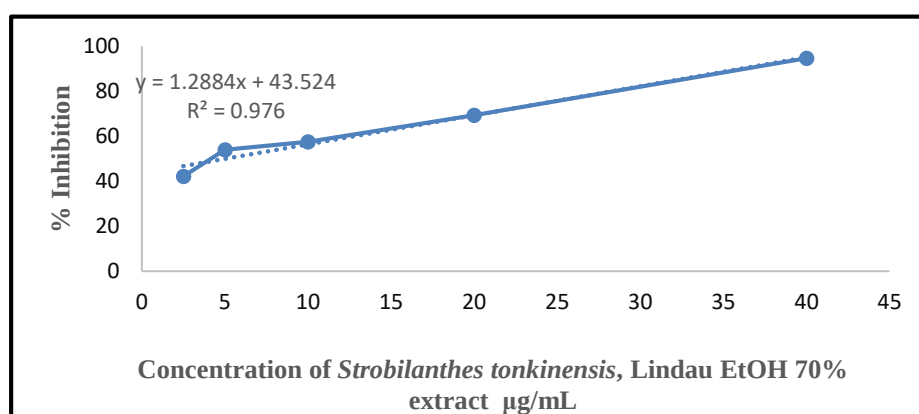


Figure 21. Percent Inhibition at Different Concentration of *Strobilanthes tonkinensis* Lindau EtOH 70% extract µg/mL

Table 6 Scavenging activity of 95% ethanolic leaves extract of *S. tonkinensis* Lindau at different concentrations

Extract	Concentration (µg/mL)	Absorbance	% Inhibition	IC ₅₀ (µg/mL)
95% Ethanol	2.5	0.132	48.031	2.925
	5	0.117	53.937	
	10	0.109	57.087	
	20	0.083	67.323	
	40	0.031	87.795	

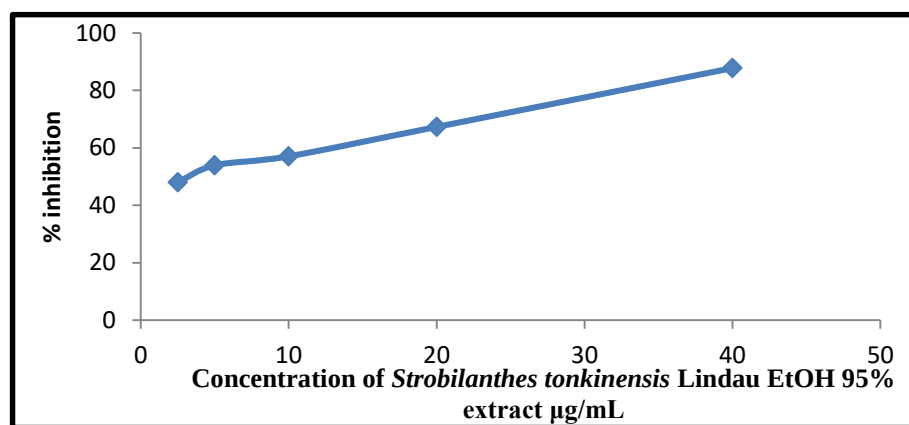


Figure 22. Percent Inhibition at Different Concentration of *Strobilanthes tonkinensis* Lindau EtOH 95% extract µg/mL

Discussion

In this study, the extraction with aqueous and 70 % ethanol from leaves of *Strobilanthes tonkinensis* Lindau and their acute oral toxicity test and antioxidant activity were carried out. The extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances. The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time and composition of the sample. Under the same extraction, temperature, solvent and composition of sample are the most important parameters. In this study, extracts of *Strobilanthes tonkinensis* Lindau were obtained by using water and 70% ethanolic extract. So, for extraction study, Reflux method was used.

The study on acute oral toxicity was performed in 6 numbers of female mice (25 ± 5 g) for each extract and by using main test of Organization for Economic Co-operation and Development (OECD) 425 guidelines (2008) because there is no previous report in the international including Myanmar about the toxicity and lethality of the leaves of *Strobilanthes tonkinensis* Lindau. So, the main test was investigated. In this toxicity study, there are no toxic signs and mortality in mice at the various doses of extract (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period. Therefore, the LD₅₀ values of *Strobilanthes tonkinensis* Lindau was found to be more than 5000 mg/kg.

In the antioxidant activity of aqueous extract and 70% ethanolic extract was determined using DPPH solution. The antioxidant activity is expressed in terms of IC₅₀ (µg/mL) values. The IC₅₀ of a compound is inversely related to its antioxidant capacity. A lower IC₅₀ value indicates higher antioxidant activity. The reference standard used was ascorbic acid. The obtained values for antioxidant activity of aqueous extract and 70% ethanolic extract by DPPH methods were ranged from 63.618 to 5.026 µg/mL. The highest activity was found in ethanolic extract and the others showed lower capacity to inhibit DPPH radicals. In comparison, ethanolic extract showed higher activity than the aqueous leaves extract of *Strobilanthes tonkinensis* Lindau.

Conclusion

Strobilanthes tonkinensis Lindau is a medicinal and aromatic plant. In Myanmar, green tea leaves are mixed with the leaves of *Strobilanthes tonkinensis* Lindau because of its strong aroma of glutinous rice. There was no information about the toxicity of *Strobilanthes tonkinensis* Lindau. According to the result, this aromatic odour may be due to the release of essential oils from the glandular trichomes present on the leaf surfaces. The information such as the presence of trichomes and crystal characters can help in identification and to detect the adulteration of other drugs in this medicinal plant. The medium lethal dose, LD₅₀ was more than 5000mg/kg body weight. Therefore, the leaves of *Strobilanthes tonkinensis* Lindau were practically non-toxic and may be relatively harmless. These leaves may be used safely in medicinal purposes.

Acknowledgements

We would like to express special thanks to Dr Kalaya Lu, Professor, Head of Botany Department, University of Mandalay for her permitting and encouraging to carry out this research paper.

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ORGANOLEPTIC EVALUATIONS, MICROSCOPICAL CHARACTERS, NUTRITIONAL VALUES, ELEMENTAL AND HEAVY METAL ANALYSIS ON POWDERED FLOWERS OF *MAGNOLIA CHAMPACA* (L.) BAILL. EX PIERRE

Ei Ei Phyu¹, Nwe Oo², Myint Khaing³

Abstract

Magnolia champaca (L.) Baill. ex Pierre belongs to family Magnoliaceae. The flower samples (tepals) were collected from aged old trees of Mount Popa in flowering period during 2022 – 2023. In this study, the organoleptic evaluations, microscopical examination, nutritional contents, and elemental and heavy metals constituents of flowers were analyzed. The organoleptic evaluation was carried out at Department of Botany, University of Mandalay in 2022. In this evaluation, the fresh tepals were yellow in colour, leathery in texture, aromatic in odour and indistinct in taste. The dried tepals were yellow in brown, brittle in texture, aromatic in odour and indistinct in taste. The colour of powdered tepals was dark brown, the texture was rough, the odour was aromatic and the taste was indistinct. The microscopical examination was carried out at Department of Botany, University of Mandalay in 2022. In this study, pitted vessels, spiral vessels, fibers, sclereids, fragment of parenchyma cells, portion of epidermal layers, tannin cells and oil cells were found in powdered flowers. Nutritional values were determined at Small Scale Industries Department, Ministry of Cooperative and Rural Development, Yangon in 2023. Carbohydrate content was the highest (63.77 %) and fat was found as the lowest nutrient (1.98 %) in the powdered flowers. Elemental analysis was performed by using Energy Dispersive X-Ray fluorescence (EDXRF) technique at Department of Chemistry, University of Mandalay in 2023. In elemental analysis, potassium (K) was the highest (66.2 %) among the elements. The heavy metal analysis was carried out at Myanmar Food Processors and Exporters Association (MFPEA), Yangon in 2023. Lead (Pb) was detected at very trace concentration. Arsenic (As), Cadmium (Cd), Chromium (Cr) and Mercury (Mg) were not found in powdered flowers. This research may be helpful in checking the purity and adulterants of powdered flowers of *Magnolia champaca* (L.) Baill. ex Pierre. These flowers may be safely used as a dietary supplement such as a form of herbal tea since it possesses rich source of nutrients and elements and lack of heavy metals.

Keywords: *Magnolia champaca* (L.) Baill. ex Pierre., organoleptic evaluations, microscopical characters, nutritional values, elemental and heavy metal analysis

Introduction

Magnolia champaca (L.) Baill. ex Pierre known as champaca is a large evergreen tree belonging to the family Magnoliaceae. It consists of 12 genera and 220 species of evergreen, trees and shrubs. It is known for its fragrant flowers and its timber is used in woodworking. The tree is native to the Indo-malaya ecozone consisting of South Asia, Southeast Asia-Indochina and Southern China. It occurs in forests from 300 meters to 1200 meters above sea level. It is wild in the forests of the Eastern sub Himalayan tract and lower hills up to 3000ft and also in Assam, Myanmar, South India and Western Ghats (Chopra *et al.* 1956).

Magnolia champaca (L.) Baill. ex Pierre is widely used in both Ayurveda and Siddha medicine. All parts of this plant provide various benefits. The different color of the flower varies according to locality. The large, scented, yellow flowers grow singly, each from base of a leaf.

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The tree starts flowering after the age of 4 – 5 years (Oyen and Xuan Dung 1999). During the flowering period, the tree is covered with thousands of golden yellow flowers with powerful and diffusive fragrance. The flower extract of *M. champaca* (L.) Baill. ex Pierre has antiulcer, antidiabetic and anti-inflammatory properties and hence used in the ailment of ulcer, skin diseases and wounds. (Nadkarni 1954).

World Health Organization (WHO) is keenly interested in the development and utilization of medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of populations in these countries (Goud *et al.*, 2005). The medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. Flowers have been used since as far back as 50,000 years in funeral rituals. Flowers are mainly used for religious purposes. The other most important use of flowers is their use in medicine and cosmetics. Previous investigations on the plant have revealed that it possesses anti-inflammatory, antimicrobial and leishmanicidal activity (Vimala *et al.*, 1997 and Takahashi *et al.*, 2004).

It has been cultivated as the shady plant and ornamental plant in Myanmar especially at Mount Popa. The large, tall and old plants of *Magnolia champaca* (L.) Baill. ex Pierre are found at Mount Popa. In the flowering time these beautiful, attractive and fragrant fresh flowers are sold in the market and roadside of this region. The preserved flowers of this plant are also sold throughout the year. The flowers of this plant have both aesthetic and medicinal values.

The aim of the present study was to evaluate the purity and safety use of the flowers of *Magnolia champaca* (L.) Baill. ex Pierre. The objectives were to evaluate the organoleptic and microscopical characters, to analyze the elemental and heavy metals constituents and to evaluate the nutritional values of the flowers of *Magnolia champaca* (L.) Baill. ex Pierre.

Materials and methods

Plant samples collection and preparation

The fresh flower samples of *Magnolia champaca* (L.) Baill. ex Pierre. were collected from Mount Popa, Kyaukpadaung Township, Mandalay Region. from July, 2022 to September, 2023. The collected flowers samples were rinsed thoroughly to remove impurities. These samples were air- dried and grinded into the appropriate size and kept in the air- tight containers to use in the studies of organoleptic evaluation, microscopical evaluation, nutritional values, elemental and heavy metal analysis.

Organoleptic evaluation of powdered flowers of *Magnolia champaca* L.

Organoleptic parameters of fresh, dried and powdered flowers samples were evaluated by using organs of senses. In this study, colour, texture, odour and taste of powdered flowers were evaluated.

Microscopical evaluation of powdered flowers of *Magnolia champaca* L.

The microscopic characters of powdered flowers were evaluated to identify the diagnostic characters. The detection of important parameters such as various cellular tissues, types of trichomes and stomata, crystals and oil cells were carried out to provide the identification of crude drugs by Metcalfe and Chalk (1950), Wallis (1967) and Evans (2009).

Elemental analysis of powdered flowers from *Magnolia champaca* (L.) Baill. ex Pierre by using Energy Dispersive X-Ray Fluorescence (EDXRF)

The qualitative elements were analyzed by using Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer technique at the Department of Chemistry, University of Mandalay in 2023. The EDX 700 spectrometer can detect a wide range of the elements from Aluminum (Al) to Barium (Ba). The required data can be produced in a few minutes and it has high degree of resolution for the spectrum evaluation. Due to its high sensitivity, the spectrometer can detect the relative concentration of elements in the percentage (%) range. The flower samples were dried at room temperature. Then, the dried flowers were ground to get fine powder. The dried powder sample (2g) were pressed into pellets and used in Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer, which produce the characteristic X-Ray spectrum of sample, consisting of the respective elements.

Heavy metals analysis by using Atomic Absorption Spectrophotometric (AAS) from powdered flowers of *Magnolia champaca* (L.) Baill. ex Pierre

Heavy metal analysis was carried out at the Laboratory of Myanmar Food Processors and Exporters Association (MFPEA), Yangon. AAS has been developed primarily for determining metals at low concentrations levels. AAS is highly sensitive for a wide range of elemental analysis which is difficult or impossible to analysis by flame photometry. It is rapid, specific and need only a small amount to analyze. This method is especially used for trace element and heavy metal analysis.

The Perkins and Elmer Analyst 800 spectrophotometer was used in this experiment. The collected samples were placed in the porcelain basin in a furnace starting at 20°C burning to 500°C and maintained at this temperature for 4 hours, and then sample ash was cooled at room temperature. After cooling, ash was digested with 10 ml of hydrochloric acid to get solution sample. This solution was carefully closed and heated at 200°C for about 10 minutes. The sample solution was cooled and filtered with filter paper. 1 mL of sample solution was added into the 100 ml volume metric flask. And then, 1 mL of this solution was diluted with 99 ml of de-ionized water and placed at the normal room temperature about 24 hours. This solution was analyzed by using atomic absorption spectrometer (AAS 800).

Nutritional values of flowers of *Magnolia champaca* (L.) Baill. ex Pierre

The flowers of *Magnolia champaca* (L.) Baill. ex Pierre were evaluated for its nutritive value at Small Scale Industries Department, Ministry of Cooperative and Rural Development, Yangon in 2024. The nutritional values have been undertaken according to the Association of Official Analytical Chemists (AOAC) method (Horwitz, 1980).

Determination of protein content

The protein was determined according to methods in carbohydrate Chemistry, 1978. Each sample was accurately weighed from 0.1 to 1 g into the digestion flask (kjedahl flask). One gram of copper sulphate and 10g of anhydrous sodium sulphate and 10 mL of concentrated sulphuric acid were added one after another. The flask was kept in an inclined position on a heating mantle and heated gently until the foaming ceases. A small amount of paraffin was added to reduce foaming. The solution was boiled vigorously until clear. The digestion flask was then cooled and about 200 mL of distilled water and 75 mL of sodium hydroxide solution were added into it. A

few zinc granules were also added. The digestion flask was connected immediately to the distillation ball or trap on the condenser, and the tip of the condenser must be immersed in a 25 mL of standard acid (0.5 N or appropriate 0.1 N) in the conical flask. Then, the flask was rotated to mix the contents thoroughly and heated immediately, until all ammonia had distilled over (at least 150 mL distillate). The receiver must be lowered down first before distillation was complete and the tip of the condenser must be washed with distilled water. The excess acid was filtrated with standard 0.1 N sodium hydroxide using methyl red as an indicator by the method of A.O.A.C (Horwitz, 1980). Protein content was then calculated by the following formula.

$$\% \text{ protein} = \frac{(x-y) \times 0.014 \times N \times 6.25 \times 100}{\text{wt. of sample (g)}}$$

Determination of fiber content

About 2g of each sample was accurately weighed and introduced into a 500 mL flask. Then, 200 mL of 1.25% sulphuric acid was added. The contents were refluxed for 30 minutes and then filtered through linen clothe in a fluted funnel. The residue was thoroughly washed with hot distilled water until the washing was no longer acidic. The residue was washed back quantitatively into the flask with 100 mL of distilled water; 100 ml of 2.5 NaOH solution were added and refluxed exactly for 30 minutes. It was then filtered thoroughly with hot distilled water. Next it was washed with 1% HCl and again with hot distilled water till free from acid. The remaining water squeezed out as far as possible. Finally, the residue was washed with a small amount of 95% ethanol. The residue was transferred quantitatively into a weighed crucible and dried to a constant weight in an oven at 105°C for three hours. It was cooled in desiccators and weighed.

The crucible and contents were ignited in a muffle furnace (600°C) until a white ash was obtained. It was cooled and weighed. The difference in two weights represents the weight of crude fiber by the method of A.O.A.C (Horwitz, 1980).

$$\% \text{ of Crude fiber} = \frac{\text{wt. of fiber (g)}}{\text{wt. of sample (g)}}$$

Determination of fat content

The fat content was determined by the Soxhlet extraction method. Sample 50 g were placed in a thimble. The neck of thimble was plugged with a little cotton wool to prevent the sample from floating out. The thimble was introduced into a Soxhlet extractor and the extractor was connected to the flask and condenser. For extraction, exactly 200 mL of petroleum ether was used. When the solvent colorless, the extraction was stopped, it took about 6 hours. The petroleum ether extract was concentrated to small volume and transferred to a weighed beaker. It was evaporated to dryness on water bath. The residue containing beaker was weighed. The difference weight of residue containing beaker was because of the weight of fat by the method of A.O.A.C (Horwitz, 1980).

$$\% \text{ non-volatile residue (fat)} = \frac{\text{wt. of residue} \times 100}{\text{wt. of sample (g)}}$$

Determination of carbohydrate

An accurately weighed 1 g of sample was placed in a round bottom flask (500 cm³), to which distilled water (200 cm³) were added. The mixture was then boiled gently under a reflux condenser for 2 – 5 hours, cooled and neutralized solution was the filtered into a volumetric flask (250 cm³). The round bottomed flask was washed several times with distilled water and the washing added to the volume matrix flask. The solution in the flask was made up to the made up to the mark with distilled water. Aliquots (3 x 10 cm³) of the Fehling's solution were titrated with the above dilute hydrolyzed solution using methylene blue as an indicator. The amount of glucose calculated from the volumetric analysis multiplied by 0.93 as in equal to the amount of starch by the method of A.O.A.C (Horwitz, 1980).

$$\text{Reducing sugar \% (Dextrose \%)} = \frac{\text{Make volume 100 or } 200 \times 0.3 \text{ first titrated volume of value} \times 0.5 \text{ HCL}}{\text{Second titrated volume} \times 20 \text{ ml of 0.5 M HCL} \times \text{sample wt. (g)}}$$

Results

Family	-	Magnoliaceae
Scientific name	-	<i>Magnolia champaca</i> (L.) Baill. ex Pierre
English name	-	Champaca
Myanmar name	-	Sagawa

Distinct characters of *Magnolia champaca* (L.) Baill. ex Pierre

Perennial tree, leaves simple, alternate, petiolate, inflorescences axillary cyme, flower bisexual, hypogynous, stamens numerous, carpels many, apocarpous, parietal placentation, fruits follicle, stipule scar.

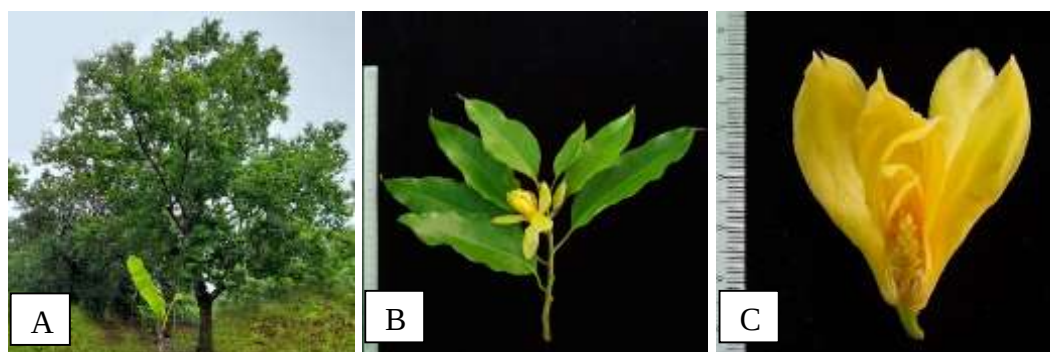


Figure 1 Morphological characters of *Magnolia champaca* (L.) Baill. ex Pierre. A. Habit, B. Inflorescence, C. L.S of flower

Organoleptic evaluation and Microscopic evaluation of powdered flowers of *Magnolia champaca* L.

Table 1 Organoleptic evaluation of tepals of *Magnolia champaca* (L.) Baill. ex Pierre.

No.	Organoleptic evaluation	Fresh tepals	Dried tepals	Powdered tepals
1	Colour	Yellow	Orange brown	Dark brown
2	Texture	Leathery	Brittle	Rough
3	Odour	Aromatic	Aromatic	Aromatic
4	Taste	Indistinct	Indistinct	Indistinct



Figure 2 Flowers (tepals) samples of *Magnolia champaca* (L.) Baill. ex Pierre.
A. Fresh tepals, B. Dried tepals, C. Powdered tepals

Microscopical evaluation of powdered flowers of *Magnolia champaca* L.

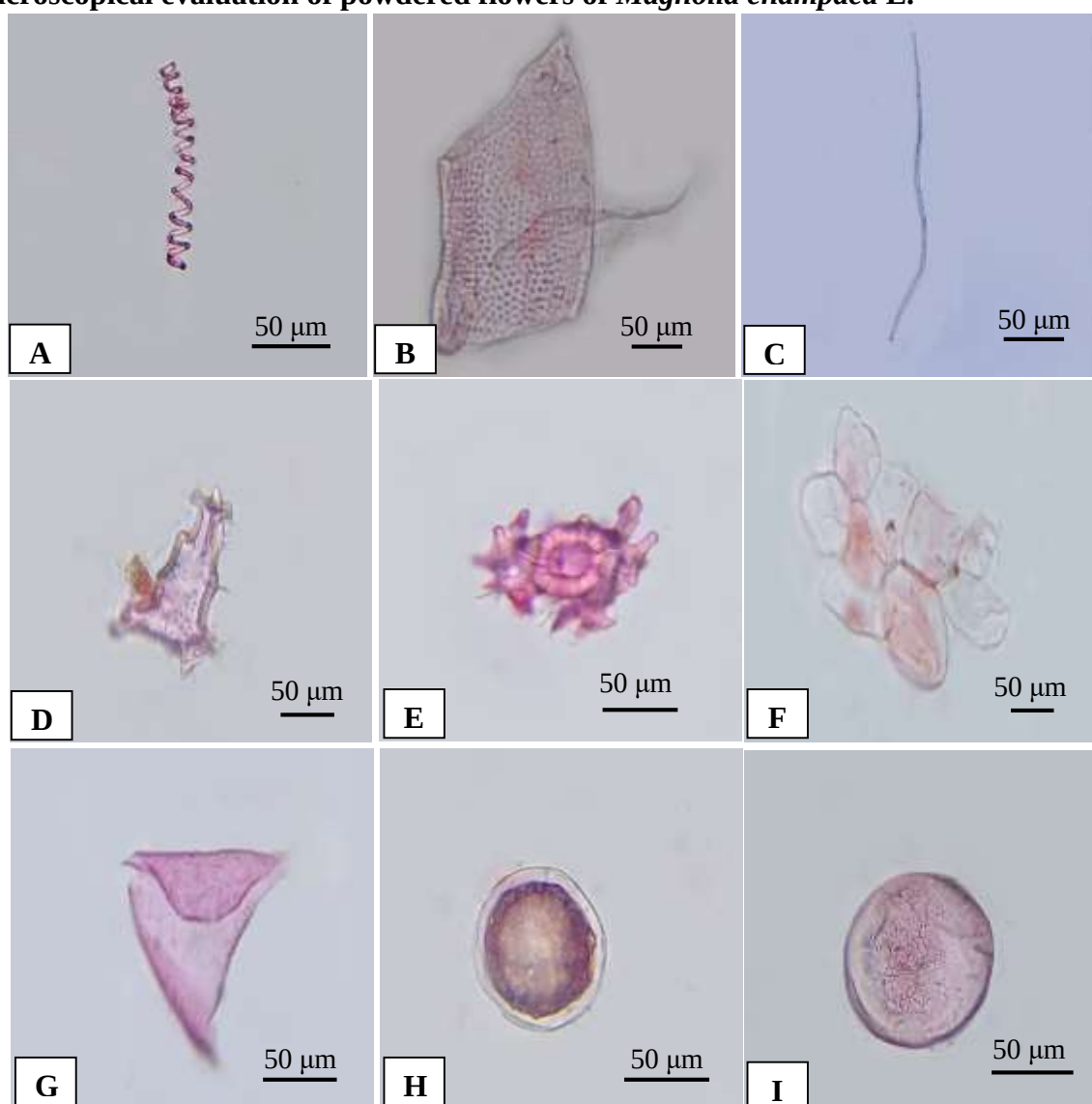


Figure 3 Microscopical characters of powdered tepals of *Magnolia champaca* (L.) Baill. ex Pierre. A. Spiral vessels, B. Pitted vessel, C. Fiber, D and E. Scelerids, F. Fragment of parenchyma cells, G. Fragment of epidermal layer, H. Tannin cell, I. Oil cell

Nutritional values of powdered flowers of *Magnolia champaca* (L.) Baill. ex**Table 2. Nutritional values of powdered flowers of *Magnolia champaca* (L.) Baill. ex Pierre**

No	Constituents	Content (%)
1	Protein	11.78
2	Fiber	17.62
3	Fat	1.98
4	Carbohydrate	63.77
5	Energy Value (Kcal/100g)	320.02

Energy Dispersive X- Ray Fluorescence Spectrometric analysis (EDXRF) of *Magnolia champaca* (L.) Baill. ex Pierre flowers**Table 3 Energy Dispersive X- Ray Fluorescence Spectrometric analysis (EDXRF) of *Magnolia champaca* (L.) Baill. ex Pierre flowers**

No	Component	Analyzed value
1	Potassium (K)	66.2
2	Calcium (Ca)	13.5
3	Magnesium (Mg)	7.13
4	Phosphorous (P)	4.14
5	Sulphur (S)	2.43
6	Aluminium (Al)	2.27
7	Silicon (Si)	1.64
8	Iron (Fe)	0.850
9	Chlorine (Cl)	0.515
10	Copper (Cu)	0.224
11	Zinc (Zn)	0.194
12	Nickel (Ni)	0.185
13	Tin (Sn)	0.157
14	Manganese (Mn)	0.153
15	Rubidium (Rb)	0.145
16	Strontium (Sr)	0.142
17	Titanium (Ti)	0.0580
18	Hafnium (Hf)	(0.0258)
19	Vanadium (V)	(0.0087)
20	Chromium (Cr)	(0.0085)
21	Molybdenum (Mo)	(0.0081)
22	Silver (Ag)	0.0040
23	Bromine (Br)	(0.0030)
24	Ruthenium (Ru)	(0.0017)
25	Carbon monoxide (Co)	ND

No	Component	Analyzed value
26	Gallium (Ga)	ND
27	Germanium (Ge)	ND
28	Arsenic (As)	ND

Atomic Absorption Spectrophotometric (AAS) analysis of *Magnolia champaca* (L.) Baill

Table 4 Heavy metals analysis of flowers of *Magnolia champaca* (L.) Baill. ex Pierre

No.	Test Parameter	Result	Unit	Limit of Detection
1	Arsenic (As)	ND	mg/kg	0.74 ppm
2	Cadmium (Cd)	ND	mg/kg	0.03 ppm
3	Chromium (Cr)	ND	mg/kg	0.09 ppm
4	Lead (Pb)	0.22	mg/kg	0.09 ppm
5	Mercury (Mg)	ND	µg/kg	0.09 ppb

Discussion

In this study, the organoleptic and microscopical characters, presence of elements, heavy metals and nutritional constituents in the flowers of *Magnolia champaca* (L.) Baill. ex Pierre were analyzed. In organoleptic evaluation, the colour, texture, odour and taste of fresh, dried and powdered tepals were examined. The colour of fresh tepals was yellow and turns brown when dried and dark brown in powder state. The texture was leathery in fresh tepals, brittle in dried tepals and rough in powdered tepals. The odour and taste of all three samples was aromatic and indistinct. These evaluations were agreed with Desale *et al.* (2022) who reported that the flowers of this plant are pale yellow to deep yellow and very fragrant. The findings from this study were also in agreement with Geetha *et al.* (2011) and Desai and Lalitha (2018) who mentioned that these powdered flowers were orange brown or dark brown, characteristic odour and gritty texture with uneven coarse particles.

In microscopical study, spiral and pitted vessels, fiber, sclereids (stone cells), fragment of parenchyma cells, fragment of epidermal layer, oil cells and tannin cells were examined in the powdered flowers. These observations were the same with Desai and Lalitha (2018) who stated that the microscopical characters of powdered flowers shows the presence of parenchymatous cells, vessels with spiral thickening, lignified stone cells and oil globules. The analysis of nutritional value of the flowers of *Magnolia champaca* (L.) Baill. ex Pierre flowers showed the presence of protein (11.78%), fiber (17.62%), fat (1.98%), carbohydrate (63.77%) and energy value (320.02%). Among them carbohydrate was found to the highest content. The findings are in agreement with Jakubczyk *et al.* (2022).

According to Energy Dispersive X-ray Fluorescence Spectroscopy (EDXRF), Potassium, Calcium, Magnesium, Phosphorous, Sulphur, Aluminium, Silicon were analyzed in the flowers of *Magnolia champaca* (L.) Baill. ex Pierre. The Potassium was the highest among the elements. Atomic Absorption Spectrophotometry (AAS) analysis showed the trace amount of lead (0.22

ppm) was present in this flower. The WHO limit for lead in herbal drugs was 10 ppm (Olanrewaju *et al.*, 2017). Arsenic (As), Cadmium (Cd), Chromium (Cr) and Mercury (Mg) were not detected in this flower. Therefore, these flowers can be used safely as a medicinal plant.

Conclusion

Magnolia champaca (L.) Baill. ex Pierre flowers have been used as a source of essential oil and in traditional medicine. Because of these uses and demand, the flowers may be adulterated. This study may be helpful to detect the adulterants and to distinguish between the closely related species of these flowers especially in the powder forms. These flowers may be safely used as a dietary supplement such as a form of herbal tea since it possesses rich source of nutrients and elements and lack of toxic heavy metals. These flowers are useful in many purposes such as in aesthetic, culture and medicine. Many kinds of medicinal plants including the old-aged trees of *Magnolia champaca* (L.) Baill. ex Pierre are grown in Mount Popa, an oasis in the desert-like dry central zone of Myanmar. The aromatic and attractive *Magnolia champaca* (L.) Baill. ex Pierre flowers are also the trademark of this area. Therefore, the conserving of these ancient, large and medicinal trees is important to protect the natural forest ecosystem in Mount Popa. For this regard long existing of *Magnolia champaca* (L.) Baill. ex Pierre trees play important role in contributing floral diversity of Mount Popa, which will be designated as “Geopark” in near future.

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ISOLATION AND STRUCTURE ELUCIDATION OF NATURAL ORGANIC COMPOUNDS ISOLATED FROM THE LEAVES OF *GNETUM GNEMON* L.

Swe Swe Aye¹, Alfani Danny Arbianto², Hyun-Jae Jang³

Abstract

The diversity of plants has led to various findings in the medicinal world. The abundance of bioactive existing in the plant is the main factor contributing to its application in pharmaceutical industries. *Gnetum gnemon* L. is an evergreen and perennial gymnosperm belonging to the Gnetaceae family. In Myanmar, it has been consumed as safety foodstuff for centuries. The aim of the present study is to identify the phytochemicals present in the leaves of *G. gnemon*. The crude methanol extract was prepared by maceration method and the obtained extract was concentrated by Rotatory evaporator. The three known constituents were isolated from crude methanol extract through repeated column chromatography separations and elucidated by using UPLC-QTOF/MS, and ¹H and ¹³CNMR spectroscopic technique. The three compounds were elucidated as (1) flavonoidal compound, Vicenin II (2) polyphenol, Piceatannol-3'-O-β-D-glucopyranoside and (3) Aglycone, Rhapontigenin glucoside by comparison with MS and NMR spectra in the previous literature.

Keywords: *Gnetum gnemon* L., Vicenin II, Piceatannol-3'-O-β-D-glucopyranoside, Rhapontigenin 3'-O-β-D-glycopyranoside

Introduction

The native range of *Gnetum gnemon* L. is Tibet to West Pacific. It is a tree and grows primarily in the wet tropical biome. In Myanmar, it is majorly cultivated and is considered as a vegetable belonging to high status. The leaves (Tanyin-ywet) are mostly used as main constituents in traditionalistic vegetable curry in Tanin-thar- ye Region. Moreover, the female strobilus, the male strobili and the unpeeled seeds (Hin-byin- thee) are used as constituents in a dish in upper regions of Myanmar. The phytochemical screening of *Gnetum gnemon* L. revealed the presence of phenol, flavonoids, alkaloids, tannin, phytosterols and carbohydrates for different extract prepared from leaves. (Preetham *et al.*, 2015). Vicenin 2 is a potent inhibitor of alpha glucoside. It has been reported to have a range of pharmacological values which includes antioxidant, hepatoprotective, anti-inflammatory and anticancer (Yang *et al.*, 2018). Stilbenoids, Piceatannol is classified as phenolic acid derivatives. It is well known for pharmacological effects such as antioxidant, antimicrobial, anti-inflammatory and antiproliferative effects (Akinwumi *et al.*, 2018). Rhapontigenin is aglycone of rhaponticin and it has to be a potent anti-allergic, anticoagulant, and anti-inflammatory compound (Roupe *et al.*, 2005). In this study, there has been no reported literature related to phytochemical components in the leaves of *G. gnemon* L. in Myanmar. Therefore, the goal of this study is to determine the chemical composition of *G. gnemon* leaves using spectroscopic techniques.

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Materials and Methods

I. Plant material Collection and Identification

The fresh aerial parts of the plant *Gnetum gnemon* L. were collected from Ye Phyu Township; Thanintharye Division, Myanmar during the flowering period of March 2019–2020. The voucher specimen was deposited at the Plant Extract Bank of Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea.

II. Preparation of plant extracts from dry leaves of *Gnetum gnemon* L.

Firstly, the leaves were washed with distilled water and were dried at 40 °C for 72 hours. The dried leaves were pulverized to a homogeneous powder using an electric blender. The powdered samples were stored in airtight containers to preserve the biomolecules present in the plant and stored at room temperature. Then, the crude plant extract was prepared by the maceration method. The dried samples (5 kg) were extracted with methanol at room temperature, filtered, and evaporated using a rotary evaporator (50°C, 250 rpm) to obtain the total extract (714.5g).

III. Isolation of organic compounds from the methanol extract from dry leaves of *Gnetum gnemon* L.

General experimental procedures

The *Gnetum gnemon* L. methanol extract (250.0g) was fractionated into five fractions using open column chromatography (C.C) using HP-20 resin. The fractionated sample (G2) that contained the targeted peak were isolated by a preparative liquid chromatography system. Furthermore, the peaks were isolated by preparative HPLC to get a pure peak. The preparative HPLC was performed using C₁₈ columns, YMC-PACK ODS-AQ-HG, (250 × 20 mm, 10 µm, YMC, Kyoto, Japan), Acclaim Polar Advantage II, (21.2 × 250 mm, 5.0 µm, Dionex, Sunnyvale, CA, USA), and Kinetex Biphenyl (20 × 250 mm, 5.0 µm, Phenomenex, Torrance, CA, USA) and MPLC system: Spot Prep II System using C₁₈ column (220g, 250 × 60 mm, 10 µm, YMC). The chemical structure of isolated compounds was elucidated using a JEOL ECZ500R [¹H NMR at 500 MHz, ¹³C NMR at 125 MHz, (JEOL, Tokyo, Japan)], and ultra-performance liquid chromatography (ACQUITY UPLC, Waters, Milford, MA, USA) equipped with a photodiode array detector (PDA, Waters) coupled to a quadrupole time of flight mass spectrometer (Micromass QToF Premier mass spectrometry, Waters) to collect spectroscopic data such as UV, MS, MS/MS, HRESIMS. AQUITY BEH C₁₈ column (1.7 µm, 2.1 × 100 mm, Waters) was used on UPLC-PDA-QToF-MS operated at 35°C with mobile phase A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient was as follows: 0.0 min (A: B = 95:5 v/v) → 1.0 min (95:5, v/v) → 8.0 min (60:40, v/v) → 8.3 – 10.0 min (0:100, v/v), → 10.30 – 12.0 min (95:5, v/v). The flow rate was 0.4 mL/min and the injection volume was 2 µL. MS data were obtained in negative ion mode under the following conditions; source temperature 110 °C, desolations temperature 350 °C, capillary voltage 2.3 kV, and cone voltage 40 V. Qualitative analysis and tentative identification of major constituents from methanol extracts of *G. gnemon* were carried out by ACQUITY UPLC (Waters) system connected to Micromass Q-TOF Premier mass spectrometry (Waters) with an electrospray ionization device, operating in the negative ion mode that were acquired using Mass Lynx software (ver. 4.1, Waters).

Results and Discussion

I. Morphological Characters of *Gnetum gnemon* L.

Scientific Name - *Gnetum gnemon* L.

Local Name - Hyinbyin, Tanyin-ywe

English Name - Melinjo

Family - Gnetaceae

Flowering and Fruiting period - October to February

Evergreen dioecious trees. Leaves simple, opposite, lanceolate in young leaves and elliptic in mature leaves, the exstipulate. Male spikes axillary or terminal, one male spike contain 5-9 involucre collars, each collar contains 12-50 male flower and 3-9 globose sterile female flowers. Male flowers with a claw-shaped perianth, stamen one, stamen adnate to the base of the perianth, synangia (anther) bifid. Female spikes axillary or terminal, one female spike contains 5-9 collars, each collars with 3-9 female flowers. Female flower globose, without perianth, orthotropous ovule, ovoid, three integuments. Seed large, drupe-like, pink or red in mature, the fleshy coats enclosing the hard seed, with longitudinally ribbed.



Figure.1 Habit of *Gnetum gnemon* L.



Figure.2 Leaves as seen



Figure.3 Male Strobilus of *Gnetum gnemon* L.



Figure 4 Female Strobilus of *Gnetum gnemon* L.



Figure 5 Fruits as seen

(1) Identification of compound 1 from the methanol extract of leaves of *Gnetum gnemon* L.

The molecular formula of compound 1 was deduced as $C_{27}H_{30}O_{15}$ from the deprotonated ion peak at m/z 593.1505 $[M - H]^-$ in HR-ESI-MS data. For the precursor ion at m/z 593, the presence of di-*C*-glucosidic linkages were assumed based on the two product ion peaks at m/z 473 $[M - H - 120]$ and m/z 353 $[M - H - 120 - 120]$ in the fragmented MS spectrum. Vitexin is a well-known, naturally occurring flavonoid that has the apigenin skeleton (Kim *et al.*, 2005). The 1H and ^{13}C NMR spectra of compound 3 were similar to those of Vitexin, except for the presence of an additional β -anomeric signal [δ_H 4.74 (H-1'') and δ_C 74.2 ppm (C-1'')]. Compound 1 was identified as Vicenin 2 based on comparisons with the NMR and MS spectroscopic data in the reported literature (Islam *et al.*, 2014 and Wang *et al.*, 2021).

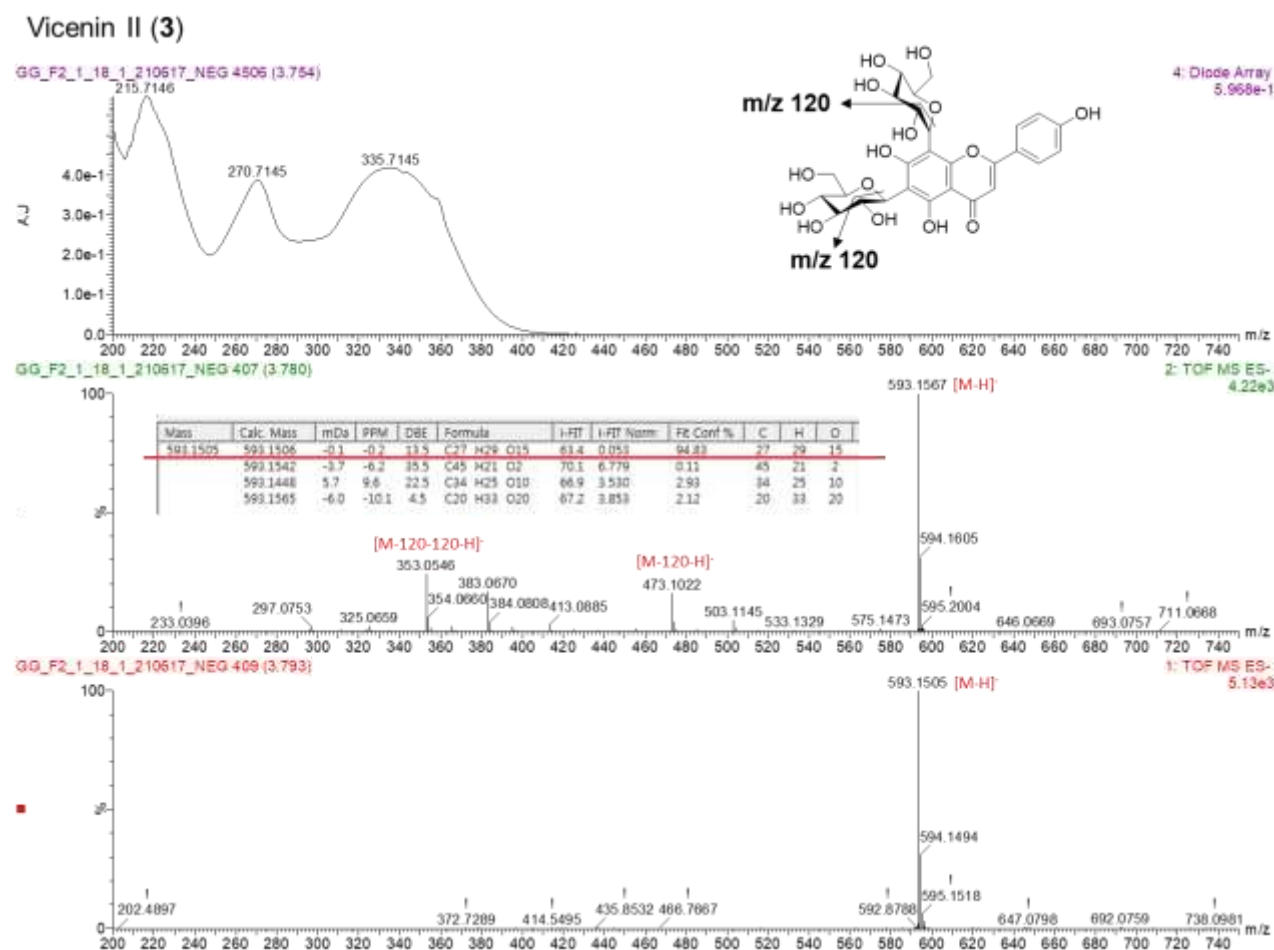


Figure. 6 UV and UPLC-QTOF-MS spectra of Vicenin II

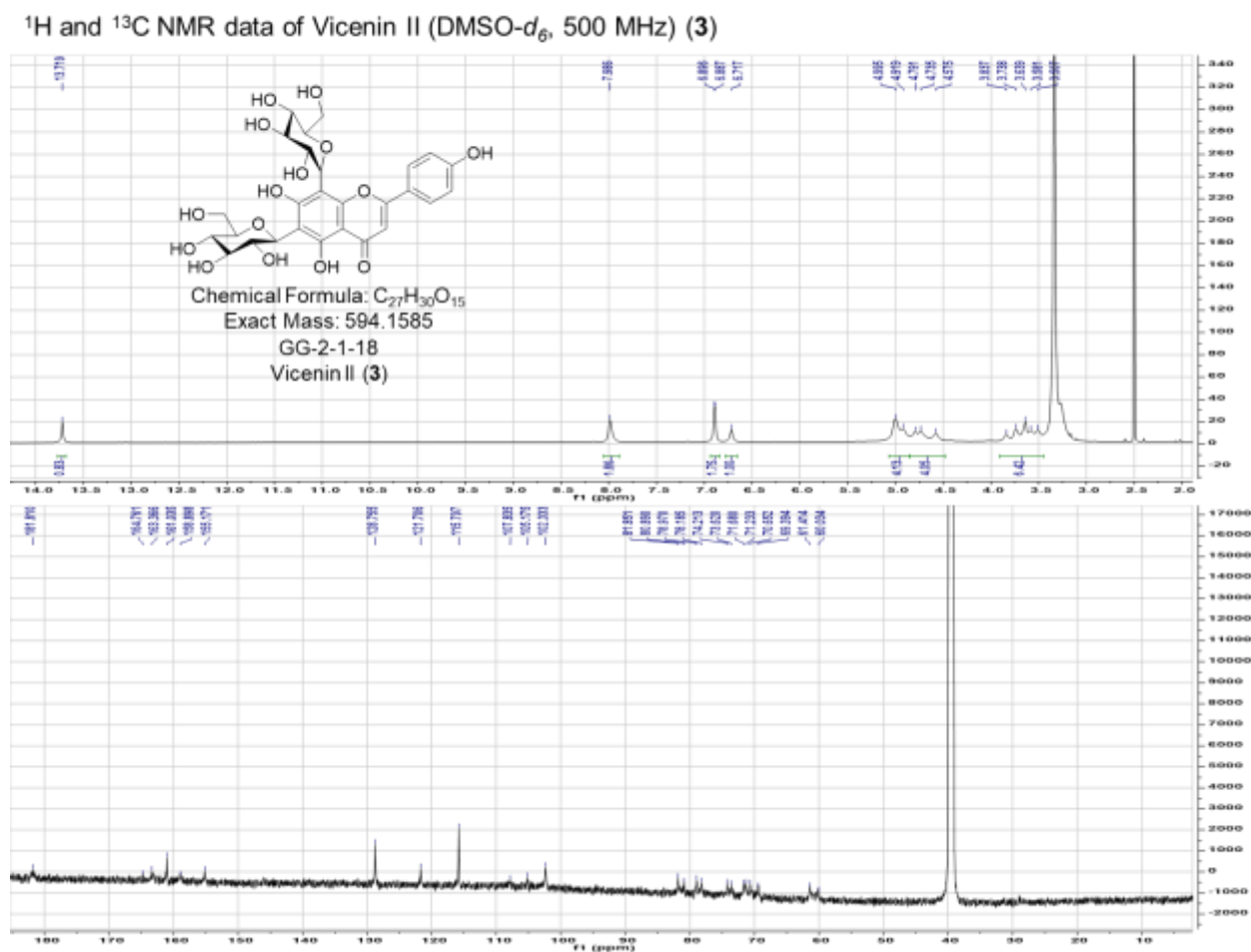


Figure.7 ¹H and ¹³C NMR (500 MHz, methanol-*d*₄) spectrum of Vicenin II

(2) Identification of compound 2 from the methanol extract of leaves of *Gnetum gnemon* L.

The molecular formula of compound 2 was determined to be C₂₀H₂₂O₉ by analyzing a pseudomolecular ion peak at *m/z* 405.1230 in the HR-ESI-MS spectrum and the fragmented MS spectrum of the parent ion at 405 produced ions at *m/z* 243 [M – hexose][–]. The ¹H NMR data of compound 2 displays *trans*-olefinic protons [δ_H 6.90, 6.85 (d, *J* = 16.0 Hz)], 1,3,4-trisubstituted aromatic ring signals [δ_H 7.08 (1H, d, *J* = 8.0 Hz); 7.05 (1H, d, *J* = 2.0 Hz); 6.93 (1H, dd, *J* = 8.0, 2.0 Hz)], 1,3,5-trisubstituted aromatic ring signals [δ_H 6.39 (2H, d, *J* = 2.0); 6.12 (1H, t, *J* = 2.0 Hz)], and glucose resonances [δ_H 4.69 (1H, d, *J* = 7.0, H-1''); 3.17–3.72 (6H, m)]. The HMBC correlation of anomeric proton (H-1'') to C-3' (δ_H 145.2) placed the glucose group at the 1,3,5-trisubstituted aromatic group. Comparison of these MS and NMR spectral data with those previously reported in the literature (Kashiwada *et al.*, 1990) suggested that compound 2 was Piceatannol-3'-*O*- β -D-glucopyranoside.

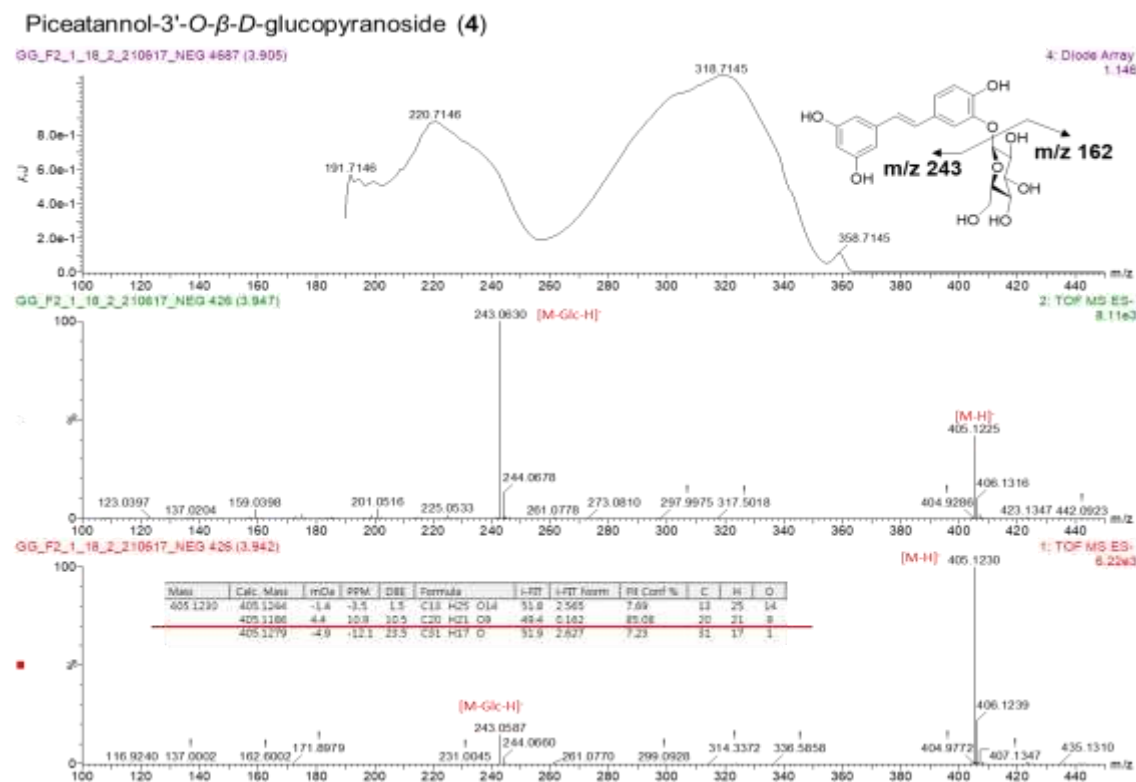


Figure 8. UV and UPLC-QTOF-MS spectra of Piceatannol-3'-O- β -D-glucopyranoside

^1H and ^{13}C NMR data of Piceatannol-3'-O- β -D-glucopyranoside (DMSO- d_6 , 500 MHz) (4)

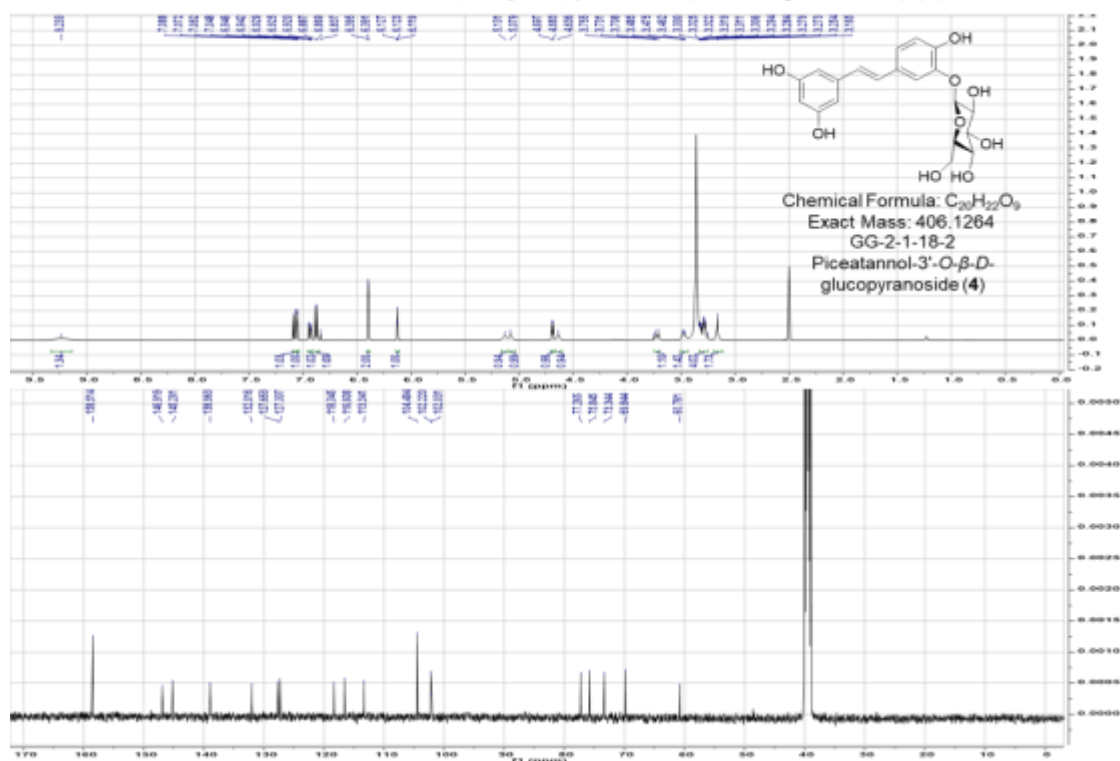


Figure 9. ^1H and ^{13}C NMR (500 MHz, DMSO- d_6) spectrum of Piceatannol-3'-O- β -D-Glucopyranoside

(3) Identification of compound 3 from the methanol extract of leaves of *Gnetum gnemon* L.

The molecular formula of compound 3 was deduced as $C_{21}H_{24}O_9$ from deprotonated ion peak at m/z 419.1371 in the HR-ESI-MS data. The UV, HRMS, 1H and ^{13}C NMR spectroscopic data were similar to those of Piceatannol-3'- O - β -D-glucopyranoside, except for additional methoxy group [δ_H 3.82, (3H, s, OCH_3 -4'); δ_C 55.7]. The position of the methoxy group was determined to be at C-4' (δ_C 149.1) based on the HMBC cross-peak between OCH_3 -4' and C-4'. Therefore, compound 3 was assigned to be Rhapontigenin-3'- O - β -D-glucopyranoside (Kashiwada *et al.*, 1990).

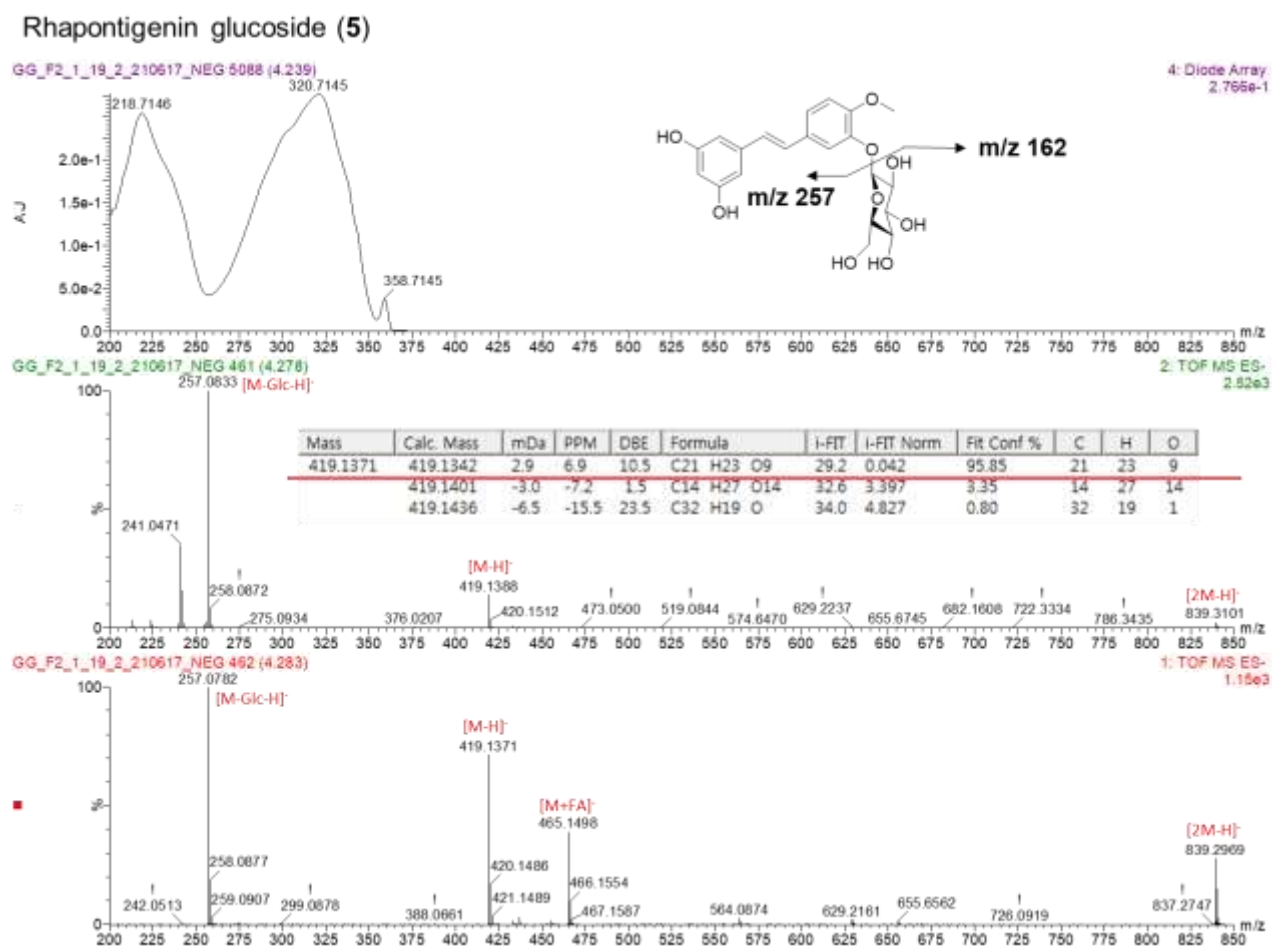


Figure 10. UV and UPLC-QTOF-MS spectra of Rhapontigenin-3'- O - β -D-glucopyranoside

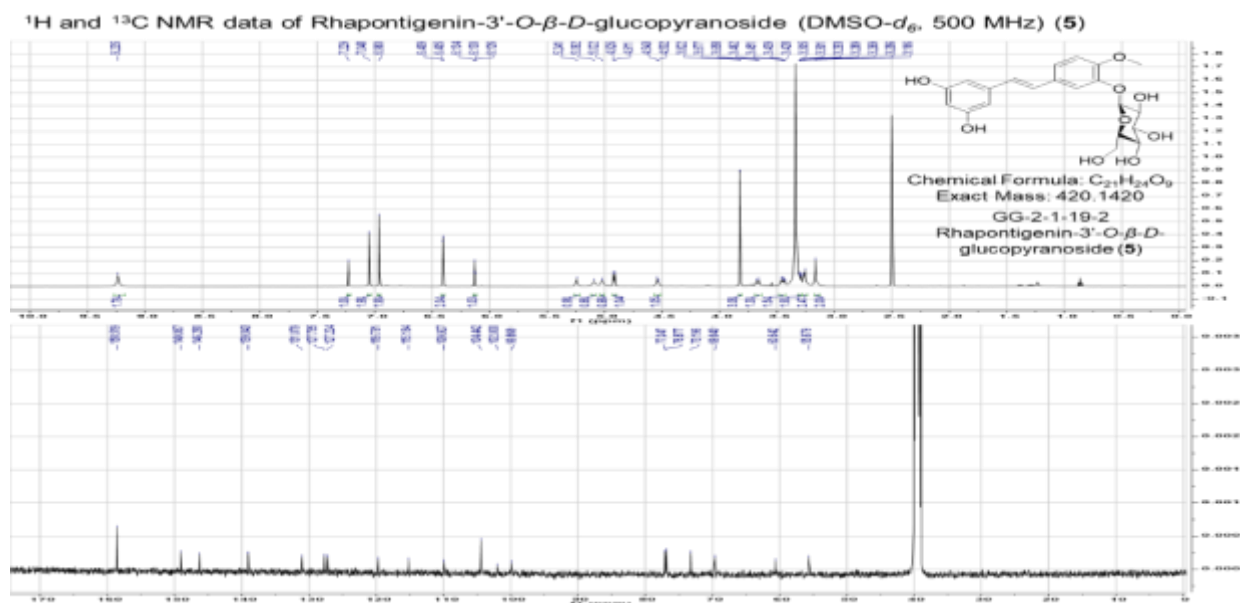


Figure. 11 ^1H and ^{13}C NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of Rhapontigenin-3'-O- β -D-glucopyranoside

Conclusion

The leaves of *Gnetum gnemon* L. was selected to isolate pure organic compounds. The isolated three compounds are Vicenin II, Piceatannol-3'-O- β -D-glycopyranoside, Rhapontigenin-3'-O- β -D-glycopyranoside. The molecular formula of compound 1, Vicenin II was deduced as $\text{C}_{27}\text{H}_{30}\text{O}_{15}$ from the deprotonated ion peak at m/z 593.1505 $[\text{M} - \text{H}]^-$ in HR-ESI-MS data, whereas the molecular formula of compound 2, Piceatannol-3'-O- β -D-glycopyranoside was determined to be $\text{C}_{20}\text{H}_{22}\text{O}_9$ by analyzing a pseudomolecular ion peak at m/z 405.1230 in the HR-ESI-MS spectrum. The molecular formula of compound 3 was deduced as $\text{C}_{21}\text{H}_{24}\text{O}_9$ from deprotonated ion peak at m/z 419.1371 in the HR-ESI-MS data.

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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH OF PERENNIAL RICE UNDER PHOSPHORUS TREATMENT

Khin Yuzana¹, Fang Liu², Yujiao Zhang³, Fengyi Hu⁴

Abstract

This experiment was explored the effect of arbuscular mycorrhizal fungi (AMF) on perennial rice 23 growth allocation under phosphorus conditions. We carried out a pot experiment of inoculating *Rhizophagus irregularis* (AM) fungus with perennial rice 23, and set up three different phosphorus (P) levels (40 μ M, 80 μ M and 160 μ M KH_2PO_4). AM fungus inoculation had significant effect on perennial rice physiological and associated gene expression compared to the control group. Plants phosphate transporter (PT) genes (*OsPT11* and *OsPT13*) were significantly up-regulated with AMF colonization under different P conditions. The inoculation with AMF showed improved plant height, plant biomass of perennial rice 23 (PR 23). In addition, phosphorus treatment also significantly increased the AMF colonization in roots of perennial rice. AMF plays a vital role in diverse functions such as soil health improvement, plant health improvement, and protection of plants against pathogen. Therefore, the molecular and functions understanding of AMF in perennial rice is very important to select an efficient species to reap the full beneficial effects from these fungi.

Keywords: Perennial rice, AMF, Phosphorus

Introduction

Perennial crops can provide effective yields and through maintaining the environment ([Glover and Regamol. 2010](#)). The main characteristic of perennial crops is no need for replanting or tillage as it can regrow after harvest. Consequently, the production of perennial rice would decrease labor costs and field management tasks (Zhang *et al.* 2018a, 2018b). Perennial crops can preserve soil biodiversity and aid the development of natural soil systems with improved soil health, high drought resilience, and long-term stability. Moreover, the perennial cropping gives a greater above-ground biomass production. Perennial rice efficiently utilizes water and nutritional resources from the subsoil. It is therefore a solution to the current climate change and depletion of nutrients and water due to intensive agriculture (Burmeister, 2021; Sanford *et al.* 2021). Lack of tillage and use of repeated pesticides may allow the evolution of pathogenic fungi, pests and weeds in host plants (Shim, 2012). In terms of soil fertility, no-tillage and low organic matter in soil along with the developed root system of plants can accumulate high carbon concentrations to the extent that nitrogen and phosphorus are imbalanced. In cases where perennial crops require nutritional support, these inputs would be optimally utilized and leveraging the capability of their root systems to capture nutrients (Qaswar *et al.* 2019). In order to prevent soil erosion, pathogens presence, and nutrient scarcity such as phosphorus, perennial rice has to be grown with inoculated microbes as biofertilizers.

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Microbial communities include a pivotal role in the functioning of plants by influencing their physiology and development (Bjorn *et al.* 2016). Among these microorganisms, arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota) are important components of soil microbial communities. AMF can exchange the nutrient via rice roots in flooded fields and AMF colonization of rice roots under wetland conditions has even been the subject of debate (Vallino *et al.* 2009; Lumini *et al.* 2011; Watanarojanaporn *et al.* 2013).

AMF is considered as an important weapon in a soil with low endogenous P (Panigrahy *et al.* 2009). Plants have developed sophisticated mechanisms to attract and interact with the fungi (Gutjahr and Parniske 2013) and P transporters have evolved and specifically expressed in infected cells (Paszkowski *et al.* 2002; Javot *et al.* 2011; Yang *et al.* 2012). Under high P conditions, AM infection is generally low, which is partly due to the P- and N-dependent synthesis of strigolactone, the plant hormone essential for the establishment of the symbiosis (Yoneyama *et al.* 2012) but also on the nitrogen status of the plant and root inherent processes that impair colonization (Javot *et al.* 2011). The rice genome contains thirteen P transporter genes in the Pht1 family (Paszkowski *et al.* 2002). Of these, ORYsa; *PHT1;1* (*PT1*), *PT2*, *PT6*, and *PT8* have been implicated in direct Pi uptake from the soil (Ai *et al.* 2009a). *PT2* has been classified as a low affinity and *PT6* as a high-affinity transporter (Ai *et al.* 2009b). The transporter genes *PT11* and *PT13* are specifically expressed in AM-colonized roots and both are required for the establishment of AM symbiosis (Paszkowski *et al.* 2002; Gutjahr and Parniske 2008). However, it was recently shown that *PT11* alone is required and sufficient for symbiotic P uptake (Yang *et al.* 2012).

The aim and objectives of the present study is to determine the interaction between perennial rice and AMF under phosphorus condition and to support for improving perennial rice productivity and sustainable agricultural development by the inoculation of AMF.

Materials and Methods

Plant and AM fungal materials

The perennial rice 23 was used in this experiment. Seeds of perennial rice were kindly provided by the breeding and foundation seed program at the School of Agriculture, Yunnan University, Kunming, China.

A commercial inoculum of AM fungi *Glomus intraradices* (*Glomus intraradices* Schenck & Smith DAOM 197198 (recently reassigned to *G. irregulare* and the *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schubler comb. nov.; Stockinger *et al.*, 2009) were selected to establish and promote symbiosis with perennial rice in greenhouse.

Experimental Design

The pot experiment was conducted in a greenhouse (24.50°N, 102.51°E) at the Institute of Resource Botany, Yunnan University. First, the preparation of PR 23 seedlings and the potting experiment were designed. Rice seeds were sterilized with 70 % (v/v) sodium ethanol solution for 15 min, and before sowing, the seeds were soaked in moist filter paper sterilized at about 30°C for 48 h until the emergence of radicle. Germinated seeds were planted in pots containing 800 ml of substrate (seedling height 12 cm ,volume 1 L, 3 plants per pot) with or without AMF (spore addition of 1000 *Rhizophagus irregularis* per pot). The substrate consisted of 75% soil and

25% sand. The field soil and sand were sterilized three times (121°C for 20 min each time). The potted plants were maintained in a greenhouse at a temperature of 26-30°C day/night and 60% relative humidity until harvest. Each treatment was replicated four times for a total of 27 pots.

Three treatments: unsterilized soil, sterilized soil without AMF inoculation (control), and sterilized soil with AMF, AMF and phosphate levels (40 µM: P1, 80 µM: P2, 160 µM: P3). After 6 weeks of planting, the fresh and dry weights of the ground and lower part of the rice were determined for the different treatment groups. The mycorrhizal symbiosis rate was calculated by staining the rice root system. Total RNA was extracted from rice roots for transcription and sequencing to isolate phosphate transporter genes (*OsPT11* and *OsPT13*).

Mycorrhizal symbiosis rate statistics

The rice root system was washed, dried, cut into 1 cm long root segments, and fixed with FAA fixative for more than 4 h. The root segments were then dried and cut into 1 cm long root segments. The root segments were removed and sequentially washed by 10% KOH solution at 90°C, and heated in water bath for 1 h; poured into KOH. After rinsing it several times in running water, it was stained by 2% hydrochloric acid for 5 min. Then, 0.05% triphenylmethyl blue staining solution was added at 90°C water bath heating for 30 min. After pouring out the staining solution, lactic acid glycerol was added. The decolorizing solution was applied several times until most of the excess dye was removed from the root segments, and the sections were placed under a microscope for observation. The AMF infiltration rate of the root segments was graded using the method.

The infection intensity M% was calculated as:

$$M\% = \frac{95 * n_5 + 70 * n_4 + 30 * n_3 + 5 * n_2 + n_1}{\text{sample total root segments}}$$

RNA Isolation, cDNA Synthesis, and qRT-PCR Assay

Frozen root and leaf samples of rice were ground in liquid nitrogen for nucleic acid extraction. The isolated RNA from the tissue powder was extracted by using Trizol RNA isolation kit (Sangon, Shanghai, China) according to the manufacturer's protocol. Subsequently, the RNA was pretreated with DNase I, followed by verification of sufficient removal of genomic DNA contamination by PCR using primers exclusively targeting genomic DNA. Quality of the extracted RNA was quantified using a Nano Drop 2000. RNA purity was estimated by calculating the A260/A280 ratio. One microgram of RNA for each sample was reverse-transcribed to synthesis cDNA with PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) (Gutjahr *et al.* 2008; Pimprikar *et al.* 2016). Quantitative real-time qRT-PCR was carried out using SYBR® Green qRT-PCR assay. The 20-µL PCR reaction contained: 0.5 µL of each primer, 1 µL of cDNA template, 8 µL of double-distilled H₂O, and 10 µL of SYBR Premix Ex Taq II (TaKaRa). The PCR procedure was as follows: denaturation at 94°C for 4 min, then 20 cycles at 94°C for 45 s, 58 or 60°C for 25 s, and 72°C for 10 s, and finally extension at 72°C for 10 min. For each gene, the experiment was repeated three times and each biological replicate was a mean of three technical replicates. Normalized expression analysis was conducted following the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen (2001), and the transcriptional levels for each gene were normalized using the reference gene. The housekeeping genes were used for the normalization of gene expression values in the respective species.

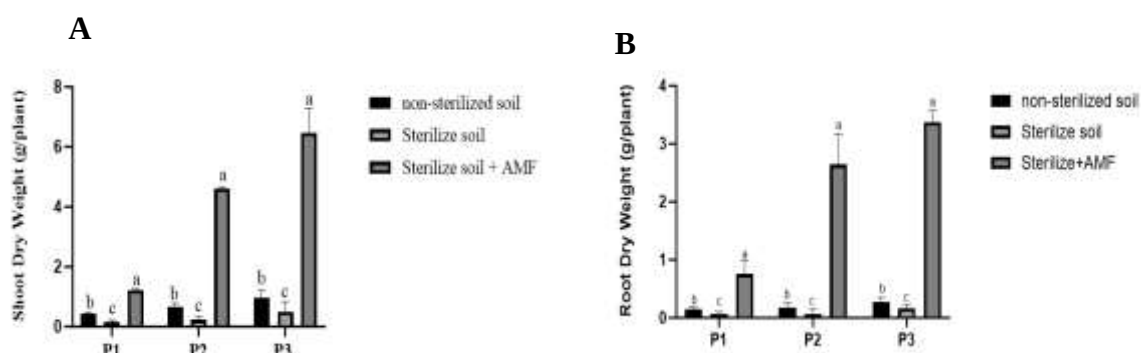
Table 1 Gene-specific primers for qRT-PCR analysis

	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>OsPT11</i>	CATATCCCAGATGAGCGTATCATG	GAGAAGTTCCCTGCTTCAAGCA
<i>OsPT13</i>	CTTCTCCATCTCCCTTGTCG	TTTTGTCGCCTAGCCAGCC
<i>Ubiquitin</i>	CATGGAGCTGCTGCTGTTCTAG	CAGACAACCATAGCTCCATTGG

Results

Analysis of plant biomass

In the present study, the biomass of perennial rice under interactive treatments of three phosphorus (40 μ M, 80 μ M and 160 μ M) concentration and AMF was performed. As Figure 1 showed, phosphorus condition showed no significant influence on shoot dry weight of perennial rice neither in non-sterilized or in sterilized soil. Whereas, under the same phosphorus condition, planting in non-sterilized soil showed higher plant shoot biomass compared to sterilized soil. Inoculation with AMF in sterilized soil has increased the shoot biomass to the highest among different treatments ($p < 0.001$). When AMF inoculation with sterilized soil, the shoot biomass reached the maximum under three phosphorus treatment, which was 87%, 93% and 95% compared to non-sterilized soil without AMF inoculation and followed by sterilized soil with non-inoculated about 25%, 57% higher than the control in non inoculated AMF with non-sterilized soil. The total biomass of root difference significantly under AMF inoculation with phosphorus addition 88% under 40 μ M, 92% in 80 μ M and 96% for 160 μ M compared to non-inoculation AMF with non-sterilized soil and sterilized soil, respectively. Analysis of the shoot and root data revealed a significant increase with AM fungi inoculation in three phosphorus than that of the non-mycorrhizal plant in non-sterilized soil and sterilized soil. A significant increase in shoot and root with AMF inoculation at three phosphorus level was observed in this experiment. To the overall, the highest shoot and root biomass increase was observed in the highest phosphorus. But under the same phosphorus levels, there was no difference in the shoot and root dry weight of perennial rice plants between non-sterile soil with and without AMF inoculation. Furthermore, mycorrhizal inoculation was resulted in significantly higher than non-mycorrhizal inoculation in sterile soil (Figure 1 A and B).

**Figure 1.** Biomass of shoot (A) and root (B) under different levels of phosphorus treatment

Note: Biomass of shoot phosphate content of rice: B. Biomass of root phosphate content of rice.

The different letters designate the parameters ($p < 0.05$)

Analysis of the plant height

One of the growth indices, plant height was investigated from the perennial rice at 42 days after inoculation with AMF (Figure 2). The plant height of AMF-inoculated rice was significantly improved as compared to that of sterilized soil without AMF inoculation under three phosphorus levels. Inoculation of AMF and P supplementation resulted in improved plant height at increase of 24%, 42% and 57%, respectively than that planted in sterilized soil with P supplement. The inoculated AMF with P supplement can reduce non-sterilized soil without AMF with P supplement about 19%, 36% and 49%. Non inoculated with sterilized soil exhibited reduced plant height 39%, 96% and 100% respectively by AMF at three phosphorus (40 μ M, 80 μ M and 160 μ M).

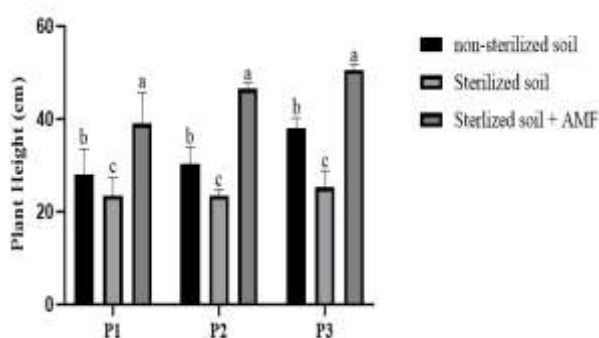


Figure 2. Comparison of plant height of rice plants under phosphorus treatment

Note: Effect of *Rhizophagus irregularis* on the plant height of PR 23 under different phosphorus level of non-inoculated and inoculated AMF. The values of each parameter labeled by different letters indicated significant differences ($p < 0.05$)

Analysis of AM fungal rate in rice

AMF was inoculated with different soil types and phosphorus levels and grown in a greenhouse. Under different P treatments, perennial rice inoculated with AMF showed highest colonization percentage (87%, 90%, and 93%). As showed in Figures 3-5, the sterilized soil inoculated with AMF with the highest phosphorus treatment level was significantly higher than the low and medium phosphorus treatments. Under the three phosphorous levels, sterilized soil with AMF showed the highest colonization rate. In all the three phosphorus level treatments, colonization rate in the non AMF inoculated and non-sterilized soil was significantly lower than and non inoculated sterilized (Figure 3).

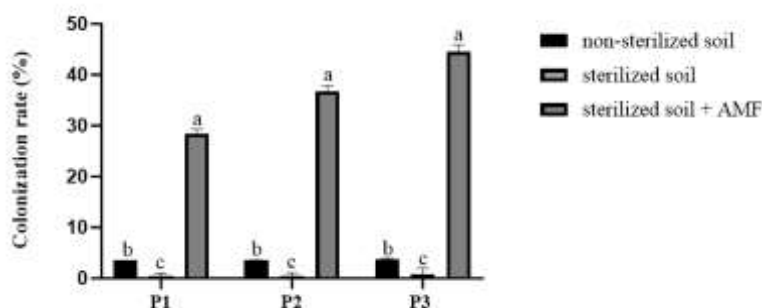


Figure 3. Comparison of root colonization rate of arbuscular mycorrhizal fungi under phosphorus treatment

Note: Effect of *Rhizophagus irregularis* on the rice root colonization rate of PR 23 under different phosphorus concentration of non-inoculated and inoculated AMF. The values of each parameter labeled by different letters indicate significant differences assessed ($p < 0.05$)

AM Fungal colonization characteristics in roots

Roots of all the perennial rice plants inoculated/non inoculated with AMF under different phosphorus conditions were examined and AMF colonization was characterized by intraradical colonization (Figure 4, A), intercellular hyphae and vesicles (Figure 4, B), intracellular hyphae with arbuscules (Figure 4, C) and Arbuscule and intercellular hyphae (Figure 4, D).

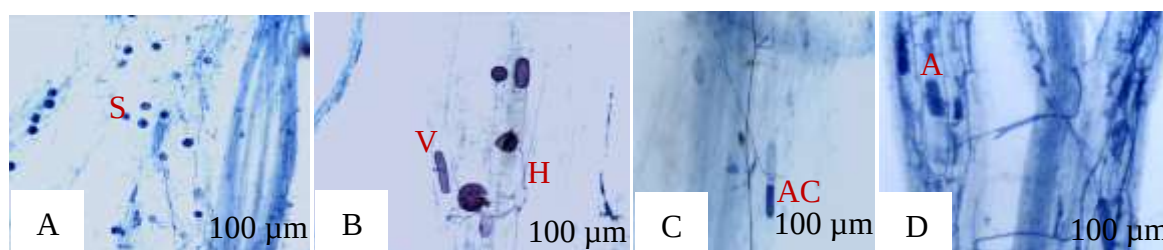


Figure 4. Arbuscular mycorrhizal (AM) fungal colonization in the rice root

Note: A- intraradical colonization; B- intercellular hyphae and vesicles; C- intracellular hyphae with arbuscules; D- Arbuscule and intercellular hyphae. Scale bars 100µm

Analysis of phosphate transporter

Expression of two arbuscular mycorrhizal fungi (AM)-mediated P uptake (*OsPT11* and *OsPT13*) were analyzed in root samples of perennial rice genotypes at 6 weeks after sowing by quantitative polymerase chain reaction. Data was generated by taking average values of perennial rice in three different soil types. *OsPT11* and *OsPT13* cDNA were isolated from the total RNA of mycorrhizal rice roots, followed by PCR amplification using primers derived from the gene sequence. The same primers were used for amplification of the structural gene. Expression of P-transporter genes was quantified in root samples. PT11 and PT13 were specifically expressed in AM-colonized roots. This study has therefore showed the relative upregulation of *OsPT11* gene by 1.16-fold, 1.30-fold and 2.2-fold in mycorrhizal groups than with the non-mycorrhizal groups under different phosphorus concentration and soil feature. Likewise, *OsPT13* has also revealed a significant regulation by 0.84-fold ($p < 0.01$), 1.03-fold ($p < 0.05$) and 2.12-fold ($p < 0.05$) under different level of phosphorus treatments (Figure 5, A and B).

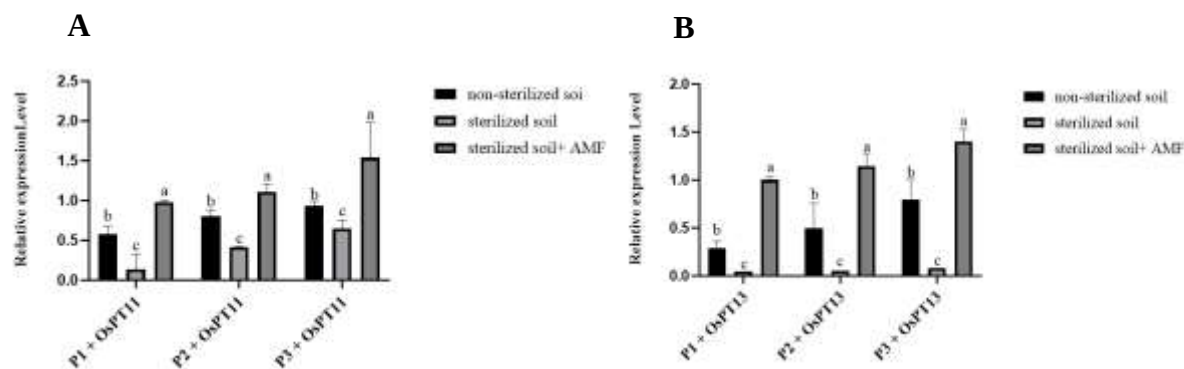


Figure 5. Relative gene expression of *OsPT11* and *OsPT13* from the root of perennial rice grown in different phosphorus levels

Discussion

Phosphorous (P) is a key essential, non-replaceable element for plant growth and development. Phospholipids mainly composed of P are components of the cell membrane. Notably, P is used in energy transfer, photosynthesis, metabolism, intracellular signaling and gene replication and expression. The low availability of P is the main constraint in much of the low input agriculture worldwide. There is a huge importance of P in several crops such as rice, barley, maize, sugar beet, and common bean. Hence growth and productivity of major crops world wide are limited by P availability.

AMF inoculation improves phosphorus acquisition, especially when the availability of soil P is low. The extending hyphae of AMF can greatly increase the absorption area and can help to solubilize P in the soil. For a species with poor root development, AMF association could enhance P uptake and promot plant growth and yield. This study is therefore demonstrating the alleviation role of AMF under phosphorus treatment in particular to an increased rice plant growth, colonization rate, shoot and root growth and upregulation of responsive genes.

In the different concentration of phosphorus, above ground and underground biomass of rice in the AMF inoculate group was significantly enhanced compared with the AMF non-inoculated group. AMF had little effect on both aboveground and underground rice biomass with phosphorus in the AMF non-inoculated with non-sterilized soil and sterilized soil. With the increase in phosphorus concentration, the effect of AMF on rice biomass gradually appeared and became more and more obvious.

In non-sterilized soil, due to the potential indigenous mycorrhiza propagules, there was significant difference than sterilized soil with non-inoculated AMF under different phosphorus stress. However, inoculated sterilized soil was significantly different from both non-sterilized and sterilized soils under three phosphorus levels.

The present study showed that the AMF-inoculated plants had higher P acquisitions, lower P uptake, increased expressions of *OsPT11* and *OsPT13* compared with non-mycorrhizal plants in non-sterilized soil and sterilized soil. The increased expressions of *OsPT11* and *OsPT13* were also in line with previous studies conducted by Paszkowski *et al.* (2002) and Glassop *et al.* (2007), which showed that the expressions of *OsPT11* were significantly up-regulated in rice associated with AMF. In addition to the higher concentration of P detected in mycorrhizal plant

tissue, the appearance of *OsPT11* in root tissue can be considered as one of the indications of symbiotic success (Paszkowski *et al.* 2002).

The *OsPTs* (*OsPT1-13*) are known to contribute to the accumulation of phosphate (Goff *et al.* 2002). The AMF symbiosis increased *OsPT11*, which was supposed to be localized in the arbuscule-containing cell (Rausch and Bucher 2001; Paszkowski *et al.* 2002). The changes of *OsPT11* and *OsPT13* only play parts of the program of taking up phosphate by the rice plant when colonized by AMF as other *OsPTs* are also responsible for the P acquisition.

Conclusion

In the present study, inoculation of perennial rice (PR 23) with arbuscular mycorrhizal species of *Rhizophagus irregularis* showed a higher performance than the non-sterilized and sterilized soils without AMF inoculation under different phosphorus stress. The importance of AMF was evident by the significant correlation between mycorrhizal colonization, physiological, and relative expression (*OsPT11* and *OsPT13*). The higher content of P in mycorrhizal plants can lead to the higher physiological, and relative expression. *Rhizophagus irregularis* could increase the phosphate transporter of rice in mycorrhizal plants and decreased in non-sterilized soil and sterilized without AMF inoculation. The increased expression of *OsPT11* and *OsPT13* were the most important factors that led to the significantly higher P concentration in plant tissues.

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INVESTIGATION ON CULTIVATION OF FOUR PERENNIAL RICE CULTIVARS IN YEZIN, NAY PYI TAW UNION TERRITORY, MYANMAR

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Abstract

One of the activities for more food production in Myanmar was approached to perennial rice cultivation. The perennial rice (PR) cultivation for Myanmar section was conducted at Yezin, Nay Pyi Taw in 2020 to 2021. The seeds of four perennial rice cultivars (PR 23, PR 25, PR 101 and PR 107) were obtained from the Research Centre of Perennial Rice Engineering and Technology in Yunnan, School of Agriculture, Yunnan University, the People's Republic of China. The experimental design was carried out using Randomized Complete Block Design (RCBD). The plants of PR cultivars from the sample plots were measured for plant height, numbers of tillers, numbers of panicles, panicle lengths, numbers of spikelets, filled grains weight, unfilled grains weight, filled grain percentages, 1000 grains weight and grain yields (baskets per acre). The tallest plant height (136.0 cm) was found in PR 101. The numbers of tillers in PR 23 and PR 25 were higher than PR 101 and PR 107. The highest filled grain percentage (98.4%) was observed in PR 23, however, the highest 1000 grain weight (31.9 g) was obtained in PR 107. The highest grain yield (105 baskets per acre) was observed in PR 107. The physicochemical properties of the cultivated soil were also recorded. In conclusion the cultivation of perennial rice cultivars can provide the sustainable production capacities for food safety in Myanmar.

Keywords: perennial rice, PR 23, PR 25, PR 101, PR 107, panicles, spikelets, tillers

Introduction

The world's population is increasing year by year and the need for food safety and food production is increasing. Food safety initiatives are being implemented in most countries around the world. Myanmar is also focusing on food safety and more food productions. Therefore, it is necessary to improve modern technologies to produce more food.

In Myanmar, perennial rice cultivation research was initiated in 2018 according to the joint collaborative research agreements between the Yunnan University, China and University of Mandalay, Myanmar. The perennial rice (PR) cultivation researches for Myanmar Section were carried out at three different climates and altitudes of perennial rice (PR) research sites, namely, Mandalay site, Nay Pyi Taw site and Pyin Oo Lwin site, for the field experiments and at the greenhouse of University Research Center (URC) of University of Mandalay as the preliminary pot experiments from 2019 to 2022. The research works are carried out by members of perennial rice team from Department of Botany, University of Mandalay. In this paper, perennial rice cultivation of Nay Pyi Taw site was described.

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Zhang *et al.* (2022) stated that the agricultural systems are need to intensify sustainably, increasing crop productivity, farmer livelihoods and soil health while using fewer resources. Those can possible when crop perennialization, the conversion of especially annual grains to perennial forms. The author reported that the successful breeding of perennial rice and assess its performance and potential. Perennial rice can get domesticated, annual Asian rice (*Oryza sativa*) was hybridized with perennial African relative *Oryza longistaminata*. In 2021, perennial rice was grown on 15,333 ha by 44,752 smallholder farmers in southern China and growing them saves 58.1% of labour and 49.2% of input costs in each regrowth cycle.

Hu *et al.* (2022) reported that the perennial cultivars produce yields comparable to commercialized annual rice (AR) varieties and maintain them for 4-5 consecutive years from a single planting. The perennial rice exhibit robust regrowth, acceptable grain and milling quality, and are strongly preferred by farmers. The author also quantified the social-economic, ecological and soil benefits attributable to perennial rice cultivation and estimated the geographical areas potentially suitable for perennial rice cultivation worldwide, based on the correlation between regrowth rate and over-winter temperatures. The innovation would help maintain food security and ecological integrity, and also inspire research on other perennial grains.

Yunnan Crop Variety Approval committee approved on Perennial rice 23(PR 23) and the certification was obtained in 2018. Therefore PR 23 is not only the first perennial rice variety approved in Yunnan Province, but also the first one in country and even all over the world. Yun Da 25 (PR 25) and Yun Da 107 (PR 107) were also approved for release already in 2020. In 2018, FAO selected perennial rice technology as International Agriculture Innovation Technology (Hu *et al.* 2022). Zhang *et al.* (2021) stated that the trials of perennial rice technology has started in Laos, Myanmar, Cambodia, Vietnam, Thailand, Bangladesh, Uganda, Cote d'Ivoire in Africa and other south and southeast Asian countries since 2018.

The aim of this research is to provide information needed for Myanmar's food security strategy and the objectives are to examine growth and yield characters of the perennial rice cultivars, to investigate the adaptation of rice cultivars on paddy fields of Myanmar.

Materials and Methods

Research site

The Nay Pyi Taw site is located near the Sein Sar Pin village, Zayarthiri Township, Nay Pyi Taw, Mandalay Region, Myanmar. It is situated at latitude 19°49'04.6"N, longitude 96°15'51.4"E and altitude 115 m.

Experimental Designs

The field experiments were carried out using Randomized Complete Block Design (RCBD) in 2020 - 2021. The field area of Nay Pyi Taw site was 918 m² and the area of each plot for each PR cultivar was 288.1 m² (8.4 m width × 34.3 m length) including 28 rows. There were 81 plants in each row. The total 2268 plants were grown in each plot.

Field Preparation and Cultivation

The cultivation of four PR cultivars (PR 23, PR 25, PR 101 and PR 107) in the first growing season at Nay Pyi Taw site were initiated and the seeds were sown in the nursery beds

on 18th January, 2020. At the same time, the soil preparation, ploughing, harrowing and irrigation were performed in the fields of PR research site. Twenty days after sowing (DAS), the seedlings were transplanted into each plot as designated (Figure 1).

All designated plots were irrigated after sowing and the subsequent irrigation was then carried out two to five times per month until the flowering stage according to the water contents of field soils. The compound fertilizers, N: P: K (15: 15:15), were treated three times to the paddy fields. For the first time of fertilizer treatment, the compound fertilizer, 100 kg ha⁻¹ was applied at the transplanting time. For the second time, the mixture of compound fertilizer, 100 kg ha⁻¹ and urea 25 kg ha⁻¹, was applied on the paddy fields during tillering. For the third time, the mixture of compound fertilizer, 100 kg ha⁻¹ and urea 50 kg ha⁻¹, was applied at the time of flowering. The methods for the fertilizer treatments were recommended by Department of Agriculture (MOAI 2015). In the regrowth seasons, fertilizer treatments were carried out two times. First time fertilizer was applied within 5 days after harvesting and the second time was applied during the initial heading stages.

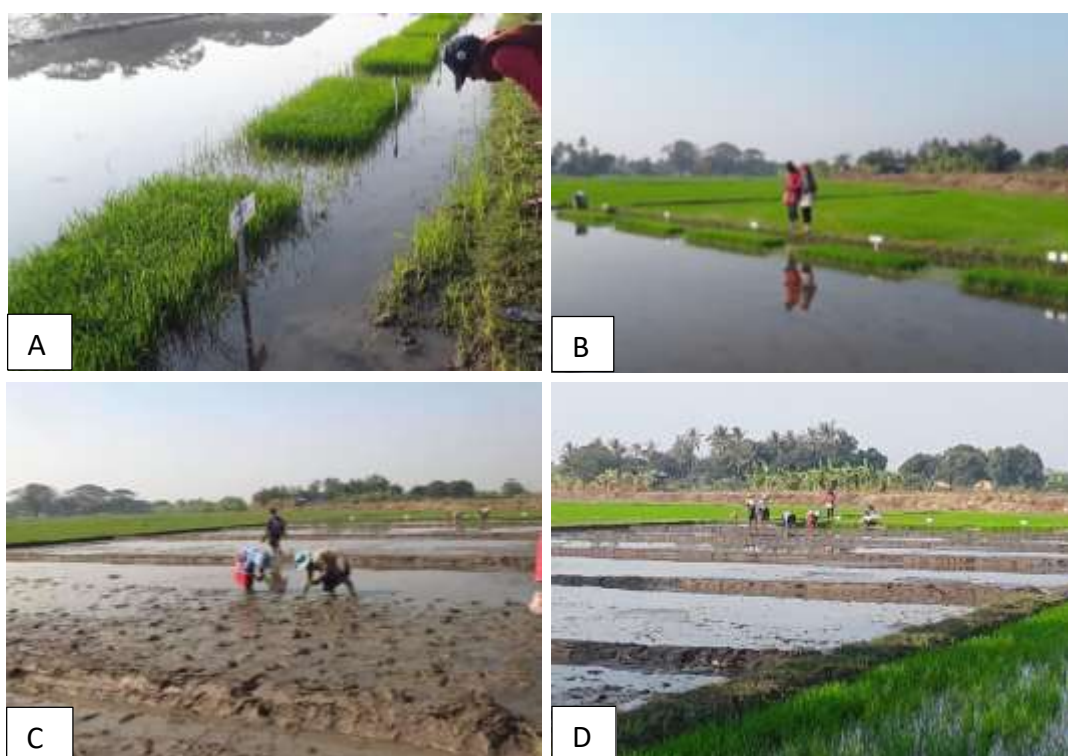


Figure 1. The cultivation of four perennial rice cultivars at Nay Pyi Taw site
(A, B) The nursery plots 20 days after sowing
(C, D) Planting of the perennial rice cultivars after field preparation

Harvesting

Harvesting was done at maturity stage when 85% of grains were changed into golden color. At harvesting time, the straw was cut off at 10 cm above the ground to maintain the consistency of new tillers arising from the rhizomes for the regrowth of rice plants to the next growing seasons.

Soil Analysis

The soil samples were randomly collected from the experimental sites before initiating the experiments. The soil samples from Nay Pyi Taw site were analyzed at the Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw, Mandalay Region.

Determination of agronomic characters and yield Components

The plant height and numbers of tillers per plant were measured at every 15-days intervals after transplanting. The numbers of panicles per plant, panicle lengths, numbers of spikelets per panicle, the filled and unfilled grains weights per panicle, filled grains percentages and 1000 grains weight per plant and yields per acre were recorded at the harvesting time. The yield sampling area for each variety was randomly selected 2 plots and each plot was 6.6 ft width x 6.6 ft length. Forty-nine plants from each plot were measured for the parameters of their agronomic characters and yield components (Figure 2).



Figure 2. Sample plot (6.6 ft width x 6.6 ft length) in PR field after harvesting

Results and Discussions

The agronomic and yield characters of perennial rice (PR) cultivation were recorded and described. The research for Nay Pyi Taw site was carried out by the cultivation of four perennial rice cultivars: PR 23, PR 25, PR 101 and PR 107. Each of the PR cultivars was measured and described for plant height, numbers of tillers, numbers of panicles, panicle lengths, filled grains weight, unfilled grains weight, filled grain percentages, 1000 grains weight (Table 1) and grain yields (baskets per acre) (Table 2). The field experiments for two growth seasons were conducted according to the designated plots and experimental designs. The physicochemical properties of the cultivated soil was also described (Table 3).

Agronomic characters

The total 9072 plants of four kinds of PR cultivars were grown at Nay Pyi Taw PR Site. Among four PR cultivars, the earliest flowering time was observed at 50 days after transplanting (DAT) in PR 107 cultivar and the latest flowering time was found in PR 101 cultivar. The tallest plant height (136.0 cm) was found in PR 101. The numbers of tillers in PR 23 and PR 25 were

higher than those in PR 101 and PR 107. The highest filled grain percentage (98.4%) was observed in PR 23, however, the highest 1000 grain weight (31.9 g) was obtained in PR 107. The lowest filled grain percentage (91.7%) and the lowest 1000 grains weight (22.6 g) were observed in PR 101. PR 23, PR 25 and PR 107 for the first growing season were harvested at 91 days after transplanting (DAT) and PR101 was 125 DAT (Table 1- 2). The conditions of four PR cultivars for first growing seasons were shown in Figure 3 – 7.

Table 1. Agronomic and yield characters of four PR cultivars at Nay Pyi Taw Site

PR Cultivars	Plant height (cm)	No. of tillers	Panicle length (cm)	Filled grains weight (g)	Unfilled grains weight (g)	Filled grains (%)	1000 grains weight (g)
PR 23	104.0	14.0	23.1	158.0	3.0	98.4	26.8
PR 25	103.0	14.0	22.1	147.0	3.0	97.8	26.7
PR 101	136.0	12.0	28.3	142.0	12.8	91.7	22.6
PR 107	109.0p	12.0	27.9	144.0	5.0	96.8	31.9

In the second growing season, the vegetative growth of PR 101 was rapidly developed and all PR cultivars were harvested at 126 days after harvesting of first growing season. Due to the limitation in travelling in COVID-19 pandemic period, the data could not be recorded in the second growing seasons.

Grain Yield

At Nay Pyi Taw site, the yield of first growing season was recorded. The highest yield (105 baskets per acre) was obtained in PR 107 and the lowest yield (60 baskets) was obtained in PR 101. The seasonal yield of PR cultivars was listed in Table 2.

Table 2. Grain yield of four PR cultivars at three sites during their growing seasons

PR Cultivars	first growing season (baskets per acre)
PR 23	98
PR 25	98
PR 101	60
PR 107	105

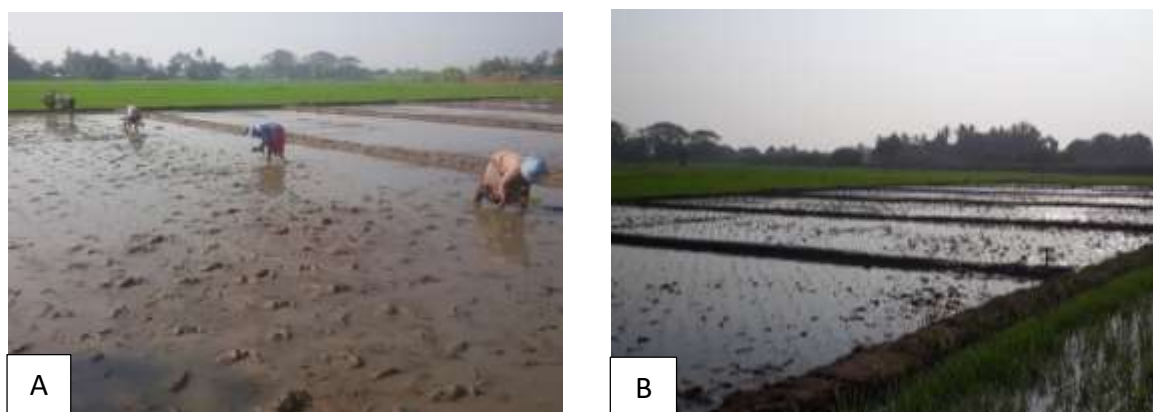


Figure 3. PR cultivation in 1st growing season at 0 DAT in Nay Pyi Taw site
(8.2.2020) (A) Transplanting to designated plots (B) After transplanting

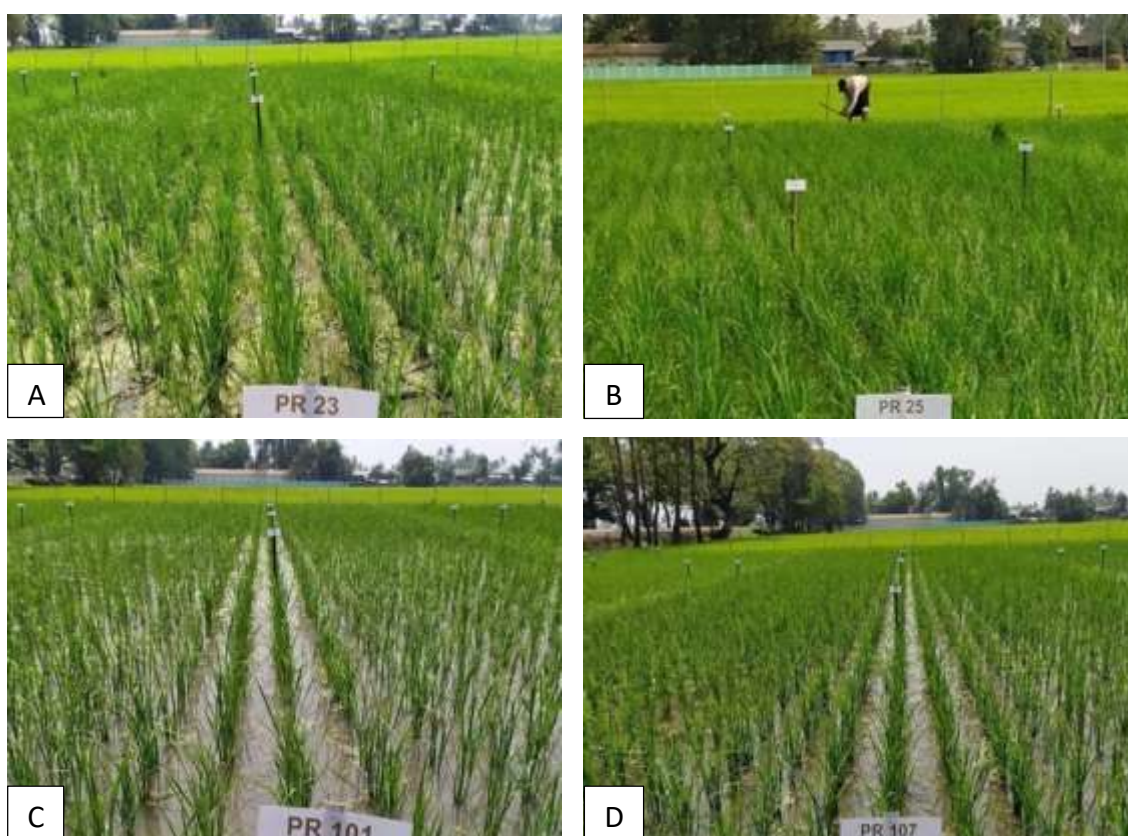


Figure 4. PR cultivation in 1st growing season at 30 DAT in Nay Pyi Taw site
(A) PR 23 (B) PR 25 (C) PR 101 (D) PR 107

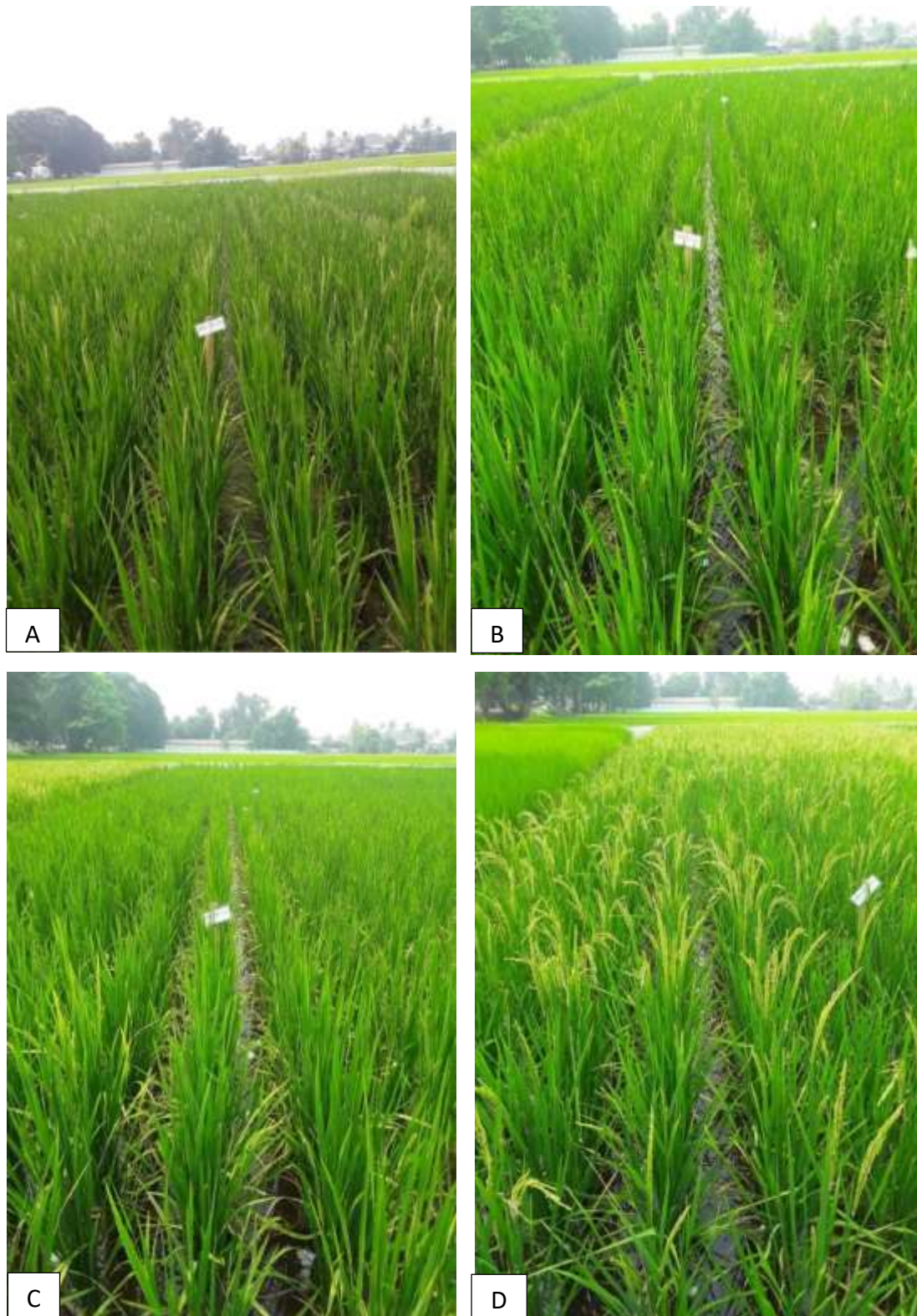


Figure 5. PR cultivation in 1st growing season at 60 DAT in Nay Pyi Taw site
(A) PR 23 (B) PR 25 (C) PR 101 (D) PR 107

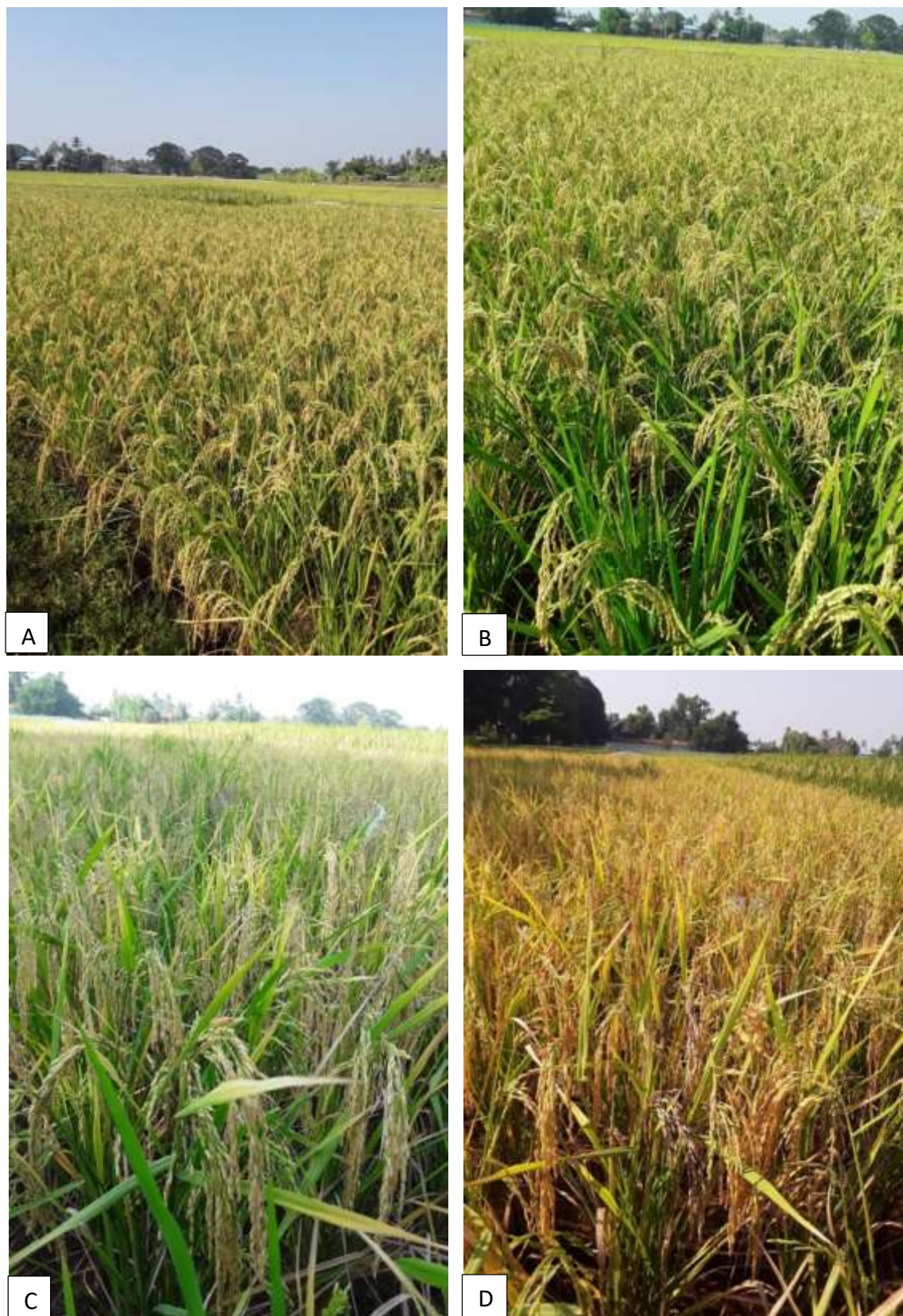


Figure 6. PR cultivation in 1st growing season at 90 DAT in Nay Pyi Taw site
(A) PR 23 (B) PR 25 (C) PR 101 (D) PR 107



Figure 7. Seeds and grains of PR produced from 1st growing season
(A) PR 23 (B) PR 25 (C) PR 101 (D) PR 107

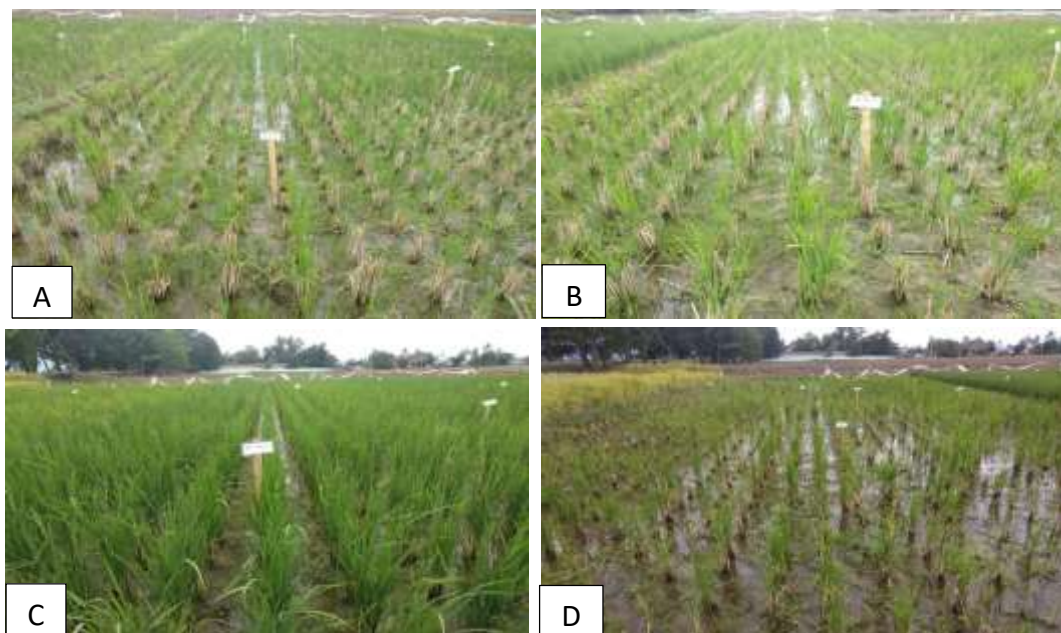


Figure 8. PR cultivation in 2nd growing season at 100 DAT in Nay Pyi Taw site
 (A) PR 23 (B) PR 25 (C) PR 101 (D) PR 107

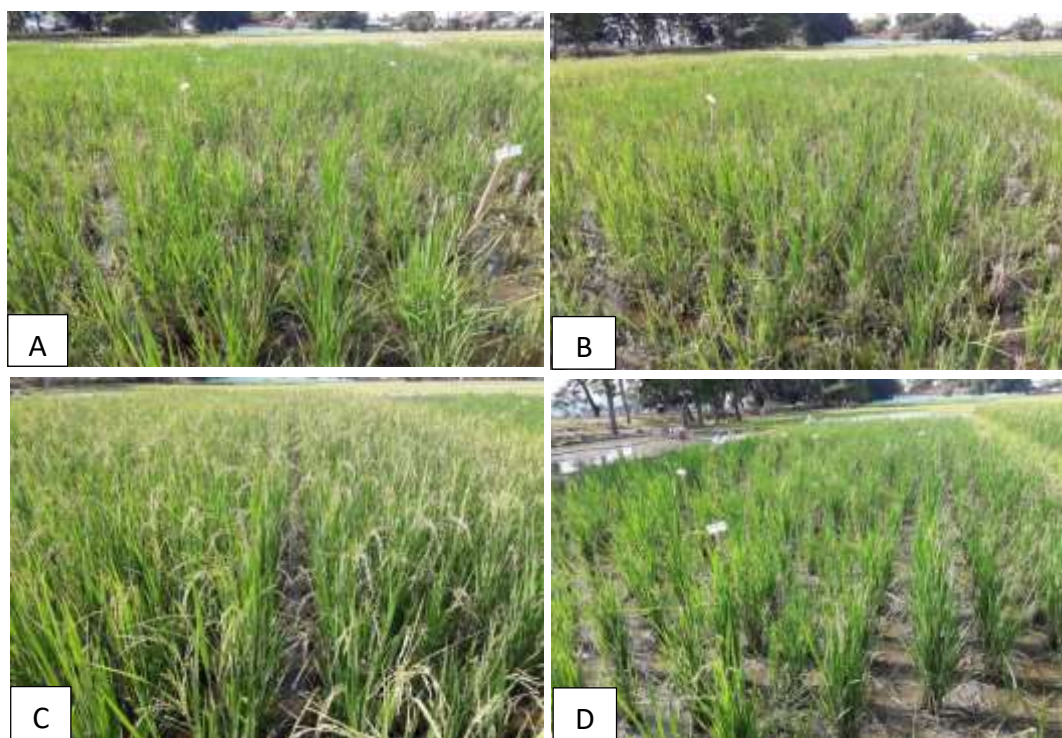


Figure 9. PR cultivation in 2nd growing season at 130 DAT in Nay Pyi Taw site
 (A) PR 23 (B) PR 25 (C) PR 101 (D) PR 107

Physicochemical Properties of the Cultivated Soil

Soil texture was found loamy sand and soil pH was 6 in Nay Pyi Taw site. The results of total nitrogen (N), available phosphorus (P), available potassium oxide (K₂O) and organic carbon contents were shown in Table 3.

Table 3. Physicochemical properties of the cultivated soil from Nay Pyi Taw Site

Location	pH	Organic matter (%)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Calcium chloride extractable SO ₄ -S (mg/kg)	Texture (%)		
							Sand	Silt	Clay
Nay Pyi Taw Site	6	1.2	47	7	74	15	83.9	10.7	5.4

Source: Department of Agricultural Research (DAR), Yezin, Naypyitaw Union Territory, Mandalay Region. Note: N – Nitrogen; P – Phosphorus; K – Potassium; SO₄-S – Sulfate sulfur

Conclusions

According to results, perennial rice cultivars were very robust and can grow rapidly in short duration. Yield and yield characters were success in rice field. It was concluded that perennial rice should be planting in large scale for food safety in Myanmar.

Acknowledgements

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ISOLATION AND SCREENING OF AMYLASE PRODUCING FUNGI FROM SOIL AT TAUNGGOO UNIVERSITY CAMPUS

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Abstract

Enzymes are the most important substances which are used today in many areas. There are different types of enzymes in the nature. Among these, amylases are one of the most significant and are widely used in the industries. Amylases are widely extracted from the microorganisms, more specifically from the fungi. This present study was aimed to isolate and screen for amylase producing fungi from two different locations of soil at Taunggoo University Campus. All together fourteen fungal isolates were obtained from two locations of soil samples. The isolated fungi were preliminary screened for amylolytic activity by growing on potato glucose agar (PGA) using gram's iodine solution. Among fourteen fungal isolates, ten fungal isolates showed positive amylase activity and four isolates showed negative amylase activity after flooding with gram's iodine solution. Soils at Taunggoo University Campus can be considered as a valuable natural source of amylase producing fungi.

Keywords - Fungi, enzymes, amylase, isolation, screening, potato glucose agar (PGA)

Introduction

Fungi are the most important microorganisms and play an important role in the decomposition of organic matter due to their degradation abilities. Fungi are important eukaryotic microorganisms and large amounts of extracellular enzymes are produced for the decomposition organic matter (Gomes., 2007). Fungi present commonly in different types of soils, indoor and outdoor air, food, agro wastes and water. Therefore, fungi are found almost everywhere (Asan., 2004). Fungi secrete different types of enzymes extracellularly and have been used for the production of more than 50% of the needed enzymes. Fungal enzymes have been used from ancient times and today in many industries, including baking, brewing, cheese making, antibiotics production, and commodities manufacturing, such as linen and leather. Furthermore, they are also used in paper production, detergent, the textile industry, and in drinks and food technology in products manufacturing ranging from tea and coffee to fruit juice and wine (Hamada, *et al.*, 2022)

Amylases are obtained from diverse sources including plants, animals, and microbes (Pandey *et al.*, 2000). Microbial amylases are more useful compared with animals and plants amylases in industrial applications. Amylase are important enzymes employed in the starch processing industries (Alva *et al.*, 2007).

Amylase production from fungi is economical because the amylase production rate is higher in fungi as compared to the other microorganisms. Amylolytic enzymes are widely produced by bacteria and fungi. Generally, fungi like *Aspergillus* species, *Penicillium* species, are widely used for the extraction of amylase (Singh, *et al.*, 2011). Enzyme production can be obtained from several microorganisms especially fungi have gained much attention because of the availability and high productivity (Pandey., 2003). The amylase has been extracted from several fungi, bacteria, yeasts and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in the industrial sectors (Gurung *et al.*, 2013).

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Materials and Methods

Samples collection

Soil sample were collected from the two locations of soil about 15 cm at Taungoo University Campus from June to August 2024. The soil sample was transferred to the sterile plastic bags and brought to the laboratory for further analysis. The soil sample was air-dried for 3 days. After drying, the soil sample was ground and one gram was weighted for serial dilution.



18°57'02.4"N 96°21'51.9"E
(SS-I, Botanical Garden)



18°57'08"N 96°21'51"E
(SS-II, Behind the Library)



Collection of soil sample

Figure 1. Map and locations for collected soil samples



Figure 2. Soil sample was dried and weighted 1 gram for serial dilution

Preparation of soil dilution and microbial culture

Isolation of soil fungi by soil dilution plating was adapted from a method by Pepper and Gerba (2004). One gram of the soil sample was added to 99 ml of sterile water and shaken well to disperse the organism. Next, 1 mL of the suspension was removed using a sterile micropipette and then transferred to a new tube containing 9 ml dilution blank. The dilution series was continued to obtain solutions with dilution factors of 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} and 10^{-9} . The medium was prepared and sterilized at 121°C for 30 minutes under the pressure of 15 lbs/sq inch. After sterilizing, the media was poured onto the sterilized petri dish for about 20 ml and waited until the medium solidified. Next, 1 ml of each soil dilution was transferred into each respective petri dish containing potato glucose agar plate supplemented with 0.1g/L penicillin using the micropipette and spread onto the plate using the sterile glass spreader. After spreading the diluted soil sample on the plates, the plates were incubated at room temperature for about (25-30°C) for a week.

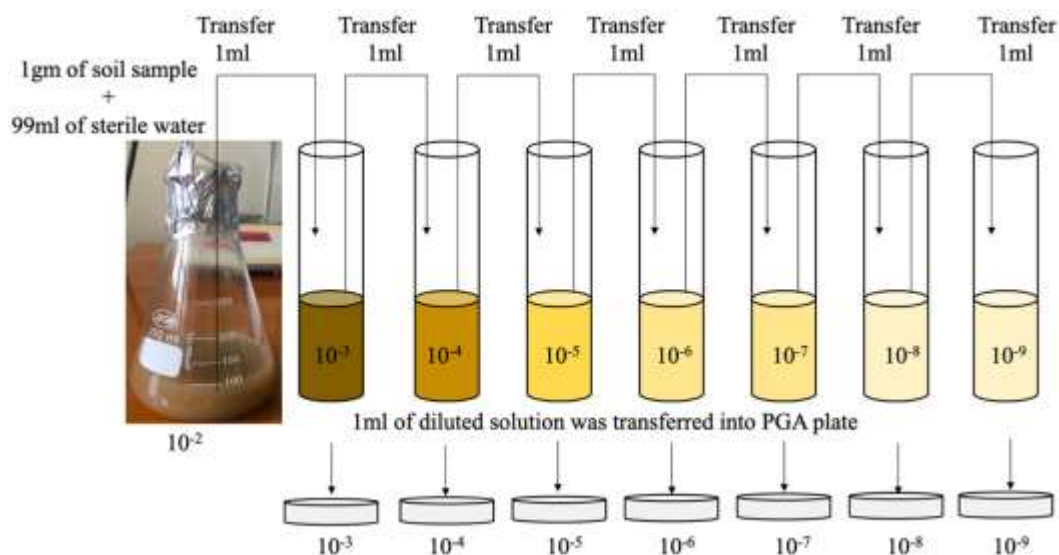


Figure 3. Serial dilution of soil samples

Isolation of fungi

The presence of fungal isolates was observed every day for 7 days. The fungi were then separated based on color and surface morphology of Potato Glucose Agar (PGA) plates. Then, in order to obtain the pure culture, the small amount of spore or hyphal tip from each isolate was then inoculated on new PGA plates supplemented with penicillin to inhibit the growth of bacteria and incubated for 5 days. The pure culture fungi were inoculated on slanted PGA for long term storage.

Macroscopical and microscopical characteristics of Fungi

For macroscopical characteristics of the fungi, colony color, texture, pigmentation are observed. As for microscopical characteristics, hyphae, conidia, fruiting bodies and conidiophores were observed and determined according the reference book of Barnett and Hunter., 1999. The fungal slides were prepared and petri dishes were examined under light microscope with magnification of 10X and 40X objective lens using the microscopic No (27) at the laboratory of department of botany.

Screening of Amylase Producing Activity

Five days old of pure fungal cultures were screened according to the method described by (Ire *et al.*, 2020). (2020). The plates were poured with iodine solution and detected the amylase enzyme producing activity. All of the pure isolates obtained were screened for actual and efficient starch degraders. Gram's iodine solution was prepared by adding 1 g iodine crystals to 2 g of potassium iodide solution and 300ml of water and kept in brown bottle and stored at room temperature (James and Chad. 2019). The iodine solution was poured into the plates containing fungi. The production of amylase is indicated by clear visible zone of hydrolysis around the fungal colonies. The fungal colony showing the zone of hydrolysis was selected correspondingly from the potato glucose agar plates for the screening of amylase. The production of amylase by isolated fungi was photographed and recorded.

Results

Isolation of the fungi from soil sample

All together fourteen fungi were isolated from two different locations of soil sample at Taungoo University Campus. Among the cultured (PGA) plates, only fungal growing plates were selected. Seven fungal isolates from soil sample (I) and seven isolates from soil sample (II) were collected. Two locations of soil samples were designated as SS-I and SS-II. All the resulting fungi were designated as isolates with respective numbers.

Macroscopical characteristics of fungal isolates

All together fourteen isolated were obtained from two soil samples. Macroscopical characteristics of seven fungal isolates from soil sample (I) are white, deep olive green, deep green, black, cotton white, pale gray, light green and seven isolates from soil sample (II) are light gray, cream color, green, blue green and light yellow colors. Three fungal isolates produce pigments on agar plates. Macroscopical characteristics and amylase producing activity of isolated fungi are mentioned in Table 1 and 2.

Microscopical characteristics of fungal isolates

For microscopical characteristics, shape of conidia, structure of fungal hyphae and fruiting body were examined under the microscope (400X) magnification. From fungi isolated from soil sample (I), oval shaped conidia are observed from two isolates, three isolates have spherical shaped conidia and the rest of the two isolates showed no formation of conidia. Large zygosporangium was observed from isolate No. 6. Two isolates such as No. 2 and 3 have hyphae with septum and aseptate hyphae were observed from the rest of five isolates. Two fungal isolates such as No. 3 and 6 showed brush-like fruiting bodies and one isolate of No. 4 has fruiting body with head at the tip of conidiophore. Fruiting body cannot be observed from the rest of four isolates.

From fungi isolated from soil sample (II), spherical shaped conidia are observed from all seven fungal isolates. Only one isolate has hyphae with septum and aseptate hyphae were observed from the rest of six isolates. Isolates No. 11 and 14 showed brush-like fruiting bodies and isolates No. 8 and 12 has fruiting body with head at the tip of conidiophore. Fruiting body cannot be observed from the rest of three isolates. Microscopical characteristics are also described in Table 3 and 4 respectively.

Amylase activity on agar plate

Among fourteen fungal isolates, four isolates (No.3, 4, 5 and 7) from SS-I and six isolates (No.9, 10, 11, 12, 13, and 14) from SS-II showed positive amylase producing activity by detecting the clear zone around the fungal colony after flooding with the iodine solution. These fungal isolates may be the promising fungal lines for amylase production. Three fungal isolates (No.1, 2, and 6) from SS-I and one isolate (No.8) do not exhibit amylase activity. Totally ten fungal isolates showed amylase activity.

Table 1. Macroscopical characteristics and amylase activity of seven fungal isolates from soil sample (I)

Isolates	Colony Morphology	Macroscopical characteristics of Fungal colony	Amylase activity on agar plate
1		Colonies characters are white and the surface of the colony looks chalky in appearance.	 Negative
2		Colonies characters are deep olive green in color and the surface of the colony shows compact mycelial growth in appearance.	 Negative
3		Colonies characters are deep green in color and white in periphery, the surface of the colony looks compact and powdery in appearance.	 Positive
4		This isolate has a rapid growth rate. The colonies are black and white in periphery. The surface texture of the colony shows powdery in appearance.	 Positive
5		Colonies characters are white cotton in color and the surface of the colony looks fluffy, velvety and rapid growth in appearance.	 Positive
6		Colonies characters are pale gray in color and the surface of the colony shows compact with white mycelial growth in periphery.	 Negative
7		Colonies characters are light green in color and the surface of the colony shows white peripheral mycelial growth in appearance.	 Positive

Table 2. Macroscopical characteristics and amylase activity of seven fungal isolates from soil sample (II)

Isolates	Colony Morphology	Macroscopical characteristics of Fungal colony	Amylase activity on agar plate
8		Colonies characters are light gray and white in periphery, the surface of the colony looks fluffy in appearance. Pigments was produced.	 Negative
9		Colonies characters are creamy in color and the surface of the colony looks compact in appearance. Pigments was produced.	 Positive
10		Colonies characters are creamy in color and the surface of the colony shows white compact mycelial growth with lobate in appearance.	 Positive
11		Colonies characters are green and the surface of the colony shows powdery and white in periphery. Pigments was produced.	 Positive
12		Colonies characters are blue green and white in periphery. The surface of the colony looks fluffy and powdery in appearance.	 Positive
13		Colonies characters are light yellow in color mycelia and black powdery at the middle and the surface of the colony looks fluffy and pale yellow mycelial growth in periphery in appearance.	 Positive
14		Colonies characters are green in color and the surface of the colony shows compact and lobate margin in appearance.	 Positive

Table 3. Microscopical characteristics of seven fungal isolates from soil sample (I)

Isolates	Colony Morphology	Microscopical characteristics of Fungal colony
1		The conidia of this fungal isolate are oval shape. Conidiophore and fruiting and conidiophore cannot be observed. The fungal hyphae are hyaline and have no septum.
2		Conidia are oval shape. Conidiophore and fruiting cannot be observed. The fungal hyphae are hyaline and have septum.
3		Conidia are small circular in shape and chain pattern. Conidiophore show in branch pattern and fruiting body with brush like structure. The fungal hyphae have septum.
4		The microscopic observation shows the presence of hyaline hyphae, conidiophores, phialides and conidia. The conidiophores were originated from the basal foot cell and terminated in a vesicle head. Conidia are spherical shape with rough surface and are located over the phialides. The fungal hyphae have no septum.
5		No conidia formation was formed. The hyphae are thin, hyaline and have no septum.
6		Conidia are small circular in shape and the fungal hyphae have no septum. Fruiting body of fungi show brush-like structure. Zygospores were also observed.
7		No conidia are observed and the fungal hyphae have no septum.

Table 4. Microscopical characteristics of seven fungal isolates from soil sample (II)

Isolates	Colony Morphology	Macroscopical characteristics of Fungal colony
8		Conidia are spherical in shape and the fungal hyphae have no septum. Conidiophore with head and fruiting body were observed.
9		Conidia are spherical in shape and the fungal hyphae have no septum. Conidiophore and fruiting body were not observed.
10		Conidia are spherical chain in shape and the fungal hyphae have no septum. Conidiophore and fruiting body were not observed.
11		Conidia are one-celled small spherical in shape, chain pattern and also appear green in color. Conidiophore show in branch pattern and fruiting body with brush-like structure. The fungal hyphae are hyaline and have no septum.
12		Conidia are spherical in shape and the fungal hyphae have no septum. Conidiophore with vesicle head and fruiting body are observed.
13		Conidia are spherical in shape and the fungal hyphae have septum. Cytoplasmic inclusion of hyaline hyphae were visualized. Conidiophore and fruiting body were not observed.
14		Conidia are small spherical in shape and chain pattern. Conidiophore and fruiting body with brush like structure are observed. The fungal hyphae have no septum.

Discussion and Conclusion

In this study, amylase producing fungi were isolated from the soil sample and screened for the production of amylase enzyme. The sub-cultured fungal colonies were studied on the basis of their colonial characteristics and examined for the amylase activity test. For the sake of fungi production of amylase, the fungal colonies were sub-cultured in potato glucose agar media and

incubated at room temperature and then gram's iodine was flooded on the media and zone of clearance was noticed which indicated the production of amylase. Totally fourteen fungi from soil were isolated and all of the fungal colony were viewed under microscope and found to be a variety of fungal isolates based on their microscopical characteristics.

According to macroscopical characteristics, the fungi isolated from SS-I showed seven different colors, chalky white, deep olive green, deep green, black, cotton white, pale gray and light green. From SS-II, colors of the fungal colony are light gray, two creamy colors, blue green, light yellow and two green colors.

For microscopical characteristics of SS-I, hyphae with septum are observed from two isolates No. 2 and No. 3 and the rest of five isolates have no septum. Brush-like fruiting bodies are observed from isolates No. 3 and No. 6. Only isolate No. 4 have fruiting body with head. Zygosporangia are observed from isolate No.6 and sexual state of fungal hyphae also occurred. Three fungi produce spherical shaped conidia, two isolates produce oval shaped conidia and the rest of the two fungi have no conidia. Seven different colony morphology are observed from SS-II and fruiting body with head are found in two isolates No. 8 and No. 12. Brush-like fruiting body are also observed from two isolates such as No. 11 and No. 14. Six fungal lines possess aseptate hyphae and only one have hyphae with septum. All of them bear spherical shaped conidia.

Among seven isolates from SS-I, four isolates showed positive amylase activity and three showed negative amylase activity. From SS-II, six fungal lines showed positive amylase activity and only one showed negative amylase activity. The fungi isolated from SS-II showed more amylase activity and can be selected as promising fungal lines for further study. Based on the results of macroscopical and microscopical characteristics, the isolated fungi may be possible different species. In the present investigation, totally fourteen fungi were isolated from different locations of soil samples at Taungoo University Campus. The results showed significant variants in morphological characteristics thus it is speculated that the soil harbors diverse fungal populations. From plate assay method, ten isolates showed amylolytic potential and four fungal isolates showed negative amylase activity.

This study can share the knowledge of the importance of different types of fungi that produced amylase enzymes applied in the industrial field. This study showed for the production of expensive amylase enzyme from the cheap sources of soil sample. This can be concluded that some soils at Taungoo University Campus can be considered as a valuable natural source of amylase producing fungi. These promising fungal isolates were further selected and screened for the production of the amylase in liquid medium in the future study. However, further studies are required for identifying and classifying soil fungi.

Acknowledgements

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STUDY ON EPIPHYTIC FERNS IN TANINTHARYI NATURE RESERVE PROJECT (TNRP) AREA, YEPHYU TOWNSHIP, TANINTHARYI REGION

Thuzar Myint¹

Abstract

A survey of epiphytic ferns was carried out in Tanintharyi Nature Reserve Project (TNRP) Area. The TNRP encompasses an area of 1,700 sq km and is located in the Dawei District Tanintharyi Region, southern Myanmar. A total of 15 species of epiphyte were collected from this area. Epiphytic ferns grow on tree trunks, on mossy tree-trunks or on branches of trees. Most species were found growing in light humus as compared to bark and heavy humus as substrate. The field survey was conducted during September, 2020 to February, 2021. Floristic study of collected specimens were classified the families, genus and species. In this survey was collected 15 species belonged to 7 genera, 4 families. Among these, polypodiaceae was the most species-rich families at the study area. Nephrolepidaceae (*Nephrolepis brownii*) was found in only one species.

Keywords: Outstanding characters, Classification, Comparison of 15 species of ferns

Introduction

Epiphytes grow on other plants and get nutrients from the air, rain, or debris. They also known as ‘air plants’, typically found in rainforests. They live symbiotically on other plants from which they obtain nutrients and moisture.

Epiphytic ferns can be divided into different groups: low-level epiphyte and high-level epiphyte. Low level epiphytes are found in moist or shady conditions. High-level epiphytes grow on the branches of the crowns of the tallest trees in the forest.

The morphological characteristic of epiphyte ferns was the erected rhizome and living in the colony. The roots of epiphytes are aerial; they do not grow into the soil but instead attach themselves to larger trees. Some epiphytes have leaves that can store large amounts of water, providing shelter and breeding grounds for various animals.

The TNRP area is characterized by undulating terrain and high elevation ranging from 15 m above sea level in the lowlands to 1,400 m at the ridge of the Thai border; the mountain range here runs from north to south while the slope rises from west to east, climbing to the top of the ridge. It consists of three reserves: the eastern parts of Kaleinaung Reserve and Heinze Reserve (about 85,725 ha) as well as Luwaing Reserve (about 84,273 ha) (TNRMP, 2013). Tropical evergreen forests, especially evergreen dipterocarp forests, occur in southern part of Myanmar. Tanintharyi Nature Reserve (TNR) was established in 2005 as a protected area (TNRMP 2009) where tropical moist evergreen forests are dominant (Pollard et al. 2014).

Totally 74 species of ferns and fern allies were collected in Tanintharyi Nature Reserve (TNRP) area. Among these, 15 species of epiphytic ferns were collected in this area.

Ferns, as a part of biodiversity, are the community which owns essential roles in the forest ecosystem. Such as the soil coverage, the manure mixer for soil nutrient, the producer for the chain food in the ecosystem, germplasm resource, and potential food and medicine resources. Thus, the main objectives of this study were to conduct a floristic study of epiphytic ferns in

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Tanintharyi Nature Reserve Project (TNRP) area, Tanintharyi Region and to provide conservation of epiphytic ferns diversity in protected areas.

Materials and Methods

Study site

The study area lies in the townships of Yephyu and Dawei, Dawei District in the northern part of the Tanintharyi Region in Myanmar. It is situated between the Dawei River and Myanmar-Thailand border in Southern Myanmar. It is located between the latitudes 14° 20' 50" and 14° 57' 55" North and between the longitudes 98° 5' 10" and 98° 31' 32" East, a total area of 1700 square kilometers (170,000 ha).

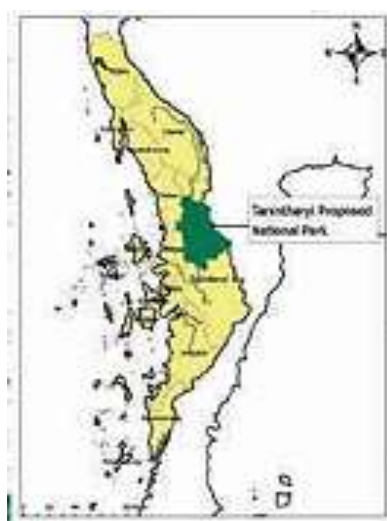


Figure 1.1 Map of Tanintharyi Region, Tanintharyi

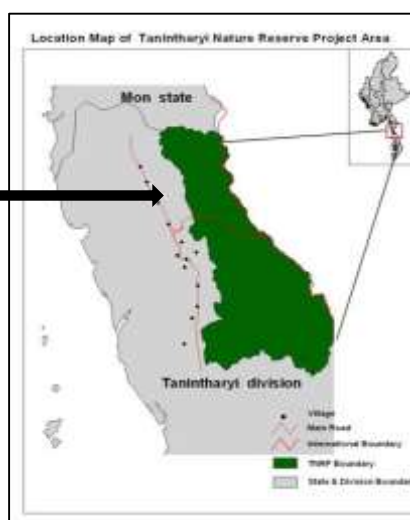


Figure 1.2 TNRP Area, Yephyu Township, Region

Materials and collecting of the specimens

Specimens are properly collected from April 2020 to February 2021. The plant specimens were collected by using along the existing trails method. The collected specimens were placed into the newspaper for drying and then placed into the press frame. Locations of specimens were determined by using GPS (GP 315, Global Positioning System device). Their habitat and some diagnostic characters of each species were recorded.

Descriptions of species and identification were done with the help of the literatures: Kholia, (2010), Tagawa & Iwatsuki, (1979-1989) and www.eFlora.org. Latest updated nomenclature with synonyms is provided based on Kew Science (www.plantsoftheworldonline.org). The classification system of epiphytic ferns of TNRP area follows that of PPG1 (2016).

Results

A total of 15 species of epiphyte were collected from different location as shown in Table 1.1 and have been classified into 1 classes, 1 orders, 4 families, 7 genera and 15 species based on the system of PPGI (Pteridophyte Phylogeny Group)(2016).

Table 1. List of epiphytic ferns from Tanintharyi Nature Reserve Project (TNRP) Area

Order	Family	Genus	Species	GPS Location
Polypodiales	Pteridaceae	1. <i>Antrophyum</i>	<i>parvulum</i>	14°09'55.5"N,98°23'40.3"E,326 m
		2. <i>Haplopteris</i>	<i>elongata</i>	14°24'07.8" N, 98°03'59.2"E,58 m
	Nephrolepidaceae	3. <i>Nephrolepis</i>	<i>brownii</i>	14°2'48" N, 98°27'55" E, 464 m
		4. <i>Davallia</i>	<i>denticulata</i>	14°03'46.8" N, 98°13'57.7" E 52 m
	Davalliaceae	5. <i>D.</i>	<i>divaricata</i>	14°03'40.8" N, 98°13'51.3" E 56 m
		6. <i>D.</i>	<i>trichomanoides</i>	14°07'49.2" N, 98°26'08.1"E 313 m
		7. <i>Drynaria</i>	<i>propinqua</i>	14°24'23.6" N, 98°10'50.3" E 33 m
	Polypodiaceae	8. <i>D.</i>	<i>quercifolia</i>	14°23'02.6" N, 98°04'44.4" E 24 m
		9. <i>D.</i>	<i>sparsisora</i>	14°07'49.2" N, 98°26'08.1"E 313 m
		10. <i>Microsorium</i>	<i>punctatum</i>	14°24'23.6" N, 98°10'50.3" E 33 m
		11. <i>Pyrrosia</i>	<i>costata</i>	14°14'55.3" N, 98°21'45.6" E102 m
		12. <i>P.</i>	<i>lanceolata</i>	14°09'57.0" N, 98°23'42.5" E325 m
		13. <i>P.</i>	<i>lingua</i>	14°17'08.0" N, 98°17'15.1" E 53 m
		14. <i>P.</i>	<i>nummulariifolia</i>	14°14'55.3" N, 98°21'45.6" E102 m
		15. <i>P.</i>	<i>piloselloides</i>	14°09'59.9" N, 98°23'54.8" E409 m

Scientific Name: *Antrophyum parvulum* Blume

Family : Pteridaceae

Common Name : Lineleaf ferns

Outstanding character

Rhizome sub-erect; scale clathrate. Fronds clustered, monomorphic; stipe appressed, forming very narrow wings; lamina spatulate, leathery, veins reticulate. Sori linear, 2 or more parallel lines along the veins, paraphyses club shaped. Spores tetrahedral-globose, trilete.

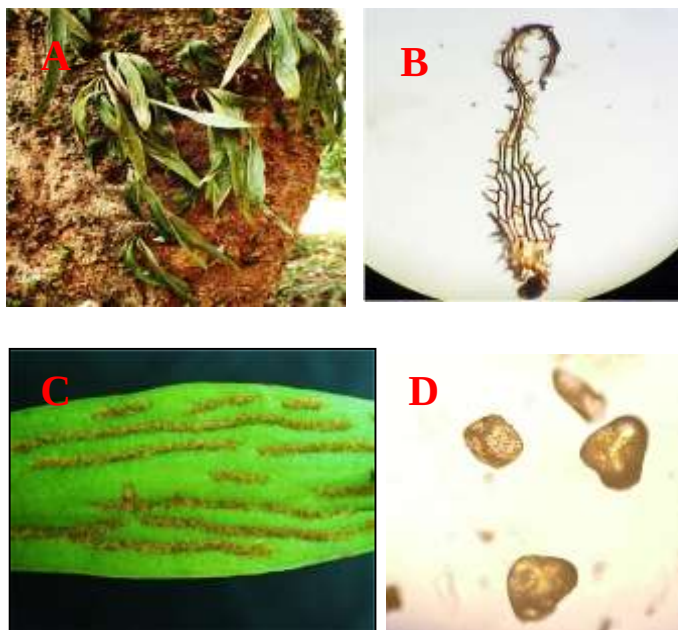


Figure. 2 A-Habit, B-Scale, C-Sori on adaxial D- spore

Scientific Name: *Haplopteris elongata* (Sw.) E. H. Crane

Family : Pteridaceae

Common Name: Tape fern

Outstanding character

Rhizome long creeping; scales subulate-lanceolate, clathrate. Fronds monomorphic, clustered at base; stipe very short; lamina simple, linear or ribbonlike, veins evident. Sori line the margin, immersed in deep groove, paraphyses many; spore monolete, bean shaped, smooth.

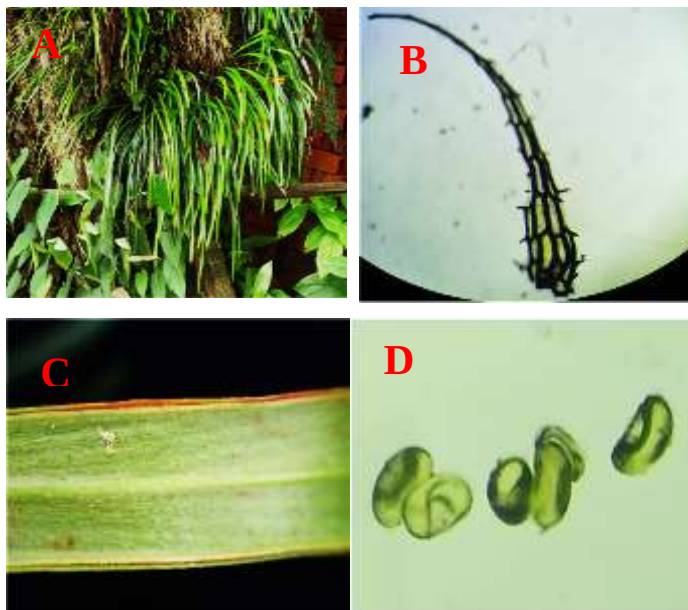


Figure. 3 A-Habit, B-Scale, C-Sori on marginal D-spores

Scientific Name: *Nephrolepis brownii* (Desv.) Hovenkamp & Miyam

Family : Nephrolepidaceae

Common Name : Not known

Outstanding character

Rhizome erect, wiry roots numerous; scales peltate, fimbriate margin. Fronds monomorphic; stipe grooved adaxially, with appressed; lamina unipinnate, pinnae 12-20 pairs, elliptic-lanceolate, articulate, veins forked. Sori bearing along the submarginal, orbicular; indusia reniform; spores oblong, monolet.

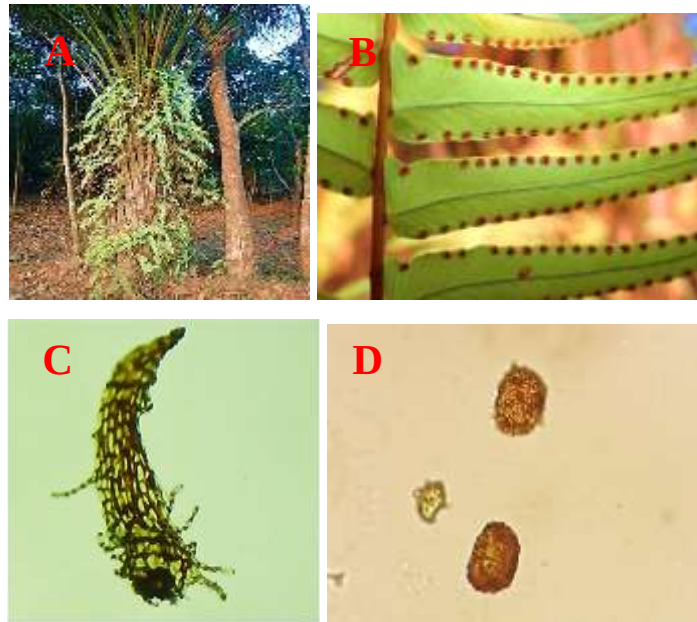


Figure. 4 A-Habit, B-Scale, C-Sori on marginal, D- spores

Scientific Name : *Davallia denticulata* (Burm.f) Mett.

Family : Davalliaceae

Common Name : foot ferns

Outstanding character

Rhizome long creeping, scales peltate. Fronds monomorphic; stipe adaxially grooved; lamina quadripinnate compound, deltoid and broadest toward base, pinnae deltoid, veins forked, false veins present. Sori at margin of lobes, indusia pouch-shaped; spores oblong, monolet.

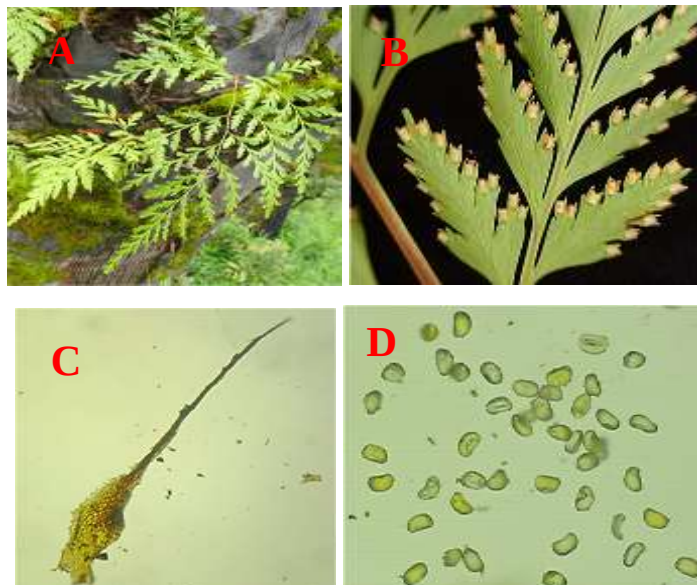


Figure. 5 A-Habit, B-Scale, C-Sori on adaxial, D- spores

Scientific Name: *Davallia divaricata* Blume

Family : Davalliaceae

Common Name : Not known

Outstanding character

Rhizome long creeping, scales subulate with long, narrowed toward apex. Fronds monomorphic; stipe adaxially grooved; lamina tripinnate compound, deltoid and broadest toward base, veins forked, without false veinlets. Sori terminal on veinlets, indusial pouch-shaped; spores oblong, monolete.



Figure. 6 A-Habit, B-Scale, C-Sori on adaxial D- spores

Scientific Name: *Davallia trichomanoides* Blume

Family : Davalliaceae

Common Name : Not known

Outstanding character

Rhizome long creeping, scales peltate base to long tail apex. Fronds monomorphic; stipe terete, lamina tri to quadripinnate, deltoid, veins forked, false veins present. Sori borne on the tip of veinlets, indusium pouch shaped; spores oblong, monolete.

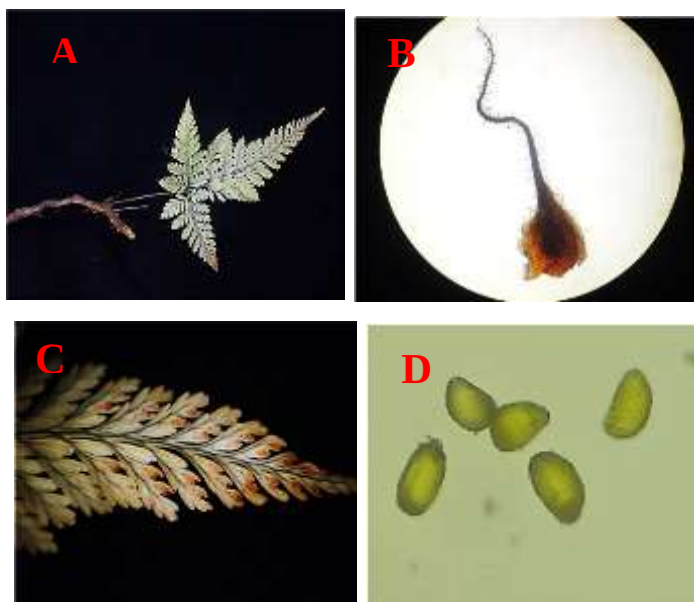


Figure. 7 A-Habit, B-Scale, C-Sori on adaxial D- spores

Scientific Name: *Drynaria*

propinqua (Wall.ex Mett)

J. Sm.ex Bedd.

Family : Polypodiaceae

Common Name : Basket ferns

Outstanding character

Rhizome long creeping; scales lanceolate. Fronds dimorphic; nest leaves sessile, ovate; foliage leaves unipinnate, pinnatifid; stipe narrowly winged, deeply lobed, pinna 10-15 pairs, lanceolate, decurrent, veins anastomosing. Sori round, two rows between costa and lateral veins; spores ellipsoid, monolet.

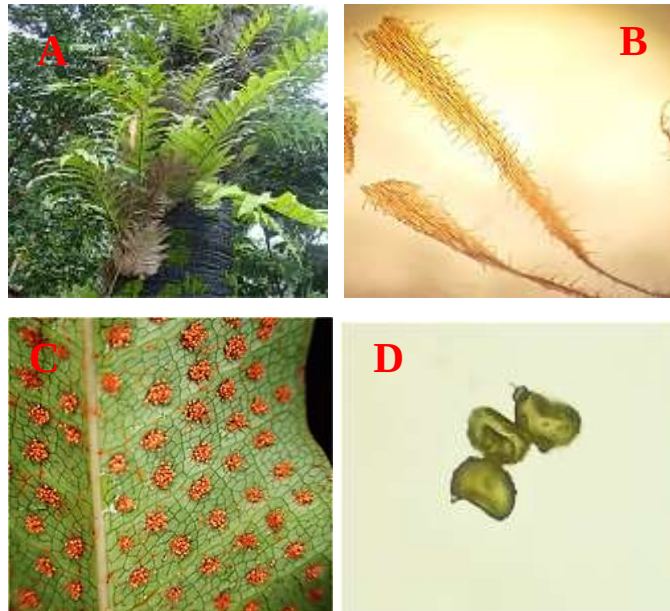


Figure. 8 A-Habit, B-Scale, C-Sori on adaxial D- spores

Scientific Name: *Drynaria*

quercifolia (L.) J. Sm.

Family : Polypodiaceae

Common Name : Basket ferns

Outstanding character

Rhizome short creeping; scales peltate, narrowing from base to tailed apex, Fronds dimorphic; nest leaves sessile, obovate, shallowly lobed; foliage leaves unipinnate, stipe winged, pinna 10-12 pairs, deeply lobed, veins anastomosing. Sori round, two rows between veinlets; spores monolet, ellipsoid.

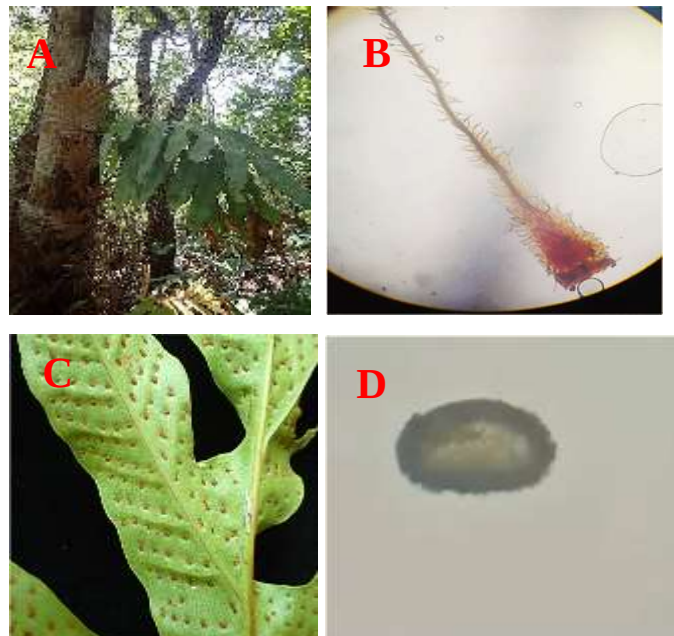


Figure. 9 A-Habit, B-Scale, C-Sori on adaxial, D- spore

Scientific Name: *Drynaria*

sparsisora (Desv.) T. Moore

Family : Polypodiaceae

Common Name : Oak-leaf fern

Outstanding character

Rhizome creeping; scales lanceolate. Fronds dimorphic; nest leaves sessile, ovate-oblong, middlely lobed; foliage leaves unipinnate; stipes narrowly winged, deeply lobed, pinna 12-15 pairs, oblong-lanceolate, veins anastomosing. Sori round, two rows between adjacent main veins; spores monolet, ellipsoid.

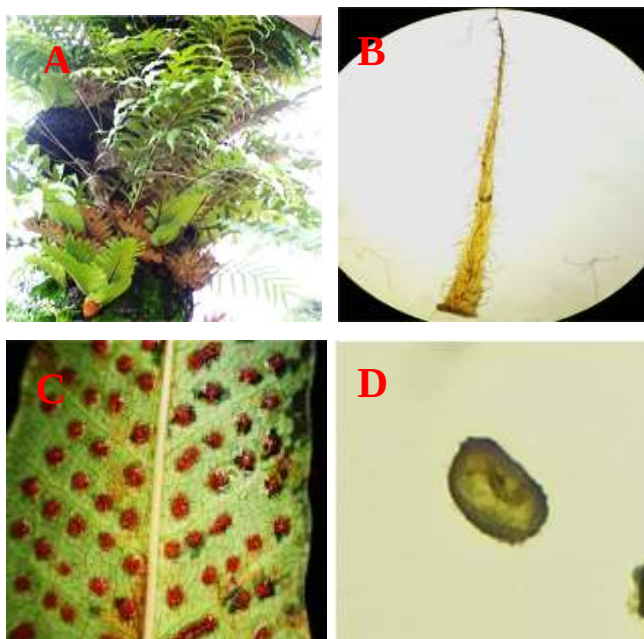


Figure 10 A-Habit, B-Scale, C- Sori on adaxial
D-spores

Scientific Name: *Microsorium*

punctatum (L.) Copel.

Family : Polypodiaceae

Common Name: Not known

Outstanding character

Rhizome short creeping; scales peltate. Fronds monomorphic, sessile; lamina simple, elliptic- lanceolate, pale green, veins anastomosing, midrib distinct. Sori small, orbicular, scattered on the upper half of the fronds; spore oblong, monolet.

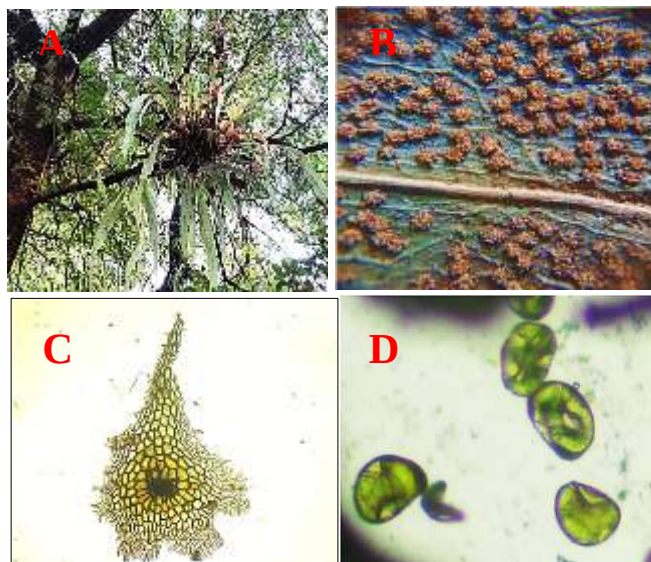


Figure 11 A-Habit, B-Scale, C-Sori on adaxial D-
spores

Scientific Name: *Pyrrosia costata*
(Wall. Ex C. Presl) Tgawa & K.Iwats.

Family : Polypodiaceae

Common Name : Not known

Outstanding character

Rhizome short creeping; scales clathrate. Fronds monomorphic; stipe terete, winged nearly to the base; lamina simple, oblanceolate, main veins distinct and pinnate, hydathodes distinct, veins anastomosing, stellate hairy. Sori superficial abaxially; spore oblong, monolet.

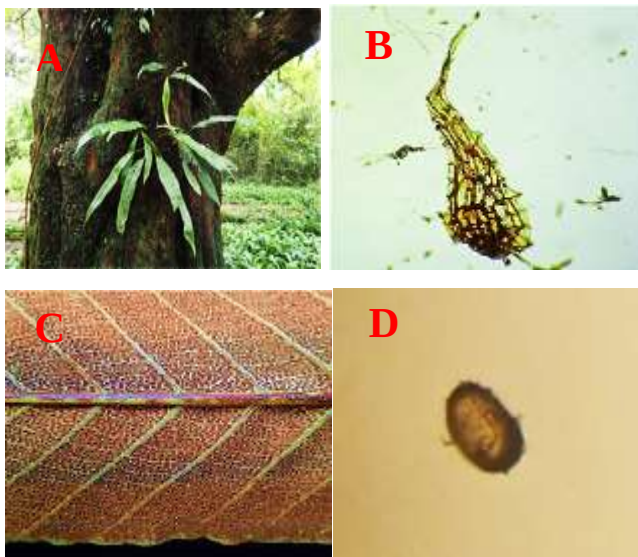


Figure 12 A-Habit, B-Scale, C-Sori on adaxial
D- spores

Scientific Name: *Pyrrosia lanceolata*
(L.) Farw.

Family : Polypodiaceae

Common Name : Japanese felt fern

Outstanding character

Rhizome long creeping, densely scaly throughout; scales peltate, margin ciliate. Fronds monomorphic; stipe terete; lamina simple, elliptic-lanceolate, veins free. Sori borne several rows on each side of main veins, the lower one densely stellate hairs throughout; spore monolet, oblong, cristae.

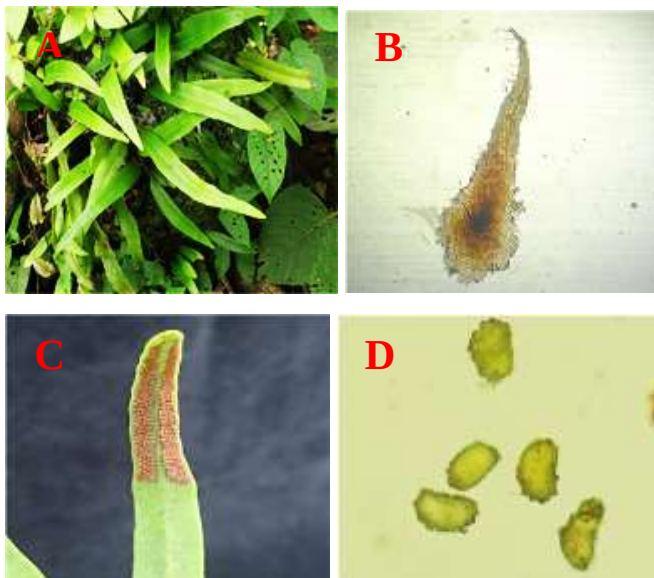


Figure 13 A-Habit, B-Scale, C-Sori on adaxial D-
spores

Scientific Name: *Pyrrosia lingua*

(Thunb.)Farw.

Family : Polypodiaceae

Common Name : Not known

Outstanding character

Rhizome long creeping; scales peltate. Fronds monomorphic; coriaceous; stipe terete; lamina simple, ob-ovate, stellate hairs, hydathodes distinct, veins pinnate. Sori borne between veins parallel, superficial; spores monolete, rugose.

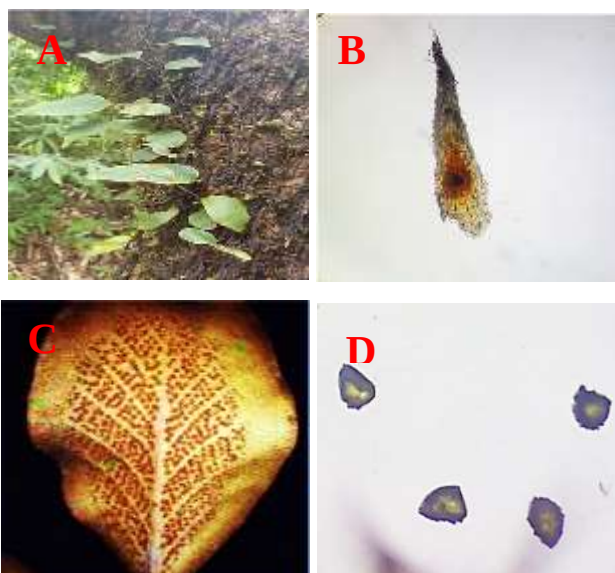


Figure 14 A-Habit, B-Scale, C-Sori on adaxial D-spores

Scientific Name: *Pyrrosia*

nummulariifolia (Sw.) Ching

Family : Polypodiaceae

Common Name : Not Known

Outstanding character

Rhizome long creeping, scales peltate, tailed apex. Fronds dimorphic; sterile leaves stipe short; lamina ovate, densely stellate hairs; fertile leaves stipe terete, lamina elliptic-ovate, veins indistinct. Sori superficial; spores ellipsoid, monolete.



Figure 15 A-Habit, B-Scale, C-Sori on adaxial D-spores

Scientific Name: *Pyrrosia**piloselloides* (L.) M.G. Price**Family** : Polypodiaceae**Common Name** : Dragon Scales fern**Outstanding character**

Rhizome long creeping, scales circular peltate, the outer hyaline and the center brown. Fronds dimorphic; stipe of sterile leaves short; lamina ovate, veins obscure, reticulate venation, both surfaces sparsely stellate hairy; fertile leaves lamina oblong, veins indistinct. Sori in two distinct rows, each row between midrib and margin; spore oblong, monolet, cristae.

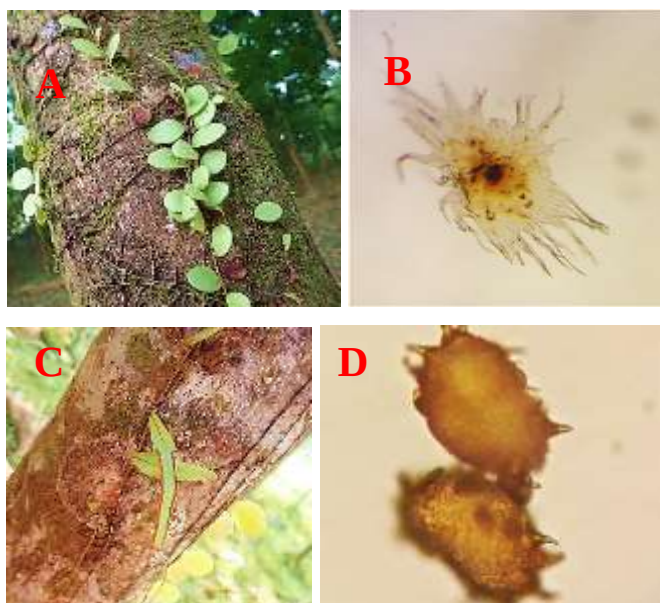


Figure 16 A-Habit, B-Scale, C-Sori on adaxial D-spores

Table 2. Comparable attributed characteristics of 15 species of epiphytic ferns in TNRP area

No	Scientific Name	rhizome	scale	frond	veins	sori	spores
1	<i>Anthrophyllum parvulum</i>	sub-erect	clathrate	monomorph	anastomosing,	margin	monolet
2	<i>Haplopteris elongata</i>	long creeping	clathrate	monomorph	indistinct	margin	monolet
3	<i>Nephrolepis brownii</i>	erect	lanceolate	monomorph	forked	margin	monolet
4	<i>Davallia denticulata</i>	long creeping	peltate	monomorph	false vein present	tip of veinlets	monolet
5	<i>Davallia divaricata</i>	long creeping	subulate	monomorph	without false vein	tip of veinlets	monolet
6	<i>Davallia trichomanoides</i>	long creeping	peltate	monomorph	false vein present	tip of veinlets	monolet
7	<i>Drynaria Propinqua</i>	long creeping	lanceolate	dimorphic	anastomosing,	two rows	monolet
8	<i>D. quercifolia</i>	short creeping	peltate	dimorphic	anastomosing,	two rows	monolet
9	<i>Drynaria sparsisora</i>	short creeping	lanceolate	dimorphic	anastomosing,	two rows	monolet
10	<i>Microsorium punctatum</i>	short creeping	peltate	monomorph	indistinct	scattered	monolet

No	Scientific Name	rhizome	scale	frond	veins	sori	spores
11	<i>Pyrossia costata</i>	short creeping	clathrate	monomo rphic	anastro mosing,	superficial	monolete
12	<i>P. lanceolata</i>	long creeping	peltate	monomo rphic	free	rows on each side of main veins	monolete
13	<i>P. lingua</i>	long creeping	peltate	monomo rphic	pinnate	between veins parallel	monolete
14	<i>P. nummulariifolia</i>	long creeping	peltate	dimorp hic	indistinct	superficial	monolete
15	<i>P. piloselloides</i>	long creeping	circular peltate	dimorp hic	indistinct	two distinct rows	monolete

Discussion and Conclusion

In the present study, 15 species belong to 4 families of epiphytic ferns collected from Tanintharyi Nature Reserve Project (TNRP) area have been described, identified and classified. Among these, Polypodiaceae had the highest number (57.1%) and followed by Davalliaceae, (21.4%), Pteridaceae (14.29%) and Nephrolepidaceae was 7.14% respectively.

Epiphytic plants usually occur on shady branches or tree trunk from low elevation to mid elevation, the most abundant genera were *Pyrrosia* which 5 species. Among these, *Microsorium punctatum*, *Pyrrosia piloselloides* (L.) M. G. Price are abundant on the tree trunk and occur more than one localities.

Two types of fronds were occurred in this area. Dimorphic fronds are observed in *Drynaria propinqua*, *D. quercifolia*, *D. sparsisora*, *Pyrrosia nummulariifolia* and *P. piloselloides* while the other species are monomorphic fronds. Erect rhizome is found in *Antrophyum parvulum* and *Nephrolepis brownii* and the rest 13 species are creeping rhizome. It is agreed with the report of Tagawa & Iwatsuki (1979-1989).

Simple leaves (fronds) were collected in *Anthrophyllum parvulum*, *Haplopteris elongata*, *Microsorium punctatum*, *Pyrossia costata*, *P. lanceolata*, *P. lingua*, *P. nummulariifolia* and *P. piloselloides*. Unipinnately compounds were found in *Nephrolepis brownii*, *Drynaria propinqua*, *D. quercifolia* and *D. sparsisora*. and tripinnately to quadripinnately compound were recorded in *Davallia denticulata*, *D. divaricata* and *D. trichomanoides*.

Among the collected families, two types of spores had been recorded in the study area. Trilete (tetradral) spores are only found in *Anthrophyllum parvulum* Blume. Monolete (bilateral) spores are observed in 14 species of plants. It is agreed by Tagawa & Iwatsuki (1979-1989), Winter & Amoroso (2003) and Kholia (2010).

In conclusion, epiphytic ferns that had been discovered from the TNRP area consist of 4 families, 7 genera and 15 species which have various morphological characteristics. TNRP area is very suitable for the growing of pteridophytes and rich resources of more species are found in this area compared different area of Yephyu Township, especially on the epiphyte.

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EFFECTS OF DIFFERENT LEVELS OF EM BOKASHI FERTILIZER ON YIELD COMPONENTS AND YIELDS OF *HELIANTHUS ANNUUS* L. (SUNFLOWER)

Nwe Nwe¹

Abstract

Sunflower (*Helianthus annuus* L.) is an important oilseed crop, but achieving optimal yields requires effective soil management practices. The use of EM bokashi, a fermented organic matter enriched with effective microorganisms, is being explored as a sustainable approach to enhance crop productivity. The study was carried out the effects of different levels of EM bokashi fertilizer on the yield components and overall yields of *Helianthus annuus* L. cv. (sunflower) under field condition. The experiment was carried out using Randomized Completely Block Design (RCBD) with replicated twice. The different levels of EM bokashi fertilizer (0, 1, 2, 3 ton acre⁻¹) were employed in this experiment. In the result, the 3 tons acre⁻¹ of EM bokashi significantly improved several yield components, the tallest plant height (67.56 cm), the maximum number of leaves (22.80) and largest single leaf area (94.60), head diameters (12.57 cm plant⁻¹), 100 seeds weight (6.73 g plant⁻¹), and total seed weight (46.32 g plant⁻¹). Consequently, the overall seed yields of sunflower were increased (1.59 tons acre⁻¹ and 33.39 baskets acre⁻¹) compared to control. The result indicated that 3 tons acre⁻¹ of EM bokashi can be beneficial soil amendment for enhancing sunflower yield components and overall productivity, offering a sustainable alternative for improving crop yields.

Keywords: EM bokashi, yield components and yields

Introduction

Sunflower, *Helianthus annuus* L. belongs to family Asteraceae and is one of the most important oil seed crops in Myanmar. Sunflower is a short duration crop and can be grown twice in a year. Sunflower plants are grown commercially in many countries such as India, China, Russia, South Africa and Myanmar (Ahmad *et al.*, 2009). Sunflower is primarily grown in Sagaing, Mandalay, Magway and Ayeyarwady regions as well as Shan State. The majority of oilseed sunflower currently grown in Myanmar are mahuyar, Doatkhit, Sunfol, Sinshwekyar-2 and Yezin-1 cultivars (Khin Mar Mar Nwe *et al.*, 2000). Among them, hybrid sunflower cultivar, Yezin-1 used in this experiment is the most popular in Magway Division.

EM is widely used as beneficial microbial inoculums for making bokashi and the use of EM helps to increase crop yields enhancing soil fertility, conserve the soil productivity, improve biological properties, and also physical amelioration of soil structures. Base material for the production of bokashi can be obtained easily around the farms, like straw, grasses, legumes, rice husk, manure, and sawdust (Vetayasuporn, 2006).

EM bokashi is the organic fertilizers help to purify the ecosystem and increase crop productivity (Alam and Seth, 2012). The use of microbial inoculants has been shown to increase the mineralization of organic materials (Pan *et al.*, 2012; Sharma *et al.*, 2014). Bokashi is organic materials that have been fermented with EM and made for good fertilizer. EM bokashi is inexpensive, capable of producing high quality products, high yield produces and preserving the environment (Higa and Kanai, 1998).

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Therefore, this study was aimed to evaluate the effectiveness of different levels of EM Bokashi fertilizer on the yield components and yields of *Helianthus annuus* L. cv. Yezin (sunflower) under field conditions.

Materials and Methods

Experimental site

The field experiment was conducted on hybrid sunflower cultivar, Yezin-1 from January to March, 2023 at the field of Botany Department, in the University of Magway.

Preparation of Making EM Bokashi Fertilizer

- (a) Bokashi basic materials, especially the dry straws were cut into small pieces. Concentrated EM solutions were mixed with molasses and water by the ratio of 1:1:18.
- (b) Firstly, spreading the small pieces of straw is stacked in layer to form a pile and the pile is sprinkled with the mixture of EM solution.
- (c) Secondly, cowdung manure was made a layer on the grasses pile.
- (d) The groundnut expeller cake was put onto the cow dung as a layer.
- (e) Chaff ashes were spread onto the groundnut expeller cake and
- (f) Put the rice bran as a layer.

This procedures are repeated until the pile was about to reach 3 feet. The pile was covered with plastic sheet to keep it air-tight. After three weeks, the compost is ready to use.



Figure 1. Preparation of Making EM Bokashi Fertilizer

Analysis of Soil Sample and Prepared EM Bokashi Fertilizer

For soil analysis, the soil from the experimental area of 6 inches depth was randomly collected from five places before sowing. The collected soil samples and the prepared EM bokashi fertilizer were analyzed at the laboratory of Land Use Department, Ministry of Agriculture, Livestock and Irrigation in Magway Division.

Experimental Design and Treatments

Field experiment was carried out by using Randomized Completely Block Design (RCBD). Each plot considered of three rows. Total of 15 plants were included in each plot. The row-to-row spacing was 60 cm and the plant-to-plant spacing was 30 cm. The size of each plot was 4 ft x 5 ft. A total of four treatments with three replications were employed. The different levels of EM bokashi fertilizer such as $T_0 = 0 \text{ ton acre}^{-1}$, $T_1 = 1 \text{ ton acre}^{-1}$, $T_2 = 2 \text{ tons acre}^{-1}$ and $T_3 = 3 \text{ tons acre}^{-1}$ were conducted.

Cultivation and Fertilization

For the study of field experiment, three seeds of hybrid sunflower, Yezin-1 were directly sown in each hole. After one week of sowing seeds, two plants were thinned and only one healthy plant was left in each hole. Different levels of EM bokashi fertilizer (0, 1, 2 and 3 tons acre^{-1}) were applied before one week of sowing seeds. During the experiment, weeding and watering were properly done whenever necessary.

Data Collection and Statistical analysis

Plant height, number of leaves, and single leaf area were collected at weekly interval. Head diameters, seed lengths and widths, 100 seeds weight, total seeds weight (plant^{-1}) and seed yields kg acre^{-1} and basket acre^{-1} were also collected at the harvesting time.

The data were analyzed by using IRRISTAT software programmed. All treatment means were compared by LSD (Least Significant Differences) at 5% level of significance.

Methods

Single Leaf Area

The leaf samples were collected and measured the length and width. The single leaf areas (LA) were computed as follow:

$$LA = K \times L \times W$$

$$LA = \text{Single leaf area}$$

$$K = \text{Adjustment factor}$$

$$L = \text{Leaf length}$$

$$W = \text{Broadest width}$$

K value varies with the shape of leaf and it is also affected by the variety, nutritional status and growth stage of the leaf. The adjustment factor, K value (0.87) for sunflower was measured according.

Yield Estimation

According to training manual of oil seed crop, yield estimation was calculated by the following formula:

$$\text{Yield (basket acre}^{-1}\text{)} = \frac{\text{Number of plants acre}^{-1} \times \text{Number of seeds plant}^{-1} \times 1000 \times \text{seed weight (g)}}{454 \times 32 \times 1000}$$

Results

Physical and Chemical Properties of Cultivated Soil

As a result, the type of the soil for the study was silt loam and moderate alkaline (pH 7.84). The moisture 3.195 % and organic carbon percentage were 2.154. The contents of N, P and K found in the soil were low levels such as 0.108 %, 1.041 ppm and 0.291 % respectively. K₂O was medium levels 13.636 (mg/100gm).

Physical and Chemical Properties of EM Bokashi Fertilizers

The results of the study showed that the chemical constituents of EM bokashi was composed of 2.281 % moisture contents, 24.251% organic matter, pH 7.08, 0.86 % N, 1.409 % P₂O₅ and 1.188 % K₂O, respectively.

Plant Height

In this experiment, plant height was obviously increased with times after 56 DAS. The plant height of sunflower had no significant differences in all treatments. Among treatments, the mean of tallest plant height (67.56 cm plant⁻¹) was obtained in plants growing in 3 tons acre⁻¹ of EM bokashi fertilizer treatment. The shortest plant height (56.32 cm plant⁻¹) was obtained in T₀ (0 tons acre⁻¹ of EM bokashi) treated plants (Table 1, Figure 2).

Table 1. Effects of different levels of EM bokashi fertilizer on plant height of *Helianthus annuus* L. cv. Yezin-1

Treatments	Plant Height (cm plant ⁻¹)								Means
	Growing Period (DAS)								
	7	14	21	28	35	42	49	56	
T ₀ (0 ton acre ⁻¹)	3.76	14.84	21.44	38.99	59.99	98.66	105.44	107.44	56.32
T ₁ (1 ton acre ⁻¹)	3.28	14.98	25.14	49.55	75.10	110.55	117.88	119.77	64.53
T ₂ (2 tons acre ⁻¹)	3.31	16.16	20.26	52.55	77.55	116.21	123.32	125.66	66.88
T ₃ (3 tons acre ⁻¹)	4.10	15.25	26.84	51.21	78.44	116.66	120.21	127.77	67.56
F-test	ns	ns	ns	ns	ns	ns	ns	ns	-
5% LSD	0.95	4.21	8.29	13.81	18.13	20.84	18.61	15.46	-
CV%	14.0	14.6	17.6	15.3	13.2	10.0	8.5	6.3	-

Mean differences within each column determined by LSD at 5 % level of significant. ns = non-significance, DAS= day after sowing seeds.

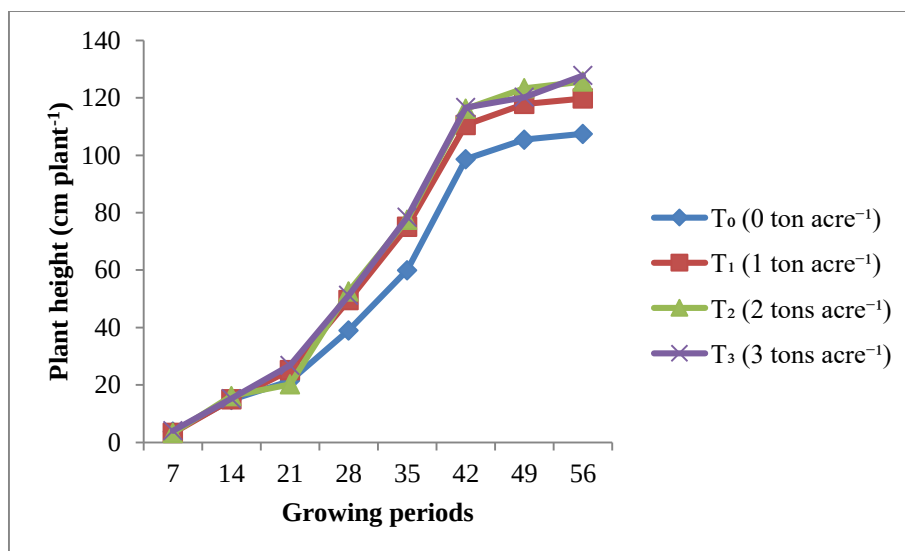


Figure 2. Effects of different levels of EM bokashi fertilizer on plant height of *Helianthus annuus* L. cv. Yezin-1

Number of Leaves

The numbers of leaves were drastically increased at 28 DAS. Statistically, the numbers of leaves were significantly differences ($P \leq 0.05$) among treatments at 7 DAS and 14 DAS. However, no significantly differences were found in all treatments after 14 DAS up to 56 DAS. The maximum mean number of leaves (22.80) was found in plants treated with 3 tons acre⁻¹ of EM bokashi fertilizer compared to control, 0 ton acre⁻¹ of EM bokashi fertilizer (21.40) (Table 2, Figure 3).

Table 2. Effects of different levels of EM bokashi fertilizer on number of leaves of *Helianthus annuus* L. cv. Yezin-1

Treatments	Number of Leaves plant ⁻¹								Means
	Growing Period (DAS)								
	7	14	21	28	35	42	49	56	
T ₀ (0 ton acre ⁻¹)	4.44	9.55	17.33	24.88	27.99	28.88	29.10	29.10	21.40
T ₁ (1 ton acre ⁻¹)	5.55	9.55	17.99	25.33	28.66	29.10	29.10	29.10	21.79
T ₂ (2 tons acre ⁻¹)	6	11.54	17.77	27.55	29.55	29.55	29.55	29.55	22.52
T ₃ (3 tons acre ⁻¹)	5.55	10.22	19.11	25.33	28.33	30.44	30.55	30.66	22.80
F-test	*	*	ns	ns	ns	ns	ns	ns	-
5% LSD	0.91	1.38	3.13	2.66	2.67	3.40	3.67	3.81	-
CV%	8.8	8.8	9.4	5.1	6.4	7.1	7.2	7.3	-

Mean differences within each column determined by LSD at 5 % level of significant.

* =significant at 5% ($P \leq 0.05$), ns = non-significance, DAS= day after sowing seeds.

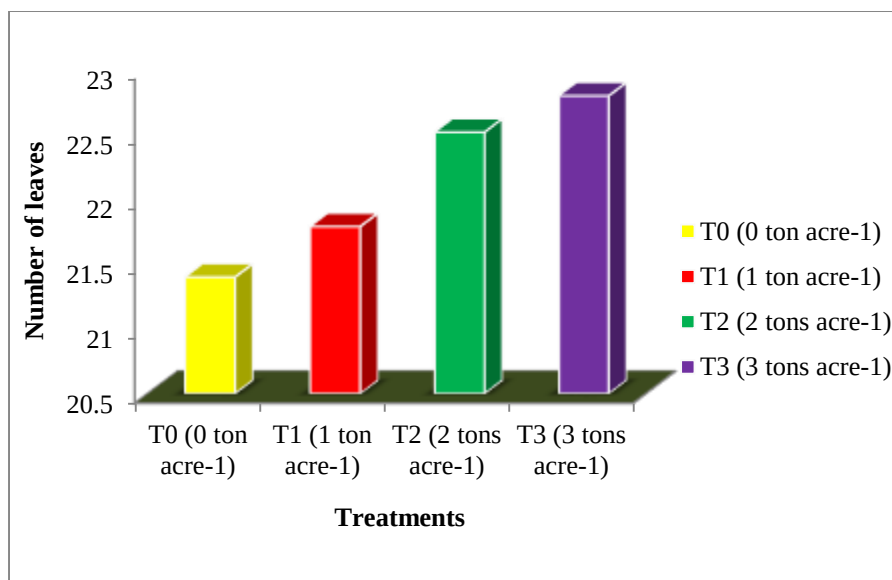


Figure 3. Effects of different levels of EM bokashi fertilizer on number of leaves of *Helianthus annuus* L. cv. Yezin-1

Single Leaf Area

The single leaf area of *Helianthus annuus* L. cv. Yezin-1 were obviously increased with times. Yezin-1 sunflower cultivar showed statistically significant differences ($P \leq 0.05$) among treatments at 7 DAS and 21 DAS. The results of mean single leaf area showed that the maximum leaf area (94.60 cm²) was obtained in the treatment of 3 tons acre⁻¹ of EM bokashi fertilizer, in contrast, 0 ton acre⁻¹ of EM bokashi (Control) was attained in minimum leaf area (66.95 cm²) (Table 3, Figure 4).

Table 3. Effects of different levels of EM bokashi fertilizer on single leaf areas of *Helianthus annuus* L. cv. Yezin-1

Treatments	Single Leaf Area (cm ²)					Means
	Growing Period (DAS)					
	7	14	21	28	35	
T ₀ (0 ton acre ⁻¹)	11.72	23.67	64.93	113.20	121.24	66.95
T ₁ (1 ton acre ⁻¹)	15.22	26.11	79.96	132.2	148.08	80.31
T ₂ (2 tons acre ⁻¹)	18.58	37.91	103.48	141.57	165.73	93.45
T ₃ (3 tons acre ⁻¹)	15.85	29.92	86.65	153.9	186.70	94.60
F-test	*	ns	*	ns	ns	-
5% LSD	4.7	11.5	24.23	45.02	52.68	-
CV%	16.3	20.7	15.4	17.7	18.0	-

Mean differences within each column determined by LSD at 5 % level of significant.

* = significant at 5% ($P \leq 0.05$), ns = non-significance, DAS = day after sowing seeds.

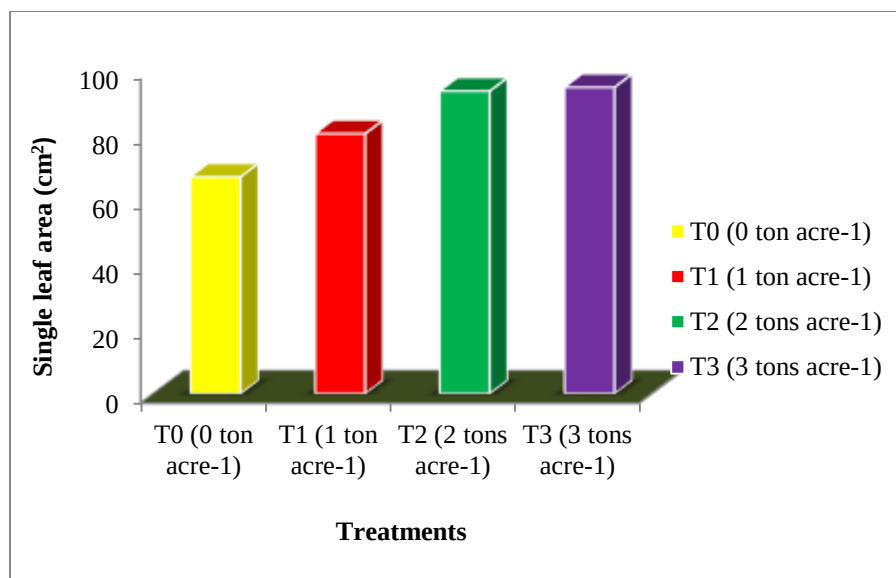


Figure 4. Effects of different levels of EM bokashi fertilizer on single leaf areas of *Helianthus annuus* L. cv. Yezin-1

Head Diameter

At harvest, the head diameters of sunflower hybrid Yezin-1 were statistically highly significant differences ($P \leq 0.01$) among all treatments. Among all treatments, the maximum head diameter (12.57 cm) was obtained in T₃, 3 tons acre⁻¹ of EM bokashi fertilizer treated plants. The minimum head diameter (8.01 cm) was observed in T₀, 0 tons acre⁻¹ of EM bokashi fertilizer (Table 4, Figure 5).

Table 4. Effects of different levels of EM bokashi fertilizer on head diameters of *Helianthus annuus* L. cv. Yezin-1

Treatments	Head Diameters (cm plant ⁻¹)
T ₀ (0 ton acre ⁻¹)	8.01
T ₁ (1 ton acre ⁻¹)	10.17
T ₂ (2 tons acre ⁻¹)	10.31
T ₃ (3 tons acre ⁻¹)	12.57
F-test	**
5% LSD	1.57
CV%	8.1

Mean differences within each column determined by LSD at 5 % level of significant.

** = significant at 1% ($P \leq 0.01$).

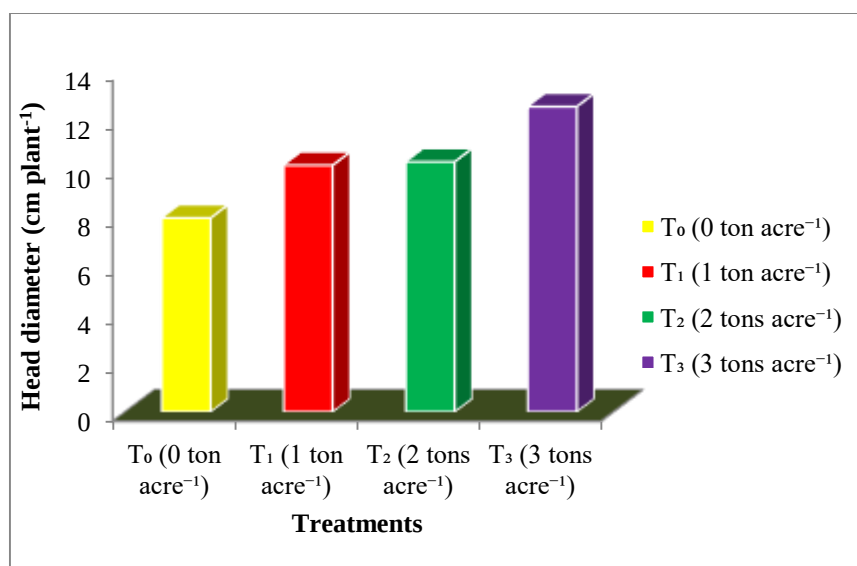


Figure 5. Effects of different levels of EM bokashi fertilizer on head diameters of *Helianthus annuus* L. cv. Yezin-1

100 Seeds Weight, Total Seeds Weight and Seed Yield

In this experiment, the 100 seed weight and total seed weight of sunflower had statistically highly significant differences ($P \leq 0.05$ and $P \leq 0.01$) at harvesting times. Among all treatments, the highest 100 seeds weight (6.73 g), and total seeds weight (46.32 g plant⁻¹) were obtained in T₃, 3 tons acre⁻¹ of EM bokashi fertilizer treated plants. The lowest 100 seeds weight (3.69 g) and lowest total seeds weight (23.94 g plant⁻¹) were obtained in T₀, 0 ton acre⁻¹ of EM bokashi treatments. Moreover, the maximum seed yield (1.59 tons acre⁻¹ and 33.39 baskets acre⁻¹) were obtained in 3 tons acre⁻¹ of EM bokashi fertilizer compared to control (0 ton acre⁻¹), which was (0.82 tons acre⁻¹ and 12.32 baskets acre⁻¹) (Table 5, Figure 6).

Table 5. Effects of different levels of EM bokashi fertilizer on 100 seeds weight, total seeds weight and seed yields of *Helianthus annuus* L. cv. Yezin-1

Treatments	100 Seeds Weight (g plant ⁻¹)	Total Seeds Weight (g plant ⁻¹)	Seed Yield (tons acre ⁻¹)	Yield (baskets acre ⁻¹)
T ₀ (0 ton acre ⁻¹)	3.69	23.94	0.82	12.32
T ₁ (1 ton acre ⁻¹)	4.72	33.14	1.13	18.41
T ₂ (2 tons acre ⁻¹)	5.29	33.34	1.14	24.16
T ₃ (3 tons acre ⁻¹)	6.73	46.32	1.59	33.39
F-test	**	*	-	-
5% LSD	0.81	11.71	-	-
CV %	8.4	18.3	-	-

Mean differences within each column determined by LSD at 5 % level of significant.

* = significant at 5% ($P \leq 0.05$), ** = significant at 1% ($P \leq 0.01$).

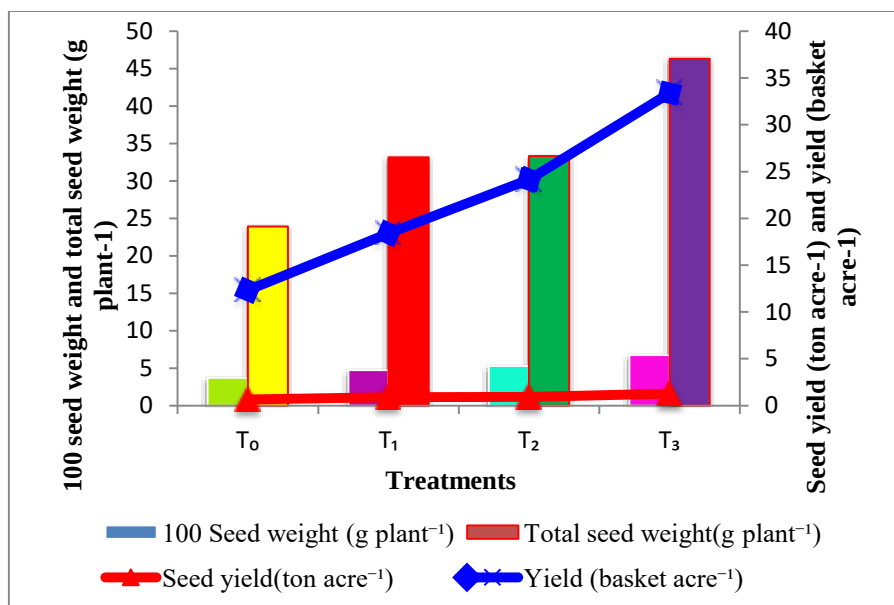


Figure 6. Effects of different levels of EM bokashi fertilizer on 100 seeds weight, total seeds weight and seed yields of *Helianthus annuus* L. cv. Yezin-1

Discussion and Conclusion

The field study were carried out to evaluate the effects of different levels of EM bokashi such as (0, 1, 2, 3 tons acre⁻¹) application on sunflower plants were provided greater yield components and yields of *Helianthus annuus* L. cv. Yezin-1.

According to the results of the study, the plant height was increased progressively over time and the tallest plant height was recorded in plants applied with 3 tons acre⁻¹ of EM bokashi helped in producing taller plant with an average height of (67.56 cm). Similar results have previously been reported on upland rice by (Haslim, 2016), in which rice husk bokashi 10 tons ha⁻¹ significantly increased plant height.

In this study, the effects of EM bokashi on the number of leaves per plant shown in (Table 2), the maximum mean number of leaves plant⁻¹ (22.52) was obtained in 3 tons acre⁻¹ of EM bokashi. Palacio-Roman and Agudelo-Esobar, (2020) mentioned that application of organic manure had significant effect on the number of leaves for sunflower.

Due to the experiment, the leaf area was significantly affected by EM bokashi fertilizer application on hybrid sunflower cultivar. The maximum leaf area (94.60 cm²) was recorded by applying 3 tons acre⁻¹ of EM bokashi fertilizer. The similar results reported by (Haslim, 2016), in which rice husk bokashi 10 tons ha⁻¹ significantly increased leaf number, and total leaf length and width.

Head diameter is one of the important yield components in sunflower. Head diameters were significantly increased with EM bokashi compare with control. Among the treatments, the application of 3 tons acre⁻¹ of EM bokashi was obtained in maximum head diameter (12.57 cm plant⁻¹). These results are similar to those of mentions of (Safari, 2003) who reported an increase in head diameters with increasing organic manure rates, this effect might attribute to positive contributions of nutrients release by organic fertilizer of plant growth.

One of the yield components characters, the 100 seeds weight is commonly used as a major determinant of sunflower yields. The different levels of EM bokashi treatments had significant effects on 100 seeds weight and total seeds weight of hybrid sunflower cultivar. After harvest, the maximum 100 seeds weight ($6.73 \text{ g plant}^{-1}$), and total seeds weight ($46.32 \text{ g plant}^{-1}$) were obtained in the plant grown in 3 tons acre^{-1} EM bokashi fertilizer in field. Klalan and Berger, (1963) also revealed that seeds weight contributes greatly to seed yields.

In order to the sunflower yields, the seed yield is mostly defined as a result of the cumulative effects of various yield components under the influence of EM bokashi fertilization. In this study, the total seeds yield was found to be ($1.59 \text{ tons acre}^{-1}$ and $33.39 \text{ baskets acre}^{-1}$) in the 3 tons acre^{-1} EM bokashi fertilizer treated plants. Similarly, (Moe Moe Ngwe, 2021) reported that hybrid sunflower cultivar Yezin-1 can be produced over 30 baskets per acre^{-1} . Nsaifabbas *et al.*, (2014) also showed that the increase in yield was due to the efficacy of bokashi and EM as fertilizer supplementing the necessary nutrients for plant growth.

In conclusion, Making EM bokashi is also easy to produce as bio-fertilizer by farmers using with the available farm-yard raw materials. Based on results of different levels of EM bokashi fertilizer application on hybrid sunflower cultivar, Yezin-1, seed yields contributing characters such as head diameter, 100 seed weight, and total seed weight were significantly influenced by the increased levels of EM bokashi fertilizer. By studying this, the level of EM bokashi fertilizer can be optimized as 3 tons acre^{-1} for the best performances in the cultivation of high yield producing hybrid sunflower cultivar Yezin-1. Therefore, 3 tons acre^{-1} of EM bokashi fertilization should be applied for hybrid sunflower cultivar. On the other hands, using the one of the bio-fertilizers, EM bokashi fertilizer with EM technology is simple, sustainable, economically viable, beneficial for farmers' livelihoods and environmentally friendly. However, further research studies should be extended in other crops to promote the organic farming system and to produce the high yield with short life spans, and good quality of organic products in the near future.

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LEAF ARCHITECTURAL STUDIES ON SIX SPECIES OF ANGIOSPERMS FROM MOHNYIN TOWNSHIP, KACHIN STATE

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Abstract

The present paper deals with leaf architectural study on 6 species belonging to 6 genera and 6 families of Angiosperms from Mohnyin Township, Kachin State. The characters studied include leaf attachment, leaf arrangement, leaf organization, laminar shape, base and apex shape, base and apex angle, margin type, leaf texture, petiole features, laminar size classes, 1°, 2°, 3°, 4° and 5° vein category, spacing, angle and areolation. In this study, the leaves were compound in 2 species and simple in 4 species. Symmetrical leaf base and petiole base pulvinate was the characteristic of *Phyllodium vestitum* Benth. Leaf margin of all species were entire, sinuous, erose and dentate. The texture of leaf was coriaceous in 5 species and membranous in 1 species. In the present research work, three types of laminar size classes (mesophyll in 3 species, macrophyll in 1 species, and notophyll in 2 species) were recorded. Primary vein category were pinnate in 4 species and palmate in 2 species. Secondary vein category were hemieucamptodromous in 1 species, cladodromous in 1 species, brochidodromous in 1 species, festooned semicraspedodromous in 1 species, and eucamptodromous becoming brochidodromous in 2 species. Intersecondary vein was present in 3 species and absent in 3 species. Tertiary vein category were irregular reticulate anastomose in 2 species, transverse freely ramified in 1 species, composite admedial reticulate in 1 species, mixed alternate and opposite percurrent in 1 species, and opposite percurrent in 1 species. Marginal ultimate venation were looped in 5 species and spiked in 1 species. A dichotomous key for the species were constructed. These resulting data showed unifying and distinguishing diagnostic characters that are good taxonomic markers for the identification and classification of flowering plants, especially in dealing with sterile plant specimens.

Keywords: Leaf Architectural, Angiosperms, Mohnyin Township, Kachin State.

Introduction

The Kachin State in Myanmar is famous for its invaluable natural resources and its rich biodiversity. Kachin State has four districts namely Myitkyina districts in the centre, Putao districts in the north, Bamaw districts in the south and Mohnyin districts in the west.

Mohnyin township is bounded by Moegaung township in the east, Homalin township in the west, Indaw township in the south and Phakant township in the north. This area lies between 24° 46' North Latitudes and 96° 22' East Longitude. The total area is 2578, 46 square miles. The elevation of Mohnyin is 333.33 meter above sea level. Indawgyi lake is one of the famous place in Mohnyin township. It has 16 miles in length and 7 miles in width. Indawgyi pagoda festival is annually held in March.

Leaf architecture was first used and described by Hickey (1973) to represent the placement and form of those elements constituting the outward expression of leaf structure, including venation pattern, marginal configuration, leaf shape and gland position (Jessica and Buof 2014).

This is aimed to study and record the leaf architecture of 6 species of Angiosperms from Mohnyin Township, Kachin state. The main objectives are to describe the leaf architecture of Angiosperms, to provide the usefulness of leaf architectural characters distinguishable in various species of Angiosperms from each other and then these valuable leaf architectural data to

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practically use in plant taxonomy and systematics, mostly especially in dealing with sterile plant specimens.

Materials and Methods

Angiosperms leaves of 6 different species belonging to 6 genera and 6 families were collected from Mohnyin Township, Kachin State. Ten mature leaf samples of each species were randomly taken from different branches of the identified plants. Plant collection was carried out during June 2022 to December 2023. Taxonomic identification of the collected specimens were determined by referring to Backer & Brink (1964-1968), Brandis (1971), Dassanayake (1980-2001), and Qi-ming & De-lin (2007-2009). All of the nomenclatural studies were checked in the websites of International Plant Names Index (IPNI) and online Botanical Database of Tropical Plants (TROPICOS). Myanmar names of collected species were referred to Hundley and Chit Ko Ko (1987) and Kress *et al.* (2003).

Fresh leaves of each species were examined under a stereomicroscope. Photographs were taking by using electric light box and stereomicroscope. For the measurement, a ruler was used in width, length and etc. while a protractor measuring angle of divergence. The laminar size class (Blade class) was measured and classified base on the result of laminar size followed by Ellis *et al.* (2009). Then the leaf architecture of these studied species were described by using the established leaf architecture characters and terminologies defined by Hickey (1973) and that of the leaf Architecture Working Group (1999). The terms massive, stout and moderate used for describing the size of the primary vein are based upon the formula suggested by Dilcher (1974).

The studied species were systematically arranged into families according to Angiosperm Phylogeny Group (APG IV) system of Byny *et.al* (2016). The arrangement of genera and species under the families were placed alphabetically. The collected leaf samples were pressed and sun dried. Then the dried leaf specimens were mounted on a herbarium sheet together with label of field data.

Results

The collected specimens 6 species belong to 6 genera 6 families were presented.

Table 1. List of the collected species from Mohnyin Township, Kachin State

Family		Species	Myanmar name
1. Fabaceae	1.	<i>Phyllodium vestitum</i> Benth.	Taw pe
2. Combretaceae	2.	<i>Getonia floribunda</i> Roxb.	Bu nwe
3. Anacardiaceae	3.	<i>Rhus semialata</i> Murr.	Chying ma
4. Polygonaceae	4.	<i>Antigonon leptopus</i> Hook&Arn	Taik- pan
5. Loganiaceae	5.	<i>Strychnos nux-blanda</i> A.W.Hill,	Khabaung gyi
6. Lamiaceae	6.	<i>Callicarpa tomentosa</i> (L.) L.	Kyun nalin

1. *Phyllodium vestitum* Benth. F.A.W.Miquel, Pl. Jungh: 217 1852. (Figure 1)

Leaflet attachment petiolulate, compound leaf arrangement alternate, leaf organization odd-pinnately compound. Blade attachment marginal. Laminar size mesophyll, 100-156 mm long and 48-77 mm wide. Laminar L:W ratio 1.8:1, leaf area 6435.56 mm². Laminar shape oblong-

lanceolate with medial symmetry and basal symmetry. Margin unlobed, sinuous with acute apex angle and acuminate apex-shape, obtuse base angle and rounded base shape, terminal apex features mucronate. Leaf texture coriaceous. Surface texture rugose. Gland absent. Petioles features canaliculate, 1.0-3.0 cm long, petiolule base pulvinulate, 1.4-2.3 cm long. Primary vein (1°) venation palmate basal actinodromous with five basal vein and compound agrophic veins, naked basal veins absent; primary vein (1°) size moderate; primary vein (1°) course slightly curved. Major secondary vein (2°) hemieucamptodromous with regular spacing; secondary vein (2°) uniform angle and decurrent attachment to midvein; secondary vein (2°) size moderate; secondary vein (2°) course abruptly upward curved; number of secondary vein along one side of 1°vein 7-10; angle between 1° and 2° vein 40°-50°; narrowly acute. Minor secondary vein (2°) simple brochidodromous. Intersecondary veins absent. Intercostal tertiary vein (3°) fabric mixed opposite and alternate percurrent, tertiary vein course sinuous; angle to primary vein obtuse; increasing exmedially angle variability. Quaternary vein (4°) fabric irregular reticulate. Quinternary vein (5°) fabric irregular reticulate. Areoles moderately developed, variable size and shape. Freely ending veinlets dichotomous branched. Marginal ultimate venation looped.

2. *Getonia floribunda* Roxb. Pl. Coromandel 1:61.1795. (Figure 2)

Leaf attachment petiolate, leaf arrangement opposite, leaf organization simple. Blade attachment marginal. Laminar size mesophyll, 136-224 mm long and 77-134 mm wide. Lamina L: W ratio 1.7 :1, leaf area 13192.2 mm². Laminar shape broadly ovate with medial symmetry and basal symmetry. Margin unlobed, entire with obtuse apex angle and acuminate apex shape, obtuse base angle and rounded base shape. Leaf texture coriaceous. Surface textures pubescent. Gland absent. Petiole features canaliculate, 0.7-1.5 cm long. Primary vein (1°) venation pinnate with one basal vein; primary vein (1°) size stout, primary vein (1°) course sinuous. Naked basal veins and agrophic veins absent. Secondary vein (2°) eucamptodromous becoming brochidodromous distally with irregular spacing; secondary vein (2°) abruptly increasing proximally angle and excurrent attachment to midvein; secondary vein (2°) size weak; secondary vein (2°) course abruptly upward curved; number of secondary vein along one side of 1°vein 7-11; angle between 1° and 2° vein 40°-60°; moderately acute. Intersecondary veins present, parallel to major secondary proximal course. Intercostal tertiary vein (3°) fabric irregular reticulate anastomose; tertiary vein course sinuous, angle to primary vein acute, angle variability inconsistent. Quaternary vein (4°) fabric irregular reticulate. Quinternary vein (5°) fabric irregular reticulate. Areoles poorly developed, random, irregular size and shape. Freely ending veinlets linear, dendritic branched. Marginal ultimate venation looped.

3. *Rhus semialata* Murr. Comm. Soc. Regiae Sci. Gott.6: 27. 1784. (Figure 3)

Leaflets attachment sessile, compound leaf arrangement opposite, leaf organization odd-pinnately compound. Blade attachment marginal. Laminar size notophyll, 66-110 mm long and 45-69 mm wide. Laminar L:W ratio 1.6:1, leaf area 3818.79 mm². Laminar shape ovate-lanceolate with medial symmetry and basal symmetry. Margin unlobed, dentate with acute apex angle and acuminate apex shape, obtuse base angle and rounded base shape. Leaf texture coriaceous. Surface texture pubescent. Gland absent. Petioles features pulvinae, 10.5-15.6 cm long. Primary vein (1°) venation pinnate with one basal vein, primary vein (1°) size stout, primary vein course straight. Naked basal veins and agrophic veins absent. Secondary vein (2°) cladodromous with regular spacing, secondary vein (2°) smoothly increasing proximally angle and deflected attachment to midvein; secondary vein (2°) size moderate, Secondary vein (2°)

course uniformly curved; number of secondary vein along one side of 1° vein 9-16; angle between 1° and 2° vein 50°-60°; moderately acute. Intersecondary veins present, distal course. Intercostal tertiary vein (3°) fabric transverse freely ramified, angle variability inconsistent. Quaternary vein (4°) fabric freely ramifying. Quinary vein (5°) fabric freely ramifying. Areoles lacking. Freely ending veinlets dendritic. Marginal ultimate venation spiked.

4. *Antigonon leptopus* Hook & Arn. Bot. Beech. Voy 208.69. 1841. (Figure 4)

Leaflet attachment petiolulate, leaf arrangement alternate, leaf organization simple. Blade attachment marginal. Laminar size notophyll, 76-99 mm long and 55-80 mm wide. Laminar L:W ratio 1.2:1, leaf area 4492.98 mm². Laminar shape ovate with medial symmetry and basal symmetry. Margin unlobed, erose with acute apex angle and acuminate apex-shape, reflex base angle and cordate base shape, terminal apex features mucronate. Leaf texture membranous. Surface texture pubescent. Gland absent. Petioles features canaliculate, 1.0-1.7 cm long. Primary vein (1°) venation pinnate with one basal vein; primary vein (1°) size stout; primary vein (1°) course straight. Naked basal veins and agrophic veins absent. Secondary vein (2°) festooned brochidodromous with decreasing proximally spacing; abruptly increasing proximally angle and excurrent attachment to midvein; secondary vein (2°) size stout; secondary vein (2°) course abruptly upward curved; number of secondary vein along one side of 1° vein 8-10; angle between 1° and 2° vein 60°-65°; moderately acute. Intersecondary veins present. Intercostal tertiary vein (3°) fabric irregular reticulate anastomose. Quaternary vein (4°) fabric irregular reticulate. Quinary vein (5°) fabric irregular reticulate. Areoles poorly developed, random, irregular size and shape. Freely ending veinlets dendritic branched. Marginal ultimate venation looped.

5. *Strychnos nux-blanda* A.W.Hill, Bull. Misc. Inform. Kew 1917.189.1917. (Figure 5)

Leaf attachment petiolate, leaf arrangement opposite, leaf organization simple. Blade attachment marginal. Laminar size mesophyll 112-211 mm long and 60-127 mm wide. Laminar L:W ratio 1.6:1; leaf area 13794.37 mm². Laminar shape ovate with medial symmetry and basal symmetry. Margin unlobed, entire with acute apex angle and acuminate apex shape, obtuse base angle and rounded base shape. Leaf texture coriaceous. Surface texture rugose. Gland absent. Petioles features canaliculate at the adaxial sides. Primary vein (1°) venation palmate; imperfect basal acrodromous with 5 basal veins. Primary vein (1°) size moderate; primary vein (1°) course markedly curved. Naked basal veins and agrophic veins absent. Secondary vein (2°) brochidodromous with irregular spacing and excurrent attachment to midvein; interior secondary veins present; major secondary vein (2°) size weak; secondary vein (2°) course sinuous; number of secondary vein along one side of 1° vein 4-11; angle between 1° and 2° vein 50°-70°; moderately acute. Minor secondary vein (2°) course simple brochidodromous. Intersecondary veins absent. Intercostal tertiary vein (3°) fabric composite admedial reticulate; tertiary vein course sinuous, angle variability inconsistent. Quaternary vein (4°) fabric irregular reticulate. Quinary vein (5°) fabric freely ramifying. Areoles poorly developed, random, irregular size and shape. Freely ending veinlets dendritic. Marginal ultimate venation looped.

6. *Callicarpa tomentosa* L. J.A.Murr. Syst. Veg. ed. 13: 153. 1774. (Figure 6)

Leaf attachment petiolate, leaf arrangement opposite, leaf organization simple. Blade attachment marginal; laminar size macrophyll, 214-360 mm long and 103-165 mm wide. Laminar L:W ratio 2.2:1; leaf area 31478.50 mm². Laminar shape ovate with medial symmetry and basal symmetry. Margin unlobed, undulate with acute apex angle and acuminate apex shape, obtuse base angle and cuneate base shape. Leaf texture coriaceous. Surface texture rugose. Gland absent. Petioles features canaliculate, 3.8-5.8 cm long. Primary vein (1°) venation pinnate with one basal vein; primary vein (1°) size stout; primary vein (1°) course straight. Naked basal veins and agrophic veins absent. Secondary vein (2°) eucamptodromous becoming brochidodromous distally with decreasing proximally spacing; uniform angle and excurrent attachment to midvein; secondary vein (2°) size moderate; secondary vein (2°) course abruptly upward curved; number of secondary vein along one side of 1° vein 10-16; angle between 1° and 2° vein 60°-70°; wide acute. Intersecondary veins absent. Intercostal tertiary vein (3°) fabric opposite percurrent, tertiary vein (3°) course straight, angle to primary vein obtuse; angle variability increasing proximally. Quaternary vein (4°) fabric mixed percurrent. Quinary vein (5°) fabric irregular polygonal reticulate. Areoles paxillate developed, distinct oriented fields. Freely ending veinlets linear, dendritic branched. Marginal ultimate venation looped.

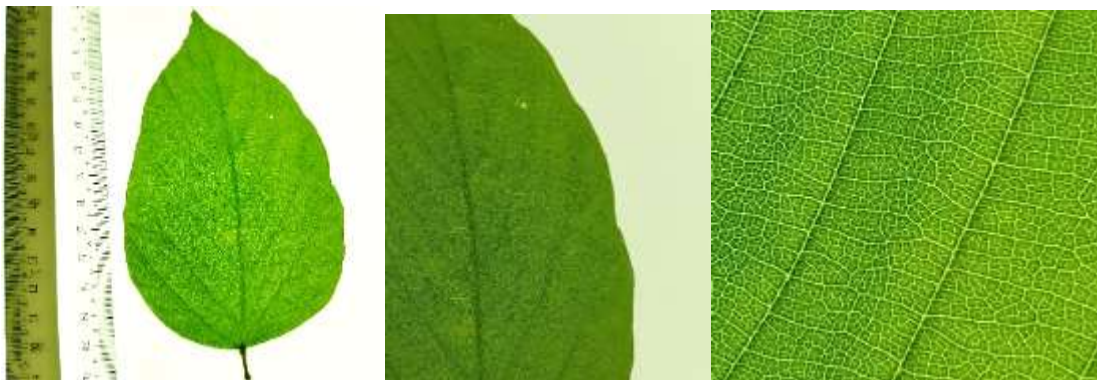


Figure 1. Laminar and leaf venation patterns of *Phyllodium vestitum* Benth.



Figure 2. Laminar and leaf venation patterns of *Getonia floribunda* Roxb.



Figure 3. Laminar and leaf venation patterns of *Rhus semialata* Murr.



Figure 4. Laminar and leaf venation patterns of *Antigonon leptopus* Hook&Arn.



Figure 5. Laminar and leaf venation patterns of *Strychnos nux-blanda* A.W.Hill,



Figure 6. Laminar and leaf venation patterns of *Callicarpa tomentosa* (L.) L.

An Artificial Key to the Studied Species

1. Leaf organization compound-----2
1. Leaf organization simple -----4
 2. Laminar size class notophyll -----3. *Rhus semialata*
 2. Laminar size class macrophyll-----3
3. Primary venation palmate; primary vein size moderate; 2° vein hemieucamptodromous becoming brchdodromous-----1. *Phyllodium vestitum*
3. Primary venation pinnate; primary vein size stout; 2° vein eucamptodromous becoming brchdodromous distally ----- 6. *Callicarpa tomentosa*

4. Leaf margin erose; leaf texture membranous -----

----- 4. *Antigonon leptopus*

4. Leaf margin entire; leaf texture coriaceous -----

----- 2. *Getonia floribunda*

5. Leaf arrangement opposite; 2° vein brochidodromous with irregular spacing -----

----- 5. *Strychnos nux-blanda*
5. Leaf arrangement alternate; 2° vein craspedodromous with irregular spacing -----

Discussion and Conclusion

The present research work deals with the leaf architectural characteristics of Angiosperms found in Mohnyin Township, Kachin State. A total of 6 species belonging to 6 genera of 6 families were collected, examined, measured and characterized based on the leaf architectural characters. The leaf architectural characters of each species were described based on the terminologies of leaf Architecture Working Group (LAWG 1999). The characters described were leaf attachment, leaf arrangement, leaf organization, leaf texture, petiole features, laminar area, laminar size class, laminar shape and symmetry, laminar ratio, base and apex angle, base and apex shape, position of petiolar attachment, margin type, lobation and vein orders.

In this study, collected species were varying characteristics of blade shape, base, apex, margin and texture. The venation patterns are also variable from species to species. Most of the studied species possess compound leaves and simple leaves. Symmetrical leaf base and petiole base pulvinate was the characteristics of *Phyllodium vestitum* Benth. The shape of leaf blade was also key characters in determining the species of flowering plant. In this research, two different lamina shape were observed and recorded. The apex shape was acuminate in all species. The base shape of the leaves were convex in *Phyllodium vestitum* Benth, cordate in *Antigonon leptopus* Hook&Arn, rounded in *Getonia floribunda* (Lam.) Roxb. and *Rhus semialata* Murr.; concave in *Strychnos nux-blanda* A.W.Hill.; cuneate in *Callicarpa tomentosa* (L.). These findings were in accordance with Backer (1964-1968), Hooker (1875-1879), Dassanayake (1980-2001) and Qi min & De-lin (2007-2009).

In the present study, most of the leaf margin were entire in 2 species; sinuous in *Phyllodium vestitum* Benth., dentate in *Rhus semialata* Murr; undulate in *Callicarpa tomentosa* (L.) L., and erose in *Antigonon leptopus* Hook&Arn. The texture of leaves were membranous in *Antigonon leptopus* Hook&Arn and coriaceous in 5 species. These findings were in agreement with Hooker (1875-1879) and Dassanayake (1980-2001).

Elis *et al.* (2009) stated that the laminar size was determined by measuring the area of the leaf. The laminar size classes were leptophyll, nanophyll, microphyll, notophyll, mesophyll, macrophyll, and megaphyll. In the present study, laminar size classes of leaf samples examined for 6 species were notophyll in 2 species, mesophyll in 3 species and macrophyll in only one.

According to Elis *et al.* (2009), the leaf venation were classified into two fundamental types namely pinnate and palmate. In this study, most of the leaves samples were pinnate venation pattern except in *Phyllodium vestitum* Benth., and *Strychnos nux-blanda* A.W.Hill.. The primary vein size was moderate in 2 species and stout in 4 species.

In the present study, major secondary vein were hemieucamptodromous in 1 species, cladodromous in 1 species, festooned semicraspedodromous in 1 species, brochidodromous in 1 species, and eucamptodromous becoming brochidodromous in 2 species. These finding were in agreement with the statements presented by Elis *et al.* (2009) and Hickey (1973).

Most of the leaves are regular secondary vein spacing in *Phyllodium vestitum* Benth.,; irregular secondary vein spacing in 3 species and decreasing proximally spacing in *Antigonon leptopus* Hook & Arn and *Callicarpa tomentosa* (L.) L. Hickey (1973) stated that angle of 2° vein divergence were narrowly acute (< 45°), moderately acute (45°-65°), wide acute (65°-80°), right angle (80-100°) and obtuse (>100°). In the present investigation, moderately acute occurred

in 4 species; narrowly acute angle observed in *Phyllodium vestitum* Benth. and wide acute in *Callicarpa tomentosa* (L.).

The tertiary vein category in the studied species have been found to be irregular reticulate anastomose in 2 species transverse freely ramified in *Rhus semialata* Murr.; mixed alternate and opposite percurrent in *Phyllodium vestitum* Benth. composite admedial reticulate in *Strychnos nux-blanda* A.W.Hill. and opposite percurrent in *Callicarpa tomentosa* (L.) species. These finding were in agreement with the statements reported by Elis *et al.* (2009) and Hickey (1973). During the present study, quaternary vein and quinary vein categories were freely ramifying, irregular reticulate and mixed percurrent. Areoles are the smallest areas of leaf tissues surrounded by veins. Paxillate developed areolation was observed in 1 species; poorly developed areolation was observed in 3 species and moderately developed areolation was found in 1 species and lacking in 1 species. Hickey (1973) reported that the veinlets into none, simple or branch. In the present study, simple or branched veinlets were observed. Hickey (1973) classified the marginal ultimate venation into looped, fimbriate and incomplete patterns. In the present investigation marginal ultimate venation exhibit great uniformity in majority of the plants. Looped marginal venation occurs in 5 species and spiked in *Rhus semialata* Murr.

Based on the resulting data, it was proved that the leaf architecture characters are good taxonomic markers in plant identification and classification. Moreover, leaf architecture has been an important topic in taxonomy, especially encountering flowering periodization, and leaves as identification tools in the flowering plants were bound to be very useful in the future.

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MORPHOLOGICAL, HISTOLOGICAL CHARACTERISTICS, PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF LEAVES OF *MESOSPHAERUM SUAVEOLENS* (L.) KUNTZE

Than Than Soe¹, Moe Moe², Naw Al Shar Phaw³, Nu Nu Win⁴

Abstract

Mesosphaerum suaveolens (L.) Kuntze known as Taw Pin Sein or Nan Saw Pin, belonging to the family Lamiaceae. The specimens were collected from Chan Mya Tharzi Township, Mandalay Region, during September 2022 to April 2023. The phytochemical results showed the presence of steroids, glycosides, phenolic compounds, tannins, saponins, α -amino acids, proteins, reducing sugars, carbohydrates and starch. Antioxidant activity was evaluated by using 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging method. The antioxidant activity of *Mesosphaerum suaveolens* (L.) Kuntze was $IC_{50} = 7.87$ mg/L. In antimicrobial activities, the different solvents chloroform, pet-ether, ethyl-acetates, ethanol, methanol and aqueous extracts of leaves were tested on five pathogenic microorganisms by using agar well diffusion methods. Among these extracts, activity was more effective on *Candida albicans* (32.2 mm inhibition zone) by the ethyl-acetates extract. The results of the studies indicated that the *Mesosphaerum suaveolens* (L.) Kuntze possesses phytoconstituents, antioxidant and antimicrobial activities. Thus, it can be utilized as a plant-derived natural product.

Keywords: *Mesosphaerum suaveolens*, Morphological, Histological, Phytochemical, Antioxidant, Antimicrobial Activities

Introduction

Mesosphaerum suaveolens (L.) Kuntze commonly known as taw pin sein or nan saw pin belongs to the family Lamiaceae (the Mint family). This family is the sixth largest angiosperm family with about 236 genera and 7173 species and has a cosmopolitan distribution. Naturally growing Lamiaceae members have been used as tea, spice and for various medicinal purposes and also used to treat fever, cough, headaches, wound healing, heart diseases and stomachaches (Ulhe and Narkhede 2013).

Mesosphaerum suaveolens (L.) Kuntze is an herbaceous plant. The word “*mesosphaeron*” comes from the Greek and Latin “*mesosphaerum*,” meaning “a type of tuberosity with medium-sized leaves,” while its specific epithet *suaveolens*, means “with a sweet fragrance” due to the aroma of essential oils exhaled by the trichomes present on its leaves. Taxonomically, *M. suaveolens* presents as botanical synonyms *Ballota suaveolens* L., *Hyptis suaveolens* (L.) Poit., and *Hyptis congesta* Leonard, with *H. suaveolens* as the most widespread synonym in scientific circles. However, the current circumscription of the genus *Mesosphaerum* P. Browne was recognized in 2012 after phylogenetic studies (Almeida 2022). This plant a shrubby, scented plant with blue or purple small flowers. The plant has been considered as a weed, distributed throughout the tropic and subtropics, common in open uncultivated areas, rocky dry substratum, on roadsides and waste grounds (Kum 2013).

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M. suaveolens (L.) Kuntze is an important source of essential oil, steroids, terpenes, alkaloids, flavonoids, phenols, and saponins. Leaves and flowers contains a wide variety of flavonoids and polyphenolic compounds and some plants species of the Lamiaceae family also contain vitamin C, vitamin E, quercetin, isorhammetin, kaempferol having the effective antioxidant property that is due to strong free radical scavenging activity. Almost all parts of this plant are being used in traditional medicine as well as it has immense of ethnomedicinal importance for treating various diseases. It was reported to be used for traditional medicine as an anticancer (Devi and Basavaiah 2023).

This plant is a very common plant in India. Indians use it the morning soup as a memory aid. Tea made from the roots of *M. suaveolens* (L.) Kuntze is used to purify the blood, and it is also used as a remedy for the “diseases” of women. It has been used as a medicinal tea in many places in Asia, and as a food and source of essential oil is South America (Sharma *et al* 2013). According to the Suganya and Sombat 2007, *M. suaveolens* (L.) Kuntze is also the edible plant which is usually applied in Thai food recipe as an appetizer because of their flavored essential oils.

In Myanmar, *Mesosphaerum suaveolens* (L.) Kuntze is an introduced species and widely distributed in roadsides and open field. This plant is one of the indigenous herbs. Its leaves are mainly used to treat respiratory diseases and diseases related to the gastrointestinal tract.

Mishra (2021) stated that *M. suaveolens* (L.) Kuntze possesses the ursolic acid which can inhibit on trascriptase-replicase and assembly of corona viral particles. And thus, this plant can be used as potent therapeutic drug against COVID-19 virus and other life- threatening RNA viruses.

Therefore, this study was aimed to study the morphological, histological characteristics, phytochemical analysis, antioxidant and antimicrobial activities of leaves of *Mesosphaerum suaveolens* (L.) Kuntze. The objectives were to observe the morphological and histological characteristics as the authentic botanical identification and to investigate the bioactive compound of this plant species.

Materials and Methods

Plant collection and identification

The specimens were collected from Chan Mya Tharzi Township, Mandalay Region during the flowering and fruiting periods from September 2022 to April 2023. After the collection, the specimens were studied and identified by using literatures such as Hooker (1875), Backer & Brink (1946) and Dassanayake (1987).The morphological and histological characteristics of *Mesosphaerum suaveolens* (L.) Kuntze were studied at the Department of Botany, University of Mandalay. For histological study, killing and fixation, dehydration, infiltration, embedding, staining and mounting were made according to Johansen’s method (1940). The parts of specimens were macerated by warming in equal volumes of 50% hydrogen peroxide solution and 50% glacial acetic acid solution according to Berlyn & Miksche (1976). Stomatal index were calculated by using the following equation (Kokate 2000).

$$S.I = \frac{S}{E + S} \times 100$$

Where,

S.I = Stomatal Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

Phytochemical analysis

The preliminary phytochemical analysis on the powder of *M. suaveolens* was carried out to determine the presence or absence of alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α -amino acids, proteins, reducing sugars, starch, carbohydrates and cyanogenic glycosides by Trease and Evans (1980) and Harbone (1984) methods. All the qualitative analysis had been undertaken at the Department of Botany, University Research Center, University of Mandalay.

Measurement of antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of the *M. suaveolens* was determined by using different concentration and ascorbic acid was used as a standard. This activity was carried out at the U Kar Kar Company Limited (Head office) in Mandalay.

Antimicrobial activity analysis

The antimicrobial activity analysis of *Mesosphaerum suaveolens* (L.) Kuntze leaves were performed by agar well diffusion method reported by Balouiri *et al.* (2016) at the microbiology laboratory in Department of Botany, University of Mandalay. Six extracts of dried powdered leaves, such as chloroform, pet-ether, ethyl-acetate, methanol, ethanol and aqueous extracts were used. Five pathogenic microorganisms such as *Candida albicans*, *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Escherichia coli* were used to investigate the antimicrobial activities of six different extracts of *M. suaveolens* (L.) Kuntze leaves.

Results

Morphological characteristics

Scientific name : *Mesosphaerum suaveolens* (L.) Kuntze, Revis 2:525.1891

Ballota suaveolens L., Syst. Not. 10.1100.1759

Family name : Lamiaceae

Myanmar name : Taw Pin Sein or Nan Saw Pin

English name : Pignut

Flowering period : September to April

Annual, erect herbs to undershrubs; stems and branches quadrangular, strongly aromatic, fistular and woody at the base, densely patent pilose with glandular, shallowly grooved, branches wide-spreading above, pubescent. Leaves simple, opposite and decussate, exstipulate.

Inflorescences axillary, capitate cyme with 1 to 5 flowers on the tops. Flowers bisexual, zygomorphic, tetramerous, hypogynous, blue. Stamens 4, didynamous, epipetalous; anthers ditheous. Ovary superior, ovoid, bilocular; style gynobasic; stigma bifid, minute. Fruits 2-nutlets. Seeds flattened, dark brown (Figure 1. A – H).

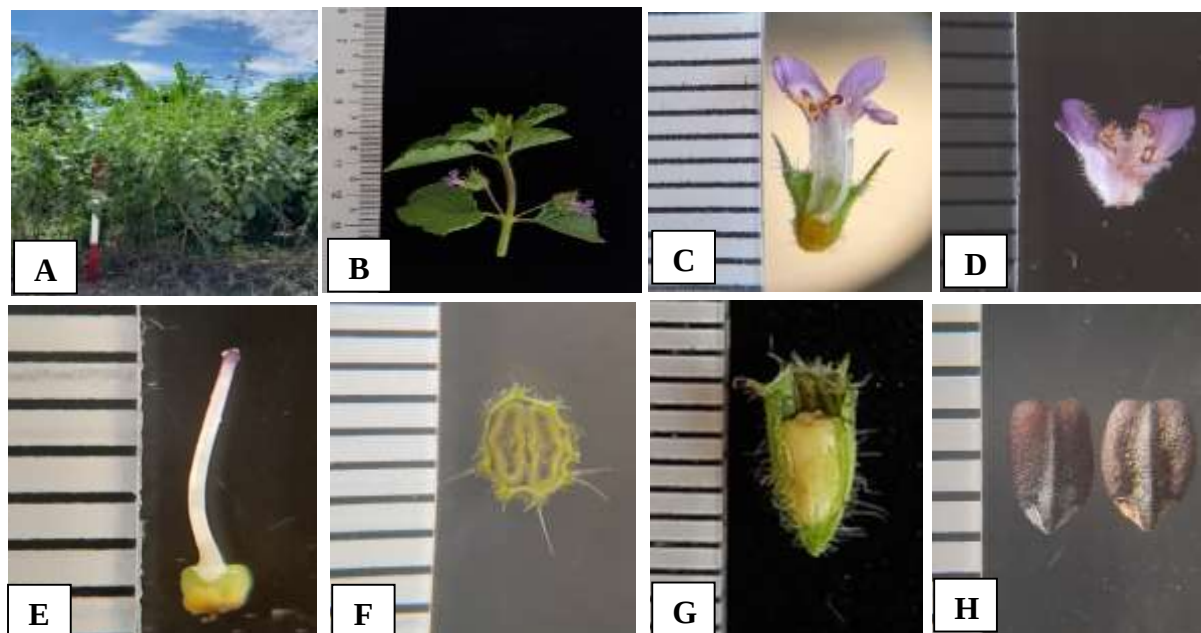


Figure 1. Morphological characteristics of *Mesosphaerum suaveolens* (L.) Kuntze

A. Habit B. Inflorescence C. L.S of flower D. Androecium
E. Gynoecium F. T.S of ovary G. Fruits H. seeds

Histological characteristics

In transverse sections, the petioles of *M. suaveolens* (L.) Kuntze were shield-shaped in outline, prominent wing at the adaxial side with capitate glandular and non-glandular trichomes. Vascular bundles were arranged as a crescentic, discontinuous, collateral type, accompanied by 3-accessory bundles present in adaxial 2-lateral prominent wings, each bundle circular in shape. In the lamina, *M. suaveolens* (L.) Kuntze. was dorsiventral with reticulate venation. Stomata types were diacytic and epidermal cell walls were wavy. The highest stomatal index were 75.00 and the lowest stomatal index were 28.58 in adaxial surfaces and the highest stomatal index were 42.86 and the lowest stomatal index were 40.00 in abaxial surfaces. The midribs were flat at the abaxial sides and convex at the adaxial sides. The stems of *M. suaveolens* (L.) Kuntze were quadrangular-shaped in outline with prominent ribs at 4-corners with deep notches in between. Vascular cambium formed a continuous ring, 1- or 2-layered. Xylem composed of vessel elements, tracheids, xylem fibres and xylem parenchyma. In transverse section of roots, they were circular-shaped in outline and periderm formation was found. Fibers, tracheids and vessel elements (thick-walled, lateral walls straight, end walls oblique or transverse, thickenings spiral, scalariform or pitted, perforation plates simple) were found in *M. suaveolens* (L.) Kuntze (Figure 3). Capitate glandular trichome, unicellular uniseriate, multicellular uniseriate and multicellular multiseriate trichomes were found through the plant (Figure 2).

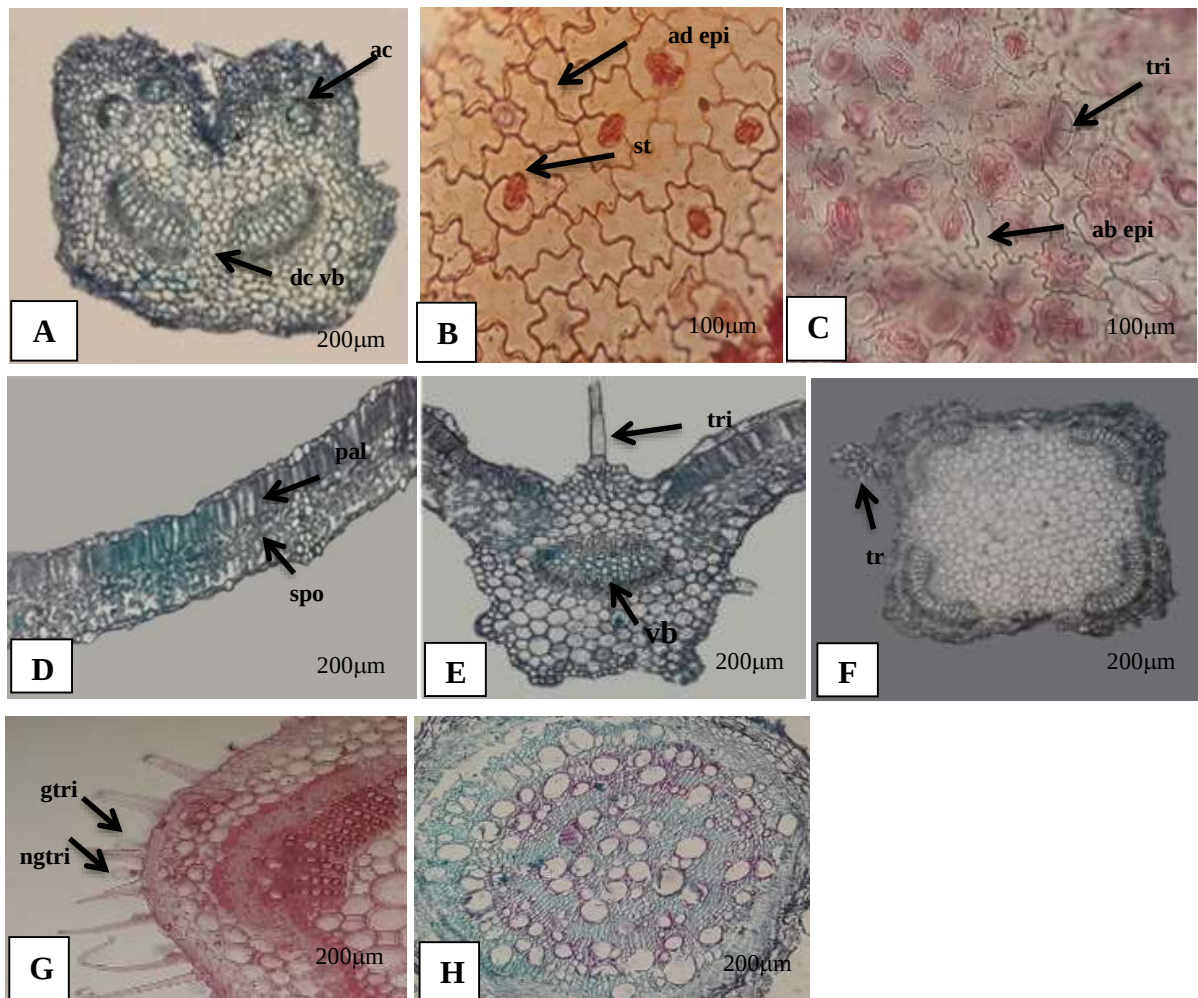


Figure 2. Internal structure of of *Mesosphaerum suaveolens*, (L.) Kuntze

- A. T.S of petiole B. Adaxial surface view of lamina
- C. Abaxial surface view of lamina showing diacytic types of stomata
- D. T.S of lamina showing palisade and spongy mesophyll cells
- E. T.S of midrib
- F. T.S of stem
- G. T.S of stem showing vascular bundle and trichome
- H. Close up view of root

(ab epi = abaxial epidermal cell, ad epi = adaxial epidermal cell, pal = palisade parenchyma cell, spo = spongy parenchyma cell, st = stoma, tri = trichome, dc vb= discontinuous vascular bundle, vb= vascular bundle)

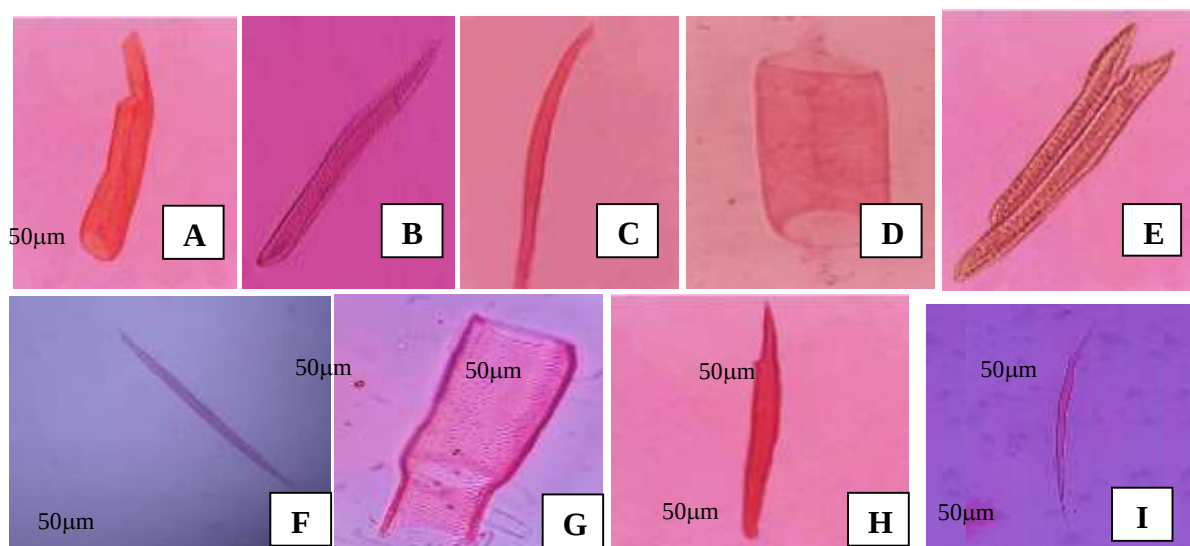


Figure 3. Macerated elements in leaves, stems and roots of *Mesosphaerum suaveolens* (L.) Kuntze

A-C Vessel, tracheids and fibers in leaves of *Mesosphaerum suaveolens* (L.) Kuntze

D-F Vessel, tracheids and fibers in stems of *Mesosphaerum suaveolens* (L.) Kuntze

G-I Vessel, tracheids and fibers in roots of *Mesosphaerum suaveolens* (L.) Kuntze

Phytochemical analysis on the leaves of *Mesosphaerum suaveolens* (L.) Kuntze

The preliminary phytochemical investigation on the powder of *M. suaveolens* (L.) Kuntze leaves was carried out to determine the presence or absence of alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α -amino acids, proteins, reducing sugars, starch, carbohydrates and cyanogenic glycosides. Carbohydrates and cyanogenic glycosides were not detected in all extracts. The results of the preliminary phytochemical analysis were shown in Table 1.

Table 1. Phytochemical analysis on the leaves of *Mesosphaerum suaveolens* (L.) Kuntze

No.	Phytochemicals	Reagent Names	Aqueous	Ethanol	Methanol	Observation
1.	Alkaloids	Mayer's reagent	-	+	+	Cream ppt.
		Wagner's reagent	-	+	+	Reddish brown ppt
		Hager's reagent	-	+	+	Yellow ppt
2.	Steroids	CHCL ₃ , conc:H ₂ SO ₄	+	+	-	Upper reddish
3.	Terpenoids	CHCL ₃ , conc:H ₂ SO ₄	-	+	+	Lower greenish yellow
4.	Glycosides	Sodium hydroxide	+	+	+	Yellow
5.	Phenolic compounds	10% Ferric chloride	+	+	+	Dark green
6.	Flavonoids	Sodium hydroxide	+	+	+	Pale green
7.	Tannins	10% Ferric chloride	+	+	+	Black
8.	Saponins	Distilled water	+	-	+	Frothing

No.	Phytochemicals	Reagent Names	Aqueous	Ethanol	Methanol	Observation
9.	α -amino acids	Ninhydrin	+	+	+	Purple spot
10.	Proteins	Millon's	+	+	+	Yellow ppt
11.	Reducing sugars	Benedict's	+	+	+	Green
12.	Starch	Iodine	+	+	+	Black
13.	Carbohydrates	Molish's	+	+	+	Purple ring
14.	Cyanogenic glycosides	Sodium picric	-	-	-	No brickish color

(+) = present, (-) = absent, ppt = precipitate, H₂O = Aqueous, EtOH = Ethanol,

MeOH = Methanol

Determination of Antioxidant Activity by DPPH Assay

In this experiment, five different concentrations (100, 50, 25, 12.5 and 6.25 mg/L) were used for aqueous extracts. Ascorbic acid was used as standard. According to the analysis of antioxidant activity, IC₅₀ value for standard ascorbic acid is 6.75 and IC₅₀ value for aqueous extract of *Mesosphaerum suaveolens* (L.) Kuntze is 7.87 as shown in Table 2, 3 and Figure 3, 4.

Table 2. % inhibition of various concentration of standard ascorbic acid

Standard Concentration	Absorbance	% Inhibition	IC ₅₀ (mg/L)
50	0.264	89.41459503	6.75
25	0.724	70.97032879	
12.5	1.123	54.97193264	
6.25	1.390	44.26623897	
3.125	1.511	39.41459503	

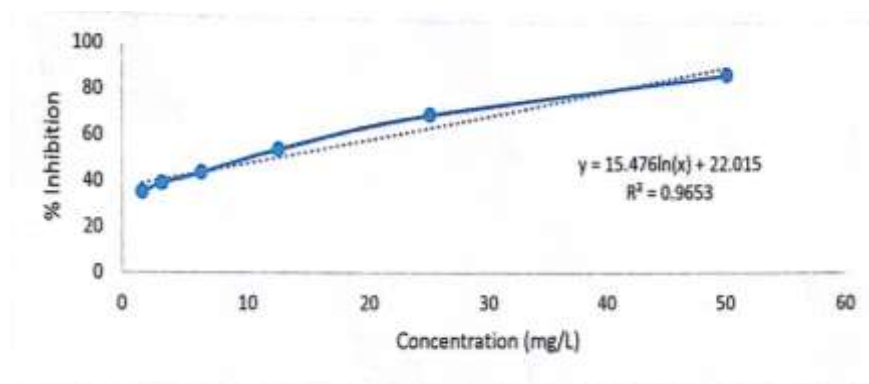
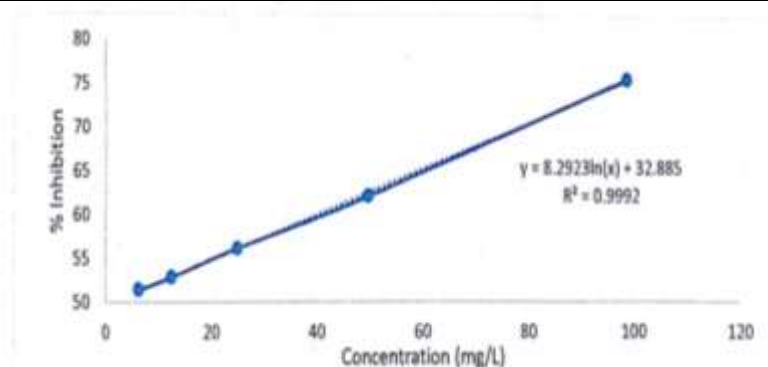


Figure 3. % Inhibition and Different Concentration of Ascorbic Acid (mg/L)

Table 3. % inhibition of various concentration of aqueous extract of *Mesosphaerum suaveolens* (L.) Kuntze

Sample Concentration (mg/L)	Absorbance	% Inhibition	IC ₅₀ (mg/L)
100	0.531	75.56374	7.87
50	0.826	61.98803	
25	0.954	56.09756	
12.5	1.025	52.83019	
6.25	1.056	51.40359	

**Figure 4. % Inhibition and Different Concentration of Leaves of *Mesosphaerum suaveolens* (L.) Kuntze (mg/L)****Analysis of antimicrobial activity**

The antimicrobial activity analysis of *Mesosphaerum suaveolens* (L.) Kuntze leaves were performed by agar well diffusion method. Six extracts of dried powdered leaves, such as chloroform, pet-ether, ethyl-acetate, methanol, ethanol and aqueous extracts were used. Ethyl-acetate extracts of leaves indicated more effective activities on all five test microorganisms than the other extracts. Aqueous extracts of leaves did not show activities on all the five test microorganisms. The results were shown in Table 4 and Figure 5.

Table 4. Antimicrobial activities of different solvent extracts of *Mesosphaerum suaveolens* (L.) Kuntze against five pathogenic microorganisms

Samples	Inhibition Zone Diameter (mm)				
	<i>Escherichia coli</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
Chloroform	9.5	16	17.3	17.3	14.5
Pet-ether	15	15.6	16.5	17.2	13.6
Ethyl-acetate	22.3	30.2	31.3	26.7	32.2
Ethanol	14.4	17.6	15	14.4	17.7
Methanol	15.1	14.8	15.1	14.6	16.5
Aqueous	-	-	-	-	-

Agar well diameter = 6 mm

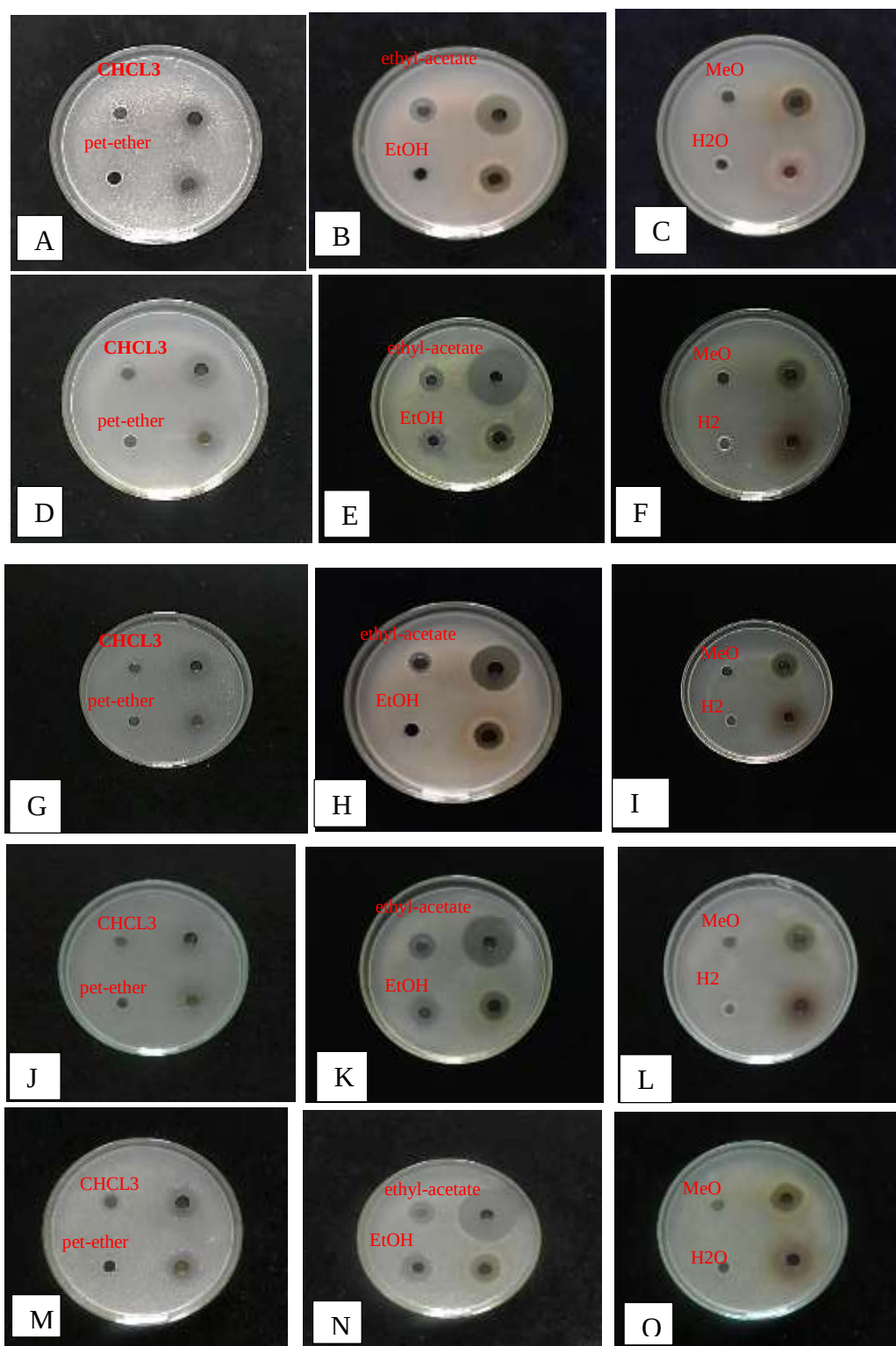


Figure 5. Antimicrobial activities of different solvent extracts of *Mesosphaerum suaveolens* (L.) Kuntze leaves against on test microorganisms
 A - C Antimicrobial activities against on *Escherichia coli*
 D - F Antimicrobial activities against on *Micrococcus luteus*
 G - I Antimicrobial activities against on *Staphylococcus aureus*
 J - L Antimicrobial activities against on *Pseudomonas aeruginosa*
 M - O Antimicrobial activities against on *Candida albicans*

Discussion

The morphological, histological, phytochemical analysis, antioxidant and antimicrobial activities of leaves of *Mesosphaerum suaveolens* (L.) Kuntze were studied in this research. This studied species *M. suaveolens* (L.) Kuntze belonging to family Lamiaceae.

According to this research, the habit of *M. suaveolens* (L.) Kuntze was annual erect herbs to undershrubs. Stems were quadrangular in shape. The flowers were blue, bisexual, zygomorphic, tetramerous and hypogynous. The stamens were didynamous. The ovary was superior and tetralocular with one ovule in each locule with axile placentation and fruit was nutlets in this species. These characters were in agreement with those mentioned by Hooker (1875), Backer (1946) and Dassanayake (1987).

In histological characters, the petiole of *M. suaveolens* (L.) Kuntze were sheid-shaped in outline with prominent wing at the adaxial side. Diacytic type of stomata are present on both surfaces of lamina. The stomatal index were 28.58 -75.00 on adaxial surfaces and 40.00 - 42.86 on abaxial surfaces. Glandular and non-glandular trichomes were present on both surfaces. Cresentic-shaped, discontinuous and collateral types vascular bundles were found in petiole. These characters were agreement with those described by Metcalfe and Chalk (1972). Metcalfe and Chalk (1957) pointed out that stem in many genera and species of labiatae are quadrangular in transverse section with well-defined groups of collenchyma in the four angles. *M. suaveolens* (L.) Kuntze was densely covered with hairs of various kinds, one of the most characteristics being the short-stalked glands with head of 1-16 or more cells. In this studies, capitate glandular trichome, unicellular uniseriate, multicellular uniseriate and multicellular multiseriate trichomes were found through the plant. Adedeji (2012) stated that qualitative foliar histological characteristics such as stomata type, trichomes type, anticlinal cell wall pattern were the most useful in species delimitation.

In this research, phytochemical analysis from three different solvent extracts such as aqueous, ethanol and methanol indicated that the leaves of *M. suaveolens* (L.) Kuntze contain various phyto-constituents alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α -amino acids, proteins, reducing sugars, carbohydrates and starch whereas alkaloids and terpenoids in aqueous extracts, steroids in methanol extracts and saponins in ethanol extracts were absent. Cyanogenic glycosides did not indicate in all extracts of the leaves of *M. suaveolens* (L.) Kuntze.

In the determination of antioxidant activities, the standard ascorbic acid exhibited antioxidant activity with IC_{50} value of 6.75mg/ L in DPPH assay. The aqueous extract of *M. suaveolens* (L.) Kuntze leaves showed 7.87 mg/L % inhibition in DPPH radical. Therefore, the IC_{50} value of this plant is near the same with standard ascorbic acid and it has high antioxidant activity.

According to the antimicrobial activity analysis of *M. suaveolens* (L.) Kuntze leaves, ethyl-acetate extracts of leaves indicated high activities on all the five test microorganisms. Among them, it was found the most effective on *Candida albicans* (32.2 mm). However, the aqueous extracts of leaves did not show activities on all the test microorganisms.

Conclusion

In conclusion, the studied morphological and histological characters of *M. suaveolens* (L.) Kuntze, were valuable to identify this species. Moreover, this plant possess the essential phyto-constituent, antioxidant properties and antimicrobial activities. And as a result, this plant can contribute as a potential plant-derived drug and as a source of other bioactive compounds to be discovered in the future.

Acknowledgements

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STUDY ON NUTRITIONAL, PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES FROM THE LEAVES OF *CHENOPODIUM ALBUM* L. (MYU)

Khin Myo Win¹, Yin Yin Kyaw², Nway Nway³

Abstract

Chenopodium album L. is known as Myu belong to Chenopodiaceae. The plant was collected from Htan tau village, Amarapura Township, Mandalay Region during March 2023. The *Chenopodium album* L. was annual erect herbs. The leaves of *Chenopodium album* L. contain nutritional values such as protein, fat, fiber, carbohydrates and organic matter. The leaves of *Chenopodium album* L. involve saponins, flavonoids, tannins, alkaloids, carbohydrates, phenolic compounds etc. In antioxidant activity, the methanol extract was higher than ethanol and watery extract. The leaves of *Chenopodium album* L. has significantly antioxidant effects. Then, antimicrobial activities of the water extract of leaves of *Chenopodium album* L. showed that the highest inhibition zone (20mm) on *Escherichia coli*, *Xanthomonas oryzae* and *Pseudomonas fluorescens*. The extracts of *Chenopodium album* L. showed good antimicrobial activities against on test organisms. The aim of the study of *Chenopodium album* (L.) leaves is to know the nutritional contents, chemical constituents, antioxidant and antimicrobial activities. It was suggested that the leaves of *Chenopodium album* L. should be eaten because it contains medicinal properties for human health.

Keywords: *Chenopodium album* L. (Myu), nutritional value, phytochemical, antioxidant and antimicrobial activities.

Introduction

Chenopodium album L. belongs to the family Chenopodiaceae (Goose foot family). *Chenopodium album* L. was originating from Western Asia. This plant was widely distributed all over the world and has about 250 species. The plant of *Chenopodium album* L. naturally grows as a weed in the field of Htantaw village, Amarapura Township, Mandalay Division during summer and winter season crops. Htantaw village lies between 21°53'45"N 96°04'11"E. This plant was found around Mandalay and Taunggyi in Myanmar. This plant is small annual herb having fleshy leaves.

The plant of *Chenopodium album* L. is a significant food crop and valuable plant in Ayurveda in tropical and subtropical countries and is abundantly used as food and herbal medicine around the world. The medicinal property of *C. album* is mainly found in its leaves and seeds. (Saini & Kant Saini, 2020).

The tender shoots of *Chenopodium album* L. are eaten raw in salad or cooked as a vegetable. The plant possesses various activities such as antibacterial, anti-inflammatory, anticancer, antiulcer, hepatoprotective, antioxidant, anthelmintic, spasmolytic and analgesic, sperm immobilizing agent, antipruritic and antinociceptive. Hence, this plant provides a significant role in the prevention and treatment of a disease. (Aman *et al.*, 2016).

Chenopodium album L. is an important medicinal plant in Ayurveda that cures leukoderma, epilepsy, fever, cough and abdominal pain. The species are cultivated as a grain or vegetable crop as well as animal feed in Asia (Ratre *et al.*, 2020). Herbal medicine is called as

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botanical medicine or phyto medicine. Phytochemical studies demonstrated that *C. album* L. methanolic extract have flavonoids, alkaloids, tannins and to a very little extent of carbohydrates and glycosides etc. (Saini *et al.*, 2019).

The leave extract of *Chenopodium album* L. was very good antioxidant effect. (Saina *et al.*, 2019). Many vegetables contain free radical inhibitors with preventive potential regarding a large number of human diseases that have been defined as nutraceuticals (Chludil *et al.*, 2008). The leaves of *Chenopodium album* L. had showed auticuicrobial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Candida glabrate* (Saina *et al.*, 2019).

Therefore, the leaves of *Chenopodium album* (L.) (Myu) were studied for the nutritional, phytochemical constituents, antioxidant and antimicrobial activities to obtain a thorough knowledge about their properties and constituents.

Materials and Methods

(a) Plant collection and identification of plant specimens

The leaves of *Chenopodium album* L. were collected from Htan taw village, Amarapura Township during March 2023. The specimen was studied for morphological characters and identified with the help of literature Backer, 1962.

(b) Preparation of Powdered sample

The leaves were thoroughly washed under tap water. The clean sample was dried in shade. Then, the leaves were coarsely powdered using a mixer grinder, sieved and stored in an air tight container for further use.

(c) Nutritional values of the Leaves of *Chenopodium album* L.

The leaves of *Chenopodium album* L. were evaluated for its nutritional values such as moisture, organic matter, Ash, crude protein, fats, crude fiber and nonstructural carbohydrate by using Association of Official Analytical Chemists (AOAC) method (Horwitz, 1980) in University of Veterinary Science, Physiology and Biochemistry Department, Yaezin.

(d) Preliminary study on Phytochemical constituents in Leaves of *Chenopodium album* L.

The leaves of *Chenopodium album* L. were studied for phytochemical constituents by various chemical tests. The presence or absence of alkaloids, saponins, tannins, reducing sugars, glycosides, starch, carbohydrates, phenolic compounds and flavonoids were studied according to method Harbone (1998).

(e) Antioxidant activities of the leaves of *Chenopodium album* L.**Screening of antioxidant activity by using DPPH free radical scavenging assay**

DPPH (1,1-diphenyl -2- picryl- hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of sample extracts. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenol in sample. DPPH (0.002 g) was thoroughly dissolved in ethanol (100 mL). This solution was freshly prepared in the brown coloured bottle and must be stored in refrigerator for no longer than 24 hours. The extracts (100 µg/mL) were prepared by dissolving 0.001 mg in 10 mL of ethanol. This solution was two-fold diluted serially with ethanol to get the sample solution with the concentration of 6.25, 12.5, 25, 50, 100 ppm. Accurately weighed 0.001 mg of ascorbic acid was dissolved in ethanol and made up the volume to 10 mL. It was used as standard solution.

Procedure

DPPH radical scavenging activity was determined by UV visible spectrophotometric method. Control solution was prepared by mixing 0.002 % of DPPH solution (1.5 mL) and 70 % ethanol (1.5 mL). The sample solution was prepared by thoroughly mixing 0.002 % (w/v) DPPH solution (1.5 mL) and the test sample solution (1.5 mL). The solution was allowed to stand at room temperature for 30 minutes. Absorbance of the solution was measured at 517 nm by using spectrophotometer and % inhibition was calculated by using the equation (Bugchi & Puri, 1998). Antioxidant analysis was performed at Chemistry Department, Yadanabon University.

$$\% \text{ Inhibition} = \left[\frac{A_{\text{DPPH}} - (A_{\text{Sample}} - A_{\text{Blank}})}{A_{\text{DPPH}}} \right] \times 100$$

where % Inhibition = percent inhibition of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The IC₅₀ (50 % inhibitory concentration) values were also calculated by linear regression excel program.

(f) Antibacterial activities of the leaves of *Chenopodium album* L.

The powdered of leaves was extracted with ether, acetone, methanol, ethyl acetate, ethanol and distilled water. For initial screening, nutrient agar was prepared according to the method described by Cruickshank (1975). Then, the extracts were tested with five pathogenic microbes.

Table 1. Test organisms utilized in the antimicrobial activities by Madigan, 2005.

No.	Test organisms	Source	Diseases
1.	<i>Aspergillus flavus</i>	-	Bronchitis
2.	<i>Bacillus subtilis</i>	JAP-0225215	Food spoilage and fever
3.	<i>Candida albicans</i>	IFO-1060	Skin infection, alimentary tract infection

No.	Test organisms	Source	Diseases
4.	<i>Escherichia coli</i>	ATCC-25922	Cholera, diarrhea and vomiting, urinary tract infections
5.	<i>Pseudomonas fluorescens</i>	-	Bacteria for leaf blight
6.	<i>Xanthomonas oryzae</i>	-	Bacteria for leaf blight

Paper Disc Diffusion Assay

Different solvent extracts of leaves of *Chenopodium album* L. were used to perform antimicrobial activities by paper diffusion method. In this method, nutrient agar was used as culture media. Two grams of nutrient agar were boiled with 75 ml of distilled water and isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10 ml of sterile growth medium. Then, they were incubated at 30°C for 72 hours on a reciprocal shaker at 200 rpm. Test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Paper disc having eight millimeter diameter were used for antibacterial assays. 0.3 ml of test organisms was added to assay medium, then poured into plates. After solidification, paper disc impregnated with broth samples were applied on the test plates and these plates were incubated for 24-36 hours at 30°C. After for 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicated the presence of bioactive compounds which inhibit the growth of test organisms.

Results

Scientific Name	- <i>Chenopodium album</i> L.
Myanmar Name	- Myu
English Name	- Goose foot; Pig Weed
Family	- Chenopodiaceae
Part used	- Tender Leaves and stems
Medicinal Uses	- Treating diarrhea and constipation
Uses	- Making salads and soup
Flowering period	- January to April

(a) Morphological characteristics of *Chenopodium album* L.

Annual erect herbs; stem and branches longitudinal ribbed. Leaves simple, alternate, exstipulate, petiolate, fleshy, cuneate at the base, irregularly dentate along the margin, acute at the apex, dark-green, with a whitish coat on the underside, mealy appearances. Inflorescences axillary or terminal cymose clusters forming panicle spike, many flowered; peduncle stout. Flower bisexual, actinomorphic, hypogynous, apetalous, greenish-white; bract minute. Calyx cup-shaped, 5-lobed; tubes long, lobes ovate-oblong, green, glabrous, persistent. Stamens 5, free, filament filiform, anther dithecous, basifixed. Carpels two, fused, ovary superior, style very short; stigma bifid. Fruit utricles, ovoid, indehiscent, within the persistent calyx, black-brown.



Figure 1. A. Habit B. Leaves C. Inflorescence D. Stamens E. Carpel

(b) Nutritional values of the Leaves of *Chenopodium album* L.

The leaves of *Chenopodium album* L. show the presence of nutritional value. The leaves are rich carbohydrates, proteins and organic matter. The content of fat is low in leaves. The results of nutritional value were shown in table 2.

Table 2. Nutritional values in the leaves of *Chenopodium album* L.

No.	Test Parameter	Results
1.	Moisture	12.7%
2.	Ash	17.2%
3.	Crude Protein	30.4%
4.	Crude Fiber	12.1%
5.	Fat	1.8%
6.	Carbohydrate	20.0%
7.	Organic matter	82.8%

(c) Preliminary study on Phytochemical constituents in leaves of *Chenopodium album* L.

According to experiment, the leaves of *Chenopodium album* L. contains alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, starch, saponin and tannins respectively. This results are shown in table 3.

Table 3. Results of Phytochemical Test for Leaf extract of *Chenopodium album* L.

No.	Test	Extract	Reagent Used	Observations	Results
1.	Alkaloids	1%HCl	Dragendroff's solution	Reddish brown ppt	+
2.	Flavonoids	EtOH	Conc HCl, Mg turning	Green color solution	+
3	Glycosides	H ₂ O	10% Lead acetate	White ppt	+
4	Phenolic compounds	H ₂ O	10% FeCl ₃	Greenish-blue solution	+

No.	Test	Extract	Reagent Used	Observations	Results
5	Reducing sugars	H ₂ O	Benedict's solution	Reddish brown ppt	+
6	Saponins	H ₂ O	Distilled water	Froth	+
7	Tannins	H ₂ O	10%FeCl ₃ ,dil H ₂ SO ₄	Yellowish brown ppt	+
8	Carbohydrates	EtOH	Fehling solution (A+B)	Red ppt	+
9	Starch	EtOH	Iodine solution	Black color ppt	+

(+) presence, ppt = precipitate

(d)Antioxidant Activities of the leaves of *Chenopodium album* L.

The leaves of *Chenopodium album* L. samples were sequentially extracted with ethanol, methanol and water. Antioxidant activity of *Chenopodium album* L. was expressed as percentage of DPPH radical inhibition and IC₅₀ values (ug/mL). IC₅₀ value of the sample was calculated from the concentration and percent inhibition curve. The results of antioxidant activity using DPPH method in sample and using Ascorbic acids as a positive control were shown in figure 4.

Determination of absorbance was carried out at wavelength 517 nm using spectrophotometer. Decrease in absorbance indicates increase in radical scavenging activity. The absorbance value of extracts was described in (Table 4).

Table 4. Absorbance of Leave extracts from *Chenopodium album* L. (Myu)

Concentration Extract	Absorbance at 517 nm				
	6.25	12.5	25	50	100
EtOH	0.001	0.011	0.012	0.013	0.014
	0.791	0.757	0.693	0.562	0.309
	0.762	0.751	0.699	0.569	0.319
	0.771	0.752	0.697	0.565	0.309
Watery	0.001	0.011	0.012	0.013	0.014
	0.805	0.751	0.695	0.601	0.431
	0.802	0.752	0.696	0.611	0.433
	0.802	0.741	0.697	0.601	0.435
MeOH	0.001	0.011	0.012	0.013	0.014
	0.841	0.781	0.705	0.579	0.228
	0.842	0.772	0.706	0.575	0.218
	0.851	0.771	0.707	0.577	0.227

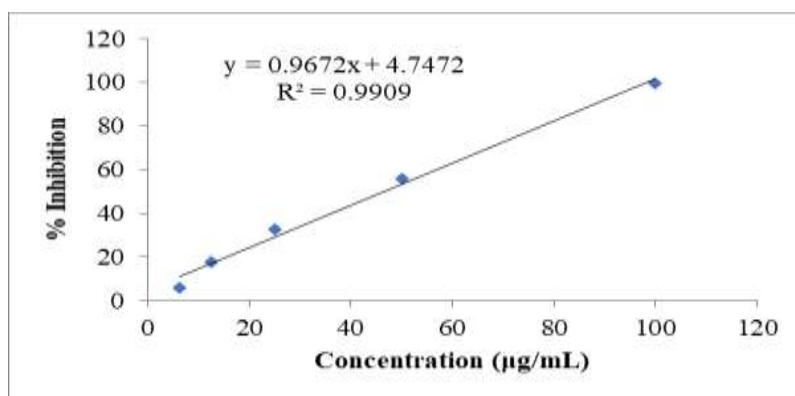
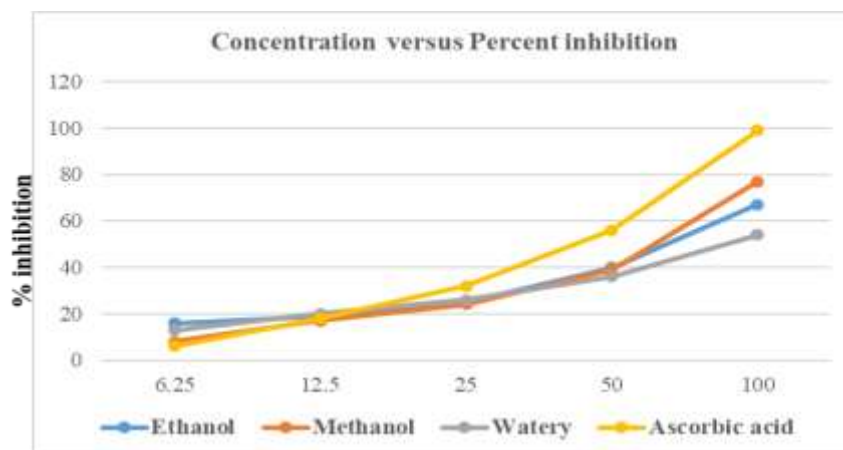
From these results, it was found that as the concentration increased the absorbance decreased, i.e., increased in concentration increased the radical scavenging activities for all samples. Free radical scavenging activities of extracts usually in terms of % inhibition were calculated.

Table 5. Radical Scavenging Activity and IC₅₀ of Ethanol, Methanol and Watery extracts of the *Chenopodium album* L. Leaves

Test Samples	% RSA (mean $\pm$ SD) in different concentration ($\mu\text{g/mL}$)					IC ₅₀ $\mu\text{g/mL}$
	6.25	12.5	25	50	100	
Ethanol	15.56 $\pm$ 0.1	18.98 $\pm$ 0.3	25.31 $\pm$ 0.3	39.72 $\pm$ 0.3	67.44 $\pm$ 0.6	68.64
Methanol	8.26 $\pm$ 0.5	16.96 $\pm$ 0.5	24.53 $\pm$ 0.1	38.67 $\pm$ 0.2	77.12 $\pm$ 0.5	62.63
Watery	12.79 $\pm$ 0.1	19.86 $\pm$ 0.6	25.62 $\pm$ 0.1	35.70 $\pm$ 0.6	54.44 $\pm$ 0.2	87.01
Ascorbic acid	5.74 $\pm$ 0.2	17.71 $\pm$ 0.1	32.41 $\pm$ 0.8	55.80 $\pm$ 0.1	99.45 $\pm$ 0.1	46.78

For the average values of radical scavenging activity, IC₅₀ values in $\mu\text{g/mL}$ was calculated by computer program called linear regressive excel program. These result were shown in (table 5) and concentration versus radical scavenging activity was plotted as shown in (figure 4, 5) and the comparison of IC₅₀ values of crude extracts of *Chenopodium album* L. was shown in (figure 6).

According to antioxidant activity, the methanol extract was lower in IC₅₀ value than ethanol and watery extracts. The methanol extract is higher in antioxidant activity than ethanol and watery extract. So, it was found that methanol, ethanol and watery extract of *Chenopodium album* were moderately good in antioxidant activities.

**Figure 4. % RSA of standard Ascorbic acid****Figure 5. A plot of % inhibition Vs concentrations of extracts of *Chenopodium album* L.**

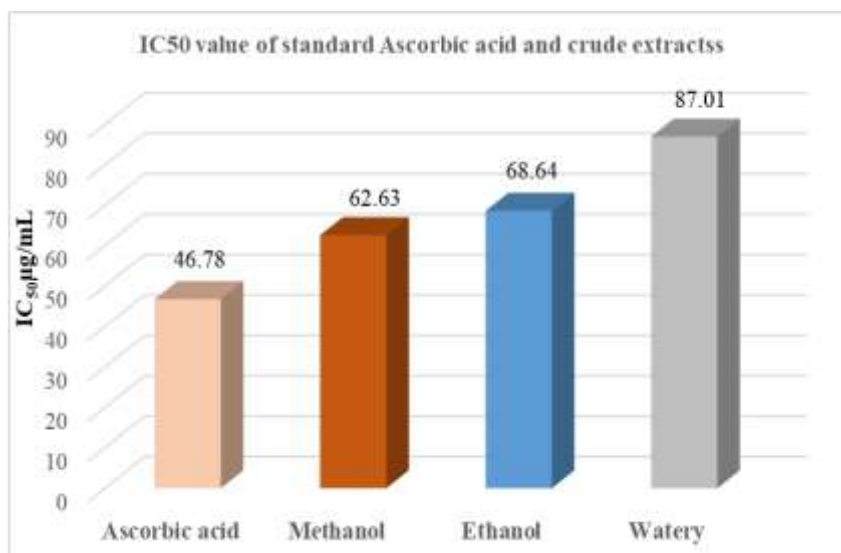


Figure 6. Comparison of IC₅₀ values of crude extracts of *C. album* L. with standard

(e) Antimicrobial Activities of the leaves of *Chenopodium album* L.

The antimicrobial activities of the leaves of *Chenopodium album* L. were carried out by various solvent such as acetyl, ethyl acetate, ethanol, methanol and petroleum-ether and water. The results of antimicrobial activities of the leaves extracts against on pathogens as shown in table 6 and figure (7). Among these, the water extract has shown the highest inhibition zone against on *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae* (20mm). The inhibition zone (16mm) was found in the water extract against on *Bacillus subtilis* and *Candida albicans* and the inhibition zone (18mm) occurred against on *Aspergillus flavous*. The acetyl extract performed antibacterial activity against on *Aspergillus flavous*, *Bacillus subtilis* and *Xanthomonas oryzae* with (14mm) zone of inhibition. The Petroleum ether extract has shown the lowest inhibition zone (8mm) against on *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans* and *Xanthomonas oryzae*. According to results, the acetyl, methanol and water extract of the leaves of *Chenopodium album* L. was effective on the tested pathogens. The water extract has been found to have significantly antibacterial activities on *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. So. the leaves of *Chenopodium album* L. have the ability to protect against pathogenic infection.

Table 6. Antimicrobial activities on various extracts of leaves of *Chenopodium album* L.

Microorganisms	Diameter of Inhibition zone (mm)					
	Acetyl	EtOAc	Ethanol	Methanol	Pet Ether	Water
<i>Aspergillus flavous</i>	14	8	8	10	8	18
<i>Bacillus subtilis</i>	14	10	10	10	8	16
<i>Candida albicans</i>	12	12	12	14	8	16
<i>Escherichia coli</i>	10	10	10	10	10	20
<i>Pseudomonas fluorescens</i>	12	12	14	14	10	20
<i>Xanthomonas oryzae</i>	14	10	12	14	8	20

Paper disc- 8mm, 8mm~12mm (+), 13mm~17mm (++), 18mm~20mm (+++)

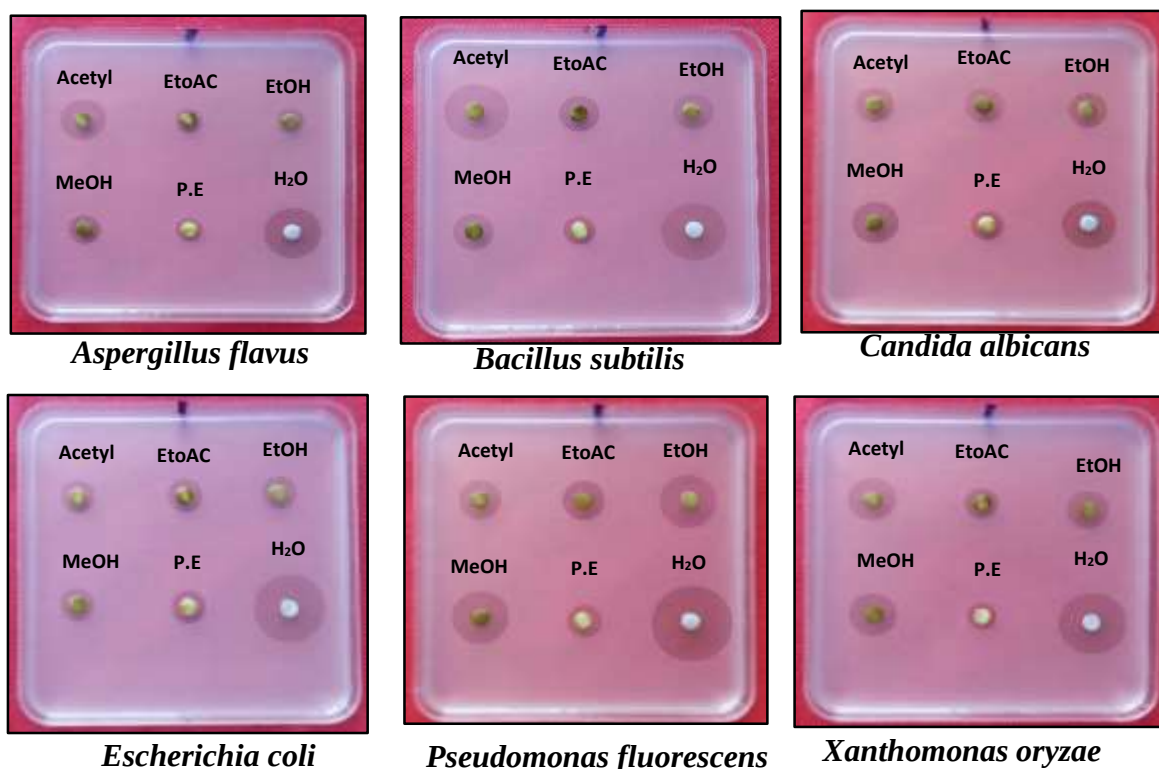


Figure 7. Antimicrobial activities of leaves of *Chenopodium album* L. on six pathogens.

Discussion

The plant of *Chenopodium album* L. belongs to the family Chenopodiaceae. The local name of this plant is Myu and the English name is goosefoot. The fresh leaves of *Chenopodium* have many beneficial medicinal properties. It is used to treat kidney stone, swelling, anemia, heart disease, jaundice and other diseases.

In the morphological study, *Chenopodium album* L. are annual erect herbs; stem and branches longitudinal ribbed. The leaves are simple, alternate, petiolate, fleshy, irregularly dentate along the margin, with whitish coat, mealy appearance on the under surface. Inflorescences axillary or terminal cymose clusters forming paniculate spike.

Pandey and Gupta (2014) reported that the leaves of *Chenopodium album* L. contain carbohydrates (40.84%), protein (28.69%), fat (4.41%), moisture 5.06%, ash 21% and dietary fiber 0.1%. In this result, the leaves contain the rich of carbohydrates (20.0%), crude proteins (30.4%) and organic matter (82.8%). The fat content is low amount (1.8%). The fiber, ash and moisture content are 12.1%, 17.2% and 12.7%. The fiber related to its indigestibility in the small intestine. The leaves should be cooked as diet for overweight people. Therefore, the leaves should be eaten raw in salads or cooked due to having nutritional values.

Arora et al., (2020) stated that the leaves of *Chenopodium album* L. possesses secondary metabolites. The different extracts of *C. album* contain steriods, triterpenoids, carbohydrates, flavonoids, tannins, saponins, proteins, alkaloids. In this study, the leaves contain alkaloids, phenolic compounds, flavonoids, saponin, tannin and etc. Alkaloids are good anesthetic agents while saponin helps the immune system and reducing the risk of intestinal cancer. The presence of flavonoids and phenolic compounds in the leaves could be responsible for their free radical scavenging activities.

Samanta and Somnath (2015) reported that revealed that ethanol extract is better than the aqueous extract for the antioxidant activity in *Chenopodium album* L.. In this study, the antioxidant activity of *C. album* L. indicated that the methanol extract exhibited the higher antioxidant potential than ethanol and watery extract. The leaves of *Chenopodium. album* L. should be consumed as antioxidant rich, vegetables for human health.

In antimicrobial activities, Saini et al., (2019) described the methanol leaf extract of *Chenopodium album* possess inhibitory activity against on *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *C. glabrata*. Al-Snafi (2019) represented that the aqueous extract performed highest antibacterial activity against on *Staphylococcus aureus* with (25 mm) zone of inhibition and the least antibacterial activity observed against on *Salmonella typhimurium* with 17.75 mm zone of inhibition. On the other hand, methanol extract displayed potential antibacterial activity all the test bacteria. In this study, the water leaf extract showed maximum activity against on *Escherichia coli* *Pseudomonas fluorescens* and *Xanthomonas oryzae* with inhibition zone of 20 mm. The methanol and acetyl extract showed that the inhibition zone (14 mm) was found the activity against on all test organisms except *E. coli*. the various extract of *Chenopodium album* L. exhibited significantly antibacterial activity against all test organisms. The Pet-ether extract was the lowest antibacterial activity was observed against on test organisms. Therefore, the antimicrobial activities of *Chenopodium album* L. showed significantly against on *Escherichia coli* *Pseudomonas fluorescens*, *Xanthomonas oryzae*, *Bacillus subtilis* and *Candida albicans*.

Conclusion

It was concluded that the leaves of *Chenopodium album* L. effects for human health. The leaves contain fibers, ash, proteins, flavonoids, carbohydrates, saponins, tannins, alkaloids. The nutritional constituents are used traditional medicines and the leaves should be eaten as curry and salad for human health. Addition the leaves of *Chenopodium album* L. have the potential antioxidant and then antimicrobial activities against on test organisms. Therefore, the leaves of *Chenopodium album* can be used to cure of various diseases. It was suggested that the leaves of *Chenopodium album* L. should be cooked and as salad because it contains beneficial nutritional contents, phytochemical constituents, antioxidant properties and against on test organisms.

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ISOLATION, PARTIAL PURIFICATION, AND APPLICATION OF FUNGAL PROTEASES FROM BEAN CROP SOIL

Phyo Wai Khaing¹, Nu Yin Aye², Pyae Phyo Hlaing³ and Nan Nan Htwe⁴

Abstract

The present study is based on the characterization of 11 and 16 isolated fungi from the bean crop soil in the Vegetable and Fruit Research Development Centre (Hlegu Township) and Myaungtagar Village, respectively. In a total of 27 isolated fungi, 13 fungi showed a clear zone around the colony to determine the preliminary protease-producing fungi. Still, the two selected fungi (V2 and M16) were found to be the highest protease producers. The two selected fungi (V2 and M16) were found as the genus *Penicillium* sp. The two selected proteolytic fungi were purified using 50% ammonium sulfate precipitation and then dialysis to obtain a partially purified enzyme. When compared with standard molecular weight markers, the relative molecular weight of *Penicillium* sp. (16) was found to be approximately $\approx < 40\text{kDa}$, but *Penicillium* sp. (2) was estimated to be 70kDa. The partial purification fold was 2.16% for *Penicillium* sp. (2), which will recover 13.05% by dialysis, but *Penicillium* sp. (16) had a fold of 2.05% which will recover 23.14% by dialysis. In the Lineweaver-Burk plot, the K_m values of *Penicillium* sp. (2 and 16) were shown to be 1.04 g%. $V_{\max}$ values were 102.04 mMmin⁻¹ of *Penicillium* sp. (16) and 100.00 mMmin⁻¹ of *Penicillium* sp. (2). In the removal of blood stain, *Penicillium* sp. (2 and 16) exhibited complete clearance of the removal of blood stain compared with the control. In the milk clotting test, two selected, partially purified proteases exhibited strong milk coagulation within about 30 minutes. In the degradation of gelatin using waste X-ray films, it was observed that 70 minutes for *Penicillium* sp. (16) and 120 minutes for *Penicillium* sp. (2) were required for complete degradation.

Keyword: Protease Fungi, *Penicillium* sp., Partial Purification, K_m and $V_{\max}$ Values, Milk Clotting, Removal of Blood Stain, Degradation of Gelatin

Introduction

Proteases (EC: 3.4.21-24 and 99; peptidyl peptide hydrolases) are enzymes that catalyze the cleavage of the peptide bond in proteins (CO-NH), liberating small chains of amino acids called peptides (Godfrey and West, 1996). Enzymes are very effective biological catalysts that accelerate almost all metabolic reactions in living organisms. Proteases are a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins and break them down into polypeptides or free amino acids.

Microbial proteases are degradative enzymes that catalyze the total hydrolysis of proteins (Haq *et al.*, 2006). The molecular weight of proteases ranges from 18-90kDa (Sidney and Lester, 1972). Microbial proteases are an important family of proteases and have an advantage over animal and plant proteases, as microbes can be cultured on a large scale in less time, and the growth conditions can easily be optimized in lab conditions. Fungi elaborate a wider variety of enzymes than do bacteria, and proteases are among the most important enzymes produced by fungi. Filamentous fungi are used in many industrial processes for the production of enzymes and metabolites (Adrio and Demair 2003).

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Proteases, one among the three largest groups of industrial enzymes, account for about 60% of the worldwide sales of enzymes (Nurullal and Fikret, 2011). The majority of the total sales (89%) of proteases are used in detergent formulation. Historically, proteases were the first to be used in laundry detergents, about two-thirds share of this industry (Mienda and yahya, 2014). They are generally used in detergents, food industries, leather, meat processing, milk clotting, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds (Paranthaman et al., 2009). The specific objectives are to determine the enzyme activity and protein content of crude protease enzyme at the optimal experimental conditions, kinetic parameters, and to investigate of protease enzyme activities and their usages.

Materials and Methods

Sample collection, isolation, and purification of fungi

Soil samples used from different environmental sources, like two different places of bean crop soil in Vegetable and Fruit Research Development Centre, Hlegu Township, and Myaungtagar Village, were collected aseptically from a 6-inch depth in sterile zip-lock plastic bags. One gram of each soil sample was serially diluted under sterile conditions. From the diluted suspension, 100 μ L was spread on potato dextrose agar (added 1% casein) medium, which was incubated at room temperature for three to five days and monitored every day for the growth of the fungal colony. Further, these fungi were sub-cultured on potato dextrose agar (added 1% casein) to obtain the pure strain (Sharma *et al.*, 2015). All isolated fungi were designated V1 to V11 for bean crop soil in the Vegetable and Fruit Research Development Centre and M1 to M16 for bean crop soil in Myaungtagar Village.

Preliminary production of proteolytic enzyme on skim milk agar (SMA) medium (Sethis and Gupta, 2015)

Preliminary screening was done by inoculating the fungal isolates on skim milk agar medium containing casein as a protein source at room temperature for one to seven days. After incubation, the protease was exhibited by a zone of proteolysis which was exhibited by a zone of proteolysis. The zone diameter was measured in cm, and the results are recorded (Abe *et al.*, 2015). The enzyme index (EI) is expressed as R/r, where R is the degradation zone diameter and r is the colony diameter (Abe *et al.*, 2015). The species that exhibits the maximum clear zone was selected for further identification.

Identification and characterization of protease fungi

The two selected fungi were examined to the genus level with taxonomic keys under macroscopic and microscopic field according to the standard method Barnett and Hunter (1960); Dube (1983).

Fermentation condition (Anson, 1938)

The 100mL of synthetic media contained casein 0.5g, dextrose 0.5g, yeast extract 0.5g, magnesium sulfate 0.02g, dipotassium hydrogen phosphate 0.1g, and sodium carbonate 0.01g at pH 8.0. After autoclaving, the respective fungi of fermentation broth were operated on a rotary

shaker at 250 rpm for 4 days at room temperature. At the end of the fermentation period, the contents were centrifuged at 10000 rpm (4°C) for 10 minutes at room temperature, and the cell-free supernatant was used as crude enzyme for the enzyme activity assay.

Protease-specific activity assay

Protease-specific activity assay was performed according to the method of Colowick and Kaplan (1955). Briefly, 1.3mL of 1% casein dissolved in 50mM potassium phosphate buffer (pH 8) was added to both sample and blank tubes and incubated at room temperature for 30 minutes. Subsequently, 0.7mL of crude protease extract was added to only the sample tube and incubated for 30 minutes at room temperature. The reaction was stopped with the addition of 3mL of 5% trichloroacetic acid (TCA) solution in both the sample and blank tubes. The mixture was then incubated for 30 minutes at room temperature. A UV-visible spectrophotometer was used to determine the absorbance value at 275nm. From the standard curve, the activity of protease samples can be determined in terms of Units, which is the amount in microgram of tyrosine equivalents released from casein per mL minute under assay conditions described by Abdilbar *et al.* (2021).

Construction of a standard curve for the determination of protein

Protein contents were determined using standard bovine serum albumin according to the method of Lowry *et al.* (1951).

Partially purified the crude enzyme

Extraction of crude enzyme from the optimum fermentation medium

The selected optimum production medium (medium II) was sterilized and then inoculated with one loop of selected protease on a shake at 200 rpm using the best fermentation period of each selected protease fungi. Extraction of each enzyme was done by simple filtration of medium using Whatman filter paper for the separation of fungal mycelia (Karthik *et al.*, 2014). The filtrate was centrifuged at 10,000 rpm (4°C) for 10 minutes. Clear supernatant was taken as crude enzyme (Sind and Hamid, 2013).

Ammonium sulfate precipitation

After centrifugation of the filtrate, the cell-free solution of the enzyme was precipitated by the addition of various ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ (30%, 50%, 60%, 70% and 80%) by continuous mixing on an ice bag using magnetic stirring. Then it was kept for an overnight period at 4°C for precipitation of protein. Again, it was centrifuged at 10,000 rpm at 4°C for 10 minutes. The precipitate in pellets was used as the crude extract of protease and examined for each protease using an enzyme assay according to the method of Huang *et al.* (2017).

Dialysis (Desalting of enzymes)

The Procedure of dialysis (Spectra/Pro®3 Dialysis Membrane, 3500 Dalton; Flat width 18mm and Length 16ft) by Anustrup (1980) is like that, firstly put the submerged fermentation under optimal conditions in centrifuged at 4,000 rpm, 15 minutes. Then, 50% ammonium sulfate was added and stirred continuously with a magnetic stirrer for 4 hours at room temperature. After 4 hours, put into the freezer for one hour to receive pellet precipitate after centrifuging at 4,000 rpm, 15 minutes. Then re-dissolved received pallet precipitate was dissolved with a minimum amount of water. After that added this solution into a dialysis bag and added the received dialysis

bag to distilled water. Then continuously stirred with a magnetic stirrer for 2 hours, every 2 hours, and distilled water 5 times. And then dialysis was continued overnight at room temperature.

Determination of the molecular weight of the partially purified protease enzyme

The molecular mass of the enzyme was determined by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), which was carried out as described by Laemmli (1970). The supernatants of protease enzyme *Penicillium* sp. (2 and 16) were partially purified with 50% ammonium sulfate precipitation by using dialysis. The molecular weight of the enzyme was estimated using a low molecular weight marker (Broad Range Unstained Protein Ladder, cat no. 26630).

Determination of the kinetic parameter

Casein was used over the concentration range 0.25% to 2% to determine the kinetic parameters K_m and V_{max} of the partially purified protease enzyme. To investigate the kinetic parameters, the best concentration of enzyme 0.7mL was added with different concentrations of casein (0.25% to 2%) as substrate. The reaction mixture was incubated at predetermined conditions (pH 8, 37°C, and within 30 to 40minutes). The Michaelis constants (K_m) and maximal velocity (V_{max}) values were determined as reciprocal absolute values of the intercepts on the X- and Y-axis, respectively, of the linear regression curve (Monika Karas and Barbara Baraniak, 2006). The measurement was made at 37°C for 30 minutes as previously determined.

Applications of the partially purified proteases

Blood stain removal activity

Activity of protease enzyme as a detergent additive was analyzed through different combinations of treatment on blood sample stain cotton fabric according to Choudhari (2012). This was studied by a washing test, seven white cotton fabric pieces (5×5cm) were stained by 60μL (0.06mL) of blood. After the dryness and stains at room temperature for seven days, they were immersed in a solution for one set of the experiments. The sets were (1) 100mL distilled water (DW) as a control, (2) 1mL partially purified protease plus 99mL DW, (3) 1mL detergent solution plus 99mL DW, (4) 1mL detergent solution plus 98mL DW and 1mL partially purified protease, (5) 1mL detergent solution plus 97.5mL DW and 1.5mL partially purified protease and (6) 1mL detergent solution plus 97mL DW and 2mL partially purified protease at room temperature for one hour. By the end of incubation, fabrics were rinsed with tap water, and the stain elimination effectiveness was determined by visual inspection of the color intensity of the cloth.

Milk clotting test

The assay of milk-clotting enzyme activity was carried out according to the standard procedure described by Alecrim *et al.* (2015). In test tubes of equal volume, cleaned of any trace of fat, 10mL of milk containing 2.6% fat and 1mL of enzyme solution were added. The test mixtures were thoroughly shaken and incubated at room temperature, and the clotting times and visual inspection of clotting were recorded at regular intervals and times.

Removal of gelatinous coating from waste X-ray film

The waste of X-ray film was done by taking (2cm by 2cm strip) 2×2cm pieces, and then these were cleaned with distilled water and wiped with a cotton swab soaked in ethanol. After drying in an oven at 40°C for 30 minutes, each of the weighty films was immersed in a flask containing 1mL of the partially purified protease in 9mL of phosphate buffer, and subsequently incubation was carried out at room temperature for three hours with a shaking speed of 200 rpm. The film was incubated in a 10mL phosphate buffer served as a control. Turbidity of the reaction mixture increased with time by visual inspection of the color. So, the changing color of each film was removed and recorded at regular intervals and time (Kumar and Dwivedi, 2019).

Results

Sample collection, isolation, and purification of fungi

Eleven and sixteen soil fungal isolates were isolated on potato dextrose agar (added 1% casein) medium from bean crop soil in Vegetable and Fruit Research Development Centre, Hlegu Township, and Myaungtagar Village, respectively. These isolated fungi were used by using serial dilution of the pour plate method at room temperature for three to five days.

Preliminary production of proteolytic enzyme on skim milk agar (SMA) medium

The purified fungal isolates were screened for the ability to produce protease enzyme on skim milk agar (SMA) medium by the streak plate method. Among all isolated fungi, 13 isolated fungi showed clear zone formations around the fungal growth, indicating the property of protease. Although only two fungi, V2 and M16, showed maximum proteolytic activity with a zone of about 2.5cm for five days and 2.9cm for six days, respectively, as shown in Figures 1 and 2.

Identification and characterization of protease fungi

In the present investigations, the selected fungal strains were characterized by the use of macroscopic and microscopic characters using standard methods. The microscopic observation based on the two selected protease fungi (V2 and M16) was identified as *Penicillium* sp. (2 and 16), as shown in Figure 1.

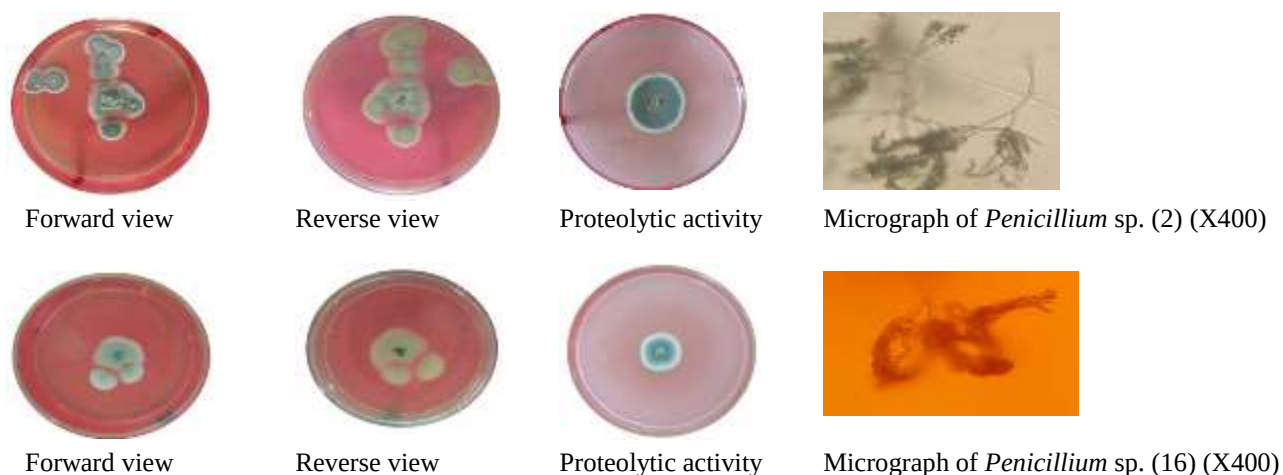


Figure 1. Morphological and microscopical characters of isolated fungi from V2 and M16

Construction of a standard curve for the determination of protein

The determination of protein by using bovine serum albumin (BSA) as a standard is a modification of Lowry's method. Before determining the protein content in the sample solution, it is necessary to construct a calibration curve by using standard protein concentration and their absorbance at 540nm. At this fixed wavelength of 540nm, the calibration curve of absorbance against the various concentrations of bovine serum albumin (BSA) was drawn as shown in Figure 3. The nature of the curve was a straight line passing through the origin, showing that Beer's law was obeyed (Mikkelsen and Corton, 2004).

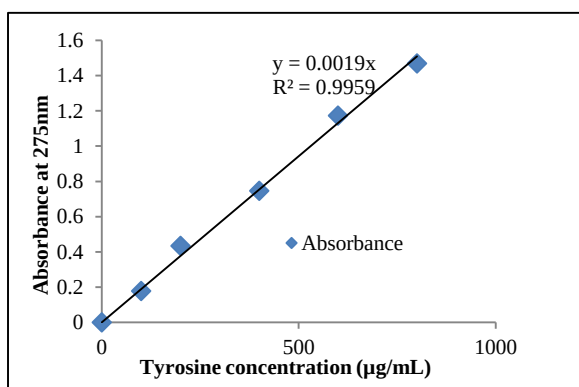


Figure 2. Plot of absorbance as a function of standard tyrosine concentration

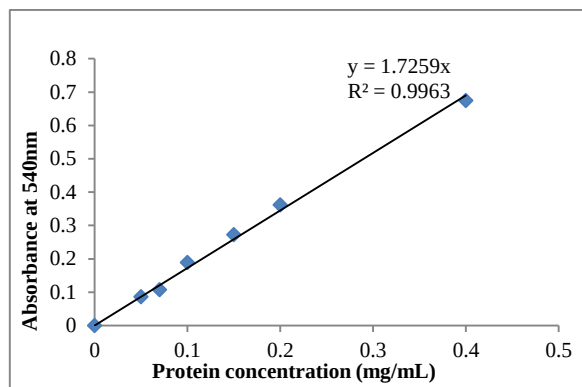


Figure 3. Calibration curve for standard protein solution

Partially purified the crude enzyme

The supernatants of two selected protease enzymes, *Penicillium* sp. (2 and 16), were partially purified with 50% ammonium sulfate precipitation followed by dialysis, as shown in Figure 4. Among the two selected proteases, the purification step for maximum protease production from *Penicillium* sp. (2) was observed in a 2-fold purification with 13.05% recovery by dialysis, and their specific activity was increased from 45.27U/mL. The *Penicillium* sp. (16) was exhibited in a 2-fold purification with 23.14% recovery, and its specific activity was increased from 32.97U/mL. Purification fold results of two selected proteases are summarized in Tables 1 and 2.



Penicillium sp. (2)



Penicillium sp. (16)

Figure 4. Purification of extracted enzymes by dialysis

Table 1. Partial purification of protease from *Penicillium* sp. (2)

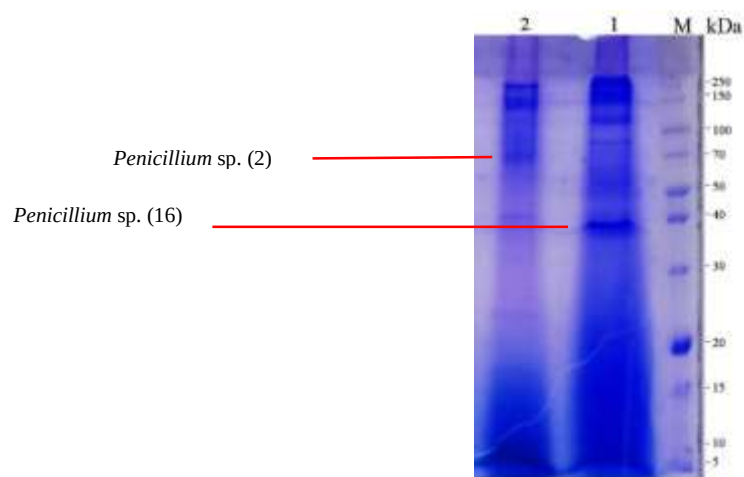
Purification steps	Total protease activity (U/mL)	Total protein (mg/mL)	Specific activity (U/mL)	Purification (fold)	Recovery (%)
Crude protease	3113.27	149.39	20.98	1	100
50% of (NH ₄) ₂ SO ₄ and Dialysis	876.53	19.36	45.27	2.16	13.05

Table 2. Partial purification of protease from *Penicillium* sp. (16)

Purification steps	Total protease activity (U/mL)	Total protein (mg/mL)	specific activity(U/mL)	Purification (fold)	Recovery (%)
Crude protease	1901.02	118.19	16.08	1	100
50% of (NH ₄) ₂ SO ₄ and Dialysis	902.04	27.36	32.97	2.05	23.14

Determination of the molecular weight of the partially purified protease enzyme

The molecular weight of partially purified protease was determined by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), using low molecular weight when compared with standard molecular weight markers. The molecular mass from *Penicillium* sp. (16) was found $\approx < 40$ kDa, and *Penicillium* sp. (2) was estimated to be 70kDa, as shown in Figure 5.



M - Molecular weight markers

Lane 1 - Purified protease from *Penicillium* sp. (16)

Lane 2 - Purified protease from *Penicillium* sp. (2)

Figure 5. Determination of molecular mass of partially purified protease by SDS-PAGE**Determination of the kinetic parameter**

In order to study the effect of various casein substrate concentrations ranging from 0.25% to 2% was conducted. The reaction rate versus casein substrate concentration curve was plotted to determine whether the enzyme obeys Michaelis-Menten kinetics, and constants were

determined from a Lineweaver-Burk plot. From the Lineweaver-Burk plot, the K_m values were found to be 1.04g% *Penicillium* sp. (2 and 16). While the V_{max} value was observed as 100.00 mMmin⁻¹ and 102.04 mMmin⁻¹ for *Penicillium* sp. (2 and 16). The results are presented in Figures 6 and 7.

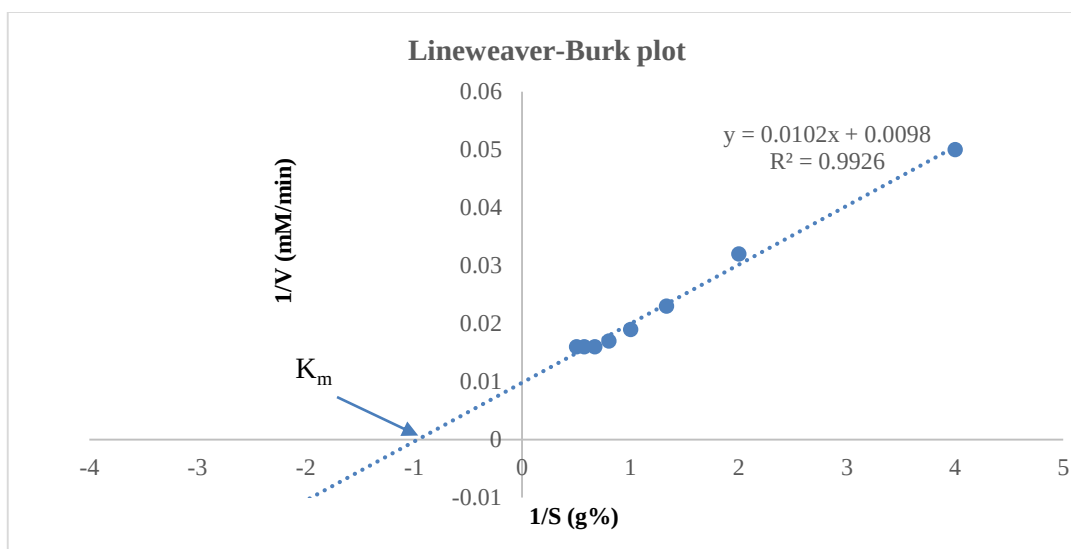


Figure 6. Kinetic plot based on the specific substrate for partially purified protease produced by *Penicillium* sp. (2) using the Lineweaver-Burk plot

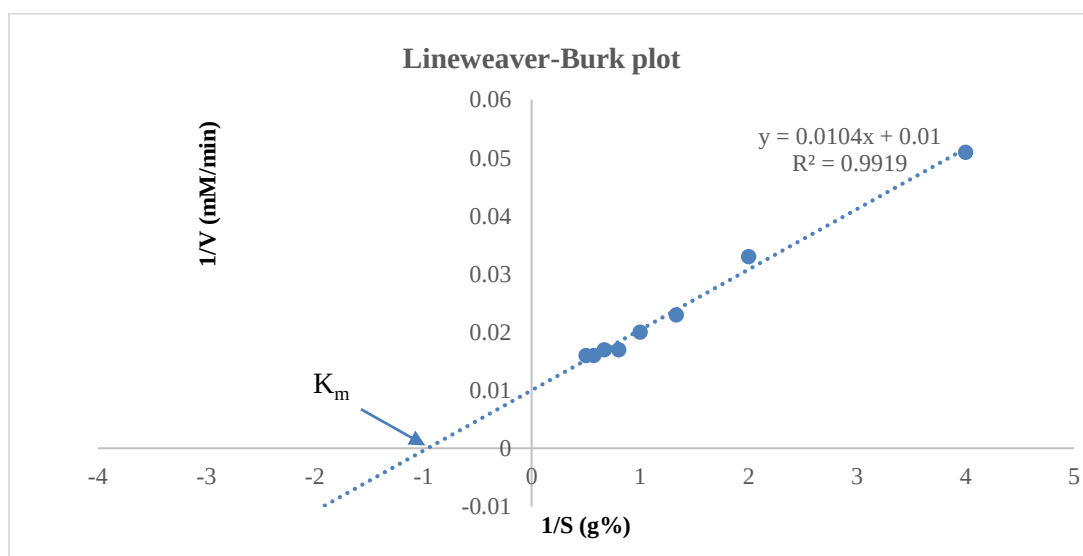


Figure 7. Kinetic plot based on the specific substrate for partially purified protease produced by *Penicillium* sp. (16) using the Lineweaver-Burk plot

Applications of the partially purified proteases

Blood stain removal activity

The washing effect of different concentrations of partially purified protease (1mL, 1.5mL, and 2mL) and cotton fabrics stained with blood was analyzed. After one hour of incubation, the blood stain cloth in 1mL of detergent solution plus 2mL of protease enzyme, for *Penicillium* sp. (2 and 16), was completely removed blood stain and *Penicillium* sp. (16) was removed blood stain by visual inspection. Only water, enzyme solution, and detergent solution were observed to have poor cleaning for protease, as shown in Table 3 and Figures 8 and 9.

Table 3. Blood stain removal activity

No.	Blood removal stain	Water	Water + protease	Water + detergent	Water + Detergent + enzyme (1mL)	Water + Detergent + enzyme (2mL)
1	<i>Penicillium</i> sp. (2)	+	++	++	+++	+++
2	<i>Penicillium</i> sp. (16)	+	++	++	+++	++++

+ = poor stain removal, ++ = medium stain removal, +++ = good stain removal, ++++ = very good stain removal



(A) Cotton pieces stained with blood (0.06mL) and dried



(B) Washed with distilled water (100mL)



(C) Washed with distilled water (99mL) + protease (1mL)



(D) Washed with distilled water (99mL) + detergent (1mL)



(E) Washed with distilled water (98mL) + detergent (1mL) + protease (1mL)



(F) Washed with distilled water (97.5mL) + detergent (1mL) + protease (1.5mL)



(G) Washed with distilled water (97mL) + detergent (1mL) + protease (2mL)

Figure 8. Result of blood stain removal activity from *Penicillium* sp. (2)

(A) Cotton pieces stained with blood (0.06mL) and dried



(C) Washed with distilled water (100mL)



(B) Washed with distilled water (99mL) + protease (1mL)



(D) Washed with distilled water (99mL) + detergent (1mL)



(E) Washed with distilled water (98mL) + detergent (1mL) + protease (1mL)



(F) Washed with distilled water (97.5mL) + detergent (1mL) + protease (1.5mL)



(G) Washed with distilled water (97mL) + detergent (1mL) + protease (2mL)

Figure 9. Result of blood stain removal activity from *Penicillium* sp. (16)**Milk clotting test**

In the determination of milk clotting enzyme, *Penicillium* sp. (2 and 16) were observed most active milk clotting enzyme within about 30minutes, as shown in Figure 10.

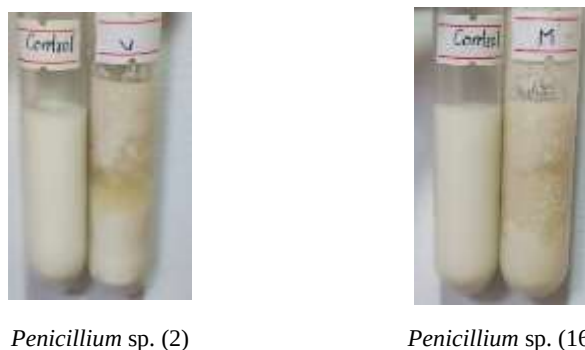


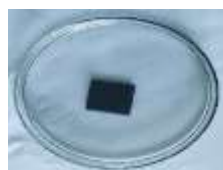
Figure 10. Milk clotting activity of the protease enzyme

Removal of gelatinous coating from waste X-ray film

In visual examination of the waste X-ray films, 1mL of partially purified enzymes of *Penicillium* sp. (2 and 16) was used at different time intervals to give efficient in-gelatin hydrolysis from waste X-ray film. As a result, the weight loss of the film was noticed after cleaning the waste X-ray film. Firstly, *Penicillium* sp. (2 and 16) were revealed to clear after 120minutes and 70 minutes, respectively, compared with the control, as shown in Table 4 and Figure 11.

Table 4. Recovery of time for gelatin hydrolysis and weight loss

No.	Strains	Duration of time at room temperature	Initial weight of X-ray sheet in grams (2 × 2) cm	Weight of X-ray film after treatment	Weight loss in grams
1	<i>Penicillium</i> sp. (2)	120 mins	0.1028	0.1005	0.0023
2	<i>Penicillium</i> sp. (16)	70 mins	0.1028	0.1005	0.0023



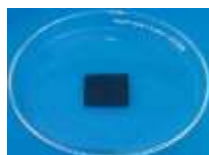
Control (10mL of phosphate buffer)



10mL of crude protease enzyme from *Penicillium* sp. (2)



10mL of crude protease enzyme from *Penicillium* sp. (16)



Control



Clearance of gelatin waste, X-ray film, *Penicillium* sp. (2)



Clearance of gelatin waste, X-ray film, *Penicillium* sp. (16)

Figure 11. Weight loss of the film during gelatin removal from used waste X-ray film

Discussion and Conclusion

The soil samples were collected from two different sites of bean crop soil from Hlegu Township and Myaungtagar Village. A total of 27 strains were screened on potato dextrose agar (added 1% casein) medium from bean crop soil. Among the 27 isolated fungi on skim milk agar medium, 13 fungi showed positive results of clear zone as protease activity, but the selected 2

fungi were found to be the high protease producers. These were subsequently identified to the genus level as two *Penicillium* sp. (V2 and M16) were observed based on their morphological characters and spore structure. The two selected proteolytic fungi, *Penicillium* sp. (2) and *Penicillium* sp. (16), were used for protease production via submerged fermentation (SmF). Casein was decomposed by the protease to produce degraded stages of protein products, which include tyrosine, among them Haldane (1965). In the present work, the collected clear filtrate (crude enzyme) was initially concentrated from 30% to 80% using ammonium sulfate. The crude enzyme formed from the use of 50% ammonium sulfate showed the best concentration with the highest activity. The molecular weight of partially purified proteases was revealed by SDS-PAGE when compared with standard molecular weight markers; the relative molecular weight of *Penicillium* sp. (16) was approximately $\approx < 40\text{kDa}$, and *Penicillium* sp. (2) was approximately 70kDa, as shown in Figure 5. This result is similar to the protease purified from *Penicillium janthinellum*, as reported 33kDa Abirami *et al.* (2011).

The specific activity of the partially purified protease increased from $20.98\mu\text{g mL}^{-1}\text{min}^{-1}$ to $45.27\mu\text{g mL}^{-1}\text{min}^{-1}$ for *Penicillium* sp. (2) and $16.08\mu\text{g mL}^{-1}\text{min}^{-1}$ to $32.97\mu\text{g mL}^{-1}\text{min}^{-1}$ for *Penicillium* sp. (16), while the protease was purified up to 13.05% for *Penicillium* sp. (2) and 23.14% for *Penicillium* sp. (16), respectively, as shown in Tables 1 and 2. Devi *et al.* (2022) reported that the 2.89-fold increase with 26.28% recovery by ammonium sulphate precipitation of protease from *Penicillium chrysogenum*.

Owing to its significant commercial application, it is important to investigate the kinetic characterization of the protease enzyme. The value of Michaelis-Menten constant (K_m) for *Penicillium* (2 and 16) was 1.04g%. The results of higher V_{max} revealed 100.00 mMmin^{-1} for *Penicillium* sp. (2) and 102.04 mMmin^{-1} for *Penicillium* sp. (16) as shown in Figures 6 and 7.

A good protease must be stable and active in detergents so that the efficiency of the enzyme can be achieved. So, in the exploitation of industrial applications, the partial purification of the protease was used to check the detergent compatibility. In the blood stain removal studies, *Penicillium* (2 and 16) obtained from a partially purified source effectively removed blood stains from cotton fabric pieces when added along with a detergent solution. Although *Penicillium* sp. (16) gives more satisfactory results than *Penicillium* sp. (2), as shown in Table 3 and Figures 8 and 9. Benlurvankar *et al.* (2016) reported that the usefulness of the protease from *Penicillium* sp. LCJ228 for removal of blood stains from cotton cloth in the presence and absence of detergents. In the presence of commercial detergents, its cleaning property indicates the possibilities to utilize these proteases in the detergent industry. The supplementation of the partially purified protease in detergent improved the cleaning of blood according to Choudhari (2012) and Suryawanshi and Pandya, (2017).

Variation in the production of milk-clotting enzyme by *Penicillium* sp. (2 and 16) was found 30 minutes, as shown in Figure 10. The filtrate possessed high general proteolytic activity because of peptonization of the curd formed. Aicha *et al.* (2012) reported that the *Penicillium* sp. obtained the best data in milk-clotting fungus enzymes. Partially purified enzyme of each protease was used at different time intervals to give efficient gelatin hydrolysis from waste X-ray film. Under the obtained condition, from protease enzyme *Penicillium* sp. (16) effectively hydrolyzed gelatin after 70 minutes from waste X-ray film. Although *Penicillium* sp. (2) revealed complete clearance of waste X-ray film after 120minutes, as shown in Table 4 and Figure 11. Abrusci *et al.* (2007) and Biswajit *et al.* (2021) reported that the gelatin layer was completely

removed, leaving the polyester film clean, and the silver was recovered in the hydrolysate. The usage of proteases has increased as detergent additives and several other applications, which include radioactive wastes like used X-ray film and the removal of gelatinous strips from film. The present isolation of radioactive wastes needs to be decomposed so that they produce less harm to living welfare (Chung *et al.*, 2019). The study of two selected enzymes can be thought of as a potential commercial good protease, and will also yield good results for future applications.

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QUALITATIVE AND QUANTATIVE PHYTOCHEMICAL ANALYSIS OF LEAVES OF *ARTOCARPUS LAMELLOSUS* BLANCO AND ITS ANTIMICROBIAL ACTIVITY

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Abstract

The plant *Artocarpus lamellosus* Blanco is an unrecorded species in Myanmar, belong to the family Moreaceae. It was collected from University of Yangon Campus, Kamayut Township. The aim of the present study on qualitative and quantitative phytochemical screening of the *Artocarpus lamellosus* Blanco and its microbial activity. In preliminary phytochemical analysis, the three different extracts such as HCL, ethanol and water extracts of leaves were found alkaloids, flavonoids, glycosides, phenolic compounds, saponins, starch, reducing sugars, carbohydrates, proteins, tannins and α -amino acid. In quantitative analysis, moisture content, acid-insoluble ash, water soluble ash. The extractive values with different solvents and ash values were also analyzed and recorded. The elemental analysis and the presence of the toxic elements of the leaves were analyzed by using Atomic Absorption Spectrophotometer (AAS). According to the result, it was revealed that moisture content (7.62%), total ash (6.24%), acid insoluble ash (75.06%) and water-soluble ash (9.73%). According to the extractive value result, the phytoconstituents of *Artocarpus lamellosus* Blanco were the highest soluble in petroleum ether whereas the least soluble in water or methanol. The nutritional value of the leaves was analyzed. Antimicrobial activities of *Artocarpus lamellosus* Blanco were investigated with seven extracts of different solvents from the powdered leaves on eight test organisms by agar well diffusion assay. Among them, antimicrobial activity on pathogenic microorganism, *Salmonella typhi* and the weakly antifungal activity was found on *Escherichia coli*.

Keyword: *Artocarpus lamellosus* Blanco (leaves) qualitative and Quantitative phytochemical analysis and antimicrobial activity

Introduction

The genus *Artocarpus* (Moraceae) consists of 50 species, mainly distributed over tropical regions of Asia. There are 15 species growing in southern China. Some *Artocarpus* members are known for their medicinal value. *Artocarpus* species have been exploited in various countries and revealed the medicinal variation possibility. As noted earlier, the most common ethnomedicinal usage was to treat wounds and ulcers as well as some skin problems. Extensive literature reported different pharmacological tests involved with the extracts and the isolated phytochemicals which exhibited anti-inflammatory, antioxidant, antimicrobial, gastroprotective, cytotoxic, anti-proliferative activities and acted as selective enzymes inhibitors. Different parts of the *Artocarpus* species are used in traditional folk medicinal practices. *Artocarpus* species as a source of essential nutrients and bioactive phytochemicals which categories it as a bifunctional food. This review compiles traditional, phytochemical and pharmacological data on different *Artocarpus* species that the plants possess pharmacological properties like, antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, Immunomodulatory, antifungal, hypoglycaemic effects, inhibits melanin biosynthesis. (Journal of Pharma Search Vol. 9 (1), 2014:7). The *Artocarpus* plants are proclaimed to be rich in phenolic compounds such as flavonoids, stilbenoids, and arylbenzofurans (Yen, *et al.*, 2017). The *Artocarpus* genus has provided diverse

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information to determine their phytochemical compounds and pharmacological activities. The relating bioactive compounds can potentially treat infection caused by microorganisms and diseases like diabetes, cancer, and malaria. *Artocarpus lamellosus* Blanco extract is as a pancreatic lipase inhibitor, and has antiobesity action, can be used for diseases such as prevention or treatment of obesity (Journal of Pharma Search Vol. 9 (1)2014:7).

The objectives of this paper are to investigate the chemical constituents of *Artocarpus lamellosus* Blanco (leaves), to examine its elemental analysis and nutritional value, and to study antimicrobial activity of the various crude extracts from the leaves of *Artocarpus lamellosus* Blanco.

Materials and Methods

Collection of the plant samples

Artocarpus lamellosus Blanco was collected from University of Yangon campus, Kamayut Township. The samples were washed, cut into small pieces and then air-dried at room temperature. When constant weight was obtained, the dried samples were pulverized by grinding machine and stored in an air tight container.

Qualitative analysis of *Artocarpus lamellosus* Blanco (Leaves)

In this investigation, the powdered sample of leaves from *Artocarpus lamellosus* Blanco was tested to find out the presence or absence of chemical constituents such as alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, reducing sugars, saponins, starch, steroids/terpenoids and tannins compounds. Qualitative and quantitative analysis were carried out in the Phytochemistry Laboratory of Botany Department, University of Yangon according to the methods of B.P. (1968); Marini Bettolo, *et al.*, (1981); Central Council for Research in Unani Medicine (1987); Trease and Evans (2002).

Test for Alkaloids

The powdered sample (3 g) was boiled with 1% HCL (50 ml) for about 20 minutes and filtered off. The filtrate was divided into two portions and test with modified Mayer's reagent, and Wagner's reagent. Treatment with the mentioned alkaloid reagents furnished alkaloid precipitates.

Test for α -amino acids

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. An aqueous portion was drop onto a filter paper, allowed to dry and sprayed with Ninhydrin reagent. The plate was allowed to dry at room temperature and then kept in an oven at 110 °C for a few minutes; purple colored spot appeared on the filter paper, indicating the presence of α -amino acids.

Test for Carbohydrates

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. The filtrate was added with (1 ml) of α -naphthol reagent solution and a few drops of concentrated sulphuric acid. A red or brick precipitate was formed which indicate the presence of carbohydrates.

Test for Flavonoids

The powdered sample (2 g) was boiled with 95% ethanol (25 ml) for about 20 minutes and filtered. The filtrate was introduced into a test tube added a small piece of magnesium and a few drops of concentrated hydrochloric acid. Boil the solution for few minutes. The presence of a pink or yellowish-brown color indicates the presence of flavonoids.

Test for Glycosides

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. The filtrate was treated with 10% lead acetate solution. A yellow color indicates the presence of glycosides.

Test for Phenolic Compounds

The powdered sample (2 g) was boiled with 1% HCL (25 ml) for about 20 minutes and filtered. The filtrate was added with 10% ferric chloride solution. Observation was made to see if dark green color appeared.

Test for Proteins

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered. The filtrate was added (1 ml) of distilled water and 5 drops of Millon's reagent. A white precipitate is formed which turns red on heating which indicate the presence of proteins.

Test for Reducing Sugars

The powdered sample (2 g) was boiled with 95% water (25 ml) 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 was formed which indicate the presence of reducing sugars.

Test for Saponins

The powdered sample (2 g) was put into a test and some distilled water was added. Then the mixture was vigorously shaken for a few minutes. Observation was made to see if frothing took place.

Test for Starch

The powdered sample (2 g) was boiled with 95% water (25 ml) for about 20 minutes and filtered, 2 drops of iodine solution were added to filtrate. Yellowish brown precipitate which indicates the presence of starch.

Test for Steroids/ Terpenoids

The powdered sample (2 g) was boiled with 95% ethanol (25 ml) for about 20 minutes and filtered. The filtrate was added (2 ml) of chloroform and (1 ml) of concentrated sulphuric acid, formation of reddish-brown color indicates the presence of steroids.

Test for Tannins

The powdered sample (2 g) was boiled with 95% water (25) ml for about 20 minutes and filtered. The filtrate was added a few drops of 5% ferric chloride solution. Black color precipitate is produced indicating the presence of tannins.

Quantitative analysis of *Artocarpus lamellosus* Blanco (Leaves)

Physicochemical investigation was determined according to British Pharmacopoeia (1965).

1. Moisture content
2. Total ash content
3. Acid insoluble ash content
4. Water soluble ash content
5. Aqueous soluble matter content
6. Soluble matter content in different solvents petroleum ether, acetone, methanol, ethanol and water.

Determination of moisture content

The moisture content of the powdered sample of the leaves was determined on an oven. The powdered samples 5 g were weighed accurately, placed in a weighed a clean dry beaker and kept in an oven at 110° C for one hour. The beaker was then removed from the oven and cooled in a desiccator at room temperature and weighed. This procedure was repeated until a constant weight of the sample was obtained.

The percentage of the moisture content was calculated by the following formula,

$$\% \text{ of total moisture} = \frac{M-m}{W} \times 100$$

M = weight of sample of weighting beaker in g before in g before drying

m = weight of sample of weighting beaker in g before in g after drying

W = weight of taken in g

Determination for Weight of Total Ash

The powered sample 10.0 g was weighted in a crucible and placed in a muffle-furnace at 550 °C until substance turned into ash. After that, the crucible was cooled and weighted. This procedure was repeated until a constant weight was obtained and the percentage of total ash was calculated.

$$\% \text{ of total ash} = \frac{M-m}{W} \times 100$$

Determination of Acid-Insoluble Ash

For the acid insoluble ash determination, the above ash was boiled for 5 minutes in 10 ml of 6% HCl. The insoluble ash was collected by filtration with filter paper. The insoluble ash was washed with water until free of acid. The acid insoluble residue together with the crucible were dried in an oven at 110 °C and weighed again. The percentage of insoluble ash was calculated.

Determination of Water- Soluble Ash

The total ash was boiled with 10ml of water for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with water and ignited to a constant weight. This

weight or insoluble matter was substrated from the weight of the total ash, to give weight of the water-soluble ash.

Determination of Soluble Matters

The powdered rhizomes 5.0 g was weighed and placed in a conical flask. It was soaked with 50 ml of water in a flask for 72 hours and overnight. Then it was filtered and the filtrate was evaporated to dryness in a previous weighed container was weighed. This procedure was repeated until a constant weight was obtained.

Antimicrobial Activities

The powered leaves were extracted with petroleum ether, chloroform, acetone, ethyl acetate, ethanol, methanol, and distilled water. The various solvent extracts were tested on eight pathogen microorganisms. Antimicrobial activities were conducted by using agar well method (Collins, 1965) at the Microbiology Laboratory of Botany Department, University of Yangon.

Test agar plates

Test organisms were *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. Each test organism (50 μ l) was added to 100 ml of assay (SM: sucrose 1.0g, malt extract 0.3 g, NaCl 0.05 g, CaCO₃, 0.01 g, agar 1.8 g, distilled water 100 ml, pH 7 poured into plates.

Agar well diffusion assay

The agar plates containing test organisms were punched to make the wells (8 mm in diameter) using sterile cork borer and filled with the stock solution (0.2 ml) and then these plates were incubated at room temperature for 24 hrs. After incubation, the diameters of the growth inhibition zones surrounding the wells were measured in mm. These zones indicate the presence of antimicrobial activities which inhibit the growth of test organisms selectively (Collins, 1965).

Table 1. Test organisms and diseases

Test organisms	Diseases
<i>Agrobacterium tumefaciens</i>	Plant tumor cell
<i>Bacillus subtilis</i>	Food spoilage and fever
<i>Candida albicans</i>	Vaginal 207andidiasis, alimentary tract infection, skin infection, sinus irritation, intense itching and sores
<i>Escherichia coli</i>	Diarrhoea and vomiting dysentery and urinary tract infection
<i>Malassezia furfur</i>	Dandruff and skin infection
<i>Pseudomonas aeruginosa</i>	Chronic lung, urinary tract infection, inflammation in bone and joints, gastrointestinal and blood infections
<i>Salmonella typhi</i>	Typhoid
<i>Staphylococcus aureus</i>	Skin diseases, wound, burns, staphylococcal pneumonia, toxic shock syndrome, bacteraemia, meningitis, osteomyelitis, endocarditis and sepsis

Results

Qualitative Analysis

In this experiment alkaloids, glycosides, phenolic compounds, steroids/ terpenoids, flavonoids, saponin, starch, reducing sugars, tannins, carbohydrates, proteins and α -amino acids were observed in the leaves. The results were shown in Table 2.

Table 2. Qualitative analysis of *Artocarpus lamellosus* Blanco

Sr.No.	Test	Extract	Test reagent	Observation	Results
1	Alkaloids	1%HCL	Mayer's reagent Wagner's reagent	orange color Reddish brown color	-
2	Glycosides	water	10%lead acetate solution	brown ppt	+
3	Phenolic compounds	1%HCL	3% Ferric chloride solution	Reddish brown ppt	+
4	Steroids/Terpenoids	EtOH	CHCL ₃ + conc:H ₂ SO ₄	Reddish brown color	+
5	Flavonoids	EtOH	Conc:HCL/Mg	Yellowish brown ppt	+
6	Saponin	Water	Distilled water	Frothing	+
7	Starch	Water	Iodine solution	Brown ppt	+
8	Reducing sugars	Water	Benedict's solution	Yellowish brown ppt	+
9	Tannins	Water	5% ferric chloride solution	Black ppt	+
10	Carbohydrates	Water	10% α -naphthol & conc:H ₂ SO ₄	Red ppt	+
11	Proteins	Water	Millon's reagent	Red ppt	+
12	α -amino acid	Water	Ninhydrin reagent	purple spot appear	+

(+) = present

(-) = absent

ppt = precipitate



Figure 1. Preliminary Phytochemical Test of *Artocarpus lamrellosus* Blanco Powdered leaves

- A. DW control, 1% HCL control and ethanol control,
- B. Test for Alkaloids and Phenolic compounds,
- C. Test for Steroid and Flavonoids and
- D. Test for Glycoside, Saponin, Starch, Reducing sugar, Tannins, Carbohydrate, Proteins and α -amino acids

Quantitative Analysis

In quantitative analysis, the solubility of the powdered leaves was the best in petroleum ether and acetone, and was moderately soluble in ethanol, and methanol. That of the powdered samples was the least in water as shown in Table 3.

Table 3. Quantitative analysis of *Artocarpus lamellosus* Blanco

Sr.No.	Physio-chemical characters	Quantity determined in %
		Rhizomes
1.	Moisture content	7.62
2.	Total ash	6.24
3.	Acid-insoluble ash	75.06
4.	Water-soluble ash	9.73
5.	Petroleum ether soluble matter	76.30
6.	Ethanol soluble matter	69.90
7.	Methanol soluble matter	64.31
8.	Acetone matter	72.58
9.	Water soluble matter	46.79

Elemental Analysis of *Artocarpus lamellosus* Blanco leaves by using Energy Dispersive X-ray Fluorescence (EDXRF) Spectrophotometer

The results of elemental analysis of *Artocarpus lamellosus* Blanco leaves revealed that Calcium (Ca) and Potassium (K) were contained in highest amount whereas Manganese (Mn), Iron (Fe) and Strontium (Sr) were present in moderate amount. Silver (Ag) and Zinc (Zn) were found in trace amount. The results were shown in Table 4

Table 4. The relative concentration of elements in the sample powder of *Artocarpus lamellosus* Blanco

Sr.No.	Element	Concentration
1.	Calcium (Ca)	67.994 %
2.	Potassium (K)	15.605 %
3.	Manganese (Mn)	9.228 %
4.	Iron (Fe)	4.695 %
5.	Strontium (Sr)	1.105 %
6.	Silver (Ag)	0.865 %
7.	Zinc (Zn)	0.508 %

Toxic elements Analysis of *Artocarpus lamellosus* Blanco leaves by using Atomic Absorption Spectrophotometer (AAS)

According to the results, Chromium (Cr), Cadmium (Cd), Arsenic (As) and Lead (Pb) were found in small amount. The results were shown in Table 5.

Table 5. Toxic elements analyzed by Atomic Absorption Spectrophotometer (AAS)

Sr. No	Test Parameter	Result	Unit	Detection Limit
1	Arsenic (As)	0.0031	mg/L	0.08 ppb
2	Cadmium (Cd)	0.023	mg/L	0.009 ppb
3	Chromium (Cr)	0.058	mg/L	0.0011 ppm
4	Lead (Pb)	0.357	mg/L	0.0063 ppb

mg/L = milligram per liter*

Nutritional Value of leaves from *Artocarpus lamellosus* Blanco

In nutritional value, protein, fiber, fat and carbohydrate were observed in the leaves. According to the experiments, the amount of fat and carbohydrate were greater than protein and fiber. The results were shown in Table 6.

Table 6. Present chemical analysis resulted by Nutritional Value

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	6.41
2	Fiber (%)	2.54
3	Fat (%)	11.9
4	Carbohydrate (%)	10.4

Antimicrobial Activities of *Artocarpus lamellosus* Blanco (leaves)

In this experiment, the petroleum ether, chloroform and acetone extracts showed no activity and the ethyl acetate, methanol, ethanol and aqueous extracts indicated high activity on *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The petroleum ether, chloroform and acetone extracts showed no activity and the ethyl acetate extract showed moderate activity and the methanol, ethanol and aqueous extracts *Candida albicans*. The petroleum ether and chloroform extracts showed no activity and the acetone extract exhibited weak activity on *Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus*. The ethyl acetate, methanol, ethanol and aqueous extracts indicated high activity on *Malassezia furfur* and *Salmonella typhi*. The ethyl acetate and methanol extracts indicated moderate activity whereas the ethanol and aqueous extracts showed high activity on *Staphylococcus aureus*. The petroleum ether, chloroform extracts of *Artocarpus lamellosus* Blanco showed no activity, but the ethanol and aqueous extracts showed high activity on eight pathogenic organisms. The acetone extract exhibited weak activity on *Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus*. The results of antimicrobial activities were shown in Table 7 and Figures 4-11.

Table 7. Antimicrobial activities of various solvents extract for the leaves of *Artocarpus lamellosus* Blanco

Test Organisms	Solvents						
	Petether	Chloroform	Acetone	Ethyl Acetate	Ethanol	Methanol	Water
<i>Agrobacterium tumefaciens</i>	-	-	-	18	26	22	20
<i>Bacillus subtilis</i>	-	-	-	20	24	20	20
<i>Candida albicans</i>	-	-	-	16	22	18	22
<i>Escherichia coli</i>	-	-	-	18	20	20	20
<i>Malassezia furfur</i>	-	-	10	18	22	22	22
<i>Pseudomonas aeruginosa</i>	-	-	-	20	22	18	22
<i>Salmonella typhi</i>	-	-	10	20	26	24	24
<i>Staphylococcus aureus</i>	-	-	10	14	20	16	22

Agar well size = 8 mm, - = no activity, 10 – 12 mm weak activity, 13 – 17 mm = moderate activity, > 18 mm = high activity

Antimicrobial activities of various extracts from the leaves of *Artocarpus lamellosus* Blanco

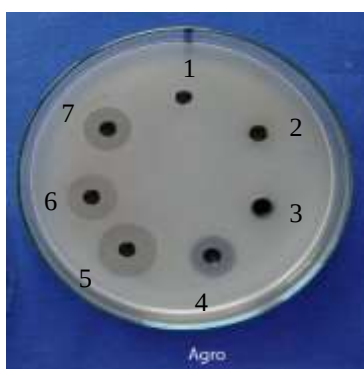


Figure. 4. Inhibitory zones against *Agrobacterium tumefaciens*

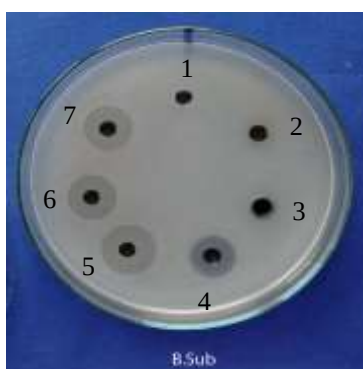


Figure. 5. Inhibitory zones against *Bacillus subtilis*

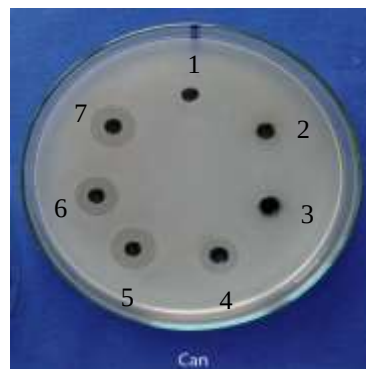


Figure. 6. Inhibitory zones against *Candida albicans*

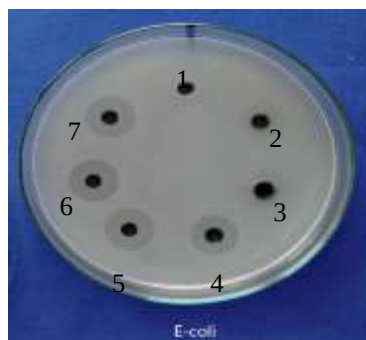


Figure.7 Inhibitory zones against *Escherichia coli*

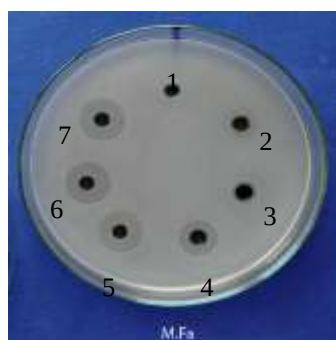


Figure. 8. Inhibitory zones against *Malassezia furfur*



Figure.9. Inhibitory zones against *Pseudomonas aeruginosa*

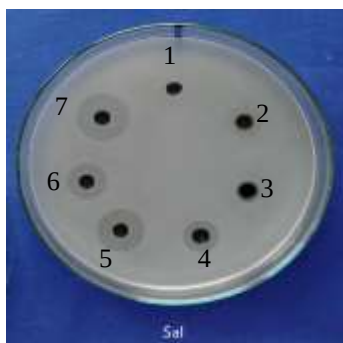


Figure. 10. Inhibitory zones against *Salmonella typhi*



Figure 11. Inhibitory zones against *Staphylococcus aureus*

- | | | |
|---------------------|------------------------|----------------------|
| 1 = Petroleum | 2 = Chloroform extract | 3 = Acetone extract |
| 4 = Ethyl acetate | 5 = Ethanol extract | 6 = Methanol extract |
| 7 = Aqueous extract | | |

Discussion and Conclusion

In this research, the qualitative and quantitative phytochemical analysis of *Artocarpus lamellosus* Blanco had been conducted. In the preliminary phytochemical study, it was revealed that the presence of twelve different physicochemicals in three different extracts. According to the results of phytochemical investigation, it indicated that the alkaloids, α -amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, saponins, starch, steroids/terpenoids, tannins and reducing sugars are presented in the leaves. *Artocarpus* species are known to produce phenolic compounds and flavonoids.

In quantitative analysis, five different solvents were used. Powdered leaves were mostly soluble in petroleum ether (76.30%) and acetone (72.58%) while they were moderate soluble in ethanol (69.90%) and methanol (64.31%), and was the least in water (46.79%). In the elemental analysis, AAS data, potassium (K), phosphorus (P), calcium (Ca) and sulphur (S) are found as macronutrient elements whereas manganese (Mn), iron (Fe), titanium (Ti), silver (Ag), Zinc (Zn) and rubidium (Rb) elements. According to this study, potassium is found to be the highest concentration in the leaves of this plant and lead is also highest concentration among heavy metals. These characters are agreed with those of WHO, 1998.

Potassium is very important mineral for the proper function of all cells, tissues, and organs in the human body. It helps regulate fluid balance, muscle contractions and nerve signals. A high-potassium diet may help reduce blood pressure and water retention, protect against stroke and prevent kidney stones. Calcium is one of the most important minerals for the human body. It helps form and maintain healthy teeth, bones, and it has key metabolic functions. A proper level of calcium in the body over a life time can help prevent osteoporosis. These minerals help our body grow, develop, and stay healthy. It is essential that the balance diet should include a wide variety of foods. Some minerals are even used to make hormones or maintain a normal heartbeat. Potassium and calcium are found in *Artocarpus lamellosus* Blanco leaves. So, it may be considered to be useful for medicinal purposes. These characters are agreed with those described by WHO, 1998.

The toxic elements such as arsenic, chromium, cadmium and lead were found as trace elements in toxic levels. Lead (Pb) concentration present in the leaves of *Artocarpus lamellosus* Blanco is 0.357mg/kg. Lethal dose of inorganic lead is 70kg (155 pound) in adult, a toxic dose is 450mg or 0.45g for 20 pound child, this works out to 160 mg or 0.16 g. The poisonous level of (Pb) for human is 60mg/kg. These constituents were not harmful on human health. Thus it could be used as traditional medicine. These characters are in agreement with those of Hartley *et al.*, 1980.

Nutritional value is important. Nutritional value relates to carbohydrates, fats, proteins, minerals, additives, enzymes, vitamins, sugar intake, cholesterol, fat and salt intake. A healthy diet throughout life promotes healthy pregnancy outcomes, supports normal growth, development, ageing, helps to maintain a healthy body weight, and reduces the risk of chronic disease to overall health and well-being. These characters are agreed with those of Magd. Zakaria, *et al.*, 2020.

In test of antimicrobial activities, the different solvent extracts of leaves inhibited *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. According to the agar well diffusion method, the ethanol and aqueous extracts from the leaves showed highly antimicrobial activities on eight pathogenic organisms. The methanol extract indicated moderate activity on *Staphylococcus aureus* and high antimicrobial activities on seven pathogenic organisms. The petroleum ether and chloroform extracts of leaves did not show antimicrobial activities on eight pathogenic organisms. These characters are in agreement with those of Cruickshank, R.J.P. (1975).

In conclusion, qualitative and quantitative analysis, elemental analysis, nutritional values and antimicrobial activities of *Artocarpus lamellosus* Blanco (leaves) were conducted. These findings are crucial to providing immense opportunities for the development of new *Artocarpus lamellosus*-based products in pharmaceutical industries, cosmetics and toxicity. The ethanol, methanol and aqueous extracts possessed higher antimicrobial activities than other solvent extracts in this study. Thus, the methanol extract or the ethanol extract from the leaves of *Artocarpus lamellosus* Blanco will be used to produce the bioactive compounds from the leaves.

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BOTANICAL AND PHYTOCHEMICAL STUDIES, NUTRITIONAL VALUE AND ANTIMICROBIAL PROPERTIES OF LEAVES OF *OPERCULINA TURPETHUM* (L.) SILVA. IN DAGON UNIVERSITY

Yin Mon Aye¹, Yee Yee Nwe², Aye Chan Myat³

Abstract

Operculina turpethum (L.) Silva. (Kyar-hin) belongs to the family Convolvulaceae. In this research work, the specimens were collected from Dagon University Campus, East Dagon Township, Yangon Region, during the flowering and fruiting periods from February to July, 2023. In this paper, the morphological characters of plant and microscopical characters of leaves were studied at the Department of Botany, Dagon University by using available literatures. In morphological study, the plant was vines, stem with 3-5 wings; leaves were ovate, pubescent; flower white; sepal persistent, carpel-2, axile placentation. The microscopical characters of leaves were conducted by free hand section method. In microscopical study paracytic type of stomata was found in both surface and non-glandular trichomes were present. The preliminary phytochemical test of powdered leaves was examined in Dagon University. Alkaloid, carbohydrate, glycoside, phenolic compound, reducing sugar, flavonoid, saponin and tannin were present. The nutritional value was examined by David Pearson and Kjeldahl method and antioxidant activity was also examined by DPPH Assay Method at the Small Scale Industry Departments in North Okkalapa Township. This powder was found to contain carbohydrates, fiber, fat, proteins, ash and moisture, and energy value 320.73 kcal. Elemental analysis of the powder was found the elements, such as Ca, K, Fe, Cu and Sr. The antimicrobial activities of three extracts of sample showed against on *Agrobacterium* spp., among them Butanol extract is most affected to the test organisms.

Keywords: *Operculina turpethum* (L.) Silva, morphological, anatomical, phytochemical, nutritional value, antioxidant activity, EDXRF and antimicrobial activity

Introduction

The plant kingdom represents an enormous storage of biologically active compounds with various chemical structures and disease preventive properties. Discovery of novel drugs depending on the pharmacological as well as pathological properties, the critical information regarding the chemical constituents is generally provided by the qualitative phytochemical screening of plant extracts (Karmakar, 2021).

Medicinal plants may provide three main kinds of benefit: health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society-wide benefits, such as job opportunities, taxation income, and a healthier labour force (Mahady, 2001).

Operculina turpethum (L.) Silva. has several medicinal purposes. Root bark and seed of this plant are used in the treatment of skin disorders such as cervical lymphadenitis, fistulas, constipation, chronic gout, fever, bronchitis, ulcers, hemorrhoids, tumors, obesity, jaundice herpes, and induced lachrimation. The use of root powder for the treatment of rheumatism, flatulence, paralysis, scorpion sting, and snake bite was also proven (Kirtikar and Basu, 1987). Leaves are reported to possess antibacterial activities and there are reports about the use of fresh juice of leaves for the treatment of corneal opacity and conjunctivitis (Sharma, 2012).

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The aim of the research is to know the pharmacological uses of *Operculina turpethum* (L.) Silva. of leaves, to verify and confirm the morphological characters of vegetative and reproductive parts, to examine the microscopical characters of leaves, to investigate some activities of this plant phytochemical studies, nutritional value, antioxidant activity, EDXRF and antimicrobial activity.

Materials and Methods

Collection and preparation of specimens

The specimens of *Operculina turpethum* (L.) Silva. were collected from Dagon University Campus, East Dagon Township, Yangon Region, during the flowering and fruiting periods from February to June 2023.

After collection, the samples of *Operculina turpethum* (L.) Silva. Plants were thoroughly washed with water and dried at room temperature about three weeks. When constant weight of the sample was obtained, the dry samples were pulverized by grinding machine into fine powder and kept in air tight bottle to study the phytochemical, nutritional value, antioxidant, EDXRF and antimicrobial activities.

Morphological identification

For morphological studies, *Operculina turpethum* (L.) Silva. Plants of all the vegetative and reproductive parts of the fresh specimens were used. These specimens were identified by using the available literatures. Myanmar name was confirmed by Hundley and Chit Ko Ko (1987) and Kress *et al.* (2002).

Microscopical studies

For microscopical studies, the samples of *Operculina turpethum* (L.) Silva. were thoroughly washed with water. The petiole, lamina, and midrib were cut by free hand section and to examine under electron microscope (Bio Blue Lab.) at the Department of Botany, Dagon University. For microscopical studies, the fresh specimens were studied according to the methods of Metcalfe and Chalk (1979), Esau (2006), Pandey (2008) and Tandon Neeraj (2011).

Phytochemical Studies of powdered leaves of *Operculina turpethum* (L.) Silva.

The preliminary phytochemical studies on the powder leaves of *Operculina turpethum* (L.) Silva. had been undertaken. The experiment was carried out to determine the presence or absence of chemical compounds, according to the method of Marini Bettalo *et al.*, (1981) and Central Council of Research in Unani Medicine (1987) at Department of Botany, Dagon University.

Nutritional values

The nutrient contents in powdered leaves of *Operculina turpethum* (L.) Silva. were analyzed by using David Pearson (1976) and protein Kjeldahl (1883) method at the Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigation, North Okkalapa Township, Yangon Region.

Antioxidant activity

Antioxidant activity of leaves of *Operculina turpethum* (L.) Silva. by DPPH Assay Method at the Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigation, North Okkalapa Township, Yangon Region.

Elemental Analysis (EDXRF)

Elemental Composition of powder leaves of *Operculina turpethum* (L.) Silva was carried by EDX-720 at University Research Centre (URC) in Yangon University.

Antimicrobial Activities

Antimicrobial Activity test of *Operculina turpethum* (L.) Silva. leaves were carried out in the Department of Botany, Dagon University. The study of antimicrobial activity was performed by paper-disc diffusion methods according to the method of Atlas (1993) by using eight test organisms. These test organisms were provided by Department of Botany, Dagon University.

Results

Morphological Characters of *Operculina turpethum* (L.) Silva

Family	-	Convolvulaceae
Scientific name	-	<i>Operculina turpethum</i> (L.) Silva
English name	-	Indian jalap
Myanmar name	-	Kya-hin
Part used	-	Leaves

Veins, stems hollow, reddish-green, 3-5 winged; latex present. Leaves alternate, simple, petiole cylindrical, puberulent; the lamina broadly ovate, the base truncate, the margins entire, the tips mucronate to acute, pubescent on both surfaces; exstipulate. Inflorescences axillary cymes, 1-2 flowered. Flower white, bracteate, bract oblong, concave, pubescent; ebracteolate; pedicellate, puberulent; complete, bisexual, actinomorphic, pentamerous, hypogynous. Sepals 2+3, 2 outer sepals ovate, inner 3 sepals, elliptic, persistent. Petals (5), broadly ovate, funnel-shaped, twisted, puberulent. Stamens 5, epipetalous; the filament white, equal, hairs at the base; the anther dithecal, basifixed. Carpel (2), ovary superior, false septum present, axile placentation; the style slender; stigma bilobed, capitate; annular disc present. Fruit capsule, enclosed with enlarged sepals, persistent depressed, globose, pubescent; the seeds white in young, black-brown in mature (Figure 1).

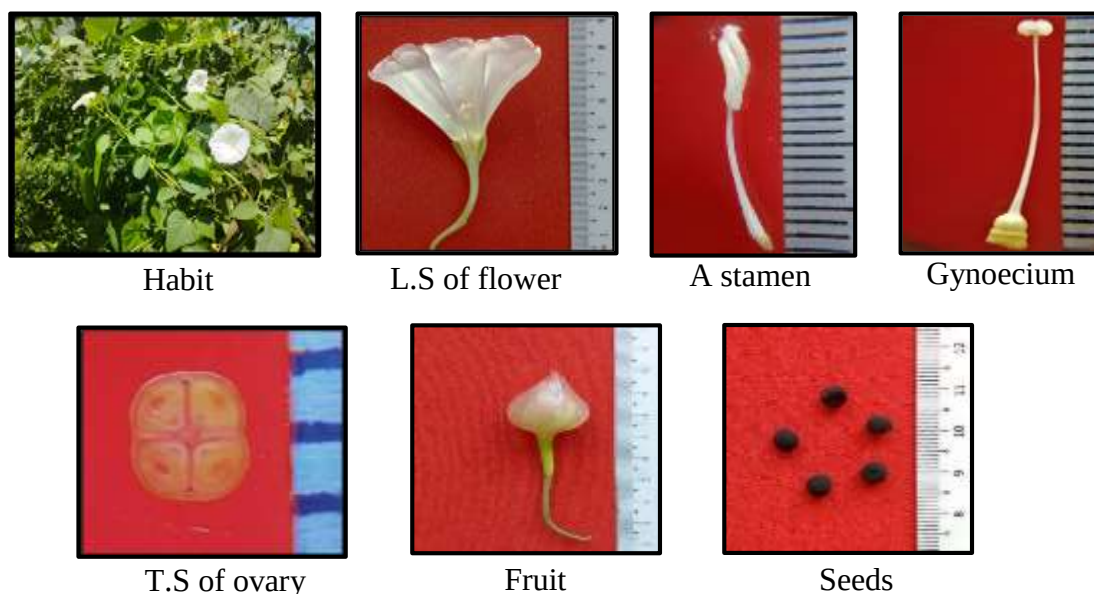


Figure 1. Morphological Study of *Operculina turpethum* (L.) Silva.

Microscopical Characters of leaves of *Operculina turpethum* (L.) Silva

Microscopical Characters of Leaves of *Operculina turpethum* (L.) was shown in Figure 2.

Lamina

In surface view, the upper and lower epidermal cells were parenchymatous, barrel shaped, thin-walled and completely arranged. Stomata were abundantly present on lower surface than upper. On both surfaces view of lamina found paracytic types of stomata. Non-glandular trichomes were present on both surfaces.

In transverse section, the cuticle layer was thin present on both surfaces. Epidermal cells were made up of one layer parenchymatous cells and oval to barrel shaped. The mesophyll layer composed of palisade and spongy parenchyma. Palisade parenchyma cells are found on upper side and two-layered. The spongy mesophyll consisted of 2-3 layers of cells and loosely arranged. The vascular bundles of lateral vein were embedded in mesophyll cells and oval in shape. They were bicollateral and open type.

The midrib

In transverse section of midrib, both surfaces were covered with thin cuticle. Uniseriate and multiseriate non glandular trichomes were present. The epidermal cells were only one layered, oval to barrel shaped and compactly arranged. The lower epidermal cells were similar to these the upper cells. Below the epidermis the cortex was differentiated into collenchyma. The collenchymatous cells below the epidermis consisted of 3-5 layers on the upper surface and 2-4 layers on the lower surface and oval or angular shape. The parenchymatous tissue composed of 5-8 layers in thickness below the vascular bundle and polygonal or rounded shapes. A few containing calcium oxalate crystals. The vascular bundles were oval in outline and embedded in the cortex. The vascular bundles were bicollateral and opened type.

Petiole

In transverse section, the petiole was basically semi-circular in outline and slightly concave in the upper side. The cuticle layer was thin. Non-glandular trichomes were present. The cortex was made up of two different types of tissues, outer collenchyma and inner parenchymatous tissues. The outer collenchymatous cells below the epidermis consisted of 3-5 layers in thickness on both surfaces. Vascular bundles embedded in ground tissue, arc shaped in outline. Vascular bundle was bicollateral and opened type and crescent shaped in outline. The phloem cells were consisted sieved tube elements and companion cells. Xylem cells were composed of vessel, tracheids, xylem fibers and xylem parenchyma.



Surface view of upper epidermis



Surface view of lower epidermis



T.S of lamina

Figure 2.a Microscopical Characters of Leaves of *Operculina turpethum* (L.) Silva.

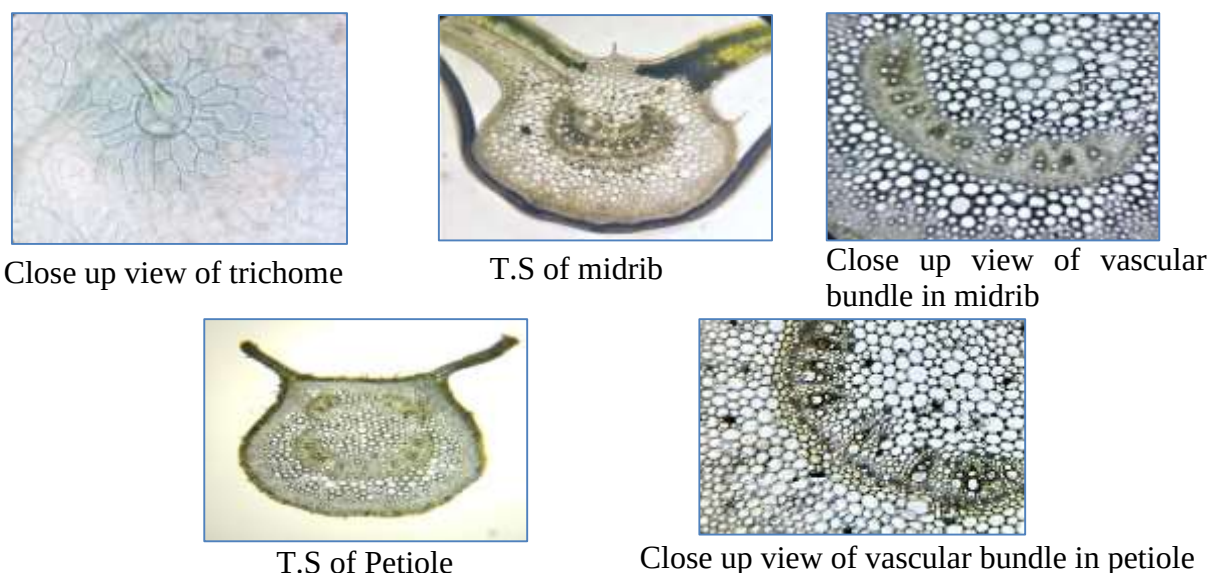


Figure 2.b Microscopical Characters of Leaves of *Operculina turpethum* (L.) Silva.

Chemical Studies

Preliminary phytochemical tests of *Operculina turpethum* (L.) Silva.

The preliminary phytochemical tests of the leaves of *Operculina turpethum* (L.) Silva. indicated the presence of alkaloid, carbohydrates, glycosides, phenolic compounds, reducing sugar, flavonoid, saponins and tannin show in Table 1, Figure 3 and 4.

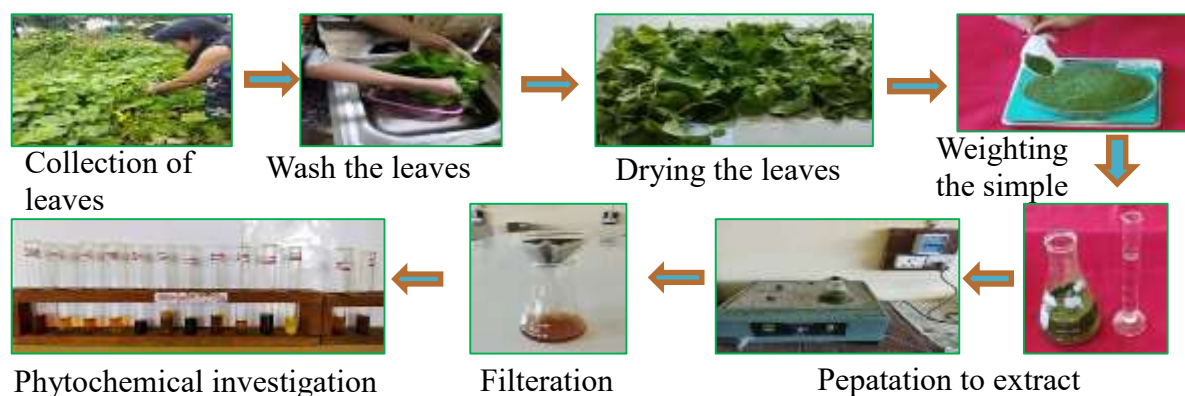


Figure. 3 Preliminary phytochemical tests of *Operculina turpethum* (L.) Silva.

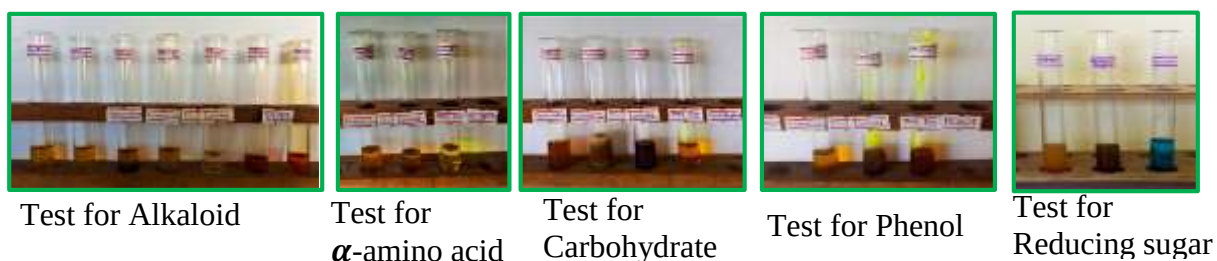


Figure 4.a Result of preliminary phytochemical tests of *Operculina turpethum* (L.) Silva.

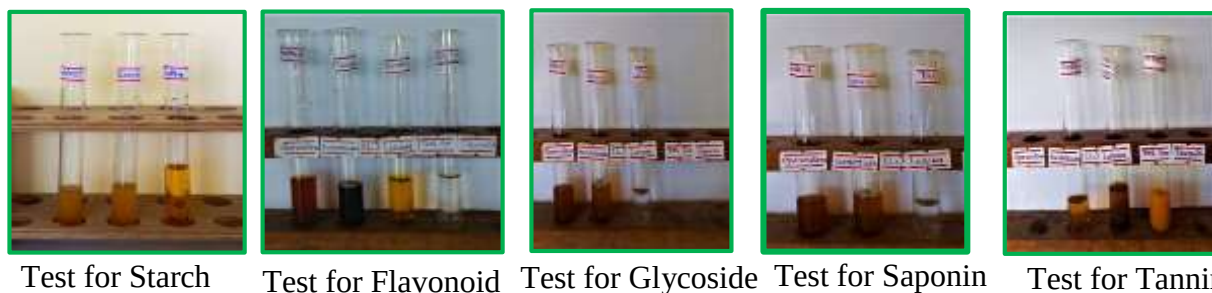


Figure 4.b Result of preliminary phytochemical tests of *Operculina turpethum* (L.) Silva.

Table 1. Preliminary phytochemical test of powdered leaves of *Operculina turpethum* (L.) Silva.

No.	Test	Extract	Test reagents	Observation	Result
1.	Alkaloid	DW	1.Mayer's reagent 2.Dragendroff's reagent 3.Wagner's reagent	No ppt. Orange ppt. Brown ppt.	- + +
2.	α -amino acid	DW	Ninhydrin reagent	No ppt.	-
3.	Carbohydrate	DW	10% α -naphthol+ Conc:H ₂ SO ₄	Creamy color	+
4.	Phenolic compound	DW	10% FeCl ₃ solution	Light brown ppt.	+
5.	Reducing sugar	DW	Benedict's solution	Brick red ppt.	+
6.	Starch	DW	Iodine Solution	Blue Brown	-
7.	Flavonoid	DW	Conc: HCl, Mg burning 10% lead acetate	Deep brown	+
8.	Glycoside	DW	10% lead acetate	White ppt.	+
9.	Saponin	DW	Distilled water	Frothing	+
10.	Tannin	DW	1%FeCl ₃	Yellowish brown ppt.	+

(+) = present (-) = absent ppt. = precipitation DW= distilled water

Nutritional values

According the result, protein, moisture, ash, fat, fiber and carbohydrate, and energy value 320.73 kcal found in leaves of *Operculina turpethum* (L.) Silva. The results were shown in Table 2.

Table 2. Determination of nutritional values from Powdered leaves of *Operculina turpethum* (L.) Silva

No.	Experiment	Percentage(%)
1	Moisture (%)	10.03
2	Ash (%)	6.89
3	Proteins (%)	13.33
4	Fat (%)	3.41
5	Fiber (%)	7.16
6	Carbohydrate (%)	59.18

Antioxidants activity

Antioxidant activity of leaves of *Operculina turpethum* (L.) Silva. by DPPH Assay Method. In this study, ascorbic acid was used as a standard, and different concentrations of extracts were used. According to the result ethanol extract of *Operculina turpethum* (L.) Silva was lowest IC₅₀ value than watery extract in Table 3.

Table 3. Determination of antioxidant activity from Powdered leaves of *Operculina turpethum* (L.) Silva

Extract	% Inhibition (mean $\pm$ SD) In Different Concentration (μ g /ml)						IC ₅₀
	50	100	200	400	600	800	
Water	32.72 $\pm$ 0.08	35.65 $\pm$ 0.08	50.24 $\pm$ 0.06	82.29 $\pm$ 0.04	93.89 $\pm$ 0.04	94.13 $\pm$ 0.04	198.34 $\pm$ 0.02
	25	50	100	200	400	600	
Ethanol	9.94 $\pm$ 0.16	20.35 $\pm$ 0.14	40.14 $\pm$ 0.10	53.55 $\pm$ 0.08	93.26 $\pm$ 0.07	94.82 $\pm$ 0.12	173.48 $\pm$ 0.04
	0.39	0.78	1.561	3.125	6.25	12.5	
Ascorbic	25.49 $\pm$ 3.6	38.07 $\pm$ 2.45	63.91 $\pm$ 0.06	86.33 $\pm$ 1.16	91.63 $\pm$ 0.33	90.17 $\pm$ 0.5	1.14 $\pm$ 1.39

Elemental Composition

Elemental Composition of powder leaves of *Operculina turpethum* (L.) Silva was carried by EDX-720 at University Research Centre (URC) in Yangon University. In this study the powdered leaves of *Operculina turpethum* (L.) Silva contain Calcium (Ca), Potassium (K), Iron (Fe), Copper (Cu) and Strontium (Sr), among them Ca is the highest content shown in Table 4.

Table 4. Elemental analysis of Powdered leaves of *Operculina turpethum* (L.) Silva

No.	Elements	Percentage %
1	Calcium(Ca)	61.77%
2	Potassium(K)	25.93%
3	Iron(Fe)	9.52%
4	Copper(Cu)	1.72%
5	Strontium (Sr)	1.07%

Antimicrobial activities

Antimicrobial activities of various crude extracts from leaves of *Operculina turpethum* (L.) Silva. by using paper disc diffusion method

In this study, the presence of antimicrobial potential in different solvent extracts of *Operculina turpethum* (L.) Silva. using eight strains of test organisms were investigated as shown in Table (5) and Figures (6,7). The various crude extracts of the leaves showed the effective on three type of test organisms. The maximum antimicrobial activity was recorded in the extract of butanol by applying paper disc method.

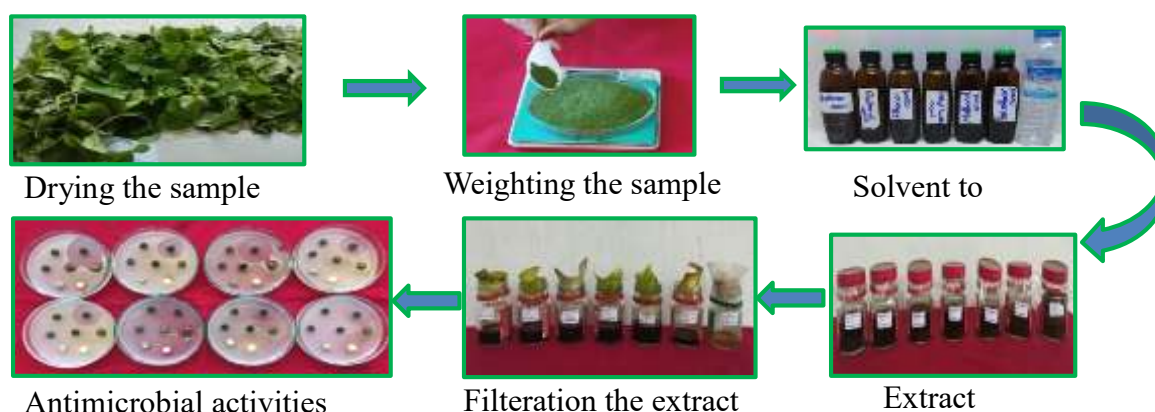
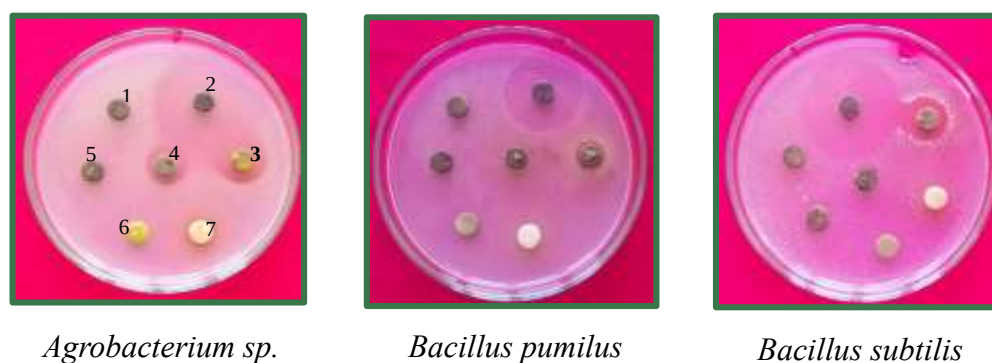
**Figure 6. Procedure of Antimicrobial Activities**

Figure 7.a Antimicrobial activity of leaves of *Operculina turpethum* (L.) Silva. with seven extracts (1. Acetone, 2. Butanol, 3. Ethanol, 4. Ethyl Acetate, 5. Methanol, 6. Pet-ether, 7. Water)

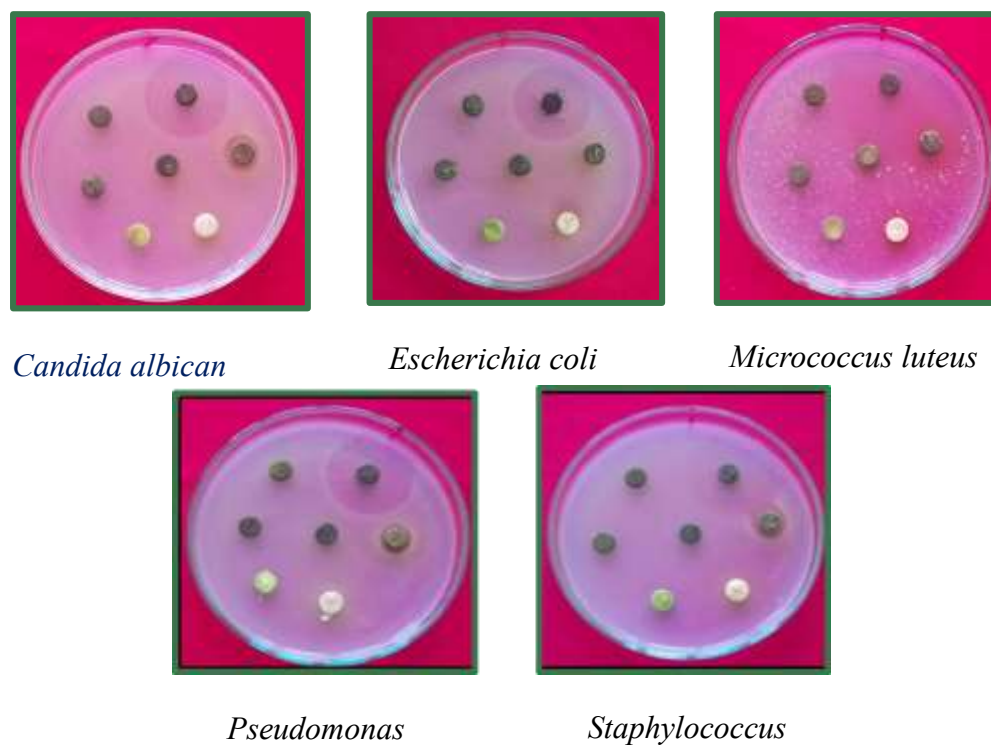


Figure 7.b Antimicrobial activity of leaves of *Operculina turpethum* (L.) Silva. with seven extracts (1. Acetone, 2. Butanol, 3. Ethanol, 4. Ethyl Acetate, 5. Methanol, 6. Pet-ether, 7. Water)

Table 5. Antimicrobial activities of leaves of *Operculina turpethum* (L.) Silva

Extracted solvent	<i>Agrobacterium</i> spp.	<i>Bacillus pumalis</i>	<i>B. subtilis</i>	<i>Candida albican</i>	<i>E. coli</i>	<i>M. furfur</i>	<i>S. aureus</i>	<i>Pseudomonas</i> spp.
Acetone	-	-	-	-	-	-	-	-
Butanol	45 mm	30 mm	40 mm	30 mm	30 mm	42 mm	32 mm	30 mm
Ethanol	15 mm	-	-	-	-	-	-	-
Ethyl Acetate	-	-	-	-	-	-	-	-
Methanol	18 mm	-	18 mm	10 mm	12 mm	25 mm	15 mm	12 mm
Pet-ether	-	-	-	-	-	-	-	-
Water	-	-	-	-	-	-	-	-

Paper disc = 6 mm

Discussion and Conclusion

In this research paper, morphological, anatomical, phytochemical studies, nutritional value, antioxidant activity, elemental analysis and antimicrobial activities of *Operculina turpethum* (L.) Silva. were studied. *Operculina turpethum* (L.) Silva. belonging to the family Convolvulaceae, was collected from Dagon University Campus in 2023.

In the present study, morphological study of *Operculina turpethum* (L.) Silva. is veins, reddish-green, 3-5 winged; latex present. Leaves alternate, simple, petiolate; the lamina broadly ovate; exstipulate. Inflorescences axillary cymes, 1-2 flowered. Sepals persistent. Petals (5), funnel-shaped. Stamens 5, epipetalous. Carpel (2), ovary superior, false septum present, tetralocular, axile placentation; the style slender; stigma bilobed, annular disc present. Fruit capsule, enclosed with enlarged sepals, the seeds black-brown in mature. Those characters are agreement with those mentioned by Dassanayake (1980), Wu *et al.* (1995) and Qi-ming (2009).

In anatomical study, in surface view of lamina, stomata are paracytic type on both surfaces, uniseriate and multiseriate non glandular trichomes were present and epidermal cells were oval to barrel shape. In transverse section of petiole and midrib, the vascular bundles were bicollateral and opened type. These characters are agreement with Metcalf & Chalk (1979); Eusa (2006), Edwin (2007) and Pandey (2008).

The preliminary phytochemical tests of powdered leaves of *Operculina turpethum* (L.) Silva. were shown alkaloid, carbohydrates, glycosides, phenolic compounds, reducing sugar, flavonoid, saponins and tannin. These data are similar to the statements describe by Trease and Evans (2002); Edwin (2007); Ali (2008), Gupta (2017), Karmakar (2021) and Pal (2024). The presence of carbohydrates and reducing sugars in this plant show the high energy content that could be utilized as a source of crude materials for pharmaceutical trades. Phenolic compounds, secondary metabolites abundantly found in both edible and non-edible plants possess biological properties of antioxidant, anti-apoptosis, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardiovascular protection, improvement of the endothelial function as well as inhibition of oxidative damage of DNA. Tannin has been reported to possess anti-carcinogenic and anti-mutagenic potential as well as antimicrobial properties.

The nutritional composition of powder leaves of *Operculina turpethum* (L.) Silva. have been analyzed. The powder was found to contain carbohydrates, protein, moisture, fiber, ash and fat. The fats, proteins and carbohydrates are vital nutrients for life expectancy. The carbohydrate contents are highest amount than others. Carbohydrates are involved the primary source of energy for all organism, plays vital roles of nutritional and structural. High carbohydrates contents suggest suitability of the plant as feed. Fats supply energy excess fat consumption result in cardiovascular disorder and aging. The present research about fats is lowest and totally safe for medicinal as well as for nutritional purpose. Fibers are found in suitable amount and good for the treatment of diseases like gastrointestinal disorder and obesity. Radha *et al.* (2021) also stated that the result of nutritional values of leaves of *Operculina turpethum* (L.) Silva.

Antioxidant activity also study, in this study IC₅₀ value of ethanol extract is lower than watery extract. According to the result ethanol extract of *Operculina turpethum* (L.) Silva had the lowest IC₅₀ value than water extract. Since the lower IC₅₀ values showed the higher antioxidant activities, the ethanol extract of sample was found to have effective free radical scavenging activity. These data are agreement with those mentioned by Neenu (2017).

The elements are helpful for our health. In this research work, the elemental analyses of *Operculina turpethum* (L.) Silva. include the calcium, potassium, iron, copper and strontium etc. Calcium is the mineral, it makes up the structures of our bones and teeth and also helps maintain typical muscle function, blood clotting, nerve transmission, and other body processes. Potassium is essential minerals. It maintains fluid levels in the body and supports the functioning of the kidneys, heart, muscles, and nervous system. Iron is a mineral that's an essential part of hemoglobin, a protein in red blood cells that carries oxygen from the lungs to the rest of our body Zafar (2010). Copper is an essential trace mineral necessary for survival. It is found in all body tissues and plays a role in making red blood cells and maintaining nerve cells and the immune system. Strontium is a natural element that plays a vital role in our bone health Prinessa and Peter (2015).

According to the antimicrobial activities investigation of leaves by using eight test organisms with seven extracts such as acetone, butanol, ethanol, ethyl-acetate, pet-ether, methanol and distilled water. Butanol, ethanol and methanol showed antimicrobial activity. Among them, butanol extract is the best antimicrobial activity (30-45 mm clear zone) and can affected all test organisms. The results were in mentioned with those described in Alam (2010), Tasleem Ahmad (2016), Gupta (2017) and Neenu (2017), but Alam (2010) stated that pet-ether extract of leaves also showed antimicrobial activity.

In conclusion, the botanical and phytochemical studies, nutritional value, antioxidant activity, elemental analysis and antimicrobial properties of leaves can give valuable information about *Operculina turpethum* (L.) Silva. Furthermore, medicinal plants contained numerous biologically active compounds which are helpful in improving the human life. Consumption of local vegetables can help overcome the problem of vitamin and nutritional deficiencies, especially for lower middle class population. Therefore, local vegetables are helpful as a source of carbohydrates, vitamins, fiber and minerals used for human health. Literature review observed that there is no clinical trials have been reported but controlled clinical trials to confirm its effectiveness and safety. It can be concluded that *Operculina turpethum* (L.) Silva. possess many nutritional, medicinal and pharmacological values and can also be a good curry in our homes.

Acknowledgements

I would like to express my warmest thanks to Rector and Pro-Rectors from Dagon University, and Dr. Tin Moe Aye, Professor and head, Department of Botany, Dagon University, for her kind and encouragement during this research work. I also thank to Dr. Thandar Oo, Professor, Department of Botany, Dagon University, for her invaluable advice.

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PHYTOCHEMICAL INVESTIGATION, NUTRITIONAL VALUES, TRACE METAL CONTENTS AND ANTIMICROBIAL ACTIVITY OF *VINCETOXICUM FLEXUOSUM* VAR. *TENUIS* (BLUME) SCHNEIDT, MEVE & LIEDE

Htay Htay Myint¹, Kyi Kyi Lwin², Kathy Myint³, Phyo Phyo Win⁴ and Naw Win Thawdar Oo⁵

Abstract

The medicinal plant *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liedt (Ma-Line-Twat) belongs to the family Apocynaceae. The specimens were collected from Yae Chan Pyin Village, Sittwe Area, during the flowering and fruiting period, from May to July, 2023. In this research, morphological characters, preliminary phytochemical tests, nutritional values, trace metal contents analysis and antimicrobial activity tests were carried out. In the morphological study, the plant was climber and milky sap present. The preliminary phytochemical tests revealed that the presence of alkaloid, glycoside, carbohydrate, reducing sugar, saponin and tannin were found; the α -amino acid, starch, phenolic compound and flavonoid were absent. In a nutritional study, it was significantly found that 38.13 % of fibres in leaves. According to the Energy Dispersive X-Rays Fluorescence Spectrophotometer (EDXRF) analysis results, chlorine was found more than other elements in leaves. The antimicrobial activity was carried out by agar well diffusion method on six types of test microorganisms. Ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on all other test microorganisms. The watery and pet-ether extracts showed the negative results on all types of test microorganisms.

Keywords: V-Flexuosum, Apocynaceae, antimicrobial activities, test microorganisms

Introduction

Plant is an important source of medicine and plays a key role in world health. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintain good health and conditions. Two-third of the world's population depends on herbal medicine for primary health care in developing countries. The reasons for this is because of their better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects (Sandberg and Corrigan, 2001).

The Apocynaceae is one of the largest and important families in angiosperm. Characteristic features of the family are almost all species produce milky sap (Wiert, 2006). Leaves are simple, opposite or whorled; flowers are large, colourful and slightly fragrant with five contorted lobes; and fruits are in pairs. Members of family Apocynaceae are rich in alkaloids, terpenoids, steroids, flavonoids, glycosides, simple phenols, lactones and hydrocarbons. Several members of this family have medicinal properties and have been in the treatment of various ailments. Most of them are consumed as food by tribal people whereas a few plants are used as source of poison.

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The native range of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede is Tropical & Subtropical Asia to Caroline Islands, China, Bangladesh, India and Myanmar etc. (Website.1).

The plants are alexipharmic; used for the treatment of urticaria, smallpox, excessive perspiration, bilious swellings, as an antidote to arsenic poisoning. Leaves are used for scabies. The leaves and roots of this plant have been used to treat jaundice and symptomatic range of bioactivities, including immune modulatory effects, free-radical scavenging (anti-oxidant), hepato-protective, anti-convulsant, anti-anxiety, anti-bacterial, anti-asthmatic, anti-inflammatory, anti-cancer, anti-amoebic, anti-psoriasis, seborrhic, and anaphylactic effects, and they are used for the treatment of leucopenia and the Schultz-Dale reaction (Mostafa, *et al.*, 2023).

The aim and objectives of present research were to know the morphological characters of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede, chemical constituents, nutritional values, trace metal contents by EDXRF and antimicrobial activities of this medicinal plant.

Materials and Methods

Collection and preparation of samples

The specimens of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede were collected from Yae-Chan-Pyin Village, Sittwe Township, Rakhine State. They were collected especially during the flowering and fruiting period from May to July in 2023. After the plant collection, both vegetative and reproductive parts of the plants were identified by using the literatures of Lawrences, 1964; Backer and Brisk 1965; Hundley and Chit Ko Ko, 1987; Dassanayake, 2000 and Kress *et al.*, 2003.

Preparation of Powdered Leaves Samples

The leaves of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede were collected from Rakhine State. The plant samples were washed with water thoroughly and dried at room temperature for 2-3 weeks. The dried samples were pulverized by grinding with a blender to get fine powdered and stored in air tight container to prevent it from moisture and air-borne contamination.

Preliminary Phytochemical Investigation from Leaves of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

For Preliminary Phytochemical test, the air-dried powdered of the leaves were tested for alkaloids, α -amino acid, glycoside, carbohydrates, reducing sugar, starch, saponin, tannin, phenolic compound and flavonoid were carried out.

Determination of nutritional values of leaves

The nutritional values of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede leaves were analyzed at the SmallScale Industries Department, Ministry of Cooperatives and Rural Development, Nay Pyi Taw. The nutritional values were determined by the method of Pearson, 1976.

Determination of elemental composition of leaves

The concentrations of elements in *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede powdered leaves were analyzed using an Energy Dispersive X-ray Florescence (EDXRF) spectrophotometer at the Universities' Research Center in University of Yangon.

Screening the Antimicrobial Activities of Different Solvent Extracts from Leaves of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

The extraction of crude drugs

For the extraction of crude drugs, the leaves powder were soaked in different solvents, such as ethyl acetate, methanol, ethanol, acetone, water and pet-ether for about three days and thoroughly shaken. Then, these solutions were separately filtered and evaporated on the water bath.

Cultivation of Test Organisms

Bacillus pumilus (IFO 905771), *Bacillus subtilis* (JAP- 0225025), *Candida albicans* (IFO- 1060), *Micrococcus luteus* (NITE 83297), *Pseudomonas* sp. (IFO 94307), and *Staphylococcus aureus* (ATCC 12877) were used for the antimicrobial activities. They were inoculated into the nutrient broth and transferred into nutrient agar media.

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 Cruickshank, 1975. The ingredients were dissolved in 100 mL of distilled water by heating in flat bottomed flask and poured into separate conical flasks. The flasks were sterilized by autoclaving at 121°C for 15 min. After autoclaving, the media were cool down to 45°C and then test organisms were added to the nutrient agar medium.

This medium poured into petridishes and solidified. After solidification, the holes were made on medium by means of a cork borer having a diameter of 6 mm. The loose agar blocks were removed from the wholes by using the sterilized wire needle.

After that about 0.01 mL of sample was introduced into agar well and incubated at 37°C for 24 hours. The inhibition zones appeared around the agar well indicating the presence of antimicrobial activities. The extracts of antimicrobial activities were measured from the zones of inhibition diameter.

Results

Morphological Characters of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

Scientific name	- <i>Vincetoxicum flexuosum</i> var. <i>tenuis</i> (Blume) Schneidt, Meve & Liede
Myanmar name	- Unknown
English name	- Snakeweed
Rakhine Name	- Ma-line-twat
Family	- Apocynaceae
Flowering and fruiting period	- May to July
Parts used	- Leaves

The plants are climbing herbs with milky latex. Leaves simple, opposite or whorled, exstipulate, petiolate. Inflorescences umbels in branched racemes, 3-7 flowered. Flowers brownish yellow, about 0.3 cm in diameter, complete, bisexual, actinomorphic, cyclic, hypogynous. Sepals 5 deeply lobed, divided almost to the base. Petals 5, united, salverform. Stamens 5, alternating with corolla lobes, epipetalous; filaments very short; anthers ditheous, sagittate, introrse, free or adherent to the stigmatic surface by a viscid fluid, connective often prolonged apically. Ovary superior, carpels 2, apocarpous; style usually 1 (with a thickened head); stigma umbel-shaped. The results were shown in Figure (1-6).

Specimen examined: Yae-Chan-Pyin Village, Sittwe Township, Rakhine State



Figure.1 Habit



Figure.2 Ventral surface of leaves



Figure.3 Dorsal surface of leaves



Figure. 4 Flower



Figure. 5 Calyx



Figure. 6 Androecium

Preliminary Phytochemical Test of Leaves from *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

The results of preliminary phytochemical test of air-dried powdered of leaves indicated that alkaloid, glycoside, carbohydrate, reducing sugar, saponin and tannin were found to be present. The α -amino acid, starch, flavonoids and phenolic compound were not present. The results were shown in Figure (7) and Table (1).



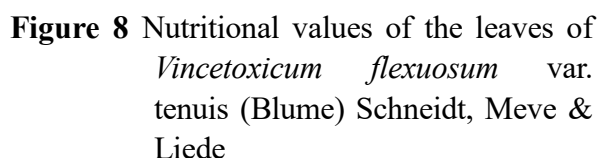
Figure 7. Phytochemical test of leaves from *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

Table 1 Preliminary Phytochemical Test of Leaves from *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

No.	Test	Extract	Test Reagent	Observation	Results
1.	Alkaloid	10% HCl	Wagner's reagent	White ppt	+
2.	α -amino acid	H ₂ O	Ninhydrin reagent	White	-
3.	Glycoside	H ₂ O	10% lead acetate solution	Pale yellow ppt	+
4.	Carbohydrates	H ₂ O	10% α -naphthol + H ₂ SO ₄ (Con:)	Red ring	+
5.	Reducing sugar	H ₂ O	Benedict's solution	Brick red ppt	+
6.	Starch	H ₂ O	Iodine solution	Brown ppt	-
7.	Saponin	H ₂ O	Distilled water	Foaming	+
8.	Tannin	H ₂ O	1% FeCl ₃ solution	Bluish black	+
9.	Phenolic compound	H ₂ O	5% FeCl ₃ solution	Brown	-
10.	Flavonoid	MeOH	H ₂ SO ₄ (Con:) + Magnesium	Dark green ppt	-

Nutritional Values and Trace Metal Contents of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

The nutritional values, such as protein, fat, fiber, ash, and carbohydrate of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede leaves were observed. The results were shown in Figure (8) and Table (2).



Elements Composition of Leaves by EDXRF Spectrometry

The content of elements in the leaves of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede was analyzed using Energy Dispersive X-ray Florescence (EDXRF) spectrophotometer analysis. It was found that chlorine was significantly present than other elements in leaves. The spectrum and spectral data were shown in Figure (9) and Table (3).



Sr.	Nutritional Parameter	Content (%)
1	Moisture	13.23
2	Proteins	18.47
3	Fat	5.531
4	Fiber	38.13
5	Ash	18.77
6	Carbohydrate	5.869

Table 3 Relative Abundance of Elements in Sample by using EDXRF

No	Element	Relative Abundance %
1	Cl	0.999
2	K	0.152
3	Ca	0.073
4	S	0.058
5	Fe	0.011
6	Mn	0.008
7	Br	0.004
8	Sr	0.001
9	Cu	0.001
10	CH	98.693

Antimicrobial Activities of Different Solvent Extracts of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

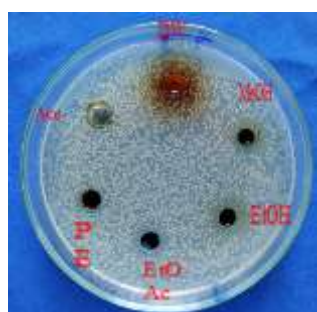
In this study, Ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on *Bacillus pumilus* and least against on *Micrococcus luteus*. The watery and pet-ether extract showed the negative results on all types of test microorganisms. The results were shown in Figure (10) and Table (4).

Table 4. Antimicrobial Activities of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

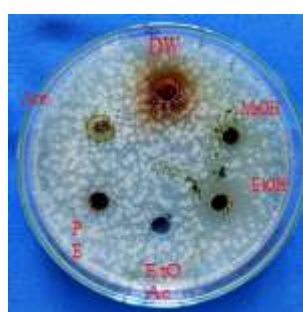
No.	Microorganisms	Size Diameter of Inhibition Zone (mm)					
		H ₂ O	MeOH	EtOH	EtOAc	Ace	P. E
1	<i>Bacillus pumilus</i>	-	11.7	13.6	-	11.4	-
2	<i>Bacillus subtilis</i>	-	10.0	19.0	-	-	-
3	<i>Candida albicans</i>	-	13.7	15.0	9.5	9.0	-
4	<i>Micrococcus luteus</i>	-	9.0	11.0	-	-	-
5	<i>Pseudomonas</i> sp.	-	9.0	12.8	-	-	-
6	<i>Staphylococcus aureus</i>	-	10.9	12.5	-	8.7	-

Key to table, 10 -12 mm = (+) weak activity, 13 -17 = (++) medium activity,

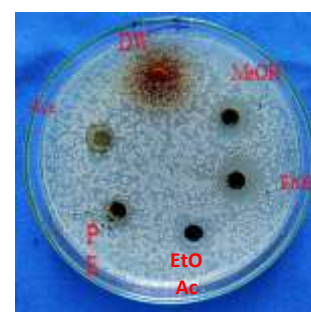
18 - 25 = (+++) high activity, 6 mm = Agar well



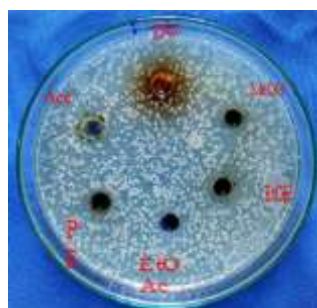
Bacillus pumilus



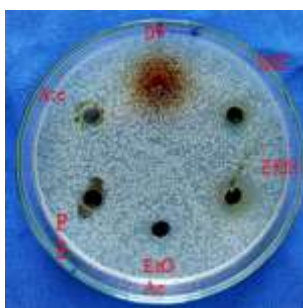
Bacillus subtilis



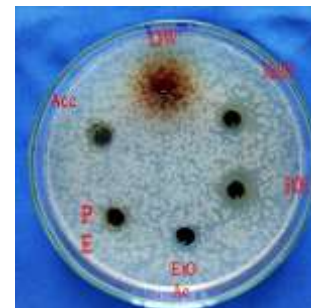
Candida albicans



Micrococcus luteus



Pseudomonas sp.



Staphylococcus aureus

Figure.10 Antimicrobial activities of leaves of *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede

Discussion and Conclusion

Medicinal plants are globally valuable sources of new drugs. In developing countries, 80 % of people are totally dependent on herbal drugs for their primary healthcare and over 25% of prescribed medicines in developed countries are derived from wild plant species (Hamilton, 2000). The secondary plant metabolites like alkaloids, flavonoids, terpenoids, glycosides, tannins etc. are biosynthesized by plants. These are active constituents in the plant and have marked pharmacological activity. These are very potent in process. Secondary metabolites are small biomolecules regarded as non-essential for the life of the producer organism (Agostini *et al.*, 2012).

The *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede plants are climbing herbs with milky latex, simple leaves, 3-7 flowered umbels racemes, brownish yellow flowers, 5-stamens and superior ovary. These characters were agreed with the Backer and Brink 1965.

People take *Vincetoxicum flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede by mouth for allergies, asthma, cancer, congestion, constipation, cough, inflamed skin, diarrhea, bloody diarrhea, hemorrhoids, tender joints, jaundice, rheumatoid arthritis, whooping cough, vomit, to cause sweating (Website.2).

Jayakumar and Karuppusamy, (2015) stated that *Tylophora* has alkaloids, flavonoids, phenols and glycosides. In this study, the preliminary phytochemical tests revealed the presence of alkaloids, glycosides, carbohydrates, reducing sugar, saponin and tannin. As exceptional α -amino acid, starch, phenolic compound and flavonoid were absent.

Ranemma *et al.*, (2017) stated that the antibacterial screening of the *T. indica* leaves extract were performed against gram positive (*S.aureus*) and gram-negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) by the disk diffusion method. In this study, ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on *Bacillus pumilus* and least against on *Micrococcus luteus*. The watery and pet-ether extract showed the negative results on all types of test microorganisms.

In the nutritional property analysis, moisture 13.23 %, proteins 18.47 %, fat 5.531%, fiber 38.13%, ash 18.77% and carbohydrate 5.869 % were obtained from the powdered leaves.

Moisture content refers to the number of water molecules that become incorporated into a food product. Moisture can enter into a product in a number of ways, it could be related to the production method of the product, the atmospheric moisture in the food production area, the packaging method of the product, or it can be related to the method of food storage. (Website.3)

Fibers appear to the lower risk of developing various conditions, including heart disease, diabetes, diverticular disease, and constipation. Proteins are need to build up muscle, blood, skin, bones, tissues and organs of the human body. A high protein intake can help with weight loss, increase muscle mass besides improve both mental and physical health. Fats are nutrients in food that the body uses to build cell membranes, nerve tissue and hormones. The body also uses fat as fuel. (Website.4)

The ash content in food refers to the minerals and inorganics left after the food sample has been heated to a very high temperature removing moisture, volatiles, and organics. Fats are

nutrients in food that the body uses to build cell membranes, nerve tissue and hormones. The body also uses fat as fuel. (Website.5)

According to the Energy Dispersive X-Rays Fluorescence Spectrophotometer analysis (EDXRF) results, chlorine was significantly present than other elements in leaves.

Chlorine is usually used to kill bacteria and other microbes in drinking water supplies and public swimming pools. Chlorine is a component of all body secretions and excretions resulting from processes of building (anabolism) and breaking down (catabolism) body tissues. Chlorine is stored to a limited extent in the skin, subcutaneous tissues, and skeleton and constitutes two-thirds of the negatively charged ions (anions) in the blood. Chlorine is readily absorbed during digestion, and similarly its rate of excretion through sweat, kidney excretion, and intestinal expulsion is high (Website.6). Potassium takes part in the ionic balance of the human body and maintains tissue excitability. Potassium is important for its diuretic nature (WHO, 2006).

In this study, ethanol extract showed the significant activities on *Bacillus subtilis* and moderate against on *Bacillus pumilus* and least against on *Micrococcus luteus*. The watery and pet-ether extract showed the negative results on all types of test microorganisms. Ranemma *et al.*, (2017) stated that the antibacterial screening of the *T. indica* leaf extracts were performed against gram positive (*S. aureus*) and gram-negative bacteria (*E.coli*, *P. aeruginosa* and *K. pneumoniae*) by the disk diffusion method.

The present research showed the significant properties of secondary metabolites, nutritional values, elemental analysis and antimicrobial activities. It was beneficial to the human health-care system and will promote natural traditional products research.

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STUDY ON MORPHO-ANATOMICAL CHARACTERS, PHYTOCHEMICAL INVESTIGATION ON LEAVES OF *SANTALUM ALBUM* L. AND ITS ANTIMICROBIAL ACTIVITIES

Aye Aye Naing¹, Aye Myat Thuzar², Seasar Wai³, Hla Theingi Lwin⁴

Abstract

Santalum album L. is belonging to the family Santalaceae which is known as Nant-thar-phyu in Myanmar and Sandalwood in English. The samples were collected from Neibainda Hill in Pyay Township during June, 2023 and February, 2024. In this study, morphological and anatomical characters, preliminary phytochemical tests, elemental analysis and antimicrobial activity of *Santalum album* L. were carried out. In morphological study, the plant is semiparasitic evergreen small tree with slender drooping branches. Leaves were simple and opposite. Flowers were complete, bisexual, regular, actinomorphic, perigynous. Perianth-segment provided with a tuft of hairs behind the stamens. Stamens 4, epipetalous, filaments long, anther dithecal, adnate, introrse. One carpel, axile placentation, one ovule, pendulous, style filiform, stigma tri-lobed, disc present, ovary half-inferior. Fruits globose drupe, seeds hard, globose or obovoid. In histological study, the epidermal cells were thin-walled parenchymatous cells and papillose formed in lower epidermis. The stomata were paracytic type and vascular bundles were collateral type and exarch. Druses-shaped calcium oxalate crystals were abundantly found in collenchyma and rarely found in parenchyma. In the preliminary phytochemical investigation, alkaloids, carbohydrates, flavonoids, glycosides, phenol, reducing sugars, saponins, starch and tannins were present and α - amino acid was absent. In the antimicrobial test, ethyl acetate extract was showed the activity on all of six microorganisms. Among them, acetone extract was showed the best activity on *Staphylococcus aureus*. Ethyl acetate showed activity on all organisms. Watery extract was showed activity on only *Candida albicans*. From the research, the findings were significance and as an authentic information could be used to future research.

Keywords: *Santalum album*, paracytic

Introduction

Man's life needs food, clothes, shelters and medicines. All of these are natural resources from plants. In a tropical country like Myanmar, the population since ancient time has household traditional remedies, which constituted mostly herbal plants category. Myanmar has richness in varieties of medicine as well as aromatic plants due to the presence of different climate zones in the country.

Medicinal plants are abundant in Myanmar. The 85% of the population live in rural areas. Most of the people use the traditional medicinal plants for the treatments on the various diseases. Although synthetic drugs and antibiotic brought about a revolution in controlling diseases, plant occupy a very significant for some important drugs. (Medicinal plants of Myanmar, 2000).

The habitat of *Santalum album* L. is eastern Indian, sandalwood is cultivated in Southeast Asia for its wood and essential oil. The trees felled throughout the year. Sandalwood's aroma has been highly esteemed in China and India for thousands of years. The wood is often burned as incense and plays a part in Hindu ritual. The heartwood is most often used in perfumery, but it also been taken as a remedy in China since around 500CE (Chevallier and Fnimh, 2016).

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The sandalwood tree, from southern India yields a fragrant white timber suitable for fine carvings and carpentry, while sandal oil, used in eastern countries for anointing the body and in the manufacture of soap and perfumes, is distilled from the yellow heartwood and roots. Sandalwood is used as incense in Hindu, Buddhist, and Muslim ceremonies and uses as joss sticks (Heywood *et al.*, 2007).

Sandalwood and its essential oil are used for their antiseptic properties in treating genitourinary conditions such as cystitis and gonorrhea. In Ayurvedic medicine, a paste of the wood is held to be useful for chest and abdominal pain. The leaves and bark are effective in treatment of dandruff, lice, skin inflammation and sexually transmitted diseases (Chevallier and Fnimh, 2016).

Leaves, fruits and seeds contain santalbic acid, palmitic acid, oleic acid, linoleic acid and sugar. Fruits yield betulinic acid and β -sitosterol and a fatty oil. Betulinic acid, β -sitosterol, glucose, fructose and sucrose from leaves. Heartwood yields essential oil which contain α , α and β -santalenes, santenol and teresantanol (Prajapati *et al.*, 2003).

Nowadays, the Republic of the Union of Myanmar also encourages for development of scientific on herbal and traditional medicine. To contribute such health policy of country, this project attempted to demonstrate regarding the evaluation and investigation of effective plants.

Thus, the aim of this study is to examine the medicinal plant scientifically, which have effective medicinal values. To fulfill this aim, the main objectives are to identify and study the morphological and histological characteristics of *Santalum album* L. to investigate the preliminary phytochemical analysis and to perform the antimicrobial activity from leaves of the plant.

Materials and Methods

Collection and Identification of *Santalum album* L.

The specimens used in this research were collected from Neibainda Hill in Pyaw Township, West of Bago Region during June, 2023 and February, 2024. Fresh specimens of the vegetative parts and floral leaves were studied. The taxonomic identification of the plant was made according to the method used in Department of Botany, Pyaw University to obtain correct family, genus and species with the help of literatures such as Handley and Chit Ko Ko (1986), Kirtikar and Basu (1935), Backer and Brink (1963), Dassanayake (1999), Kress *et al.*, (2003). The histological characters of the plant were identified by according to the references such as Esau (1965), Metcalfe and Chalk (1950), Lawrence and Cathy (1988), Pandey and Chadha (2000) and Trease and Evans (1999).

Preparation for histological Studies of *Santalum album* L.

The histological characters of fresh specimens were examined by preparing free hand sections with the razor blade. The specimens were cleared with chloral hydrate solution. The safranin solution was used for staining. Phloroglucinol and con. HCl were used for lignin test. The presence of calcium oxalate crystals was tested by using 80% sulphuric acid.

The collected specimens were washed and cut into small piece then air dried at room temperature. After that, these were homogenized by grinder to get powder and stored in air tight containers for histological, phytochemical studies, elemental analysis and antimicrobial test.

Preliminary phytochemical Analysis on leaves of *Santalum album* L.

The preliminary phytochemical investigation on the leaves had been undertaken. The experiment was carried out to determine the presence and absence of alkaloids, α -amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch and tannin according to the method of Marini Bettalo *et al.*, (1981), Central Council of research in Unani Medicine (1987) and Harbone (1989) at Department of Botany, Pyay University.

Antimicrobial activities of various solvent extracts from leaves of *Santalum album* L.

The various solvent extracts were tested on six pathogenic microorganisms. Antimicrobial activities were conducted by using paper disc diffusion method (Davis and Stout, 1971) at the Microbiology Laboratory, Botany Department, Pyay University. Test organisms and diseases were shown in Table (1).

Extraction and test organisms

The dried powdered sample was extracted with petroleum ether, chloroform, acetone, methanol, ethanol, ethyl acetate and water by percolation method. Crude extracts of various solvents of leaves were tested on six microorganisms such as *Bacillus pumilus*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Staphylococcus aureus*. The test organisms used in this study were obtained from the Microbiology Laboratory, Department of Botany, Pyay University and conducted for determination of the antimicrobial activities.

Preparation of sample for the test of antimicrobial activity

Nutrient agar was prepared according to the method described by Davis and Stout, 1971. Nutrient agar was used as basal cultural medium for the bacteria test organisms. Each test organism (50 uL) was added to 100 ml of assay medium and then poured into plate.

Shake well for homogeneously

After solidification, paper disc (6mm) impregnated with extracts (20 μ l paper disc) were applied on the test agar plate and incubated for a day. After that, clear zones (inhibitory zones) surrounding the disc were measured. These zones indicate the presence of antimicrobial metabolites which inhibit the growth of test organisms.

Table 1 Types of test organisms, their respective code numbers and diseases

No.	Test organisms	Code number	Diseases
1.	<i>Bacillus pumilus</i>	IFO 90571	Food poisoning
2.	<i>Candida albicans</i>	NITE 09542	Skin infection, vaginal candidiasis
3.	<i>Escherichia coli</i>	AHU 5436	Cholera, diarrhea
4.	<i>Micrococcus luteus</i>	NITE 83297	Skin disease
5.	<i>Pseudomonas fluorescens</i>	IFO 94307	Urinary tract infection, dermatitis
6.	<i>Staphylococcus aureus</i>	ATCC 12877	Skin disease, food poison, wound infection

Results

Scientific name	-	<i>Santalum album</i> L.
Family	-	Santalaceae
Common name	-	Sandalwood
Myanmar name	-	Nant-thar-phyu

Outstanding Characters of *Santalum album* L.

The plant *Santalum album* L. is semiparasitic evergreen small tree with slender drooping branches, reaching up to 18cm in height. Leaves were simple and opposite, leaf blade elliptic-lanceolate, glabrous, apice acuminate, base acute, margin undulate, petiolate, exstipulate. Inflorescence axillary and terminal, short paniculate cyme. Flowers bracteate, ebracteolate, pedicellate, complete, bisexual, regular, actinomorphic, perigynous. Tepals (4), synsepalous, valvate, campanulate, tepaloid (dark brownish red), adnate to the base of the ovary. Perianth-segment provided with a tuft of hairs behind the stamens. Stamens 4, epipetalous, opposite to the tepals, filaments long, anther ditheous, adnate, introrse, longitudinal dehiscence. Carpels (3), tricarpeal, syncarpous, axile placentation, ovules 2-3, pendulous, style filiform, stigma trilobed, disc present, ovary half-inferior. Fruits globose drupe, purple black with ribbed endocarp and seeds hard, globose or obovoid.

Flowering and fruiting period from December to March.

Histological characters of *Santalum album* L.

Lamina

The lamina of *Santalum album* L. was typically dorsiventral, venation reticulate, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System

Composed of epidermal cells, there are stomata and trichome in epidermal tissue system. In surface view, the cells of both surfaces were parenchymatous, thin and straight-walled. The upper epidermal cells were rectangular in shaped. The lower epidermal cells were barrel in shaped. Paracytic type of stomata were present on lower surfaces and papillose were formed. The guard cells were reniformed in shape and contained abundant chloroplasts. In cuticle layer, the upper is thicker than the lower. These are shown in figure 2.

Ground Tissue System

The mesophyll consisted of palisade and spongy parenchyma. The palisade mesophyll was made up of 3 layers. Vertically cylindrical cells, which are closely packed with one another. They contain numerous chloroplasts. The spongy mesophyll composed of 3-5 layers. They were rounded in shape and loosely arranged. Intercellular spaces were present among them. The oil cells were embedded in mesophyll tissues. These were shown in figure 2.

Vascular Tissue System

Vascular bundles were embedded in mesophyll. They were very small in size, ovate to elongate in shape, collateral type and exarch, the phloem tissues were very small in size. The xylem tissues were made up of 3-5 layers. These are hexagonal in shape. The xylem consisted of fiber and spiral tracheid. These were shown in figure 2.

Midrib

In transverse section, slightly convex in the upper surface and very concave in the lower surface, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System

In surface view, the cells were irregular in shaped. In transverse section, the epidermal cells were barrel in shape single layer. The cuticle layers of both surfaces were thin. They were shown in figure 3.

Ground Tissue System

The cortex was made up of collenchyma and thin-walled parenchyma cells. In the above of vascular bundle, thin-walled parenchymatous cells were composed of 3-8 layers and collenchymatous cells were composed of 3-5 layers. In the below of vascular bundle, the collenchymatous cells were found in three patches and 2-9 layers in each. The parenchymatous cells were round in shape and 3-10 layers. The rosette crystals were abundant in collenchymatous cells and rarely found in parenchymatous cells. They were shown in figure 3.

Vascular Tissue System

The vascular bundle was crescent shaped, collateral and close type. The phloem tissues were small. The xylem tissues were exarch and made up of 3-9 layers and 20-22 rays. These were hexagonal in shape. The xylem composed of fiber, spiral tracheid and pitted vessels. They were shown in figure 3 and 5.

Petiole

In transverse section, flat in upper surface and two horns in laterals, very concave in lower and distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System

In surface view, the cells were parenchymatous, thin-walled and irregular shaped along the length of the petiole. In transverse section, the epidermal cells were barrel in shape. The cuticle layer was thin-walled. They were shown in figure 4.

Ground Tissue System

The cortex was made-up of two different types of tissues, parenchyma and collenchyma. In the above of vascular bundle, the parenchyma tissues were made up of 5-10 layers and the collenchyma tissues were made up of 3 paths and 2-4 layers in each. In the below of vascular bundle, the parenchyma tissues were made up of 3-7 layers, the collenchyma tissues were found in under of lower epidermal cells and made up of 2 layers and 3 paths of 3-9 layers in below the vascular bundle. The rosette-shaped calcium oxalate crystals were abundant in parenchyma tissues and rarely found in collenchyma. They were shown in figure 4.

Vascular Tissue System

The vascular bundle was crescent shaped in outline, collateral and close type. The phloem cells were small size. The xylem tissues were exarch, made up of 5-11 layers and 25-27 rays. The xylem consisted of fiber and spiral tracheid pitted vessels. They were shown in figure 4 and 5.

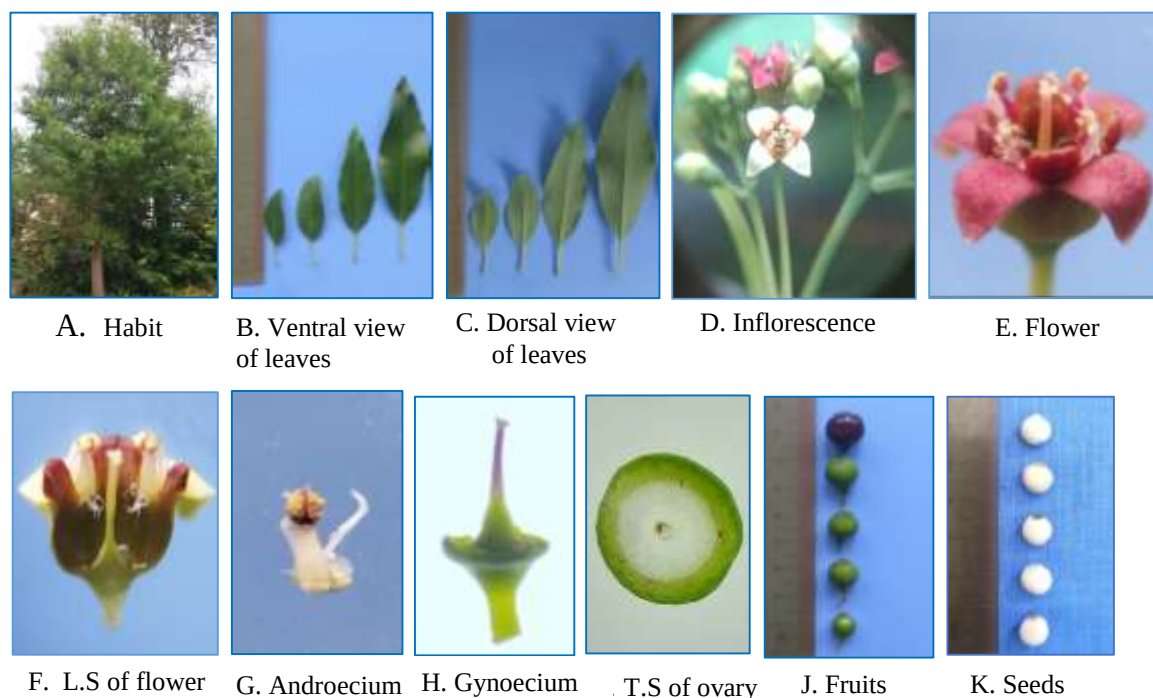


Figure 1 Morphological features of *Santalum album* L.

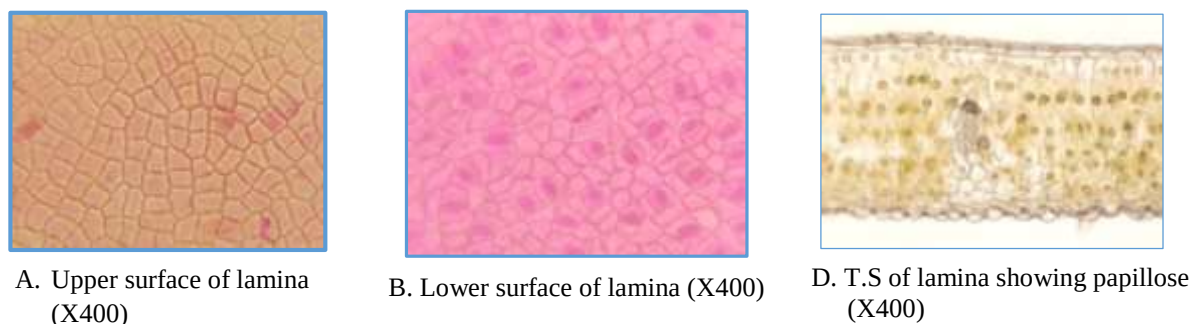


Figure 2 Histological Characters of lamina of *Santalum album* L.

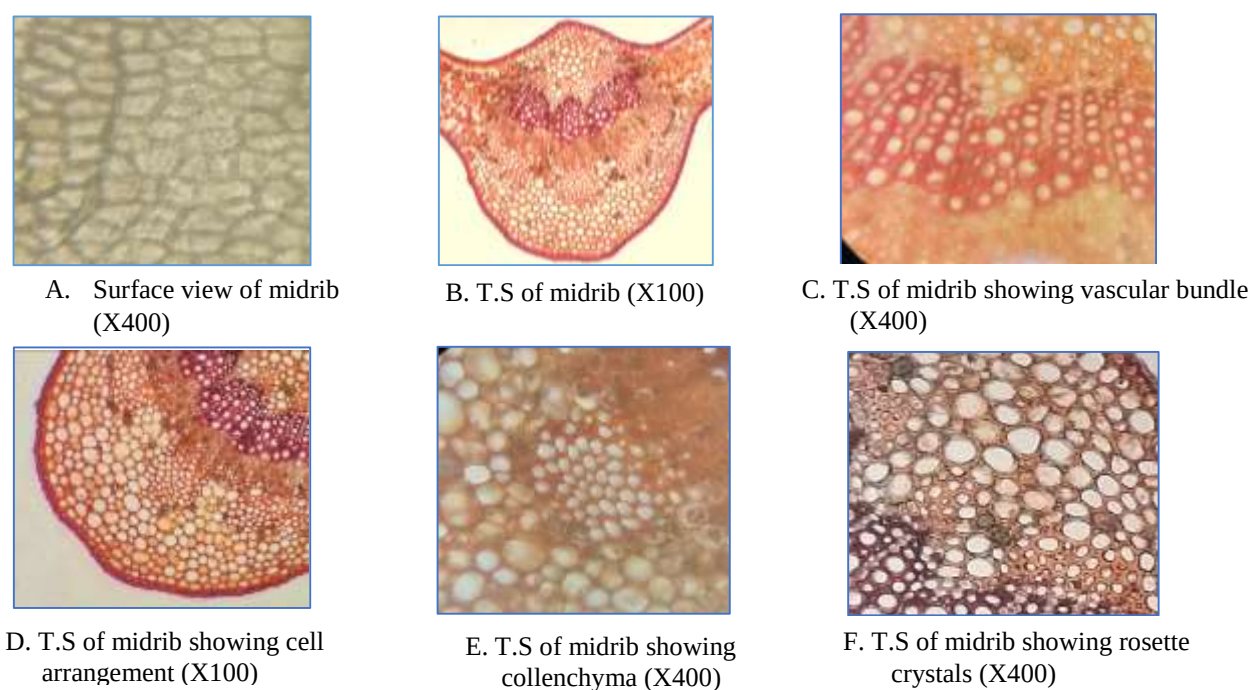


Figure 3 Histological characters of midrib of *Santalum album* L.

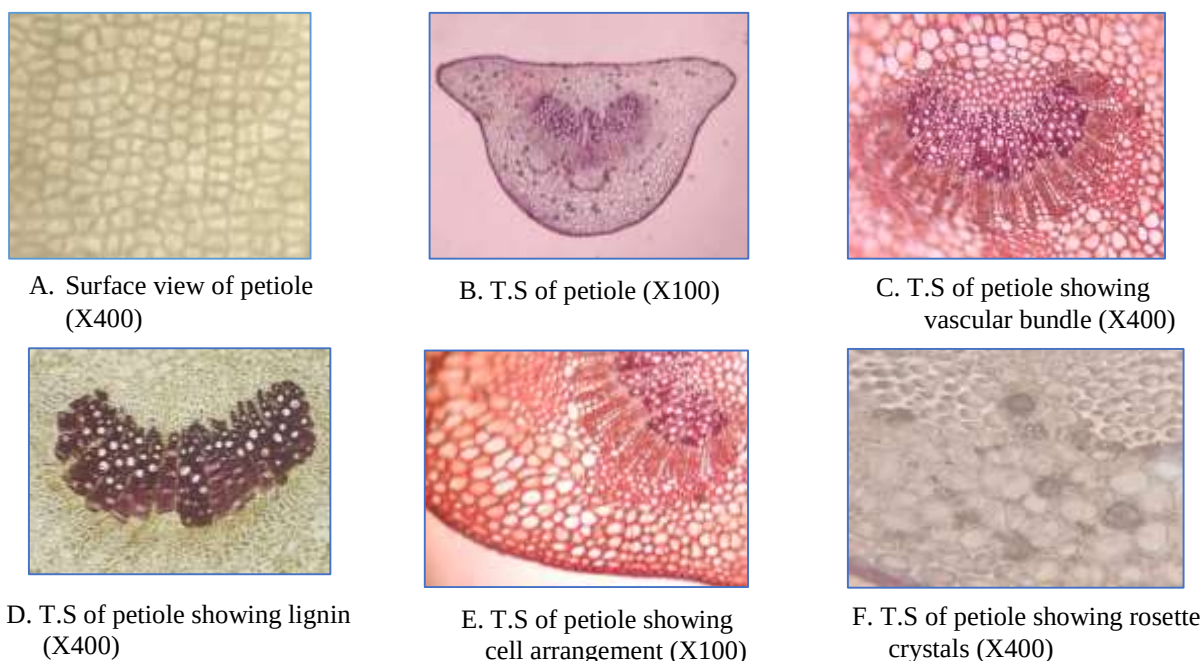


Figure 4 Histological characters of petiole of *Santalum album* L.

Diagnostic features of leaves powder of *Santalum album* L.

Sensory characters

Colour	Odour	Taste	Texture
Yellowish green	Astringent	Astringency	Fibrous

Microscopical characters of powdered leaves of *Santalum album* L.

Vessels, tracheids and fragments of sample were present in powdered leaves of *Santalum album* L. Vessels were found as pitted and tracheids were found as fibre, and spiral thickening. Fragments of sample were found as rosette crystals.

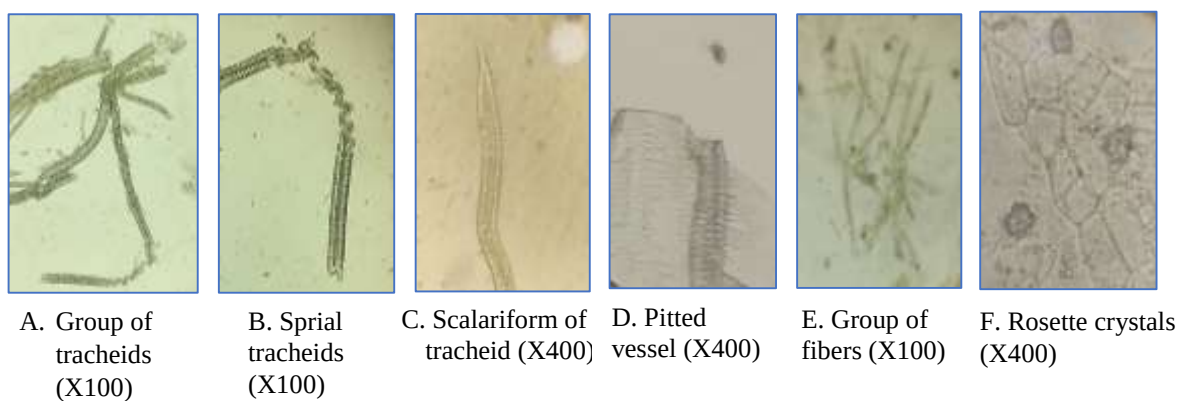


Figure 6 Microscopical Characters of Powdered Leaves of *Santalum album* L.

Preliminary phytochemical study of the dried powdered leaves of *Santalum album* L.

The preliminary phytochemical tests of the dried powdered leaves of *Santalum album* L. were indicated that the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenol, reducing sugars, saponins, starch and tannins. α - amino acid, was absent. The results were shown in table 2.

Table 2 Preliminary phytochemical tests of the leaves of *Santalum album* L.

No	Test	Extract	Test reagent	Observation	Results
1	Alkaloids	D/W	1.Dragendroff's reagent	Orange ppt	+
			2.Mayer's reagent	Cream ppt	-
			3.Wagner's reagent	Reddish brown ppt	+
2	α - amino acid	D/W	Ninhydrin reagent	No color change	-
3	Carbohydrates	D/W	10% α -naphthol + H ₂ SO ₄ (conc:)	Red ring or pink colour	+
4	Flavonoids	D/W	Mg /HCl (conc:)	Pink colour	+
5	Glycosides	D/W	10% lead acetate solution	White ppt	+
6	Phenol	D/W	10%FeCl ₃ , solution	Deep blue ppt	+
7	Reducing Sugars	D/W	Benedict's solution	Yellow/red ppt	+
8	Saponins	D/W	Distilled water	Frothing	+
9	Starch	D/W	I ₂ solution	Blue black colour	+
10	Tannins	D/W	1%FeCl ₃ solution	Yellowish brown ppt	+

(+) Present, (-) Absent, (ppt) Precipitate

Antimicrobial activities of different solvent extracts from leaves of *Santalum album* L.

Screening of antimicrobial activities of various extracts were carried out by using seven solvents such as acetone, ethyl acetate, ethanol, methanol, pet ether, chloroform and water. The results were shown in table 2 and figure 8.

Table 4. Inhibition zone exhibited by different extracts from leaves of *Santalum album* L. against six microorganisms

Solvent	Organisms					
	<i>Bacillus pumilus</i>	<i>Candida albicans</i>	<i>Escherichia coli</i>	<i>Micrococcus luteus</i>	<i>Pseudomonas fluorescens</i>	<i>Staphylococcus aureus</i>
Acetone	12.1 mm	15.3 mm	-	15.1mm	14.7 mm	18.8 mm
Ethyl acetate	12.0 mm	14.8 mm	10.1 mm	12.1 mm	15.3 mm	15.3 mm
Ethanol	10.8 mm	11.8 mm	-	13.0 mm	-	15.4 mm
Methanol	10.4 mm	13.0 mm	-	12.0 mm	-	14.7 mm
Pet ether	8.8 mm	13.6 mm	-	10.6 mm	12.0 mm	15.8 mm
Chloroform	-	13.5 mm	-	9.7 mm	-	8.4 mm
Water	-	9.6 mm	-	-	-	-

Paper disc – 6mm

According to this experiment, the leaves extract with ethyl acetate showed effective antimicrobial activity on all different microorganisms but watery extract showed activity on only *Candida albicans*. Among them, acetone extract showed the highest activity on *Staphylococcus aureus*.

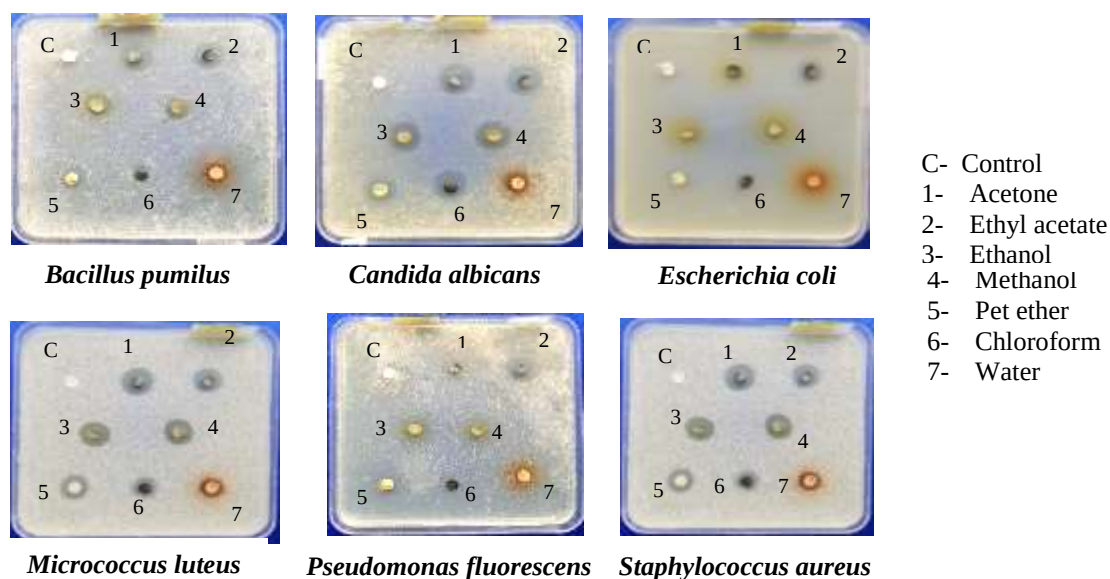


Figure 9 Antimicrobial activity from leaves extracts of *Santalum album* L. on six

Discussion

In this research work, morphological, histological characters, preliminary phytochemical investigation, elemental analysis and antimicrobial activities from leaves of *Santalum album* L. have been undertaken.

The morphological characters of *Santalum album* L. are agreement with mentioned by Kirtikar and Basu (1935), Handley and Chit Ko Ko (1986), Dassanayake (1999), Kress *et al.*, (2003) except ovule characters.

Although Backer and Brink (1963) stated that 2-3 ovules and Dassanayake (1999) stated that 2-4 ovules, only 1 ovule observed in this study. Style filiform, stigma tri-lobed, disc present, ovary half-inferior. Fruits are globose drupe, purple black and seeds are hard, globose or obovoid. These are agreement with Kirtikar and Basu (1935), Handley and Chit Ko Ko (1986), Dassanayake (1999), Kress *et al.*, (2003).

In histological studies, the characters of lamina, midrib and petiole are agreed with mentioned by Metcalfe and Chalk (1950).

In Preliminary phytochemical studies, carbohydrates, flavonoids, glycosides, phenol, reducing sugars, saponins, starch and tannins were present. In alkaloid test, showed the reaction with Dragendroff's reagent and Wagner's reagent, not showed with Mayer's reagent. α - amino acid was absent.

In the antimicrobial activities, the leaves extract with ethyl acetate showed effective on all different microorganisms but watery extract showed activity on only *Candida albicans*. Among them acetone extract showed the highest activity on *Staphylococcus aureus*. Anyhow, all the different solvent extracts showed activities on *Candida albicans*.

Conclusion

From the results, it can be concluding that leaves of *Santalum album* L. showed the presence of many secondary metabolites and phytochemical constituents, which could be used as a source of traditional medicine. It can help to local people such as this plant has medicinal value for curing the various diseases and could be utilized the efficiency for authentication of Myanmar traditional medicine systematically.

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OPTIMIZATION FOR THE FERMENTATION CONDITIONS OF SELECTED SOIL FUNGUS *CURVULARIA* SPP

Swe Zin Win¹, Thidar Oo², Khin Thida Swe³

Abstract

In the study, ten different fungi were isolated from different soil samples. It was collected from three different places in Bye Da Yaw Village in Sagaing Township, Sagaing Region for the isolation of soil fungi during May, 2022. Isolated soil fungi were tested by using five test organisms. Ten fungal strains showed antibacterial activities against the five test organisms during the preliminary screening. Among them, soil fungus SZF-02 showed the highest antibacterial activities on *Escherichia coli* (19.54mm) during the confirmatory screening. Therefore, this fungus SZF-02 was selected for further investigation such as antibacterial activities, effect of different fermentation media, effect of pH and temperature. In the present study, the maximum production of antibacterial metabolite was observed in different fermentation media with FM-1 showed distinct inhibitory zones of (22.00 mm) against *Escherichia coli* under pH 5 showed the best inhibitory zones of (21.60 mm) and incubation temperature 23°C showed the highest inhibitory zones of (21.91 mm). The microscopically characters of selected fungus SZF-02 was preliminary studied. On the basis of the morphological, microscopical characters and references key, the selected fungus SZF-02 was identified as *Curvularia* spp. Present study concludes that, soil fungus SZF-02 (*Curvularia* spp) serve as a potential source for further investigation will produce antibacterial metabolite.

Keywords: Antibacterial activities, different fermentation media, pH, Temperature

Introduction

Fermentation can be defined as a process in which biochemical changes are brought about in organic substances (carbohydrates, proteins, and fats) through the action of enzymes which are produced by microorganisms. Fermentation processes, the precursor of the specific products are not added in the medium, carbon and nitrogen sources present in the medium during their metabolism may initiate the biosynthesis of precursors that regulate the metabolism and influence the end products synthesis. Nutrients type and their concentrations in the medium play an important role in commencing the production of primary and secondary metabolites as limited supply of an essential nutrient can restrict the growth of microbial cells or product formation. Generally, carbon and nitrogen sources present in the medium can influence the metabolite production (Elibol, 2004).

Medium optimization is still one of the most critically investigated phenomenon that is carried out before any largescale metabolite production, and possess many challenges too. Before 1970s, media optimization was carried out by using classical methods, which were expensive, time consuming, involving plenty of experiments with compromised accuracy. Nevertheless, with the advent of modern mathematical/statistical techniques, media optimization has become more vibrant, effective, efficient, economical and robust in giving the results. For designing a production medium, the most suitable fermentation conditions (e.g., pH, temperature, agitation speed, etc.) and the appropriate medium components (e.g., carbon, nitrogen, etc.) must be identified and optimized accordingly. Further, by optimizing the above said parameters,

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maximum product concentration could be achieved (Gupte and Kulkarni, 2003; Franco-Lara *et al.*, 2006; Wang *et al.*, 2011).

Optimal fermentation conditions are very important for maximal productivity of metabolites. (Phay, 1997).

The aim of the study is to the isolation and antimicrobial activities of isolated soil fungi from the collected soil samples, to study the Fermentation conditions of soil fungi for the production of antimicrobial metabolites and to identified the genus of selected soil fungus (SZF-02).

Materials and Methods

Collection of Soil Samples

Three different soil samples were collected from three different places at Bye Da Yaw Village in Sagaing Township, Sagaing Region During May, 2022. The soil texture, soil pH and soil moisture % were measured at Department of Agriculture (LAND USE). Soil interpretation of results was made in Mandalay Township.

Isolation of Soil Fungi from Chemical Treatment Dilution Method (Hayakawa and Kobayashi, 2005)

The collected soil sample was air-dried at room temperature. The soil sample was grounded and sieved. 2g of sieved soil was put into test tube and then 4 mL of sterilized water was also put into the tube containing soil samples. 14 mL of 70% of methanol solution was then added into the tube containing soil suspension and was shaken for 1 minute and diluted with sterile water. The dilution series were cultured on low carbon source medium such as glucose yeast extract agar medium (GYA).

Preliminary Study for Antibacterial Activities by Agar Well diffusion Assay(Collin, 1965)

The isolated soil fungi were cultured on potato glucose agar medium for seven days. The isolated fungi were inoculated into seed medium and incubated at 27°C for three days. Five percent of seed medium were transferred into the fermentation medium. The fermented broth was used by agar well diffusion assay method (Collin 1965) to check the antibacterial activities against five test organisms. These test organisms were supported by Department of Biotechnology of Patheingyi University for the cooperation research. Cork borer was used to make the wells (8mm) in diameter for antibacterial assays. The fermented broth (20µL) was impregnated and allowed to dry.

The assay medium for the antibacterial activities test was prepared and one percent of test organism was added to assay medium, then poured into plates. After solidification, agar well impregnated with samples (fermented broth) were applied on the agar plates and incubated at 27°C for 24-36 hours. Clear zones (inhibitory zones) surrounding the agar well indicated the presence of bioactive metabolites which inhibit the growth of test organisms. Five test organisms, *Agrobacterium tumefaciens*, (NITE 09678), *Escherichia coli* (AHU5436), *Pseudomonas fluorescens* (IFO94307), *Staphylococcus aureus* (AHU8465) and *Bacillus pumilus* (IFO90517) were used in agar well diffusion assay.

Basal Media Used for the Antibacterial Activities of isolated Soil Fungi (NITE, 2004)

<u>Seed medium</u>		<u>Fermentation Medium</u>		<u>Assay Medium</u>	
Glucose	0.5 g	<u>(Basal medium)</u>		Glucose	1.0 g
Yeast extract	0.3 g	Glucose	2.0	Polypeptone	0.3 g
KH ₂ PO ₄	0.01 g	Yeast extract	0.3 g	KNO ₃	0.1 g
KNO ₃	0.1 g	KNO ₃	0.1 g	Agar	1.6 g
Soluble starch	0.3 g	K ₂ HPO ₄	0.01 g	DW	100mL
MgSO ₄ 7H ₂ O	0.01 g	CaCO ₃	0.1 g	pH	6.5
DW	100mL	DW	100 mL		
pH	6.5	pH	6.5		

Utilization of Four Different Fermentation media for Isolated Fungus SZF-02**FM 1**

Glucose	2.0 g
Sucrose	2.0 g
Polypeptone	0.3 g
KNO ₃	0.1 g
K ₂ HPO ₄	0.01 g
CaCO ₃	0.1 g
DW	100 mL
pH	6.0

FM 2

Sucrose	2.0 g
Yeast extract	0.3 g
Polypeptone	0.3 g
KNO ₃	0.1 g
K ₂ HPO ₄	0.01 g
CaCO ₃	0.1 g
DW	100 mL
pH	6.0

FM 3

Glycerol	2.0 g
sucrose	1.0 g
Yeast extract	0.3 g
KNO ₃	0.1 g
K ₂ HPO ₄	0.01 g
CaCO ₃	0.1 g
DW	100
mL	
pH	6.0

FM 4

Maltose	2.0 g
Yeast extract	0.3 g
Polypeptone	0.3 g
KNO ₃	0.1 g
K ₂ HPO ₄	0.01 g
CaCO ₃	0.1 g
DW	100 mL
pH	6.0

Distinguished Characters of the Pathogenic Bacteria *E. coli*

The selected bacterium was cultured on peptone glucose agar medium. After 24 hrs of incubation, plates were observed for microscopic characteristics such as gram-negative and microscopic characters such as facultative anaerobic, rod shape and coliform bacterium of the genus *Escherichia*.

Different Fermentation Media on Selected Isolated Fungus SZF-02 (Omura 1985)

In the preliminary study, the effect carbon source (0.5g) and nitrogen source (0.3g) were used in basal medium for the study of antibacterial activities. Different carbon and nitrogen sources were replaced in basal medium for the production of antibacterial metabolites against *E. coli*.

Determination of Effects of pH on Fermentation by Isolated Fungus SZF-02

To determine the effect of pH for the production of secondary metabolite by isolate fungi SZF-02 in the fermentation media with different pH. The experiment was carried out individual at various pH 4, 5, 6, 7 and 8 incubations respectively. Adjustments of the pH were done by addition of hydrochloric acid and sodium hydroxide to achieve acidity and alkalinity respectively were incubated at room temperature.

Determination of Effects of temperature on Fermentation by Isolated Fungus SZF-02

The optimization temperature of fermentation medium (FM-1) of SZF-02 were carried out four different temperature viz. 21 °C, 23 °C, 25 °C and 27°C and incubated for 7 days. Antibacterial activities were tested by using agar well diffusion assay method.

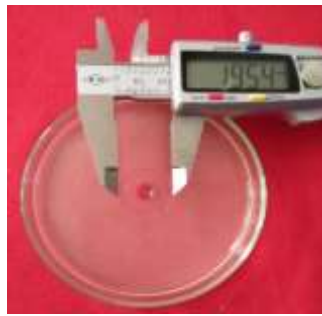
Identification of Distinguished Characters of Selected Fungus SZF-02

The morphological and microscopical characters of fungus SZF-02 was observed by the methods of Ando and Inaba, 2004, Domsch and Gams, 1993, Watanabe, 2002 and Barnett, 1960 with the help of Olympus Microscope.

Results

Screening of Antibacterial Activities of Isolated Soil Fungi

In this study, total of ten fungi colony was isolated from three different soil samples. All fungal strains showed antibacterial activities against the five test organisms during the preliminary screening. Among them, soil fungus SZF-02 showed the highest antibacterial activities against *Escherichia coli* (19.54mm) during the confirmatory screening.



Fermentation periods 5 days

Agar well = 8mm

Test organism = *E. coli*

Figure 1. Antibacterial activities of selected fungus SZF-02

Effects of Different Fermentation Media of Isolated Fungi SZF-02

In the present study, five kinds of fermentation media were used. According to the results of antibacterial activities, fermentation medium FM-1 showed the highest inhibitory zone of (22.00 mm) in diameter.

Table 1. Basal Medium and Various Fermentation Medium of Isolated Fungus SZF-02 against *Escherichia coli*

Medium	Inhibitory zone diameter (mm)
Basal	12.55
FM-1	22.00
FM-2	20.40
FM-3	19.29
FM-4	20.17

Agar well= 8mm

Test organisms- *E. coli*

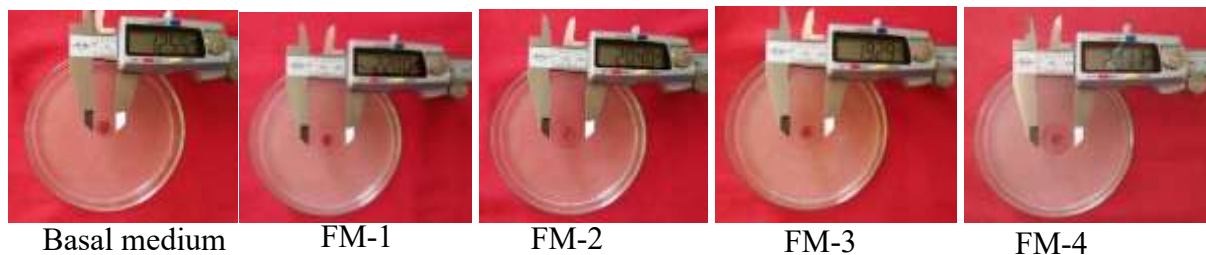


Figure 2. Effect of different fermentation media of isolated fungus SZF-02

Effects of pH of Isolated Fungus SZF-02

The optimization of pH of the fermentation broth for antibacterial metabolite production was done by carrying out the fermentation at eight different pH values pH 4, 5, 6, 7 and 8, incubations. The antibacterial activities assayed by culture against test *E. coli* was found maximum at pH 5 as the inhibitory zone of 21.60 mm.

Table 2. Effect of pH on fermentation media

Isolated fungi SZF-02	
pH	Inhibitory zone (mm) and fermentation period (5 days)
4	17.31
5	21.60
6	21.18
7	20.61
8	18.04

Agar well = 8mm

Test organism = *E. coli*



pH 4

pH 5

pH 6

pH 7

pH 8

Figure 3. Effect of pH on fermentation of SZF-02 against *E.coli*

Effect of Temperature on Fermentation of SZF-02

The optimization temperature for antibacterial metabolite production was carried out at four different incubation temperatures 21°C, 23°C, 25°C and 27°C incubated. Thus 23°C is the most suitable temperature (21.91mm) for the antibacterial metabolite production.

Table 3. Effect of incubation temperature on fermentation media

Temperature (°C)	Inhibitory zone (mm)
21	12.55
23	21.91
25	19.05
27	15.15

Agar well = 8mm

Test organism = *E. coli*

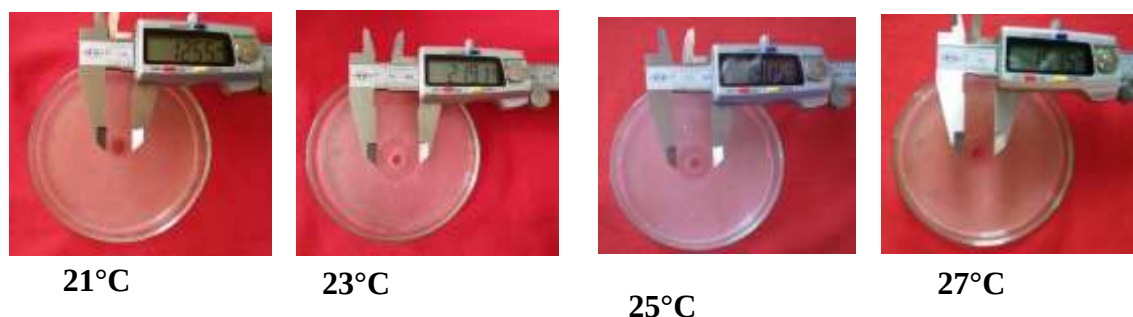


Figure 4. Effect of temperature on fermentation of SZF-02 against *E.coli*

Preliminary Study on the Morphology and Microscopical Characters of SZF-02 (Ando and Inaba, 2004)

After 5 days cultivations, it was observed that colonies on potato glucose agar medium at 25°C. Colony is dark brown, woolly surface, reverse is dark. In microscopical characters, hyphae are septate darkly pigmented. Conidiophore are brown, erect simple or branched with bend or twisted and holding brown conidia. Conidia are large, porosporous, subellipsoidal, mostly 4-celled, darker brown in 2- central cells, especially curved, larger in the penultimate cells, with indistinct hilum basally.

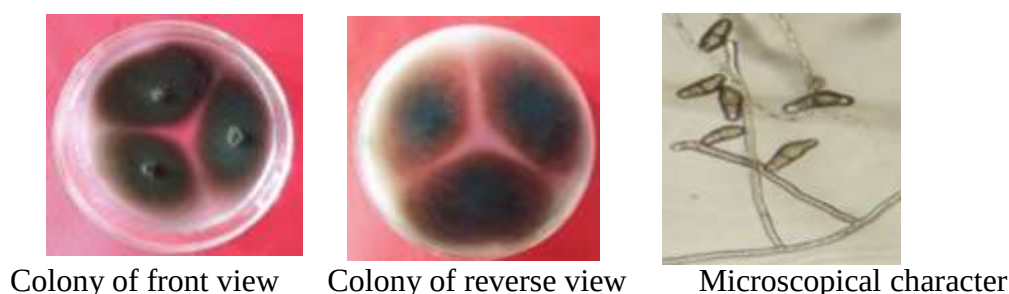


Figure 5. Morphological and Microscopical characters of SZF-02 on PGA medium

Discussion and Conclusion

Stanbury *et al.*, 1999, also reported the quality of fermentation media is very important as it provides nutrients and energy for growth of microorganisms. In this study, ten different fungi were isolated from different soil samples. It was collected from three different places in Bye Da Yaw Village in Sagaing Township, Sagaing Region during May, 2022 by using soil dilution method (NITE, 2004).

Ten different soil fungi were isolated and their antibacterial activities were tested by using five test organisms. All fungal strains showed antibacterial activities against the five test organisms during the preliminary screening. Among them, soil fungus SZF-02 showed the highest antibacterial activities (19.54mm) on *Escherichia coli* during the confirmatory screening (Figure 1) Therefore, fermentation conditions were investigated for the production of antibacterial metabolites. This isolated fungus SZF-02 was the most promising and was selected for detailed study such as antibacterial activities, effect of different fermentation media, effect of pH and effect of various temperature. In the present study, effect of different fermentation medium with FM-1 showed distinct inhibitory zones of ingredients like each 2.0 g glucose and sucrose and polypeptone proved to be the best fermentation and showed the highest antibacterial activities (22.00mm) against *Escherichia coli* (Table 1 and Figure 2).

SZF-02 showed that, fermentation medium containing (SZF-02) pH 5 is the best for the production of antibacterial metabolite (21.60 mm) (Table 2 and Figure 3). The effect of incubation temperature 23°C showed antibacterial activity (21.91mm) (Table 3 and Figure 4) were optimized for the fermentation condition. The microscopical characters of selected fungus SZF-02 was preliminary studied. Soil fungus SZF-02 may be genus *Curvularia* sp. Based on the distinct microscopic characters of hyphae, conidiophore and conidium.

The result of antibacterial activities, different fermentation media, effect of pH and temperature on SZF-02 will be investigated for further extraction and purification of antibacterial metabolites against *E. coli*. According to Literature, the present studies on antibacterial metabolite with expectations which had the potential source to provide a therapeutic agent to the treatment and control of bacterial infections used for clinical and food industrial. Therefore, SZF-02 (*Curvularia* sp.) could be a potent source of antibacterial natural products. In this study, screening of fungus SZF-02 (*Curvularia* sp.) revealed the potential sources for further investigation will produce antibacterial metabolite.

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QUALITY OF UPPER EOCENE COAL SEAMS EXPOSED IN THE DATHWEKYAUK AREA, TAMU TOWNSHIP, SAGAING REGION*

Thawng Lian Mung¹

Abstract

The research area is mainly composed of clastic sedimentary rocks and coal seams of Upper Eocene Pondaung and Yaw Formation. The total (6) representative coal samples collected from differences coal seams in the Nyaungpin stream section, near Minthami village, Tamu Township. Six coal samples were used to determine the quality or grade of coals. The coals of the study area constitutes macerals of Vitrinite group (76% to 91%), Liptinite group (0.4 % to 2.8%) and Inertinite group (8% to 17%) respectively. The vitrinite reflectance analysis was carried out on a MPV- Leitz microscope by means of reflected white light using oil immersion objective. The proximate analysis was carried out on the coal analysis instruments, Ollital, Model - JZLR – 9000B. The results of vitrinite reflectance value of coal seam are range from 0.56 and 0.64. The proximate results of coal seams are composed of moisture- 1 % - 9 %, volatile matter – 42% - 45%, Ash – 3% - 11%, fixed carbon – 42% - 51% , calorific value – 10989 - 13261 Btu/lb. Both the vitrinite reflectance values and proximate analysis values were indicate that the coal rank of coal seams fall into “Sub bituminous A and B” rank. The classification based on the German and American Society for Testing Materials (ASTM) coal classification scheme. Subbituminous coals of the study area are economically important because those are used as fuel for electric power generation and industries. Coal samples were analyzed in laboratories at Yamaguchi Univeristy, Japan, Pengujan tek MIRA, Bandung, Indonesia and Georesources Mineral Analysis Laboratory, Yangon, Myanmar.

Keywords: Coals, vitrinite reflectance, proximate analysis and subbituminous.

Introduction

The study area is located about 6 miles east of Minthami village, Tamu township, Sagaing Region. The area is emplaced between the latitudes N 23° 51' and longitude E 94° 18' and on one inch topographic map of Burma Survey Map and it occupies parts of Map sheet No. 84 I/5. The general structural trend of the study area is nearly NNE - SSW direction. This area is a mountainous and forested region and the ranges are running NE-SW direction. The location map of the study area is shown in Figure (1). Some geologists were studied the Chindwin basin. Aung Khin and Kyaw Win (1968, 1969) published two papers concerning with the geology, paleogeography and hydrocarbon prospects of Burma Tertiary Basins including Chindwin Basin. Bender (1983) briefly described the geology of the Chindwin Basin.

Kyaing Sein et.al (1983) carried out the geological map of Tamu Mawleik- Phaungpin Area, (D.G.S.E, unpublished report). Thawng Lian Mung (2011) carried out the M.Res (Thesis) on sedimentology of the Dathwekyauk coal measures, Upper Chindwin Basin Tamu Township, Sagaing Region.

The aims of this study are to identify the vitrinite reflectance values, proximate analysis values and to determine the grade or quality of coal.

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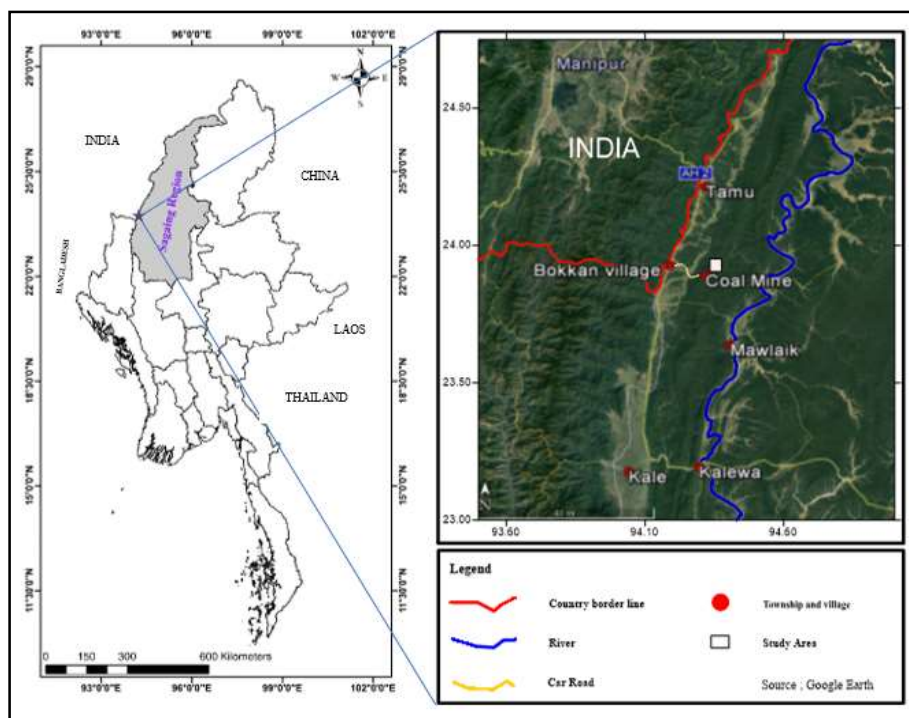


Figure 1. Location map of the study area.

General Geology

Regional Geology of the Study Area

Geomorphologically, as well as tectonically, Myanmar can be subdivided into four major tectonic provinces which are north-south trending linear belts, these are from east to west (1) Shan-Tanintharyi Block (2) Central Cenozoic Belt (3) Western Fold Belt and (4) Rakhine Coastal Belt. In the Early Cretaceous, India Plate started drifting northward and Ceno-Tethys subducting beneath Southeast Asia. Initial “soft” collision between the India Plate and Eurasian Plate occurred during the Paleocene (Wandrey, 2006), and “hard” collision occurred during the Middle Eocene (Metcalf, 2006). The tectonics of the Central Basin and Western Ranges of Myanmar is determined by the subduction of the Indian Plate beneath the Burma Plate (Brunnschweiler, 1966; Win Swe, 1981). There are at least three basins in the Central Cenozoic belt. The three basins from north to south are the Chindwin Basin, Minbu Basin and Irrawaddy Embayment. The Chindwin basin is fore-arc basin associated with the Tertiary NE directed subduction zone of NW Myanmar. The Chindwin basin is located between the Indo-Burma Ranges in the west and Wuntho Massif mass in the east. The Eocene rocks are exposed in larger linear belts on the western limb of the Chindwin syncline and in two disconnected smaller belts on the eastern limb of the same syncline. They are composed of alternating sequences of sand and shale units. The upper part of the Eocene sequence in the Chindwin basin is made up of non-marine deltaic deposits which locally contain commercial coal seams in Thitchauk area near Kalewa and Dathwekyauk area to the west of Yuwa village. (Win Swe, 2012). Structurally, the Chindwin basin is bounded to the west by Kabaw fault system and to the east by Sagaing strike-slip fault. Towards the East, Chindwin Basin is separate from the Back-Arc basin by the “Igneous Wuntho Massif”. (Bender, 1983).

Stratigraphy and Lithology

A thick succession of thin bedded to massive sandstone and shale with minor conglomerate of Eocene age are extensively exposed in a linear north – south trending belt. The Eocene strata of this belt are mainly made up of molasse sedimentary rocks. The present research area is mainly composed of upper Eocene units, Pondaung Formation and Yaw Formation, see Figure 2. These Formations are mainly made up of sandstone, siltstone, shale, mudstone, siderite bands, coaly shale and hard coal seams. In the study area, trough - cross bedding, ripple - cross lamination, parallel lamination and planar - cross beddings are the most common internal structures of the sandstone outcrops. The siderite nodules are common in the carbonaceous mudstone. Twenty coal seams are present in the study area. The thicknesses of coal seams are range from 5 cm to 3 m. (Thawng Lian Mung, 2011).

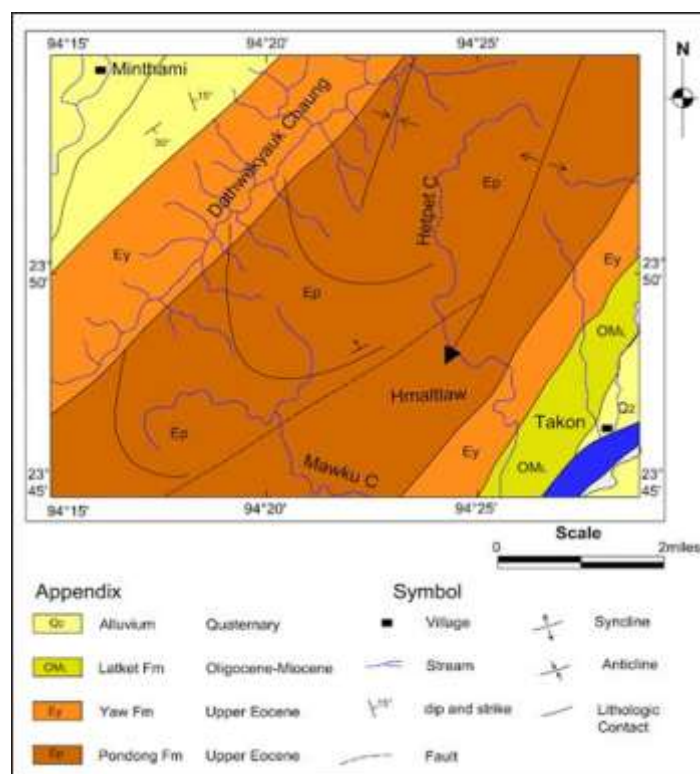


Figure 2. Geological and Location Map of the Study Area. (modified after Kyaing Sein et al.,1983).

Methods of the Present Research

Field Works and Sampling

Coal samples were collected from the coal seams of the Upper Eocene Strata. The samples were collected from the surface exposures in the present investigation area. A total six representative coal and shaly coal samples were obtained from measured stratigraphic section along the Nyaungpin stream sections. The samples were collected from base to top as benches of a channel of the coal beds in order to provide a vertical section of the beds.

Laboratories Works

The samples collected from the field area were analyzed in Laboratories at Section of Geology, Faculty of Education, Yamaguchi University, Japan, Laboratorium Pengujian tekMIRA, Bandung, Indonesia and Laboratory of Georesources Mining Co.ltd, Yangon Region, Myanmar. In laboratory, the briquettes were prepared and used to describe the vitrinite reflectance and maceral analysis. The vitrinite reflectance analysis was carried out on a MPV-Leitz microscope by means of reflected white light, using oil immersion objectives (10x and 50x). Maceral constituents of each coal sample were determined by counting at least 500 points per sample under reflected 'white' light. The reflectance was measured according to Austrilia Standard 2856 and ASTM (2009). See figure (3). The proximate analysis was carried out on the coal analysis instruments, Ollital, Model-JZLR – 9000B. The proximate analysis of coal is an assay of the moisture, ash, volatile matter, and fixed carbon as determined by series of prescribed or standard test methods.

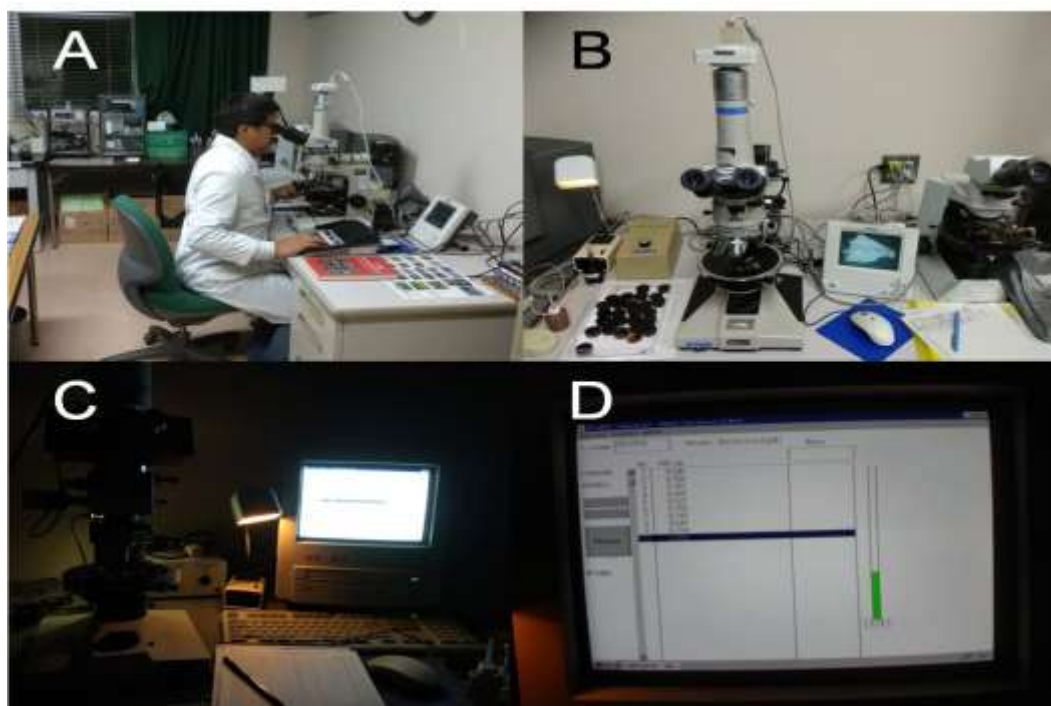


Figure 3. (A, B) showing maceral analysis and (C, D) showing vitrinite reflectance analysis. (At Yamaguchi University, Japan.)

Mode of Occurrences of Coal Seams

There are sixteen coal seams present in the investigated area. But six representative coal seams and samples were used for this research work. The thickness of coal seams ranges between (8) cm to (300) cm. The coal seams are well crop out along the Nyaungpin stream section (N 23° 53' 03'', E 94° 16' 36'' to N 23° 53' 50'', E 94° 17' 04''). Megascopically, coals samples are generally dark brown to black colored and appeared brittle. The coal bearing formation is composed of shale with sandstones and siderite bands. The coal seams are underlain by mud, siltstone and overlain by sandstone. The dip amount of coal seams are varies from 38° to 60° and the dip direction showed 110° to 132° (E to SE) direction. See figure 4).



Figure 4. Coal seams of the study area.

Results and Discussion

Maceral Composition (%)

All the three maceral groups (vitrinite, liptinite and inertinite) have been determined in the coal samples. The distribution of various macerals and maceral group is shown as a relative percentage. **The vitrinite group** predominants for all of the coal seams and its percentage varying between (78 to 91 vol.%). The vitrinite maceral subgroup is mainly composed of detrovitrinite/desmocollinite, telovitrinite/ telocollinite, collinite and telinite. The dominance of collinite and telinite gives a clear indication that the coal is derived from the woody tissues of roots, stems and leaves which composed of cellulose and lignin. (Stach, E., 1982). See figure (5).

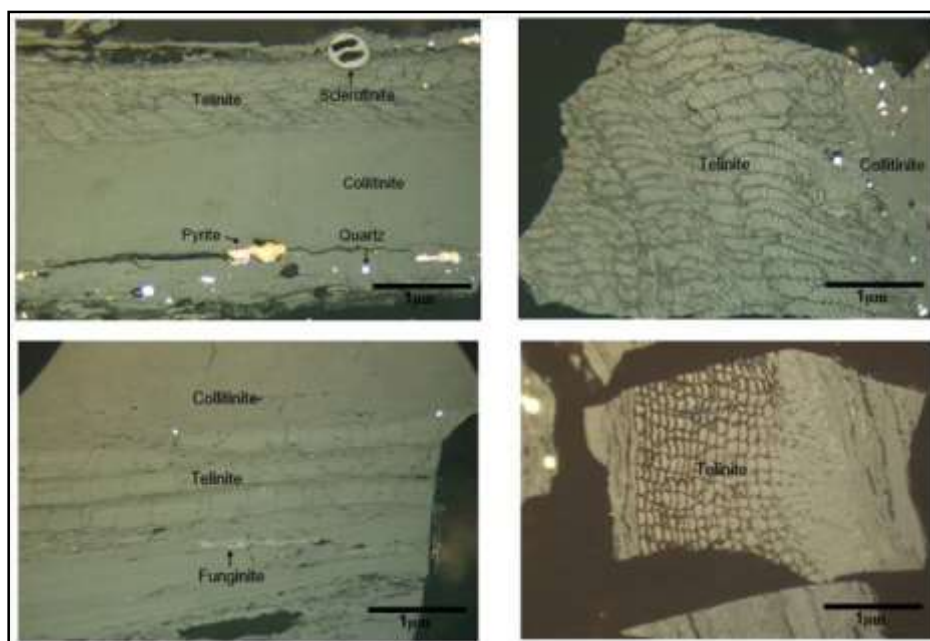


Figure 5. The vitrinite macerals of coal in the study area.

The liptinite group ranges from (0.4 to 2.8 vol.%) in all samples. The sub macerals are mainly represented by sporinite, cutinite and resinite. Cuticles occur not only on leaves and needles but also on shoots, stalks and thin stems. When regarded as a maceral, the resistant cutine layers are termed “cutinite”. See figure (6).

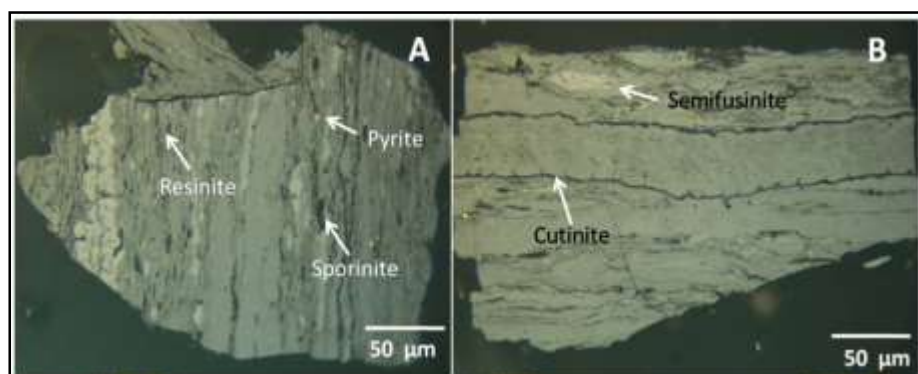


Figure 6. Liptinite macerals in coal. (A). Resinite (small lenses, round to oval or round in shape) and sporinite; (B). Cutinite show that thin to thick wall structures.

The component of **Inertinite group** is (8% to 17 vol%) and sub macerals content are represented by fusinite, semifusinite, sclerotinite and micrinite. Semifusinite always shows lower reflectance than fusinite. The cell structure of semifusinite is mostly less well preserved than that of fusinite. The sclerotinites occur in the form of tabular, cellular or non-cellular hyphae which have grown together in various irregular forms. See figure (7).

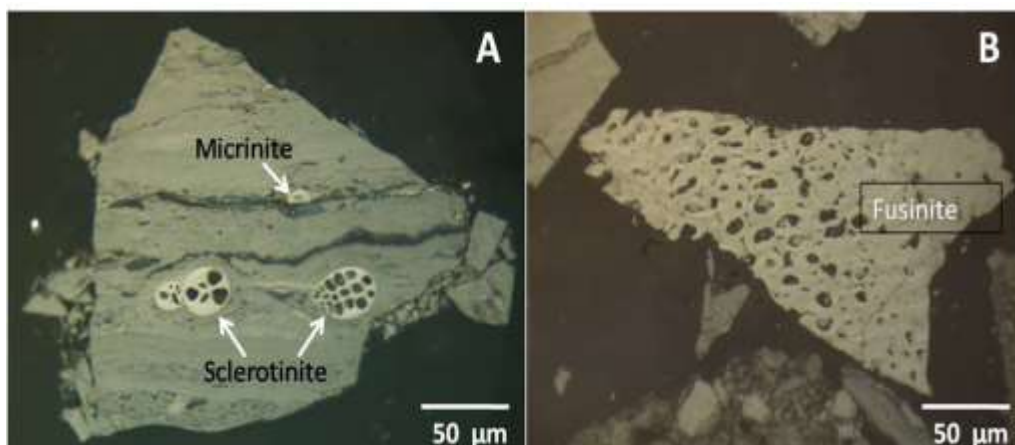


Figure 7. (A, B) showing submacerals of Inertinite Group.

Minerals Matter

The mineral matter is generally composed as (1% to 5 vol%). Clay Minerals may occur in various forms in vitrinite. Pyrite can occur as veins, irregular nodules and filling cell cavities. See Figure (8).

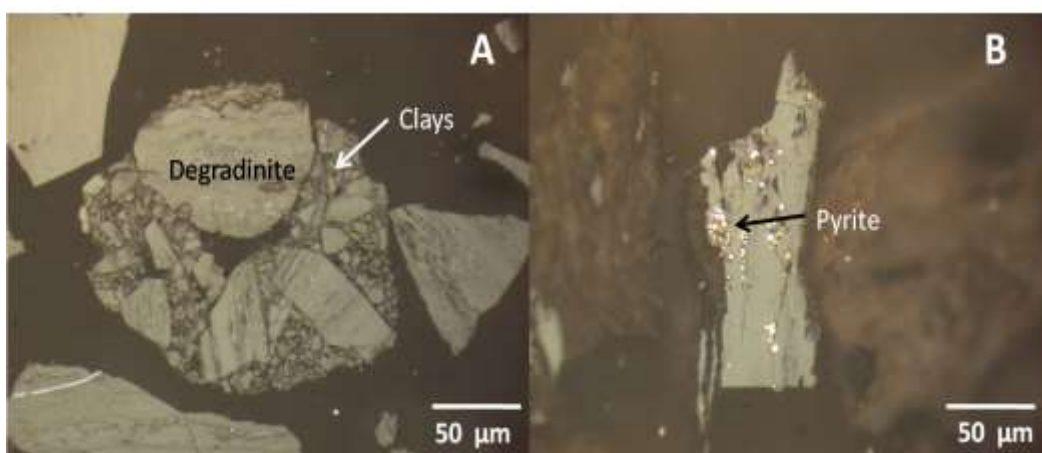


Figure 8. (A). Clays minerals and (B). Pyrite minerals.

Vitrinite Reflectance Analysis

The reflectance was measured according to Australia Standard 2856 and ASTM. The details of reflectance analysis results have been summarized in Table 1. The mean vitrinite reflectance value range between 0.56% to 0.64%, placing the coal in the low to medium rank category, based on the American Society for Testing and Material (ASTM) coal classification scheme. (Diessel, C.F.K., 1992).

Table 1. Vitrinite reflectance values (%) of coal in the study area.

Sample No	NC- 1	NC - 4	NC - 9	NC - 15	NC - 16
Maximum value (%)	0.69	0.63	0.64	0.59	0.59
Minimum value (%)	0.60	0.56	0.55	0.54	0.53
Mean Maximum Reflectance value (%)	0.64	0.59	0.59	0.56	0.56

Proximate Analysis

The proximate analysis of coal is an assay of the moisture, ash, volatile matter, fixed carbon and calorific values. The proximate analysis is one of the physical properties of coal. The calorific value is a direct indication of the heat content (energy value) of the coal. The calorific value can be used to evaluate the effectiveness of beneficiation processes. The volatile matter (d.a.f. basis) of the Dathwekyauk coals ranges from 42.11% to 45.97% with a mean of 44.5%. The ash ranges from 3 % to 11.6 % with a mean of 7.5%. The fixed carbon ranges from 42.4 % to 51.4 % with a mean of 48 %. The calorific values range from 10989 Btu/lb to 13261 Btu/lb with a mean of 12073 Btu/lb. The results of the proximate and calorific values are summarized in Table 2.

Table 2. Proximate analysis results (%) of coal in the study area.

Sample No	Moisture (%)	Volatile Matter (%)	Ash (%)	Fixed Carbon (%)		Volatile Matter (%)	Ash (%)	Fixed Carbon (%)	Calorific Value Btu/lb
Wet Basis						Dry Basis			
NC - 1	0.95	45.09	3	50.96		45.52	3.03	51.45	13261
NC - 4	2.08	43.77	6.60	47.55		44.7	6.74	48.56	12593
NC - 11	9.39	41.65	10.5	38.46		45.97	11.59	42.45	10989
NC - 12	6.28	39.46	7.4	46.86		42.11	7.9	50	11934
NC - 15	8.48	40.34	7.45	43.73		44.07	8.14	47.79	11592

Coal Rank of the Study Area

To classify coal, the vitrinite reflectance values, calorific values and proximate analysis values are needed. The mean vitrinite reflectance value range between 0.56 % to 0.64 %. The volatile matter (d.a.f basis) range between 42.1 % to 45.9 %. The calorific values are 10900 Btu/lb to 13000 Btu/lb. Under the German and American classification schemes, both of the vitrinite reflectance values and proximate analysis values were indicate that the rank of the research area coal seams was fall into “Subbituminous A and B”. See Table 3.

Table 3. The German and North America classifications and their distinction on the basis of different physical and chemical rank parameters. (Stach.E., 1982).

Rank  		Reff Rm Oil	Vol. M d.a.f %	Carbon d.a.f %	Bed Moisture
German	USA				
Torf	Peat	0.2	68	ca 60	ca 75
Weich Braunkohle	Lignite	0.3	60		
			56		
			52		
Matt	Sub. Bit	C 0.4	48	ca 71	ca 25
Glanz	B	0.5			
	A	0.6	44	ca 77	ca 8 -10
Flam	C	0.7			
Gas flamrr	B	0.8	40		
	A				
Gas	Medium Vol. Bituminous	1.0	32	ca 87	
		1.2	28		
Fett	Low Volatile Bituminous	1.4	24		
Ess		1.6	20		
		1.8	16	ca 91	
Mager	Semi Anthracite	2.0	12		
			8		
Anthrazit	Anthracite	3.0	4		
Meta Anthrazit	Meta Anthracite	4.0			

Conclusion

The research area is mainly composed of Upper Eocene units, Pondaung Formation and Yaw Formation. These Formations are mainly composed of sandstone, siltstone, shale, carbonaceous shale, coaly shale, shaly coal and hard coal seams. Megascopically, coal samples are generally dark brown to black coloured and appear brittle. Petrographically, the maceral Vitritine group (78% to 91 vol%), liptinite group (1% to 2.8 vol.%) and Inertinite group is (8% to 17 vol%). The mineral matter is generally composed as (2% to 8.6 vol%). Telovitrinite, detrovitrinite, semifusinite, sclerotinite, alginite, fluorinite and cutinite are the common macerals. The mean vitritine reflectance value range between 0.56 % to 0.64%. The calorific values are 10900 to 13000 Btu/lb. Coals of the research area are Sub-bituminous A and B, based on German and North America coal classification. The Subbituminous coals of the study area are

economically important because those are used as fuel for electric power generation and industries.

Acknowledgements

The author is grateful to Dr. Than Zaw Oo, Rector of West Yangon University, for allowing us to do this research. The author wishes to express sincerely gratitude to Dr. Toh Toh Win Kyi, Professor and Head of Department of Geology, West Yangon University for her permission, advices and instruction. Finally, the author would like to be grateful to the AUN/SEED-Net and Japanese International Cooperation Agency for financial support.

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BRACHIOPODA FROM TAUNGNYO FORMATION, MAWLAMYINE AREA, MON STATE*

Ye Yint Aung¹

Abstract

This paper reviews the brachiopod fossils from the Taungnyo Range, Mon State, Myanmar. I describe here, Late Devonian to Early Carboniferous brachiopods including; *Punctospirifer scabricosta*, *Tropidoleptus carinatus*, and *Tetracamera subcuneata* were found in the study area. According to the discovery, each species possesses different shell outline and surface morphological features. Fossil specimens are well-preserved in the mudstone and argillite (C_{1t}²) unit of Taungnyo Formation. The fossil brachiopods are absence C_{1t}¹ and C_{1t}³ units. Among them, all taxa are first described in Myanmar but previously named in the world.

Keywords: Brachiopoda, Taungnyo Formation, Taxonomic Classification, Devonian, Carboniferous, Paleocology, Mon State

Introduction

Carboniferous to Early Permian rocks are well exposed along the Taungnyo Range. There have been a few studies, the most works have been done on the sedimentological field. Paleontological and sedimentological purpose of Taungnyo Formation described by Clegg (1953), Nyunt Htay (1979), Kyaw Min (1997), Moe Zat (2006), Nay Zar Zar Linn Aung (2006), Kyi Pyar Aung *et al.* (2014), Ye Yint Aung (2016), Moe Moe Lwin (2017), and Nandar Myint Maung *et al.* (2020). Although the detail paleontological analysis of brachiopod fossils are less detailed in this area. Therefore, the present to identify systematic paleontology of brachiopods in the Taungnyo Formation.



Figure 1. Location map of the study area. (A) Location of Mon State.

(B) Detailed map of the Taungnyo Range and fossil localities marked by red star.

Aims and Objectives

The aim of this paper to identify the systematic study and taxonomic classification of brachiopod faunas. The objectives of this work include to describe the various lithology, to review and update of the fossil specimens, and to determine the stratigraphic position of the study area.

* Second Prize (2024)

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Location, Material, and Methods

The study area is located about 4.8 km from southeast of Mawlamyine Township, Mon State (Fig 1). It is also situated in the one-inch topographic map sheet no. 94H/11 and UTM map sheet no. 1697-11. I have taken 16 days long field investigation in 2016. The detailed field investigation was being done in order to know the different rock units and to collect the complete fossil specimens. The measurement of stratigraphic sections was used by the Jacob's staff methods wherever the appropriate localities. Also, the fossils were being systematically collected during the stratigraphic measurements. The fossil specimens were collected carefully packed together with tissue papers, cottons, and rubber bands. Fossils were selected and photographed with a cannon camera. The mapping was used by GIS software.

Regional Geologic Setting and Stratigraphy of the study area

The Taungnyo Range is a part of the Shan Tanintharyi Belt and trending nearly N-S structural direction. Regionally, Taungnyo Range is mainly covered by the Paleozoic strata with preserved brachiopods and other marine invertebrate faunas in this area. The regional geological map of the study area is shown in Figure 2.

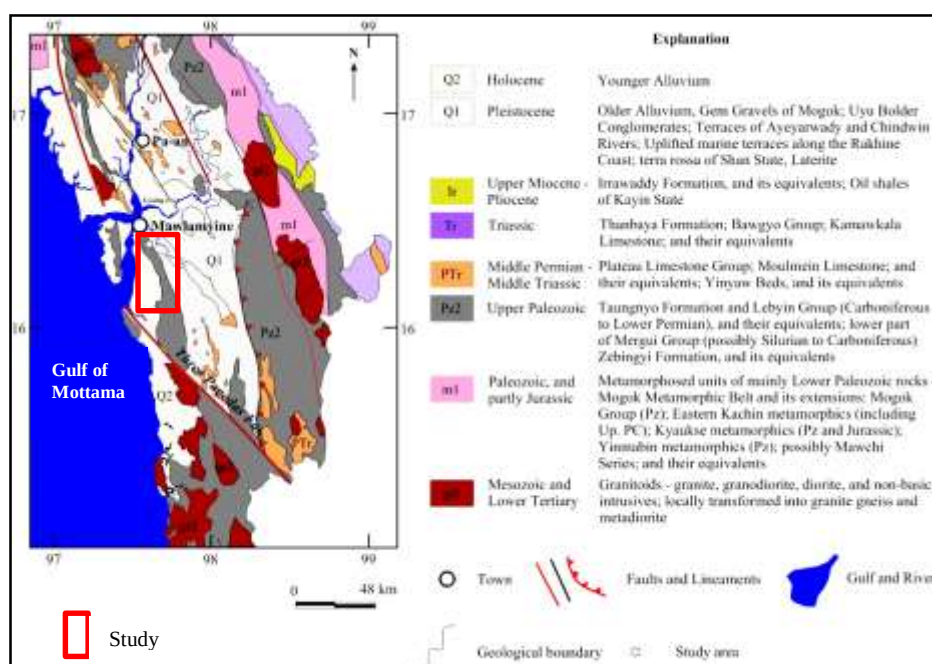


Figure 2. Regional Geological map of the study area

(cited from geological map of Myanmar, MGS, 2014)

The study area consists of clastic sedimentary rocks and limestone occurred as belts striking NW-SE direction. The Early Paleozoic rock of Taungnyo Formation and the Permian rock of Moulmein Limestone Group are widespread in the study area.

Leicester (1930) was first proposed the Taungnyo series from a rocks sequence of sandstones, quartzites, shales, and slates exposed along the Taungnyo Range. And then, the contributors include Pascoe E. H. (1959) and Brunnschweiler (1970) used the term as a Taungnyo Group. Taungnyo Formation consists of sandstones, shales, slates, siltstones, argillites, and mudstones (Figure 3). Sandstone is mostly quartzose sandstone, arenite, pebbly greywacke,

and quartzwacke (in Pascoe, 1930). Shale is pink, purple, reddish, and yellowish color. It has been subdivided into three major lithologic units; C_{1t}^1 unit, C_{1t}^2 unit, and C_{1t}^3 unit.

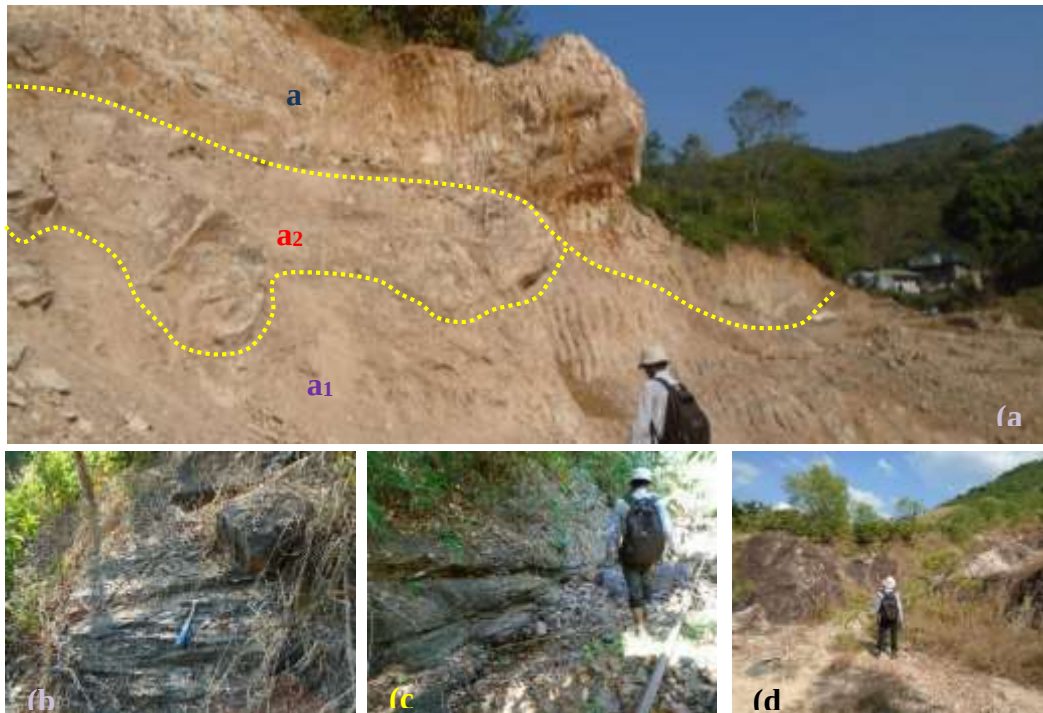


Figure 3. Rock units of Taunggyo Formation; (a) C_{1t}^1 unit, [a₁] = Intercalated with shale and mudstone [a₂] = mudstone [a₃] = sandstone (b) Black slate of C_{1t}^1 unit (c) fossiliferous mudstone and argillite of C_{1t}^2 unit (d) thick bedded quartzo sandstone and friable sandstone of C_{1t}^3 unit

Brachiopods were mainly preserved fossiliferous mudstone and argillite of C_{1t}^2 unit of Taunggyo Formation in the study area. Some brachiopods and associated fossils occur in the Taunggyo Formation are mentioned by Kyaw Htin Khaing (2001), Zaw Win (2009), Ohmar Soe Yin *et al.* (2018), and Hai Peng Xu *et al.* (2021). The systematic work on the fossils and their collection from the following measure section Figure 4.

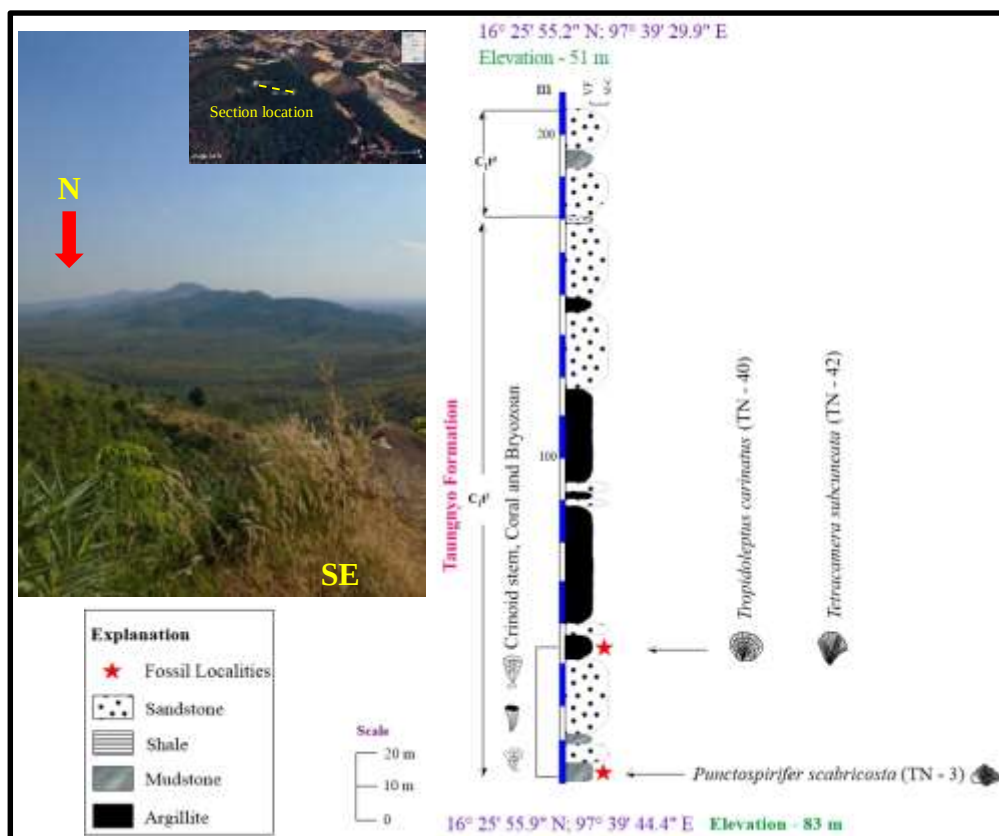


Figure 4. Stratigraphic measure section and distribution of fossil in the Taungnyo Formation

The Taungnyo Formation is overlain by Moulmein limestone (Permian age) with an unconformity. Moulmein Limestone Group is exposed as isolated hills on the alluvial plains at the eastern and western part of the Taungnyo Range. Moulmein Limestone Group is mainly composed of carbonate sedimentary rocks with a general trend of NW-SE direction.

Systematic Paleontology

Morphological terminology and classification of brachiopod follow by *Treatise on Invertebrate Paleontology* (Moore R. C., 1965).

Phylum BRACHIOPODA Dumeril, 1806

Order SPIRIFERINIDA Ivanova, 1972

Suborder SPIRIFERINIDINA Ivanova, 1972

SuperFamily SPIRIFERINADEA Davidson, 1884

Family SPIRIFERINIDAE Davidson, 1884

Genus *Punctospirifer* North, 1920

Punctospirifer scabricosta White, 1860 (Fig 5)

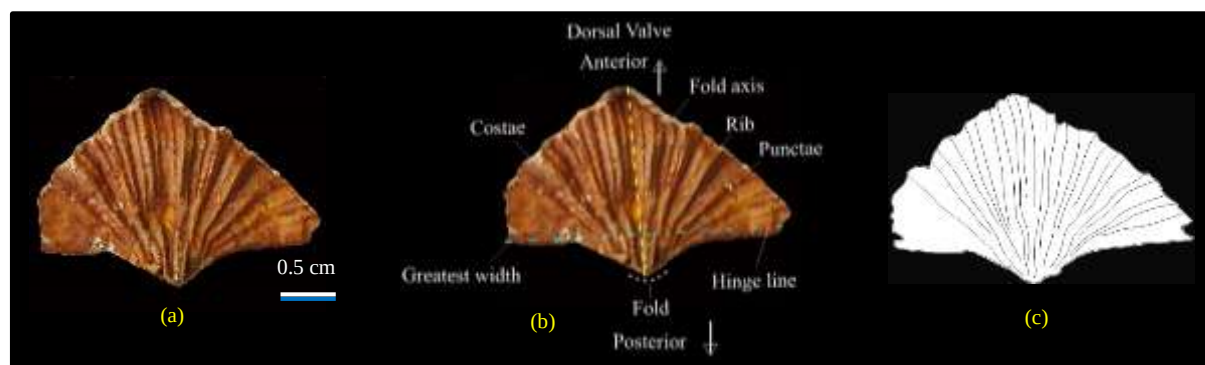


Figure 5. *Punctospirifer scabricosta* from C₁t² unit of Taungnyo Formation. (a) Exterior view of dorsal valve complete specimen, (b) terminology of the fossil specimen (YUDG-MlmTn 3; X 3), (c) Sketch of the shell outline

Material—External shell (YUDG-MlmTn 3)

Type Locality—West of Shwekyatkyā Monastery, **Southeast** of Pokukku monastery.

Diagnosis—Shell small, slightly ventribiconvex, slightly wider than long, greatest width along hinge line; the outline of brachial valve sub-semicircular; ventral interarea of moderate height, to nearly orthocline; beak moderately incurved over interarea, notothyrium open, triangular; dorsal interarea not developed; fold and sulcus wide, smooth, sharply-delimited, each flank with about 4 undivided plications which decrease in size laterally; surface of both valves marked by distinct capillae and numerous, closely-spaced growth lines, giving shell a lamellose appearance; shell substance sparsely punctate; dimensions of a complete specimen: Ventral valve with short dental lamellae and median septum; dorsal valve with cardinal process of comb-like cross section and low median ridge along fold; crural plate lacking (Figure 5).

Description—Shell medium sized, distinctly triangular in shape, strongly transverse, widest at hinge, strongly, sub-equally biconvex; Shell with large, densely spaced punctate. Specimens are maximum length of 10.7 mm, width 27.7 mm. Pedicle valve strongly extended laterally, characterized by strong, sub-rounded sulcus, not flaring; sulcus somewhat deeper than lateral grooves; valve with 3 or 4 sub-angular to sub-rounded, simple lateral plications; plication bounding sulcus slightly broader, higher than lateral plications, lateral extremities without plications, flat, smooth. Pedicle valve is interior with strong hinge teeth, median septum; remainder of characters of both valve interiors unknown.

Discussion—This species is a common element of the *Calvustrigistruther fordii* assemblage of the eastern faces, although it is not usually found in large numbers. The Banff specimens agree in all external respects with authentic specimens from the midcontinent. Allan, Warren, and Rutherford (1932, p. 239), Brown (1952, p. 103), and Nelson (1960, Pl. 5, Figure 19) previously reported this species from the Jasper Park region.

Order TEREBRATULIDA Waagen, 1883

SuperFamily ENTELETOIDEA Waagen, 1884

Family TROPIDOLEPTIDAE Schuchert, 1896

Genus *Tropidoleptus* Hall, 1857

Tropidoleptus carinatus Conrad, 1839 (Figure 6)



Figure 6. *Tropidoleptus carinatus* from C_{1t}² unit of Taungnyo Formation. (a) Exterior view of ventral valve complete specimen, (b) terminology of the fossil specimen (YUDG-MlmTn 3; X 2.8), (c) Sketch of the shell outline

Material—Internal shell (YUDG-MlmTn 40)

Type Locality—Occurs in the Pokukku Chaung and near the **Northeast** of Pokukku Monastery.

Diagnosis—Rounded and oval shape, curl growth line, sharp, large, depressed, non-carinate *Tropidoleptus carinatus* with low, short, and thin dorsal median septum and low rounded costae.

Description—The shell is very low, planoconcave, weakly unisulcate, rather thick-walled, with cardinal extremity obtuse and never mucronate. The size is unusual and very large for the genus; the largest specimen examined attains 27.3 mm width. The shell wall is densely exopunctate. The ventral valve is transversely oval, 20.2 mm of the maximum length. The lateral margins are evenly rounded; the anterior margin is only weakly curved. The ventral valve is gently and evenly concave in a transverse profile and weakly and unevenly convex in a lateral profile, with the maximum convexity and depth in the posterior third (Figure 6).

Discussion—Specimens from various stratigraphical levels may be referred to typical subspecies *T. carinatus* (Conrad, 1839). The size of the specimens does not exceed 25 mm and falls into usual size range of the type subspecies. The specimens of the Upper Pragian (sample H 1052) age have fine dorsal median septum perfectly developed already in very small individuals (pl. 5, Fig 19). Inner periphery of these small shells bears bundles of delicate radial ridges. Adult specimens are some 20 mm wide and have weakly convex ventral valve devoid of distinct carination. Specimens from stratigraphically higher beds, referred to the Upper Emsian, Emsian/Eifelian boundary interval and Eifel a have commonly smaller shells, with width about 15 mm. The ventral valve is considerably convex and, in some specimens, also distinctly carinate.

Family TETRACAMERIDAE Likharev in Rzhonsnitskaja, 1956

Genus *Tetracamera* Weller, 1910

Tetracamera subcuneata Hall, 1858 (Cited by Butts, 1922) (Figure 7)

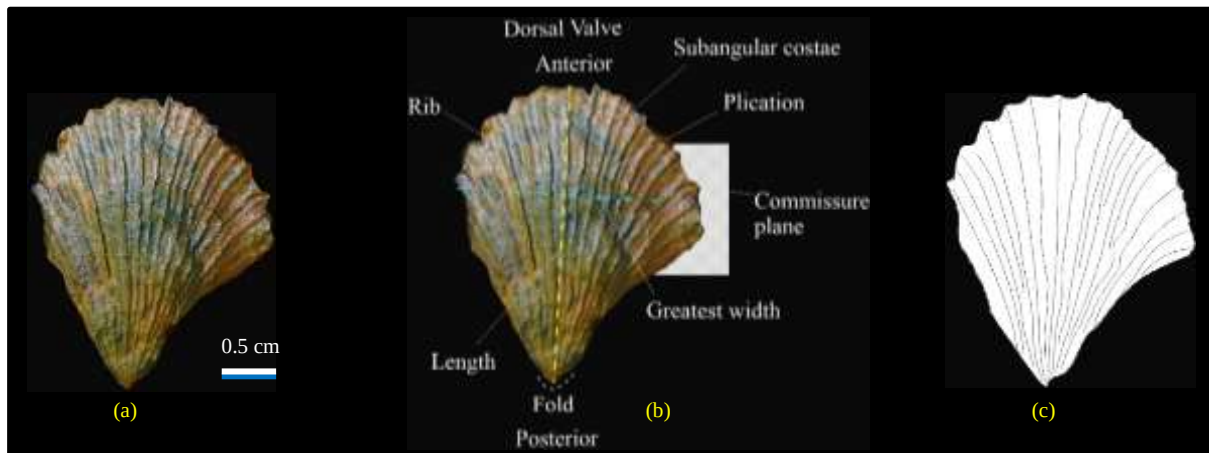


Figure 7. *Tetracamera subcuneata* from C₁^{t2} unit of Taungnyo Formation. (a) Exterior view of dorsal valve complete specimen, (b) terminology of the fossil specimen (YUDG-MlmTn 42; X 2), (c) Sketch of the shell outline

Material—External shell (YUDG-MlmTn 42)

Type Locality—Occurs in the Pokukku Chaung and near the **Northeast** of Pokukku Monastery.

Diagnosis—Small or medium-sized; acute apical angle; commissure plane or weakly uniplicate; costae sub-angular; capillae may develop. Septalium open; lateral lamellae support hinge below dental sockets and rest on floor of brachial valve on either side of median septum.

Description—Shell rhynchonelli form and elongated outline, small or medium size, triangularly sub-ovate to sub-pentagonal in outline, the medial fold, and sinus poorly developed or nearly obsolete except towards the front, the lateral and anterior margins of both valves deflected so as to stand at nearly right angles to the plane of the valves, both valves marked by simple, sub-angular or rounded plications which reach to the beak. The maximum width is 26 mm and length is 28 mm (Figure 7).

Discussion—The species included in this genus were referred to *Camarophoria* by Hall and Clarke, and usually have been so referred since the publication of their work. The shells do not possess the characteristic cruralium of *Camarophoria*, besides possessing other characters wholly foreign to that genus. The most essential generic characters are the presence of the buttress plates connecting the dental lamella with the inner surface of the outer shell wall, and the presence of the supplementary lamella in the rostra portion of the brachial valve in addition to the median septum. The species *T. subtrigona* which is here included in the genus differs in a rather fundamental manner from the genotype, not only in the general form of the shell but in the arrangement of the internal lamella, and should perhaps be made the type of a distinct genus but the discussion of these characters is reserved for treatment under the specific description.

According to the detail measurements, maximum length and width of *Punctospirifer scabricosta* (10.7/27.7mm), *Tropidoleptus carinatus* (20.2/27.3 mm), and *Tetracamera subcuneata* (28/26mm) as shown in (Table 1) and (Figures 8-11).

Table 1. The Measurements of brachiopod fossils from the Taungnyo Formation, Mon State, Myanmar. Abbreviations: ML = Maximum Length, MW = Maximum Width, SC = Space of Costae

Taxon	Specimen No.	Measurements (in mm)						
		ML	MW	Space of Costae SC - 1 to SC - 15				
<i>Punctospirifer scabricosta</i>	YUDG-MlmTn 3	10.7	27.7	5.1	2.2	3	3.8	4
				3.6	2.9	2.9	2.5	4.2
<i>Tropidoleptus carinatus</i>	YUDG-MlmTn 40	20.2	27.3	2.4	2.5	2.5	2.4	2.4
				2.3	2.6	2.4	2.5	2.7
<i>Tetracamera subcuneata</i>	YUDG-MlmTn 42	28	26	1	0.8	1.2	1	1.3
				1	1	1	1	1.2
				1.2	1.4	1.7	1.3	-

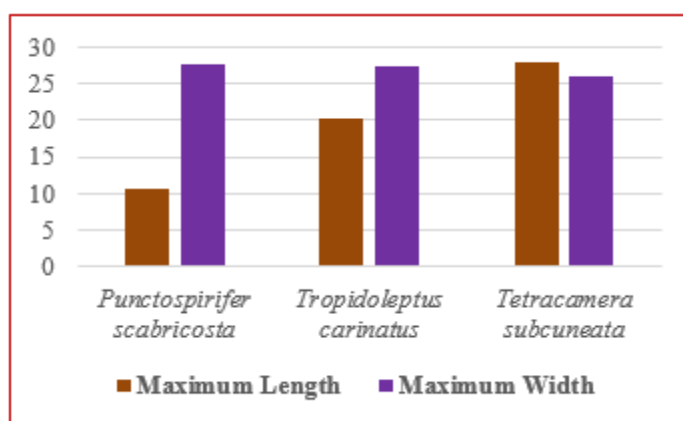


Figure 8. The columnar chart showing maximum length and maximum width of brachiopods

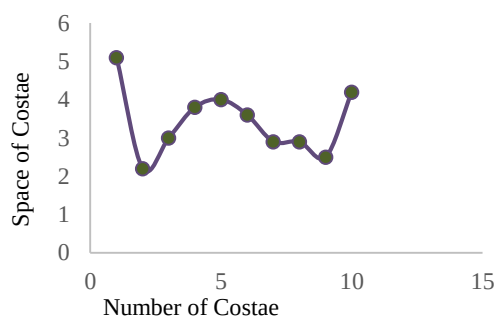


Figure 9. Scatter line and markers of costae measurement; *Punctospirifer scabricosta*

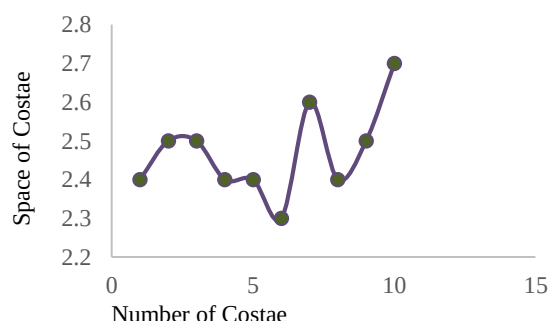


Figure 10. Scatter line and markers of costae measurement; *Tropidoleptus carinatus*

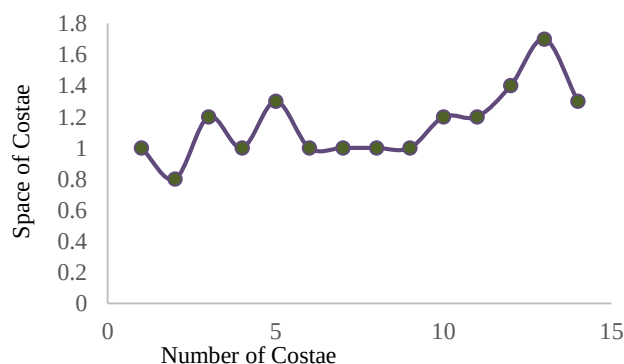


Figure 11. Scatter line and markers of costae measurement; *Tetracamera subcuneata*

Paleoecology

In this section, the discovery of brachiopod fossils is mostly sessile epibenthic, epifaunal, suspension feeders, and dwellers illustrated in Table 2. Some may have become deposit feeders and dwellers. The Devonian to Carboniferous seas were dominated by the most brachiopods. The Early Carboniferous brachiopods include; *Punctospirifer scabricosta* and *Tetracamera subcuneata* are display compressed geometries which facilitated suspension on fluid substrates and may have benefited respiration under lower oxygen tension. Late Devonian - Carboniferous brachiopod such as *Tropidoleptus carinatus* is live only in the sea, and most species avoid locations with strong current wave. Now, brachiopods are live mainly in cold water and low light. The occurrence of Late Devonian brachiopod fauna from inhabited deep water soft bottom settings located presumably below the photic zone (Szulczewski 1971; Biernat and Racki 1986a).

Hence, the abundant faunas are indicated deposition in a normal salinity and clear water. Another explanation suggests that the lack of shallow water facies, presence of distinct grading, and resemblance of Bouma's sequence strongly indicates the Taungnyo Formation were deposited in submarine fan (Day Wa Aung, 2014). Therefore, the depositional environment of Taungnyo Formation is probably submarine fan. These macrofossils found within the mudstone and argillite was considered when determination the depositional setting during the Middle Devonian time.

Table 2. Ecology of the brachiopod faunas in the Taungnyo Range

Formation/Group	Age	Taxon	Localities and Coordinates	Ecology
Taungnyo Formation	Early Carboniferous	<i>Punctospirifer scabricosta</i>	Road cut 16° 25' 51.5" N 97° 39' 44.7" E	Stationary epifaunal suspension feeder
	Late Devonian- Carboniferous	<i>Tropidoleptus carinatus</i>	Stream section 16° 25' 56.9" N 97° 39' 38.9" E	Ubiquitous dwellers
	Early Carboniferous	<i>Tetracamera subcuneata</i>	Stream section 16° 25' 56.9" N 97° 39' 38.9" E	suspension feeder

Results and Discussion

This study mainly based on the morphological characteristics, three different species recognized and find-out the first recorded paleontological data from the Taungnyo Formation. The three brachiopods species were recognized to their morphological features and done by good identification. According to the findings, the fossil brachiopods was discovered colonizing of other fossils such as crinoid stems, corals, and bryozoans in the C_{1t}² unit which it located in very narrow site. Among these fossils, usually common and abundant brachiopod faunas in the study area. The brachiopods were collected from two different localities and fossils distribution sites in the northern part of the Taungnyo Range (Figure 12).

Morphologically, a comparison of different shell outlines is clearly. *Punctospirifer scabricosta*; width is wider than length and the ribs are distinct larger than other species. *Tropidoleptus carinatus* is rounded-shape and *Tetracamera subcuneata* is elongated shell outlines. These species differ from the Zweekabin Range brachiopods of Taungnyo Group. The observed brachiopod faunas are mainly formed by solitary and both of complete pedicle and brachial valve. Until recently, the systematic investigation is less and also paleontological workers are limited. It is remaining the more detail studies of paleontological evidence. This paper is a contribution to the taxonomic classification of the brachiopod fauna and suggest that the paleoecology and geological age of Taungnyo Formation. Moreover, Middle Devonian brachiopods such as *Fimbrispirifer venustus* and *Ovetensispirifer ovetensium* are described by Ye Yint Aung & Chit Sein (2022). Therefore, the age of the Taungnyo Formation is probably Middle Devonian age and should be amendment the geological age according to the discovered brachiopod assemblages.



Figure 12. Showing fossil sites of the C_{1t}^2 unit of Taungnyo Formation in the Taungnyo Range

Conclusions

The present paper describes three brachiopods species and study detail morphological features. Different type of shell outlines and their morphological features are clearly distinct. This study mainly focused on fossil brachiopods and analyzed here from the C_{1t}^2 unit of Taungnyo Formation.

I collected fossil specimen number (YUDG-MlmTn 3), (YUDG-MlmTn 40), and (YUDG-MlmTn 42) in the study area. Faunal content of the brachiopods, especially *Punctospirifer scabricosta*, *Tropidoleptus carinatus*, and *Tetracamera subcuneata* are mentioned in this paper. These fossils are found at the northern portion of the Taungnyo Range especially which are located in the Pokukku Chaung section and along the footpath of Shwekyatkya Monastery.

The recent discovery of fossil sites deserved to be designated as geoheritage site, Taungnyo Range geopark, and regional geologic significance. Furthermore, these latest findings are important for geological age of Taungnyo Formation and one of the significantly recorded of Myanmar Geosciences.

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Conflict of Interest (COI)- The author declares no conflict of interest in this paper.

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PALEOECOLOGY OF HOLOCENE FORAMINIFERA SPECIES IN SMART ISLAND, MYEIK TOWNSHIP, TANINTHARYI REGION, MYANMAR

Hnin Hnin Maung¹, Myint Swe² and Htet Akar³

Abstract

The study area is located in Myeik City, the Tanintharyi Region of Myanmar. It is situated in the extreme south of the country on the coast of an island, the Andaman Sea. Myeik Archipelago lies off the far south coast of Myanmar. Samples from the Smart Island and Two-Face Island generally contained medium sand and fine sand, with minor silt, lime, and mica flakes. The (3) specimens are collected from Smart Island and Two-Face Island. All the dried residues were sieved into three fractions with mesh sizes of 30, 60, and 90. After sieving the samples, the dry samples were examined under a binocular microscope, and all the foraminifer specimens were found to be well preserved. The present study aims to focus on the distribution of foraminifer assemblages in the Andaman Sea, which is part of the complex geological setting. This result is mainly focused on the paleoecology of Holocene Foraminifers. The study areas reveal that (116) genera have been recorded belonging to (145) species. There are (36) Holocene index fossils that occurred in the study area. *Textularia conica* occurred in the area between 1000 and 3500 m of water depth, and *Eponides* sp., *Lenticulia* sp., and *Textularia* sp. arise in the deeper inner shelf to middle shelf. *Bolivina* sp and *Bulimina pupoides* are befallen in the outer shelf. Smart Island is the most populated and diverse area in the study area. According to this evidence, Smart Island is deeper than Two Face Island. Therefore, the western part of the study area is deeper than the eastern part of the study area. An attempt has been made to correlate the Bengal Basin fauna with the known Holocene fauna from the Atlantic and Pacific regions. This research has implications for the paleoceanography of Andman Sea.

Keywords: Smart Island and Two-face Island, 36 Holocene index species, brackish to abyssal plain.

Introduction

Myeik is a city in the Tanintharyi Region of Myanmar, located in the extreme south of the country on the coast of an island on the Andaman Sea in figure (1). Myeik Archipelago lies off the far south coast of Myanmar. It's comprised of hundreds of islands that have coral reefs and beaches. Myeik is the largest city in the Tanintharyi Region and is the regional headquarters of Myanmar Navy's Tanintharyi Regional Command. The area inland from the city is a major smuggling corridor into Thailand. The results of this study indicate a significant ongoing change in the water environmental characteristics of the Andaman Sea. benthic foraminifers are abundant around the Smart Island and the Two Faces Island.

Purposes of Study

The main purpose of the study areas are as follows:

- (1) To identified the Foraminifera species
- (2) To interpret the relationship between the distribution of benthic foraminifera and environmental conditions
- (3) To determine the age of the Foraminifera
- (4) To reconstruct the Holocene paleoceanography and sedimentary environments

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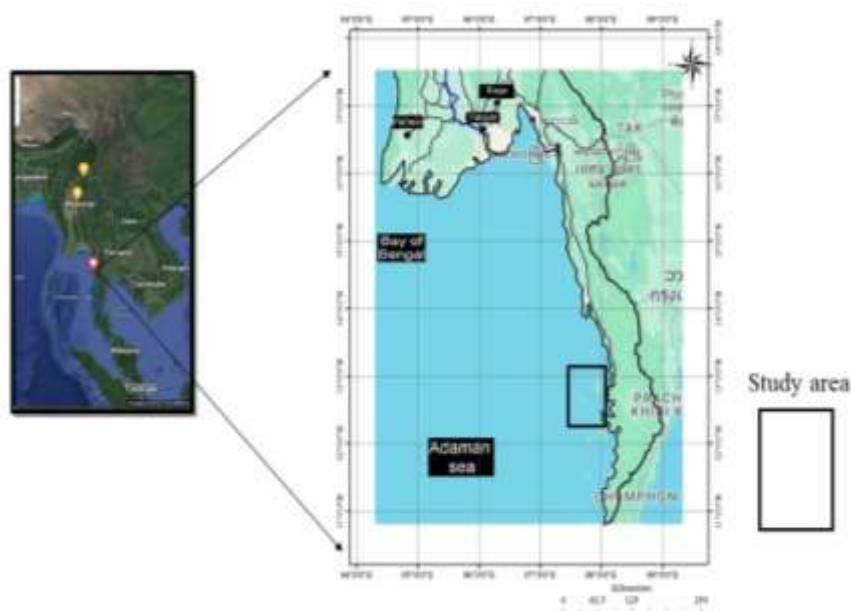


Figure 1. Location Map of the study area

Previous Works

Chit Saing and Tin Myint (1971) studied a total of (157) foraminifer species collected from the shallow water sediments of the Zalattaung area, located about a mile west of the Thandwe (Sandoway), Bay off Bangle of Rakine Coast. The samples were grab samples, all of which were collected by personnel from the Burma Navy Hydrographical Department on board “Thutayti”.

Ko Yi Hla (2009) carried out several studied on surface sediments of the Ayeyarwady continental shelf, such as sediment distribution, sediment dispersion, the relationship between tidal influence and sediment supply. He mainly focused on the detailed study of recent foraminifer species present in the seafloor sediments based on 22 grab samples.

Khin Mar Than (2023) carried out a marine survey along the Bay off Bangle the Rakhine Coast, Ayeyarwady Delta Shelf, and Tanintharyi Coast, and collected 26 sediment sample of Kasten Core. A total of (175) species had been identified and described in her Ph.D thesis.

Methods of Study

The beach samples were collected at low tide during the daytime from the constant depth (1.5m). The beach samples were collected from the east to west of the Smart Island and Two face Island in figures (2 and 3). All the wet samples were (about 50g) treated with water containing a little amount of soda ash and detergent and left for a day. These samples were again washed over the steel sieve of 150 mesh thoroughly. The residue obtained was dried and stored into sealing plastic bags. The dried residue with a weight of 10 gm was sieved again through mesh 30, 60, and 90 respectively. The beach sand samples from northern most part including Smart Island are rich in a larger number of Holocene foraminiferal fossils, all of which are well preserved. Handling by means of a sable hair brush is convenient in picking the residues under binocular microscope.



Figure 2. Field group photo in Smart Island (Par pant Kyun)



Figure 3. Location of the smart island

Regional Geologic Setting

Tanintharyi Region is composed of a north-south trending, narrow, mountainous, coastal stretch of the mainland part of Myanmar. The territory is about 150 km (93 miles) north of Aceh in Indonesia and is separated from Thailand and Myanmar by the Andaman Sea. It comprises two island groups, the Two-Face Island (Two Sand Line Beach) and the Smart Island (Par Pant Kyun) islands. Two Face Island is located 46 miles away from Myeik City, and Smart Island is located 51 miles away from Myeik City. The study area lies in the Shan-Tanintharyi Block and the southern continuation of the Tanintharyi Ranges. The regional geological map of the study area is shown in figure (4).

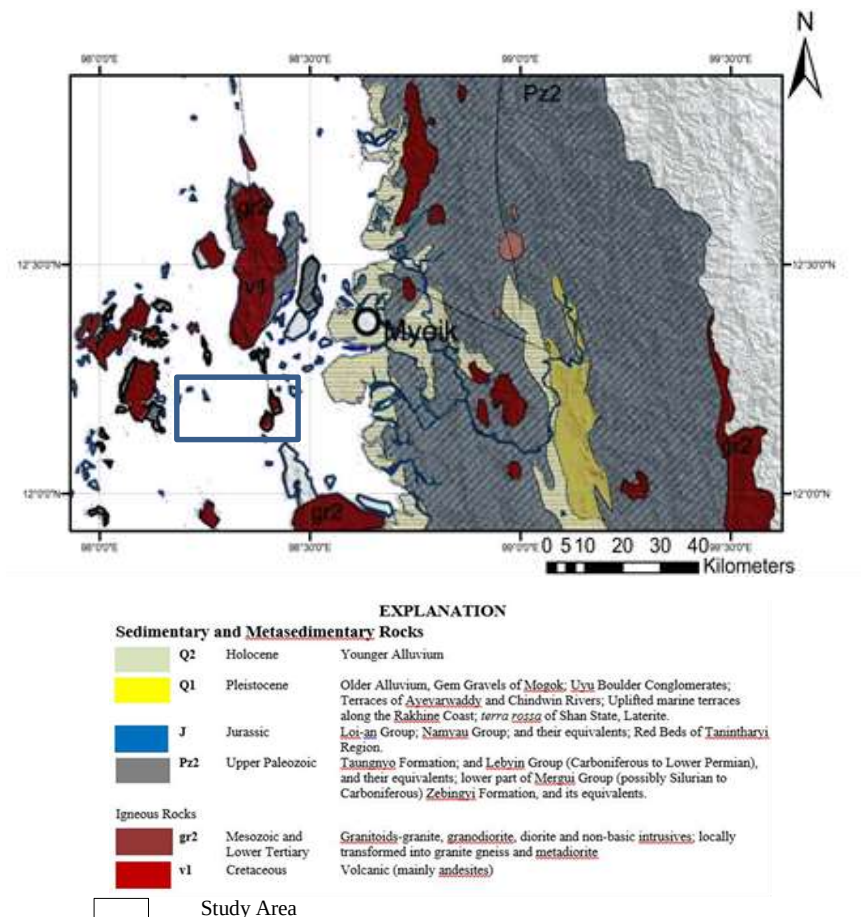


Figure 4. Regional Geological Map of the Study Area (after MGS, 2014)

Faunal Analysis

The present study has revealed that (116) genera have been recorded, belonging to (145) species in Table (1). The sediment in the areas are mostly composed of medium sand, although some sample collected near the shore contain mixtures of detrital grains such as silt, lime and mica flakes. An impressive form of the specimens contain 22%, with cylindrical form and lenticular form species dominating at about 60%, followed by arenaceous fauna at approximately 10%, and fusiform foraminifers at about 25%. The biserial foraminifera are founded minor amount. Some arenaceous species are *Bolivina robustus bardy*, *Bolivinita quadrilateral Schwager* and *Textularia fuliacea*, *Elphidium advenum*, *Quinqueloculina cultrate*, *Ammobaculites agglutinans*. These have also occurred in the study area, with a high salinity and Ph value.

Populations and their Distribution in Relation to Environmental Factors

The study report that *Hanazawa nitidula* var.1 is the highly populated in the smart island. *Nonion stelligerum*, *Quinqueloculina seminulum linnefcus*, *Rectoglandulina glans* are the lowest populated in the study area. The total population of species in Smart island is (141) species, northern part of Two-face island is (93) species and (58) species is in the southern part of Two-face island in Table (1). The total diversity of species in Smart island is (67) species, northern part of Two-face island is (64) species and (39) species in the southern part of Two-face island. All of the above evidences, Smart island is the most populated and diversity in the study area in figures (5) and (6). According to the Isreasky (1949) described that, if the continuous decrease in species number basin becomes continuously shallowing and continuous increase in species number, basin becomes continuously deepening. According to this evidence, Smart Island has deeper than Two-face Island. Therefore, the western part of the study area has deeper than the eastern part of the study area.

Quinqueloculina Lamarchia is abundant in the study area between 0-20 m water depth and occurred in inner shelf. *Nonion stelligerum* is also occurred in inner shelf. *Elphidium crispum* is dominates in the area between 0- 100 m water depth. *Bolivina antiqua* is dominates in the area between 100-1000 m water depth and found in outer shelf. *Textularia conica* occur in the area between 1000- 3500 m water depth.

In the study area, 41 species occurred in the shallow marine. *Quinqueloculina*, *Nonionella*, *Textularia*, *Lenticulina*, *Eponides* and etc. occurred in the inner continental shelf. Some species such as *Lenticulina*, *Bulimina*, *Bolivina* etc. occurred in the middle continental shelf to outer continental shelf. *Pygro* species sentient in abyssal plain (by Berggren and Boersma, 1978). So, all of the above species are distributed from outer continental shelf to abyssal plain in figure (7).

Table 1. Showing the distribution of foraminifera species in Andman Sea, Myeik Township

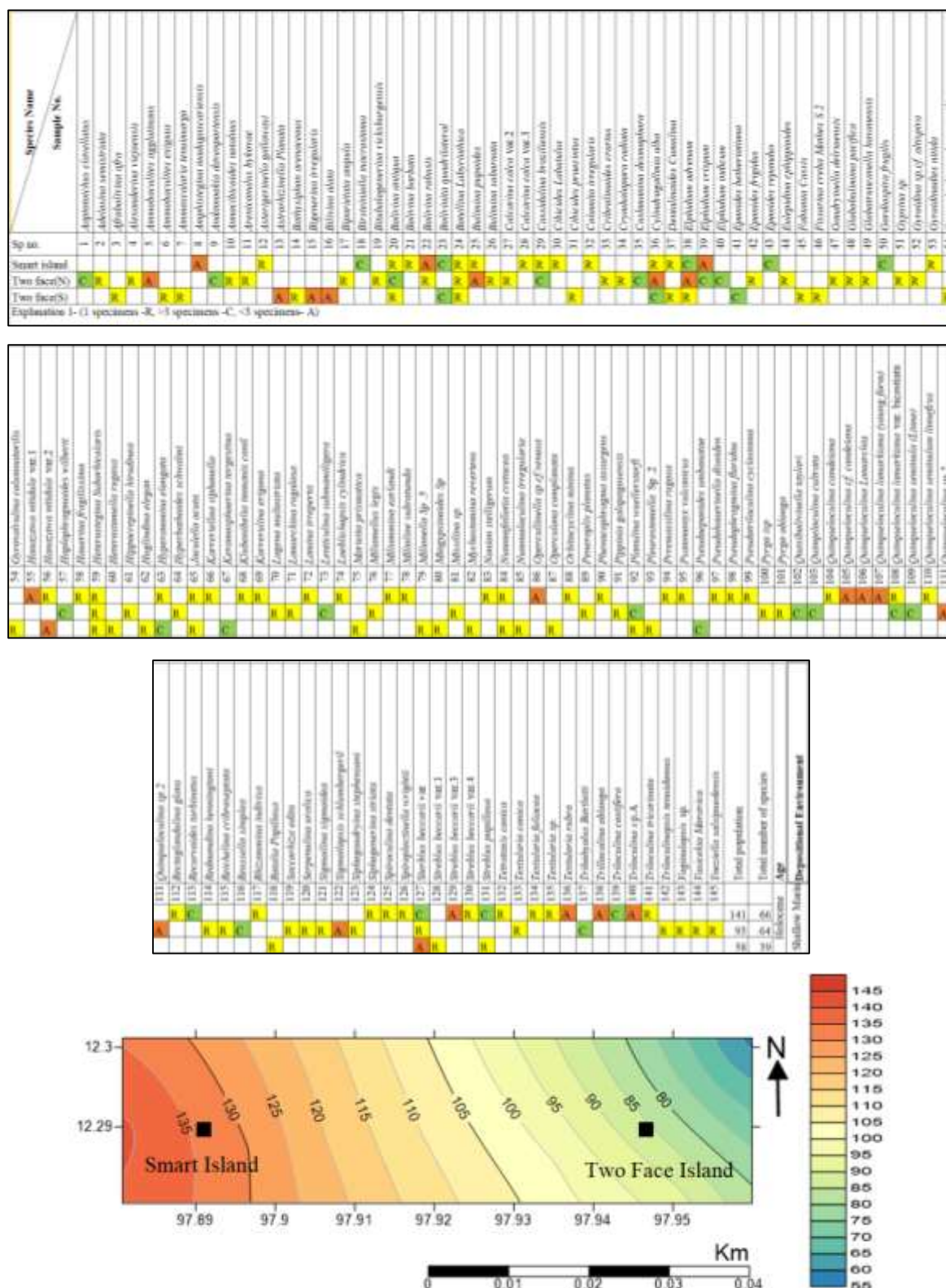


Figure 5. Population density of the Smart Island and Two Faces Island

Conclusion

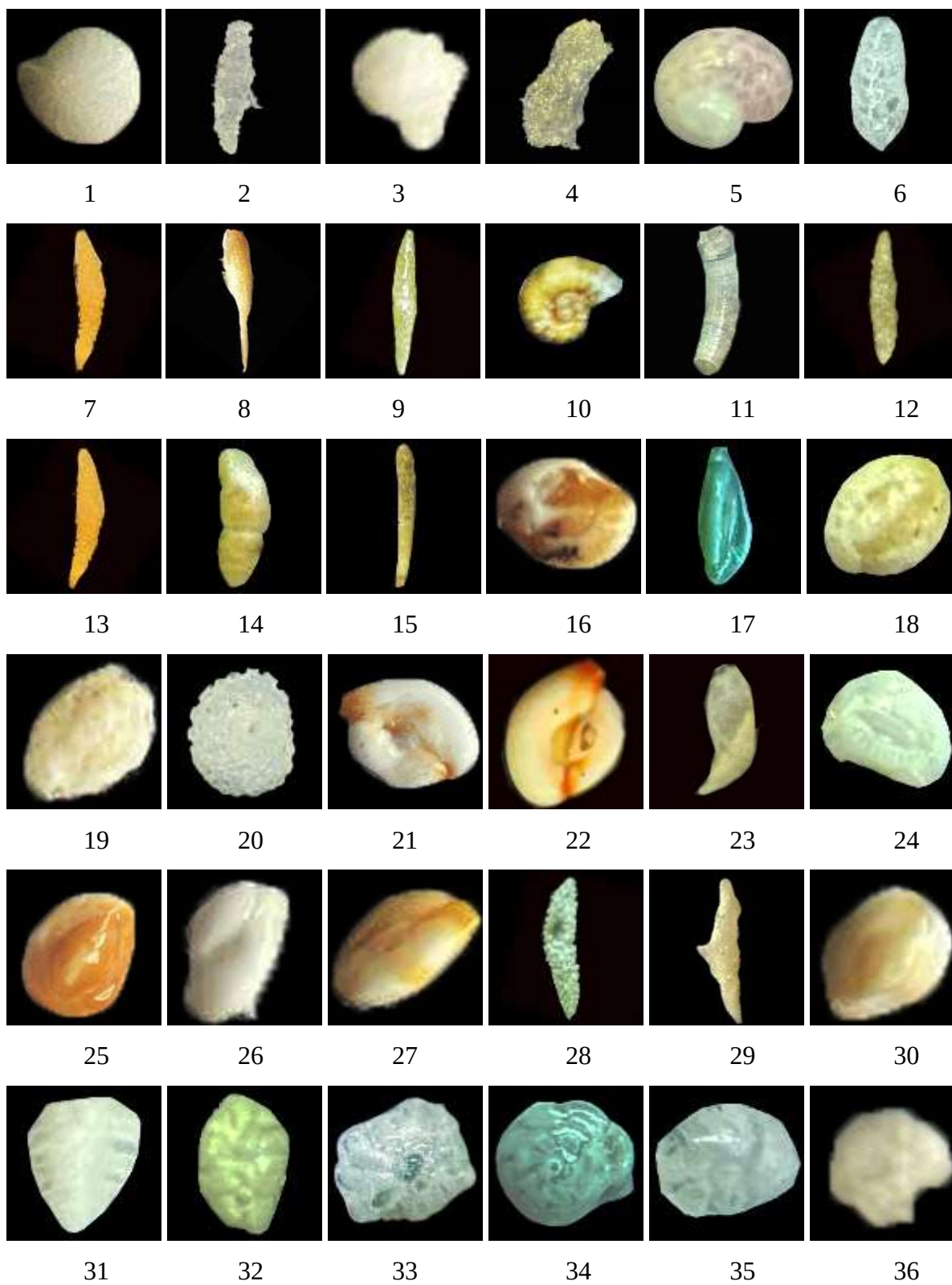
Myeik is a city in the Tanintharyi Region of Myanmar, located in the extreme south of the country on the coast of an island on the Andaman Sea. The research aims to improve understanding of the taxonomy and paleontological aspects of foraminifers in the study area. There are (116) genera, belonging to (145) species. There are (36) Holocene index fossils are recorded in the study area. Smart Island is the most population and species diversity in the study area. According to this evidence, Smart Island has deeper than Two-face Island. Therefore, the western part of the study area has deeper than the eastern part of the study area. *Textularia conica* occur in the area between 1000 to 3500 m water depth and *Eponides* sp., *Lenticulia* sp., *Textularia* sp. are occurred in the deeper inner shelf to middle shelf. *Bolivina* sp and *Bulimina pupoides* are occurred in the outer shelf. In the study area, most of the species are occurred in inner shelf to abyssal plain. An attempt has been made to correlate the Adman Sea fauna with the known Holocene fauna from the Atlantic and Pacific regions.

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1mm

Explanation of Plate I

1. *Alexanderina viejoensis*
2. *Ammoscalaria tenuimargo*
3. *Annocibicoides notalhus*
4. *Astrorhizinella planata*
5. *Birsteiniolla macrostoma*
6. *Bolivinita quadrilutera*
7. *Botellina labyrinthica*
8. *Cushmanina desmophora*
9. *Cylindrogallmia alba*
10. *Gordiospira frugilis*
11. *Hippocrepinella hirudinea*
12. *Hyperammina elongata*
13. *Jaculella acuta*
14. *Korrerulina erigona*
15. *Loeblichopsis cylindrica*
16. *Miliamellus legis*
17. *Miliammina earlandi*
18. *Milioline subrotunda*
19. *Missilina* sp.
20. *Mychostomina revertens*
21. *Pippinia galapagosensis*
22. *Prremassilina rugosa*
23. *Psammonyx vulcanicus*
24. *Pseudohauerinella disoiden*
25. *Pyrgo* sp.
26. *Pyrgo oblonga*
27. *Quinqueloculina seminula*
28. *Rhizammia indivisa*
29. *Saccorhiza edita*
30. *Sigmoilina sigmoidea*
31. *Spiroplectinella wrightii*
32. *Streblus beccarii* var
33. *Streblus beccarii* var.3
34. *Streblus beccarii* var.4
35. *Streblus papillosa*
36. *Trihohyalus Bartletti*

CHARACTERISTICS AND GENESIS OF LOW SULFIDATION EPITHERMAL GOLD DEPOSIT AT KYAUKMYET PROSPECT, MONYWA MINING AREA, CENTRAL MYANMAR: IMPLICATIONS FOR CONCEPTUAL GENETIC MODEL

Toe Naing Oo¹, Agung Harijoko² and Lucas Donny Setijadji³

Abstract

The Kyaukmyet prospect is situated in the Monywa mining area, Central Volcanic Belt (CVB), central Myanmar. The main objective of this paper writing is not only to describe the characteristics of low sulfidation epithermal deposit but also to modify the conceptual genetic model of research area. Gold mineralization hosted within rhyolite unit as colloform-crustiform quartz veins associated with gold and base metal (Zn and Pb) sulfide. The primary metallic minerals, in order of abundance, are pyrite, chalcopyrite, galena, sphalerite and gold; gangue minerals are mainly quartz, adularia, calcite, sericite, illite/smectite, and chlorite/epidote. The main types of wall-rock alteration comprise silicic, argillic and propylitic alterations. Microthermometric measurements of primary LV/VL fluid inclusion assemblages in quartz shows that the ore-forming fluids belong to low temperature (160-300°C), low-salinity (0.4–2.1 wt% NaCl equiv.). Estimated trapping hydrostatic pressures are 16 to 24 bars, corresponding to entrapment depths of 170 to 260 m below the paleowater table. The Kyaukmyet prospect is recognized as a low-sulfidation epithermal mineralization derived from a fluid source during a phase change in temperature and pressure interpreted as being related to fluid boiling. According to the characteristics of several varieties including wall rock alteration, ore mineralization and fluid inclusion analysis, our study may reveal to be classified deposit type and implication for conceptual genetic model at Kyaukmyet prospect, Monywa mining area.

Keywords: Wall rock alteration, Ore mineralization, Low-sulfidation, Boiling, Genetic model

Introduction

Gold mineralization at Kyaukmyet prospect is located in the western part of Sagaing Fault, Monywa mining area, central Myanmar (Figure 1). This area is northeast 3 km faraway from well-known Monywa mining area (high sulfidation type Cu deposit), central Myanmar. Small scale local gold rush and artisanal working were started since last thirty years ago. This area is also a part of the Central Volcanic Belt (Mitchell et al., 2011, Mitchell et al., 2012, Maung Maung Naing, 2008) which famous for their primary metallic minerals production and distinct geological feature. Generally, The Central Volcanic Belt (CVB) with a NW-SE trend is one of the most important metallogenic belts in Myanmar hosting numerous Cu ± Au±Ag porphyry, skarn deposits (Mitchell et al., 2011, Knight and Khin Zaw, 2015) but locally epithermal gold mineralization (including research area) is also spread along this belt (Figure 1). The geology and characteristics of the Kyaukmyet prospect indicate that it is a volcanic–subvolcanic hydrothermal deposit (Win Min Htet, 2008, Knight and Khin Zaw, 2015).

However, the prospect is still under exploration and lacks quantitative systematic research. The ore forming fluids characteristics and the gold mineralization processes is still

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poorly constrained. Here, we present the first detailed description of gangue and ore mineral assemblages, and outline the alteration and mineralization hosted by the Kyaukmyet prospect.

Combining these data with result of fluid inclusion microthermometric, our study provides insights into the genesis of the Kyaukmyet prospect and may have implication for the regional exploration of gold deposits in the Monywa mining area.

Geological Setting

Myanmar is located in a tectonic setting covering several metallogenic belts, with a high potential for the occurrence of economic tin-tungsten, lead-zinc-silver and gold-copper deposits (Figure 1). In the recent past in Myanmar, there were only small artisanal mines exploiting near surface oxidized zones for gold and copper. No systematic exploration for gold and copper had been carried out using modern exploration techniques; several gold and copper deposits cropping out remained unexplored. Myanmar has an enormous endowment of mineral deposits including resources of antimony, lead, zinc, copper, gold, tin, tungsten, chromium, nickel and precious jade, rubies, sapphires and diamonds. These mineral deposits are found within specific belts or provinces, and were formed during specific metallogenic epochs during the Phanerozoic (Gardiner et al., 2015). Copper and gold belts occupy the central portion of Myanmar, in the Central Volcanic Belt and west of the Sagaing Fault (Figure 1).

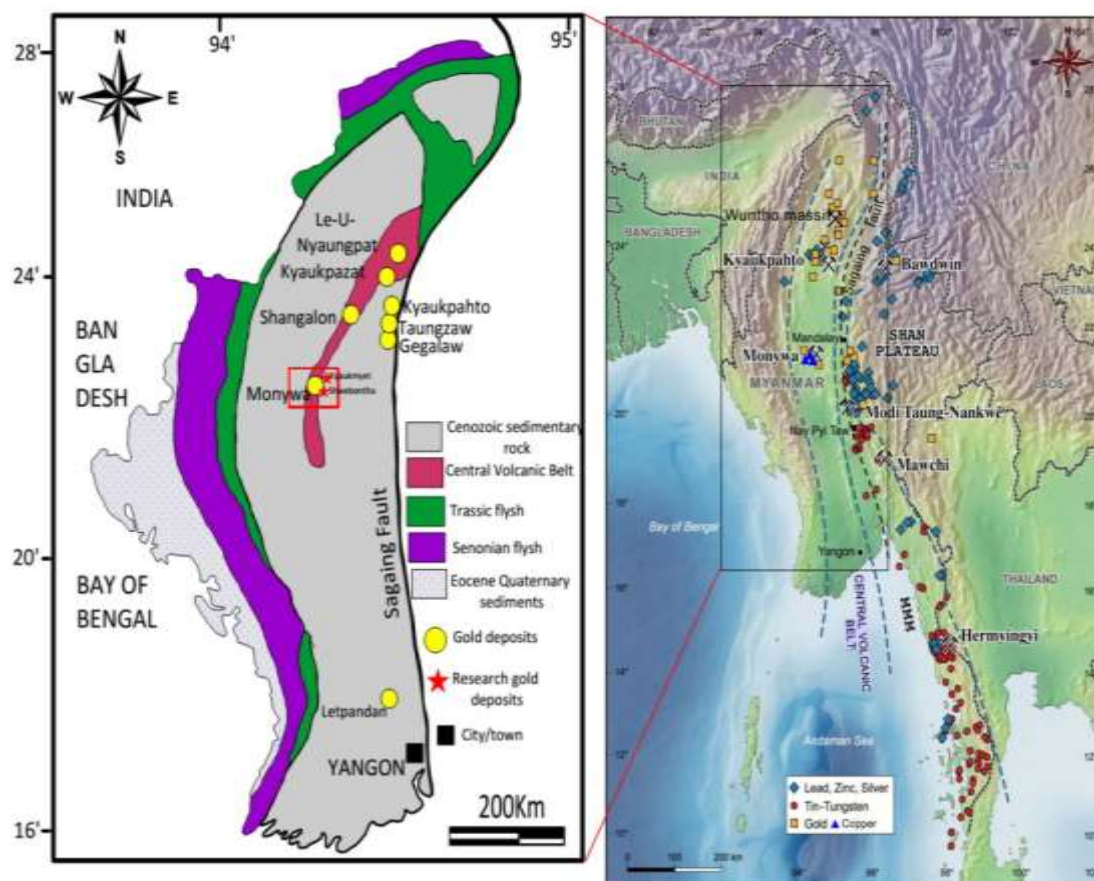


Figure 1. Map illustrating the location of N-S trending Central Volcanic Belt with some primary gold deposits (Modify after Gardiner et al., 2015).

Central Volcanic Belt is generally represented by a discontinuous belt of both plutonic and volcanic rocks. The Central Belt of the region is endowed with abundant precious and base metal resources and has been a source of gold and copper. Porphyry style Cu-Au and its related

epithermal Au deposits (e.g Shangalon, Le-U-Banmauk, Kyaukpazat, and Monywa mining area) are found along the Central Volcanic Belt. The Monywa mining area is located in the Central Volcanic Belt, within the central part of Myanmar (Figure 1). In the Monywa mining area, volcanic rocks are mainly intruded by the Upper Cretaceous age of granodiorites (Mitchell et al., 2011). In general, the basement rocks are mostly distributed by volcanic rocks including andesite, quartz andesite porphyry, dacite, and rhyolite in the Magyigon Formation (Figure 2). Most copper-gold (high-sulfidation Cu type) and gold-silver (low-sulfidation type) and base metal deposits between the Chindwin River and Powintaung sandstone are mainly generated by the Oligocene-Miocene Magyigon Formation (Figure 2).

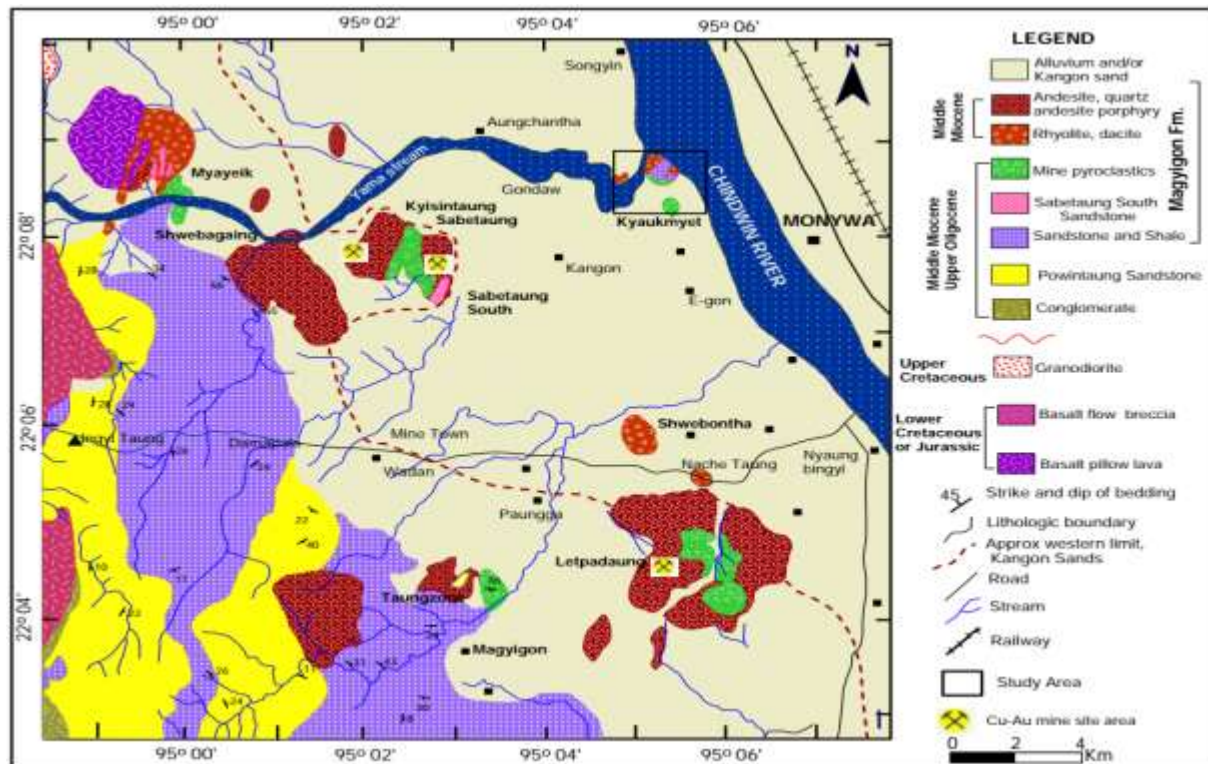


Figure 2. Simplified regional geological map of Monywa mining area and the black rectangle is the Kyaukmyet prospect (Modified after Mitchell et al., 2011).

Research Methods

Methods conducted in this research comprised of petrography, SEM-EDX and fluid inclusion method. A total of fifteen (15) samples were taken from the surface outcrops in the Kyaukmyet prospect, Monywa mining area: hydrothermally altered rock (7 samples) and mineralized quartz vein (8 samples). Ten samples were prepared for thin sections, doubly-polished thin sections, or polished sections. Thin sections and polished sections were determined petrographically to examine the primary and secondary (alteration) mineral assemblages. A detailed study on ore microscopy of polished thin sections using both transmitted and reflected light were performed to observe ore mineral assemblages and textural relationships. Some of the ore minerals were confirmed again by scanning electron microscopy with energy dispersive X-ray (SEM-EDX). Subsequently, a total of 5 representative ore samples were selected for fluid inclusion analysis in order to determine ore formation temperature and salinity. All laboratory analyses were done at the Department of Earth Resources Engineering, Kyushu University, Fukuoka, Japan.

Results and Discussion

Wall Rock Alteration

The hydrothermal mineralogy and spatial association of different alteration assemblages in the Kyuakmyet prospect was studied by detailed petrographic examination. Three main hydrothermal alteration types are distinguished in the Kyuakmyet: silicic, argillic and propylitic alterations (Figure 3). Silicic alteration is covered with mineralization vein but the groundmass is strongly silicified. There is a texture of bladed quartz, carbonate±adularia vein (Figure 3a,b). They are commonly exhibited lattice bladed calcite replaced by quartz, adularia and pyrite. Argillic alteration mostly occurs in rhyolite rock (Figure 3c). Argillic alteration is pervasive and altered-outcrops are generally ash white colour on the weather surface and soft (Figure 3c). Argillic alteration is characterized by the replacement of plagioclase by illite/smectite, quartz and minor amount of pyrite (Figure 3d). At locations that relatively distance to the mineralization, outcrops of propylitic alteration were found in the study area. As can be seen in Figure 3e, an indication of the propylitic alteration occurred in altered rhyolite which is shown by the greenish color of the outcrop. The greenish colour represents chlorite and epidote, the key minerals of propylitic alteration (Corbett and Leach, 1998). Under the microscope, the presence of chlorite, epidote, and sericite that selectively altered or replaced the groundmass plagioclase was observed in thin section (Figure 3f).

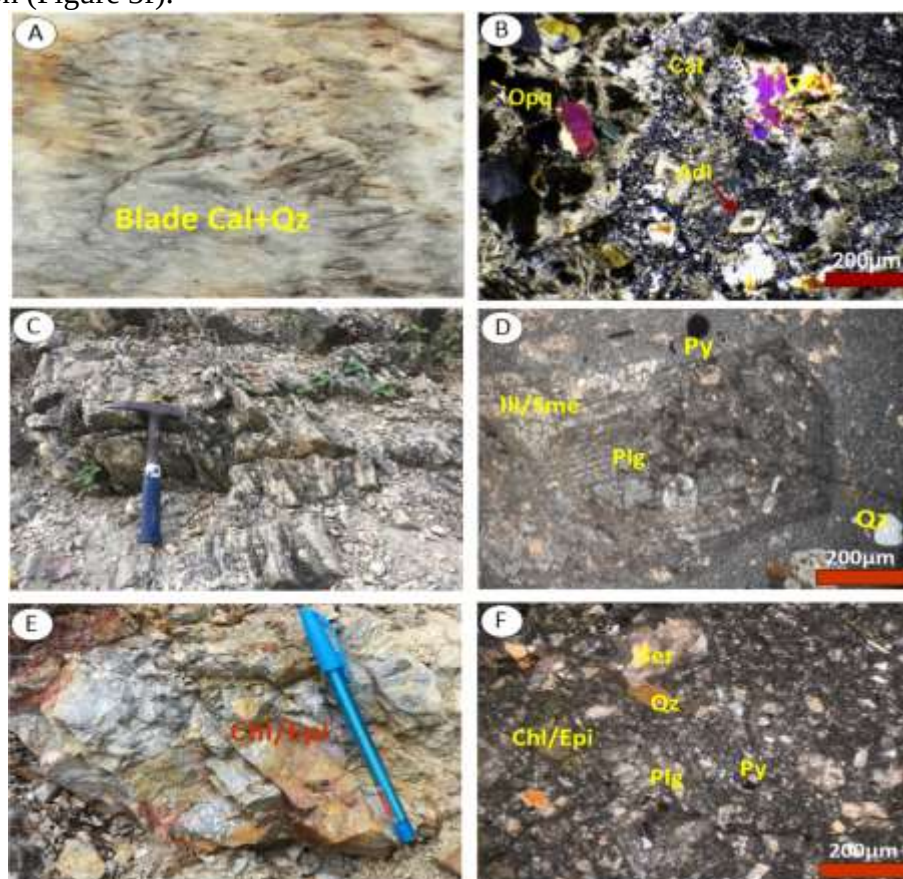


Figure 3. Outcrops of (a) the silicic alteration, (c) argillic-altered rhyolite rock and (e) propylitic altered rhyolite rock, (b,d,f) The results of petrographic examination of alteration mineral assemblages. Abbreviations: Py-pyrite, Adl-adularia, Chl-chlorite, Epi-epidote, Ser-sericite, Ill-illite, Sme-smectite, Qz-quartz, Cal-calcite, Plg-plagioclase, Opq-opaque

Mineralization Characteristics

The mineralization within the Kyaukmyet prospect is hosted by massive altered rock and gold-base metal-bearing colloform-crustiform quartz–sulfide veins, both of which are hosted by rhyolite rock of the Magyigon Formation. Based on megascopic and microscopic observations, the veins are made up of silica minerals particularly quartz and sulfide minerals (pyrite, chalcopyrite, galena, sphalerite and electrum/gold). The texture of the veins based on megascopic observations consist of colloform-crustiform texture of gold-base metal bearing quartz veins (Figure 4). Mineralization veins mostly occur as replacement and fracture filling types in rhyolite rock unit.

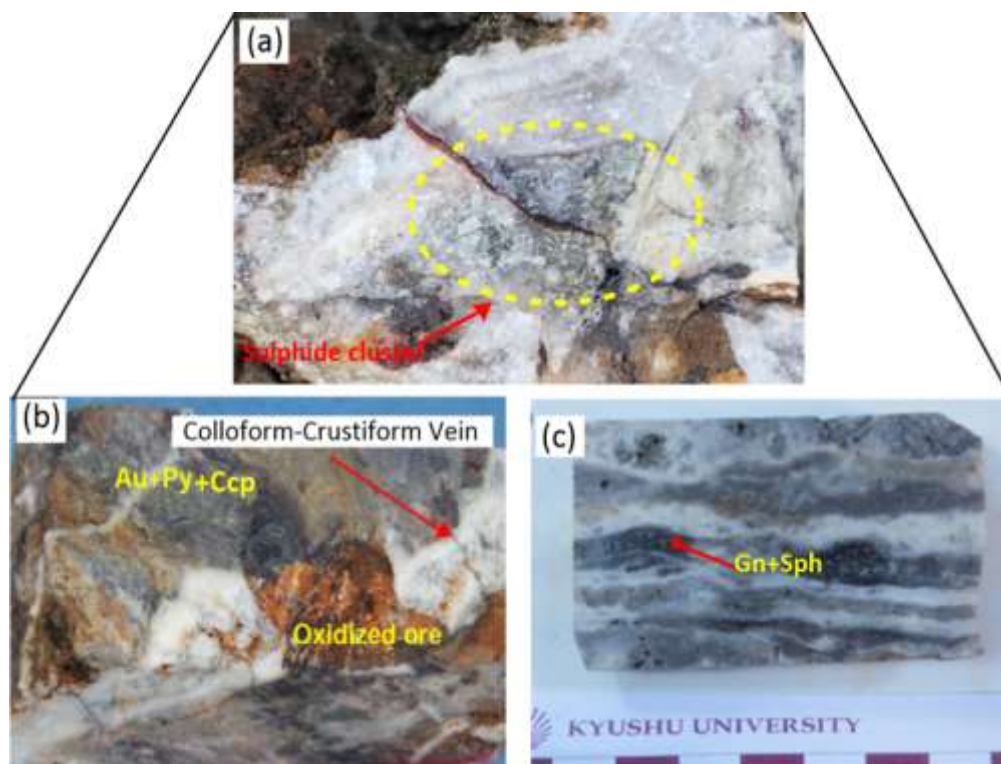


Figure 4. (a) Outcrop nature of symmetrical concentric banding of colloform-crustiform quartz vein texture (b,c) Hand specimens showing colloform-crustiform textures of gold-base metal bearing quartz veins in rhyolite host rock at Kyaukmyet prospect.

Megascopic observations and ore microscopic observations show that the principal ore minerals present in the study area include: pyrite (FeS_2), galena (PbS), sphalerite (ZnS), chalcopyrite (CuFeS_2) and gold (Au). Among them, pyrite is the most dominant sulfides in the mineralized veins and host rocks. It displays euhedral (cubic form) (Figure 5a,c), pale yellow to yellowish white. Chalcopyrite occurs as anhedral and irregular grain as well as enclosed in euhedral pyrite crystal (Figure 5). Sphalerite is observed as anhedral grains. It is grey and shows internal reflection. Sphalerite appears to have replaced (Figure 5b). Galena exhibits light grey color and anhedral form. It occurs as a mineral that replaced pyrite (Figure 5b). Electrum observes as free grains or locked in the euhedral pyrite crystal (Figure 5c). Generally, electrum grain is less than 80% Au with larger size between 100-200 μm (Figure 5d).

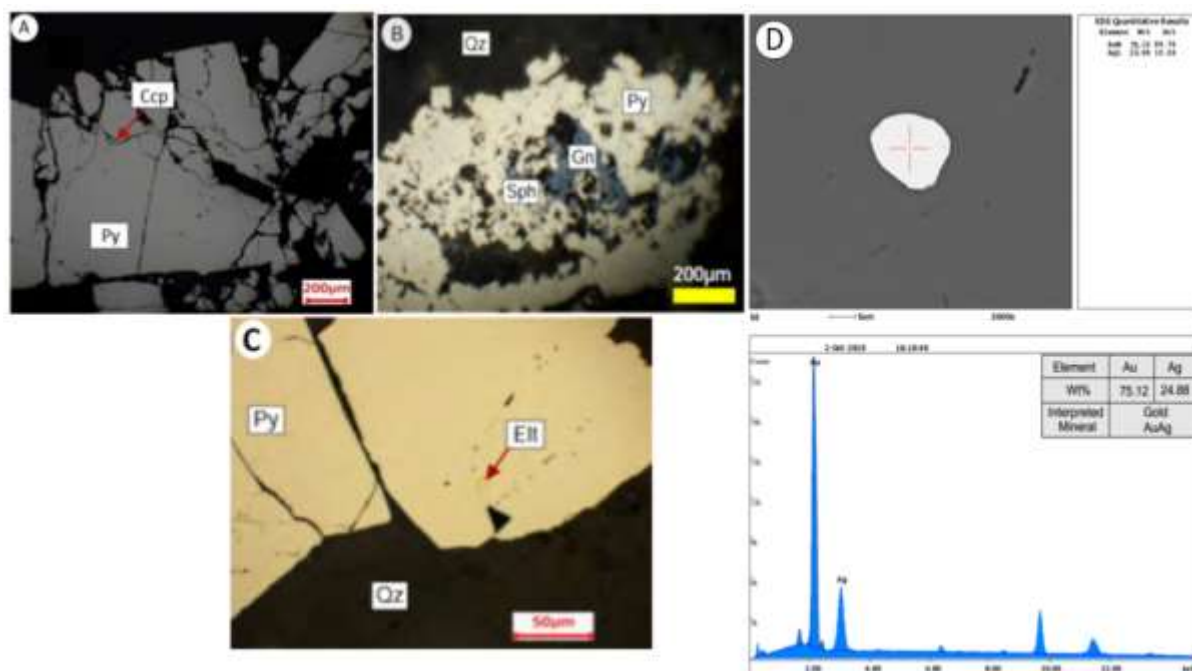


Figure 5. (a-c) Common primary metallic mineral assemblages, (d) Back scattered image of electron grain with elemental compositions at Kyaukmyet prospect. Abbreviation; Py-pyrite, Ccp-chalcopryite, Sph-sphalerite, Gn-galena, Elt-electrum, Qz-quartz.

Fluid Inclusion Microthermometry

Microthermometric measurements were carried out for fluid inclusions in quartz veins from the Kyaukmyet prospect. Two types of fluid inclusions were classified in quartz in the gold-base metal bearing colloform-crustiform veins. They are liquid-rich inclusions with consistent liquid-to-vapor ratio and coexisting liquid-rich and vapor-rich fluid inclusions (Figure 6).

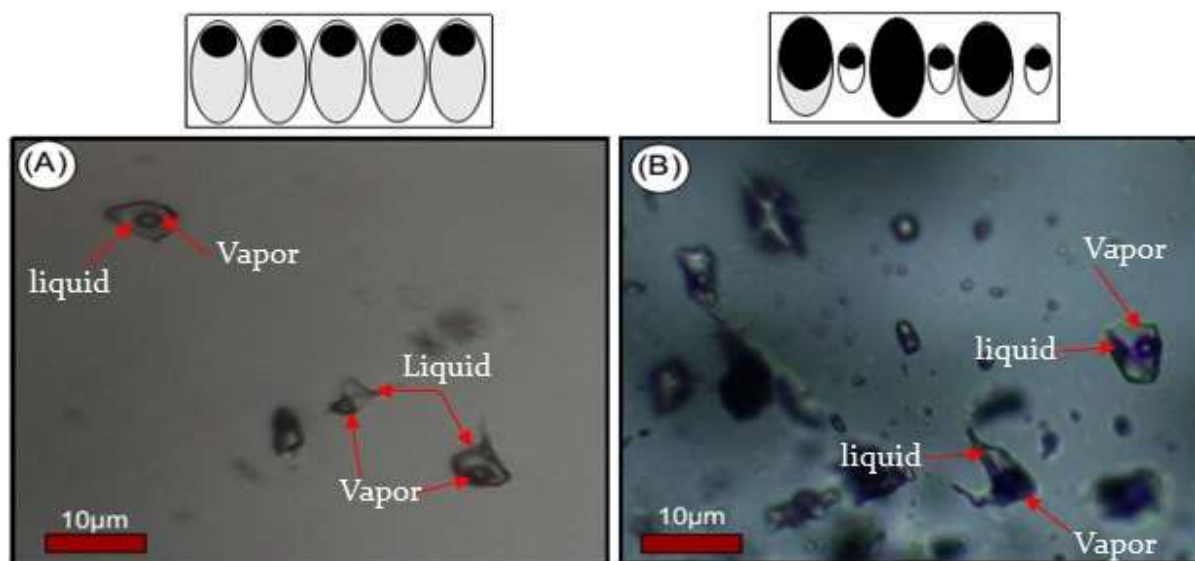


Figure 6. Fluid inclusion types observed in quartz at Kyaukmyet prospect (a) containing liquid-rich inclusions with consistent liquid-to-vapor ratio, indicative of non-boiling, (b) coexisting liquid-rich and vapor-rich fluid inclusions, indicative of boiling condition.

Characteristics of the ore-forming fluids in quartz

The fluid inclusion data are shown in Figure 7. The homogenization temperature of fluid inclusions in quartz have between 160 and 300 °C, with an average temperature of 230 °C. The salinities of fluid inclusions in quartz ranged from 0.4 to 2.1 wt% NaCl equivalent.

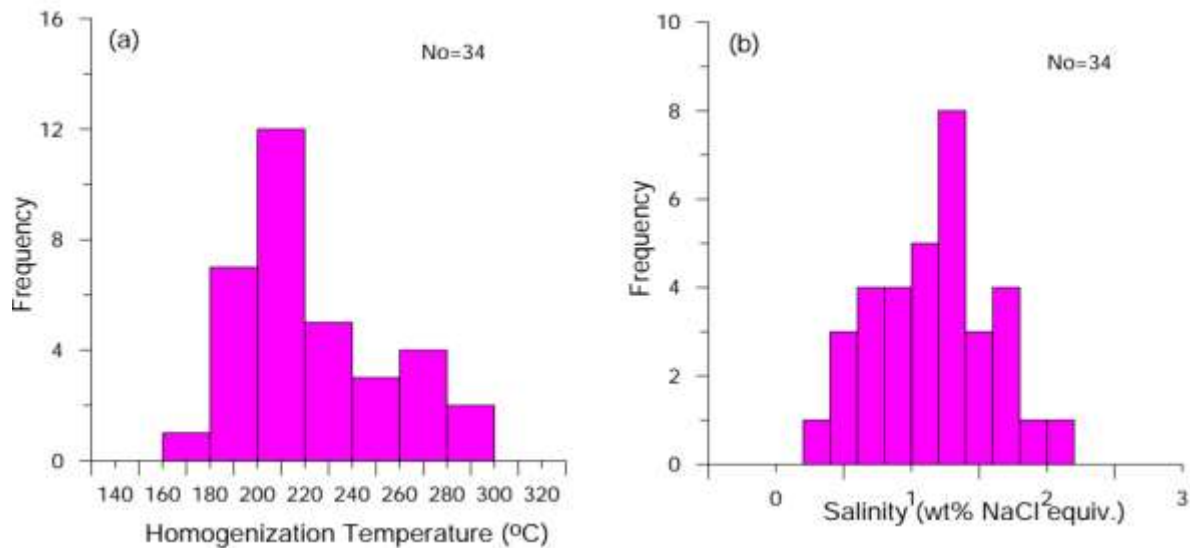


Figure 7. Histograms of homogenization temperatures and salinities for fluid inclusions in colloform-crustiform texture of gold-base metal bearing quartz veins at Kyaukmyet prospect.

Plotting these fluid inclusions data on a bivariate diagram of salinities (wt% NaCl equivalent) versus homogenization temperature (Wilkinson, 2001) indicates that the mineralization within the Kyaukmyet low-sulfidation epithermal gold deposit makes a recording the fluid boiling indicators (Figure 8a).

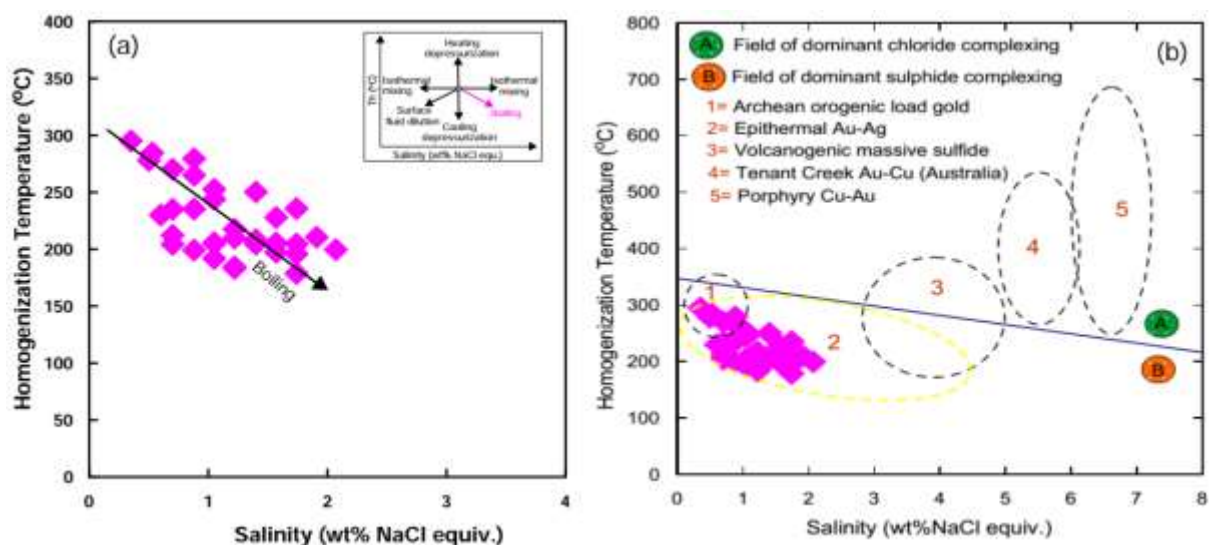


Figure 8. (a) Correlation of homogenization temperature versus salinity (wt% NaCl equiv.) for fluid inclusion assemblages (Wilkinson, 2001), (b) Homogenization temperature versus salinity diagram of the Kyaukmyet prospect for comparison stabilities of chloride and sulfide complexes with respect to temperature (Pirajno, 2009).

In a subsequent study, the results obtained from fluid inclusion salinity and homogenization temperature studies were plotted on homogenization temperature versus salinity diagram of hydrothermal mineralization systems (Large et al. 1988) in Figure 8b. The diagram had five distinct areas which were 1 -Archaen orogenic gold 2-epithermal gold-silver 3-volcanogenic massive sulfide 4-Australian Tenent creek Cu-Au deposits and 5-porphyry Cu-Au deposits respectively. As it was illustrated in the diagram, all fluid inclusion analysis data are located within the second field which represents epithermal Au-Ag deposit where the dominant transport for metal was as sulfide complexation. It is further indicated that the causes of deposition from sulfide complexes are: 1) pressure release and boiling; 2) oxidation, which decreases the sulfide contents and the pH (Pirajno, 2009) (Figure 8b).

Moreover, the first peak salinity ranges are also not different (0.4 to 2.1 wt% NaCl equiv.) and it assumes a mean salinity 1.25 wt.% NaCl equiv. of salinity on the curve. The formation temperature estimated from the T_h data can be used to determine the formation depth of the veins. Boiling point curve of (Hass, 1971) was applied (Figure 9) and the minimum formation depth is estimated at 170-260 m for the Kyaukmyet prospect below the paleowater. This depth is equivalent to hydrostatic pressure around 16 to 24 bars for the Kyaukmyet prospect (Figure 9). The resulted paleodepths and pressures estimation at Kyaukmyet prospect reveal that it formed within low sulfidation epithermal system of Sillitoe and Hendenquist (2003) that generated at depths in 100-1000m and pressure less than 10 MPa (100 bars).

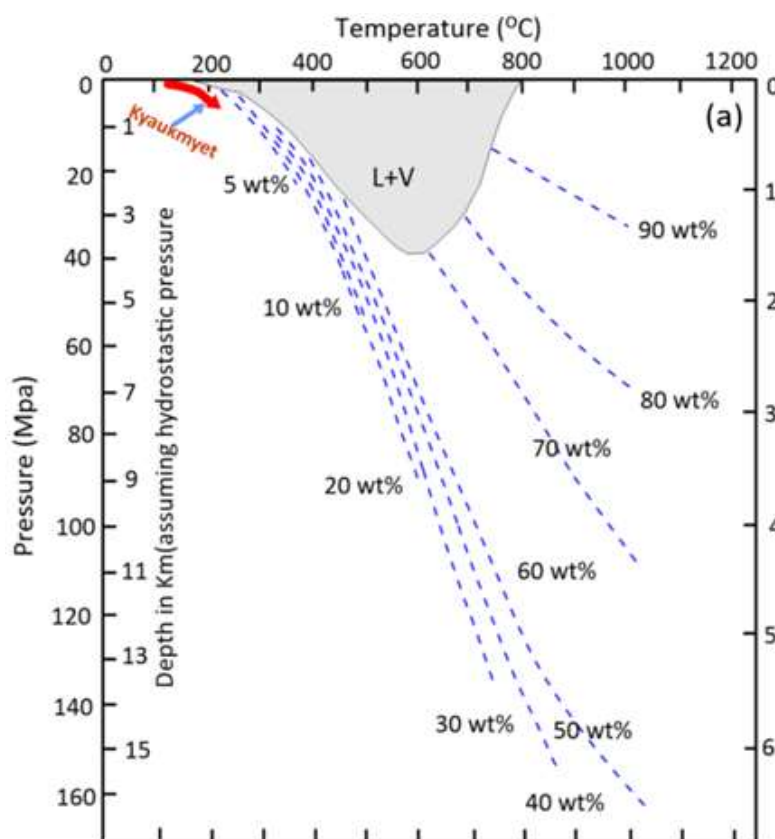


Figure 9. Estimation of paleodepth and pressure for fluid based on (Hass, 1971).

Conceptual Genetic Model

One of the main objectives of this research is to develop a genetic model of epithermal gold mineralization system at the Kyaukmyet prospect, Monywa mining area. Based on the nature and characteristics of hydrothermal alteration, ore and gangue minerals assemblage, quartz vein textures, fluid inclusions petrography and microthermometric data including formation temperature, pressure, salinity, estimate formation of paleo-depth as well as other relevant geological setting, the genetic model for the formation of epithermal gold mineralization system in the Kyaukmyet prospect was established (Figure 10).

At the stage of ore mineralization, hydrothermal fluid phase exsolved and concentrated ore-forming metals and volatiles during the ascent, followed by crystallization, and cooling of magma (Hedenquist and Lowensten, 1994). Along the mineralized ore zones of rising fluids (as a result of pressure release), the fluids are heading to higher elevation where it can be boiling as well as mixing with circulating meteoritic fluids. In the stage of mineralization, gold-base metal bearing colloform-crustiform quartz veins of the Kyaukmyet prospect was precipitated in shallow level by both of fluid boiling and fluid mixing processes. In fact, those mineralized veins formed by changing of fluid conditions where mixing of two fluids and boiling are the principal mechanism of gold and base metals deposition process in this low-sulfidation epithermal deposit. Successive changing of the fluid conditions by fluid boiling and fluid mixing resulted in repetitive layers in mineral assemblages such as quartz, carbonate, adularia and sulfides. At this phase, gold is precipitated as electrum. In addition, hydrothermal alteration zones are also occupied as silicification as well as argillic alteration zone by interaction with wall rocks and nature of hydrothermal fluids. Genetic model for the development of the epithermal gold mineralization system in the Kyaukmyet prospect was based on the emplacement of an reduced, near neutral pH, and dilute fluids formed by the input of magmatic components into deep circulating groundwaters level and are typically associated with calc-alkaline to alkaline magmatism, in volcanic arcs low sulfidation epithermal system (Figure 10), which is similar to the developments of low sulfidation epithermal system proposed by Hedenquist et al., 2000.

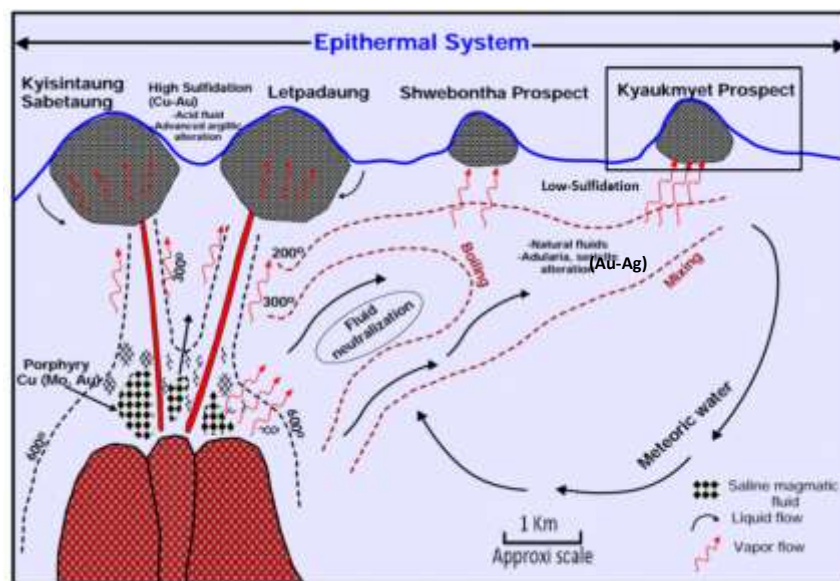


Figure 10. Conceptual genetic model for the formation of epithermal gold mineralization system at Kyaukmyet prospect, Monywa mining area, central Myanmar (Modified after Win Min Htet, 2008).

Concluding Remarks

Detailed field observations and previous exploration results show that ore mineralization found as gold-base metal bearing colloform-crustiform quartz veins texture which are mainly hosted in rhyolite rock unit. The alteration mineral assemblages of the Kyuakmyet prospect comprise quartz, adularia, sericite, illite, smectite, chlorite, epidote and calcite, and the primary sulfide minerals are pyrite, chalcopyrite, sphalerite, galena and gold. Those quartz vein textures and mineralogy in this research belong to typical characters of a low-sulfidation epithermal system. Based on the fluid inclusion petrography, the coexisting liquid-rich and vapor-rich fluid inclusions are observed as boiling indicator. The boiling of hydrothermal fluid is believed as a cause of primary metallic minerals deposition. Microthermometric fluid inclusion analysis portrays that ore mineralization occurred at the temperature of 160-300° C, the low salinity of 0.4-2.1 wt.% NaCl equiv., the minimum hydrostatic pressure of about 16-24 bars, which is equivalent with 170-260 m below paleodepth. Therefore, according to the characteristics of wall rock alteration, vein textures and ore mineralization as well as paleodepth in the Kyuakmyet prospect is classified as a low sulfidation epithermal system as well as useful concept for exploration targeting of ore mineralization at Kyaukmyet prospect, Monywa mining area.

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ASSESSMENT ON THE STABILITY OF SPILLWAY SLOPE CUTTING AT THE THA-HTAY HYDROPOWER PROJECT, THANDWE TOWNSHIP, RAKHINE STATE

Aung Kyaw Myat¹, Hlaing Myo Nwe² and Myint Myint Soe³

Abstract

The present research is carried out slope geology, geotechnical characteristics of rocks and slope stability analysis of spillway slope cutting at the Tha-Htay Hydropower Project, Thandwe Township, Rakhine State. This area is tectonically situated at the Central part of the Rakhine Coastal Belt. The rocks comprise the medium to thick bedded, indurated and moderately jointed sandstone intercalated with thinly laminated, fissile, partly carbonaceous shale and mudstone of Ngapali Formation (Middle Eocene). Most of the rock units are trending nearly NW-SE in direction. The fracture system shows as closely spaced and moderately to highly jointed nature in the map of lineament analysis. According to geotechnical investigation, the rocks exposed along the spillway slope cutting site govern fair, poor and very poor rock classes respectively where the rock classes display CL and CM rock classes in this spillway slope site. Therefore, the supporting system should be installed with wire-mesh and rock bolt covering together shotcrete and systematic drained pipes. Moreover, some of the slope failures can be observed by kinematic analysis. Slope monitoring systems should be prepared on these slope sites. Hence, the selection of systematic supporting systems should be done on surface geological, geotechnical properties of rocks combined with design modeling.

Keywords: Slope geology, geotechnical investigation, slope stability

Introduction

Tha-Htay Dam is a zoned earth dam which will supply 110 kW in total power of electric current for the country. The rocks in this area are composed of sandstone, siltstone, shale and mudstone of Ngapali Formation (Middle Eocene in Age) with volcanic rocks. This area is tectonically situated at the Central part of the Rakhine Coastal Belt.

Location

The study area is situated at Tha-Htay Chaung, Thandwe Township, Rakhine State. This area bounded between North latitudes, 18° 38' 10" to 18° 38' 50" and East longitudes, 94° 22' 40" to 94° 23' 30" which is shown in Figure (1).

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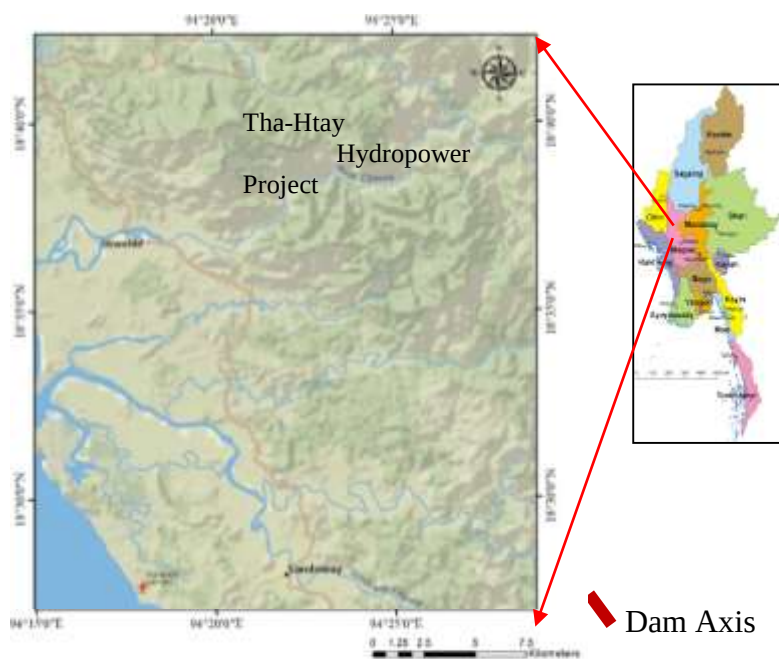


Figure 1. Location map of the study area

Physiography and Drainage

Two drainage patterns such as dendritic pattern and sub-trellis pattern are governed in this area. The dendritic pattern occurred in the Northern and Southern parts of the area. The sub-trellis pattern is mostly governed along the Tha-Htay Chaung which is illustrated in Figure (2). Therefore, the rocks in this area are composed of the alternated units of competent and incompetent rocks. According to the physiographic analysis, there are three physiographic terrains such as a highly elevated area, moderately elevated area and flat plain area. Highly elevated area is found in the North-Eastern part and flat plain area occurs in the South-Western part of the area. The study area is occupied in the moderately elevated area which is depicted in Figure (3).

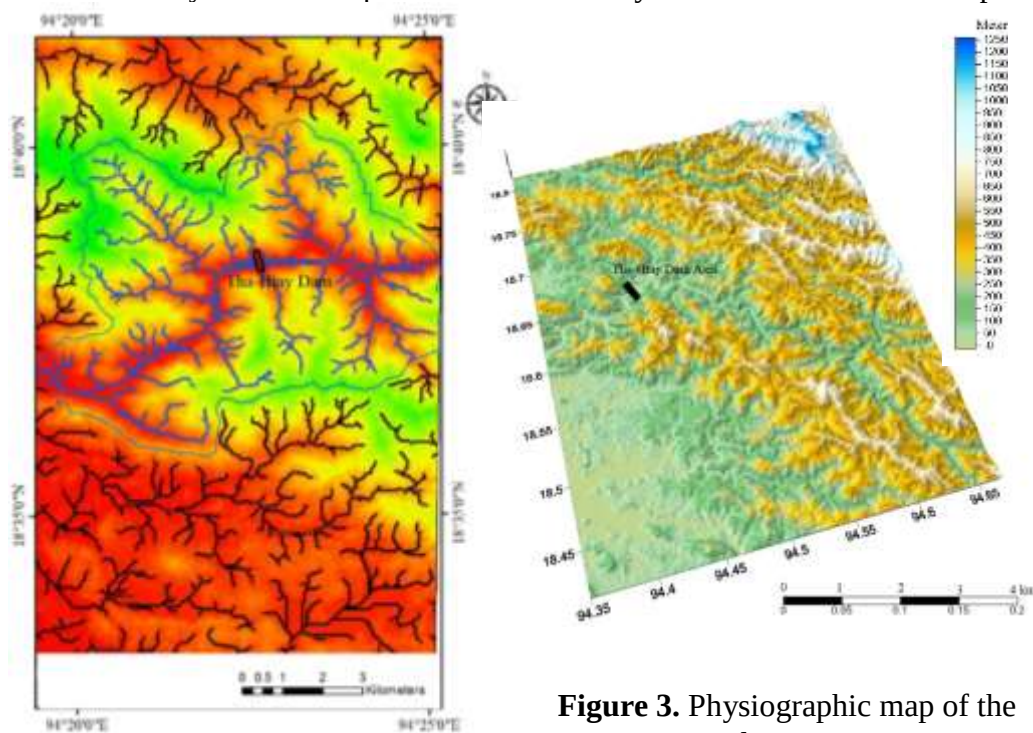


Figure 3. Physiographic map of the study area

Figure 2. Drainage map of the study area

Regional Geologic Setting

The research area lies within the Central part of the Rakhine Coastal Plain. The rocks are regionally composed of sandstone, siltstone, shale and mudstone of Ngapali Formation and Gwa Formation with some volcanic rocks.

Gwa Formation (Early Eocene) is mostly found in many places in the main Rakhine Yoma. It is mainly of indurated shale with thin sandstone beds and occasionally alternation sequences are present.

Ngapali Formation (Middle Eocene) is mainly exposed on the Ngapali Beach and in the vicinity of the main Rakhine Yoma. The rocks are composed of sandstone, shale with limestone, conglomerate, mudstone and siltstone occasionally interbedded with thin to medium bedded greywacke. The regional geological map is explained in Figure 4.

Lineament Analysis

Three main structural units are classified on the basis of the structural subdivision, topographic boundaries and distribution of the lineament system.

Structural unit (I) is observed at the North-Eastern part of the research area in the upstream catchment area of Tha-Htay Hydropower project area. This unit is characterized by the NE-SW trending regional pattern and the fracture system is widening in nature. This feature displays poorly to moderately jointed and slightly oblique to regional structural trends.

Structural unit (II) is located in the Central part of the area especially in the dam site area. This unit is characterized by NW-SE and NE-SW, partly randomly E-W trending and the fracture system can be assumed as closely spaced and moderately to highly jointed in nature.

Structural unit (III) is the same as Structural Unit (I) but it occurs in South-Western part of the area. This area is characterized by low topographic terrain which is displayed in Figure 5.

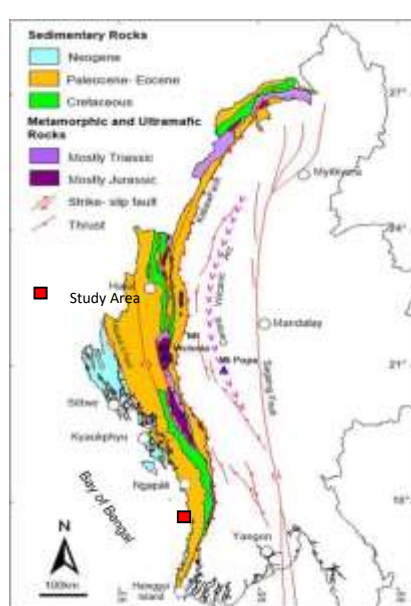


Figure 4 Regional geological map of the study area (After Myanmar Geological Map 2014 in Kyi Khin et al., 2017)



Figure 5. Lineament map of the study area based on satellite image

Aim and Objectives

The research aims to examine the geotechnical characteristics of rocks and slope stabilization of the spillway slope cutting. The objectives are

- To explore geology and fracture systems of the rocks
- To examine the geotechnical properties of rocks
- To evaluate slope stabilization by kinematic analysis
- To select suitable stabilization method

Methodology

Geological and geotechnical investigations are carried out based on the nature of rocks and discontinuities at the spillway slope cutting. Fracture system analysis has been done on the lineament as well as field observation.

The strength of rocks has been measured by a uniaxial compression test. Rock quality designation (RQD) was converted by three-dimensional volumetric joint count in the field which is described by Palmstrom (2005). $J(v)$ formula is $J_v = 1/S_1 + 1/S_2 + 1/S_3$ and so on plus $N_r/5$ where N_r is a random joint on outcrop.

Rock mass rating measurements for rock slopes have been made by RMR classification of (Bieniawski, 1989) which is principally composed of six basis parameters such as rock strength, rock quality designation (RQD), spacing of joints, condition of joints, groundwater condition and orientation of discontinuities. The qualification of geological strength Index (Hoek et al., 2013) is calculated on the formula of $GSI = 1.5 J_{cond} (89) + RQD/2$.

Kinematic analysis for rock slope stability is carried out to know failure types of slopes which is described by Goodman (1998), Jeongi and Kulatilake (2001) and Yousif et al. (2013) which is based on relationship of discontinuities of slope, slope inclination and friction angle of rock.

Results and Discussions

In this research, slope geological mapping, volumetric joint count, rock quality designation (RQD), rock mass rating (RMR), geological strength index (GSI) and kinematic analysis of the rocks had been done at the spillway slope.

Layout of the Spillway Slope

The spillway slope cutting dips to the south with 160° - 180° in direction and the general slope inclination is nearly 60° facing to the south. The high of a spillway slope is 170 m which is shown in Figure 6A and 6B.



Figure 6. (A) Spillway location on google map (B) layout map of the study slope at the spillway slope cutting

Geological Investigation of the Study Area

The rocks comprise the medium to thick bedded, partly thin to medium bedded, bluish grey to grey, indurated and moderately jointed sandstone intercalated with thinly laminated, fissile, partly carbonaceous shale and mudstone of Ngapali Formation (Middle Eocene). Most of the rock units are trending nearly NW-SE in direction. In the Northern part of the Dam Axis, the rocks are plunging nearly to the North that is remarked by dipping of rock units.

The Ngapali Formation can be divided into three sub-units based on the composition of rock units. Subunit-1 is exposed at the eastern part of the study area and the rocks comprise with light grey to yellowish brown, partly reddish brown, moderately jointed and weathered, thin to medium bedded sandstone intercalated with thinly laminated shale and mudstone which is shown in Figure 7. Subunit-2 is exposed at the Central and South-western parts of the project area and the rocks consist of light grey to grey, partly bluish grey, moderately jointed, thick bedded to massive, turbiditic sandstone intercalated with thinly laminated shale and mudstone which is shown in Figure 8. Subunit-3 is exposed at the Western and Central parts of this area. The rock unit is composed of thinly laminated, dissected, highly friable carbonaceous shale and mudstone interbedded with light grey to grey, thin bedded sandstone which is proved in Figure 9. The geological map is illustrated in Figure 10.



Figure 7. Thin to medium bedded sandstone with shale and mudstone at the Upstream

Figure 8. Thick bedded to massive sandstone with shale and mudstone at the Dam Axis

Figure 9. Thinly laminated, shale and mudstone with sandstone at the downstream

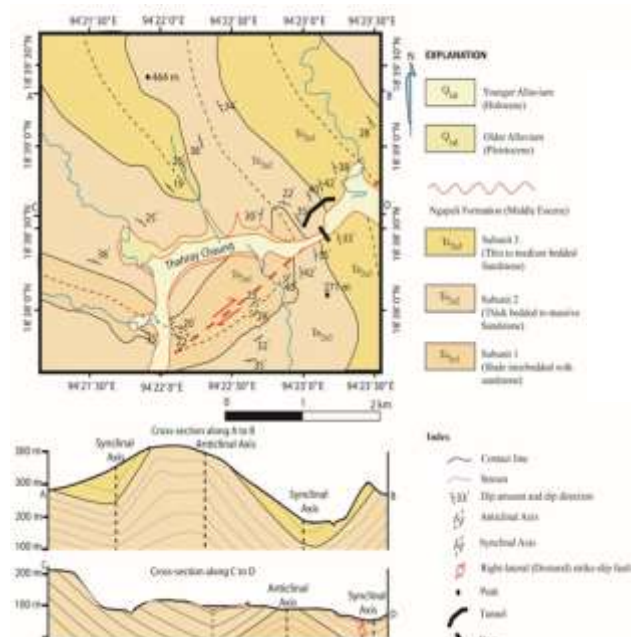


Figure 10. Geological map of the study area

Engineering Properties of the Rock at the Studied Slope Site

Rock Mass Characterization Analysis

The study spillway site is located at the side of left abutment along Dam Axis where the rocks are exposed grey to dark grey colored, partly yellowish-brown colored, medium to thick bedded, highly jointed sandstone intercalated with thinly laminated, soft, shale and mudstone. Most of the rocks are dipping to the North and North-West and z-shaped drag folds occur in the mudstone layers. The rocks exposed on the slope can be divided into three zones as Zone I, Zone II and Zone III based on the natures of rocks as well as rock mass characterizations which are described in (Figure 11) and Table 1.

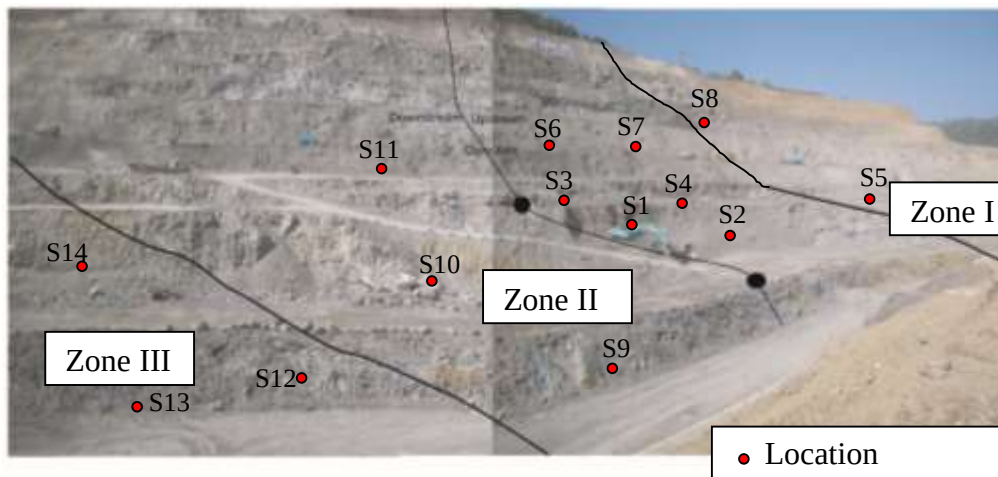


Figure 11. Zonation on rock mass characterization at the spillway slope

Table 1. Rock Mass Characterization of the spillway slope site

No	Jv	RQD (%)	RMR	GSI	Rock Class	ϕ value based on RMR	Possible Failure
S1	16.9	59	60	54	CM	25° to 35°	Plane
S2	21	44	57	51	CM	25° to 35°	Wedge, Topple
S3	19	52	53	50	CM	25° to 35°	Plane, Wedge
S4	19	51	53	50	CM	25° to 35°	Wedge
S5	22	44	48	46	CM	25° to 35°	Wedge, Topple
S6	21	44	57	53	CM	25° to 35°	Plane, Wedge
S7	18	56	57	51	CM	25° to 35°	Wedge
S8	15	64	50	53	CM	25° to 35°	Wedge
S9	19	54	45	44	CM	25° to 35°	Wedge
S10	19	51	57	48	CM	-	-
S11	25	32	24	33	CL	-	-
S12	29	21	48	39	CL	-	-

No	Jv	RQD (%)	RMR	GSI	Rock Class	ϕ value based on RMR	Possible Failure
S13	28	22	21	32	CL	-	-
S14	29	19	21	31	CL	-	-

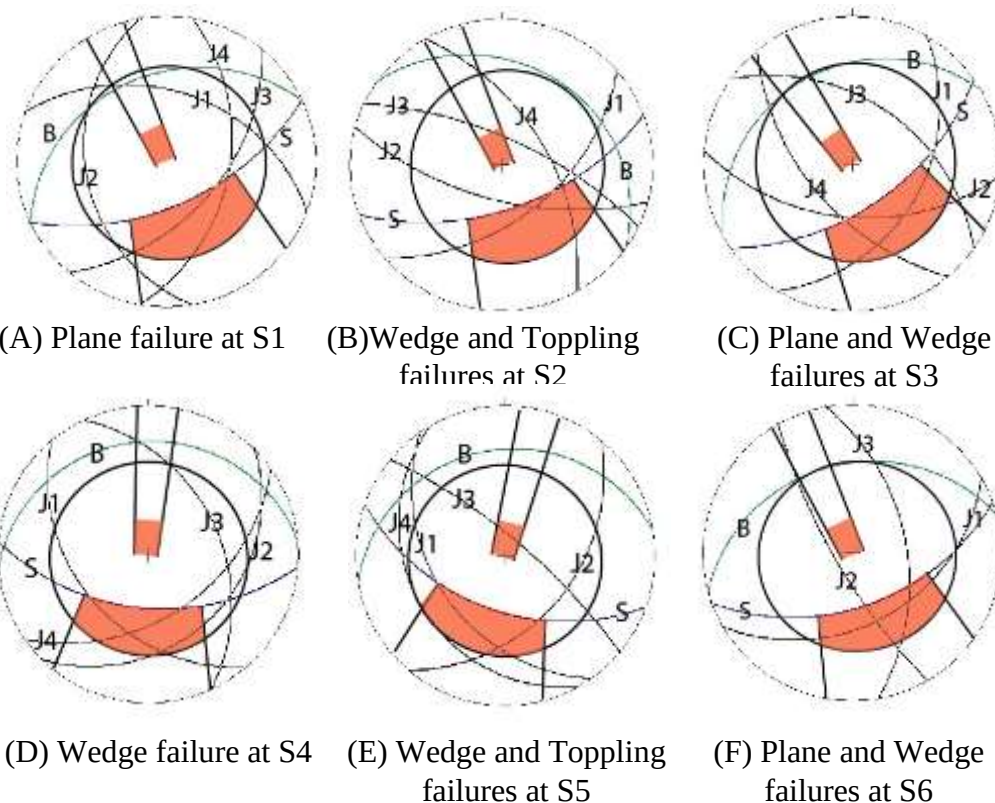
According to rock mass characterization analysis, the rocks exposed at Zone I are fine to medium grained, thin to medium bedded, indurated, moderately to highly jointed, yellowish-brown colored sandstone (8-90 cm in thickness) intercalated with thinly laminated shale and mudstone. In this zone, most of the rocks have fair rock class according to the Rock Mass Rating (RMR) values and CM rock class shows in GSI values.

Zone II occurs at the Central part of the study slope site which is mostly governed with fine to medium grained, medium to thick bedded, moderately to highly jointed, bluish grey to grey colored sandstone intercalated by thinly laminated shale and mudstone. Most of the sandstones are thick bedded in nature and they have moderate to high strength. In this zone, the rocks comprise a fair rock class as RMR value, except two locations where groundwater flows out and most of the rocks are CM rock class in GSI values.

The Westernmost part of the spillway slope site is known as zone III where the rocks are composed of thinly laminated shale and mudstone intercalated with thin to medium bedded, bluish grey to grey colored, highly jointed sandstone. Most of the slope site is covered with mudstone. In this zone, the rocks comprise a poor rock class as RMR value and Most of the rocks are CL rock class in GSI values respectively.

Kinematic Analysis for Slope Stability

The spillway slope cutting is located at the right side of the Dam Axis which is composed of moderately indurated sandstone with clay and mudstone. Nine slope locations are selected to study the kinematic analysis. According to this study, these slopes can be plane failure, wedge failure and toppling failure which are described in Figure (12).



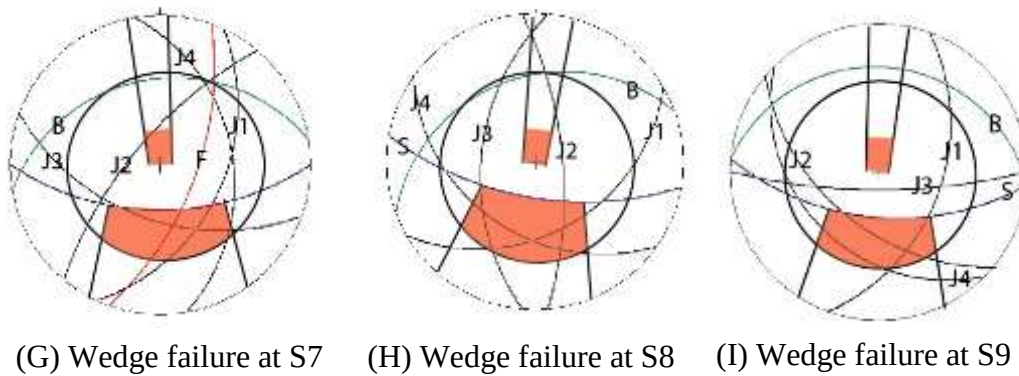


Figure 12 A-I Kinematic analysis of each slope site on the relationship of the discontinuities and slope face parameters

Conclusion and Recommendation

The rocks composed in this area are generally fine to medium grained, medium to thick bedded, partly thin to medium bedded, indurated, moderately to highly jointed sandstone with thinly laminated, dissected, carbonaceous shale and mudstone. Hence, two different strengths of rocks are composed as the alternate layers. This condition is not suitable for slope stability in the rainy season because carbonaceous shale and mudstone are composed of clay minerals which are easily decomposed in rainy water.

The bedding nature of rocks and spillway alignments are generally suitable for this Dam Construction. But the false bedding planes such as discontinuities are unsuitable for slope stability at spillway slope cutting sites. Moreover, two faults occurred in this area and one of the faults, a thrust fault was found in the spillway slope cutting site. Hence, the depth of the failure plane should be confined with the borehole drilling process. Most of the rock bolts should be installed until the rock bolts reach the bottom of the failure plane.

According to Rock Mass Rating (RMR) analysis, the rocks exposed along the spillway slope cutting site govern fair, poor and very poor rock classes. The rock classes display CL and CM rock class in this spillway slope site by the Geological Strength Index analysis. For GSI value based on the structural behavior of rocks, the rocks exposed in this slope should be supported systematically. Therefore, the supporting system should be installed with wire-mesh and rock bolt covering together shotcrete and systematic drained pipes. Furthermore, there were two or more places where groundwater is flowing out continuously. In these places, special treatments should be given with drainage systems and rock bolts where subsurface fracture systems and nature of rocks should be done to explore more detail.

According to kinematic analysis for slope stability, some of the slopes can be failures. Therefore, slope monitoring systems should be prepared on these slope sites. Overall surface geology and engineering properties of the rocks are pointing out the conditions that require a systematic supporting system. Hence, the selection of systematic supporting systems should be done based on surface geological, geotechnical properties of rocks combined with design modeling.

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ASSESSMENT OF THE INFLUENCE OF HYDRAULIC AND GEOCHEMICAL FACTORS ON AQUIFER EVALUATION IN THE KYAUKPADAUNG AREA

Min Han Nyein¹, Zin Nwe Khaing², Nay Nwe Myo³, Hnin Ei Hlaing⁴

Abstract

The research area is located in Kyaukpadaung Township, Nyaung-U District, Mandalay Region. The area is located between North latitude 20° 41' 56" to 21° 02' 7" and East longitude 95° 02' 58" to 95° 30' 37". The two stratigraphic units are 1) the Irrawaddy Formation (Late Miocene-Pliocene), and 2) the Pegu Group (Mid-Miocene). The Irrawaddy aquifer is the main aquifer of the research area. Chemical Analysis is important to specify the characteristics of groundwater. The concentration of hydrogen ions (pH) is between 6.67 and 7.77. The total dissolved solids are a maximum of 650 ppm. Electrical conductivity (E.C) is not more than 1000 μ mho/cm. The water type is classified by the methods of TDS, Piper, and Kurlov. According to the TDS, Piper, and Kurlov's method, the research area is the freshwater types and the research area can be classified into 2 types and 4 types. The concentration of Bicarbonate ions is widely distributed in most of the water of the studied region and the amount present in groundwater is relatively higher than other anions. The specific yield of the groundwater is 1000 to 2000 gallons per hour for 4-inch diameter wells and the depth of the water-bearing horizon ranges from 40 ft to 270 ft in the Irrawaddy aquifer. Calculated by the pumping test, the transmissivity of the aquifer (KD) is 202 m²/day. Calculated by the recovery test, the transmissivity of the aquifer (KD) is 43.94 m²/day. The irrigation water types were analyzed by the methods of SAR, and MAR. According to these methods, the SAR method gets the 5 water types and the MAR gets suitable and permissible water types. The groundwater of the research area is evaluated as suitable for drinking water, domestic uses, and irrigation water.

Keywords: TDS, Piper, Kurlov's, and SAR

Introduction

Location, Site, and Accessibility

The research area is situated in Kyaukpadaung Township, Nyaung-U District, Mandalay Region. The area is located between North latitude 20° 41' 56" to 21° 02' 7" and East longitude 95° 02' 58" to 95° 30' 37". The total coverage of the area is about 1,797 square kilometers. Figure (1) shows the location map of the research area. The population of the study area is about 261,089 (census 2019). The elevation of the research area lies above 300 mean sea level. The area is easily accessible during the whole season. The drainage pattern of the study area is the Dendritic Pattern. Figure (2) shows the drainage map of the research area. This area has two seasons. There is a dry season (mid-November – mid-May) and a wet season (remainder of the year). Annual rainfall sets during the May to October monsoon period. The study area receives rainfall from the southwest monsoon which moves from the Bay of Bengal and Andaman Sea. The average annual rainfall is about 638 mm (25 inches).

General Geology

The three stratigraphic units that make up the current region and its surroundings are showed on the geological map (2014): 1) Alluvium (Holocene), 2) Irrawaddy Formation (Late Miocene-Pliocene), and 3) Upper Pegu Group (Mid-Miocene), also describes the geological succession and hydrogeological significance of the study area's aquifer.

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The research area is part of the Minbu basin, which is a part of the western margin of Central Myanmar. It is almost entirely made up of Cenozoic sediments, mostly sandstones and clays or shales. Paleogene-Neogene sediments, deposited in shallow marine, fluvial, and deltaic environments, comprise most of the study area. The layers can be separated into four lithologic units.

Sedimentary Rocks

3. Quaternary Deposits (Pleistocene)
2. Irrawaddy formation (Late Miocene - Pliocene)
1. Upper Pegu Group (Oligocene-Miocene)

Igneous Rock

1. Igneous Rocks (Pre -Tertiary)

The best exposures of these rock units can be seen in the whole area. The present area includes many sedimentary structures such as calcareous pellets. Like concentrations, root-like structures, nodules, and cross-bedding. Figure (3) shows the Regional Geological Map of the research area.



Figure 1. Location map of the research area

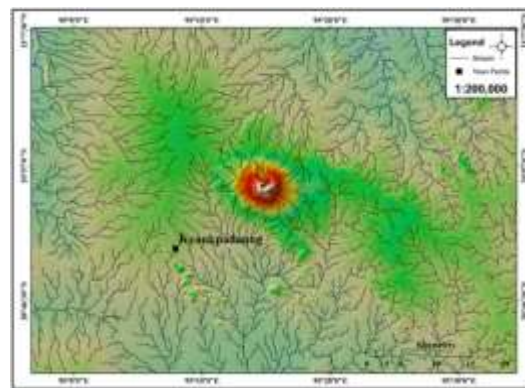


Figure 2. Drainage map of the research area

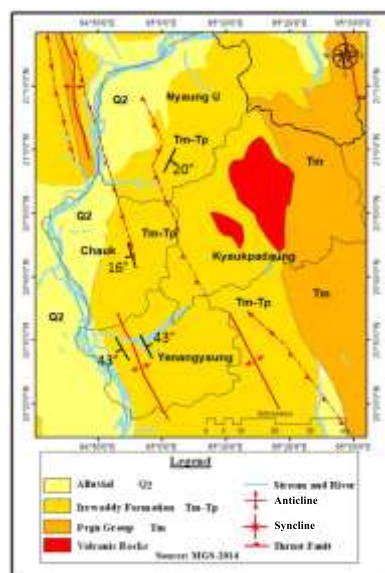


Figure 3. The Regional Geological Map of the research area.

Material and Methods

Purpose of the study

The purpose of this research is described as follows;

1. To classify the groundwater types
2. To analyze the quality of groundwater
3. To determine the hydraulic characteristics
4. To assess the suitable amount of drinking water and irrigation water

Method of the study

There are five main sections to achieve the objectives of this research.

1. To examine cation and anion parameters
2. To classify TDS, the Piper Diagram Method, and Kurlov's Method
3. To calculate the Pumping test and Recovery test
4. To compute the Sodium Adsorption Ratio (SAR)
5. To compute the Magnesium Adsorption Ratio (MAR)

Chemical analysis parameters of the research area

Chemical Analysis is important to specify the characteristics of groundwater. Determination of pH, total dissolved solids (TDS), electric conductivity E.C, dissolved cations of Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺, and Fe⁺⁺, and dissolved anions are classified in the laboratory. Table (4) shows the result data of the chemical analysis parameters in the research area. Figures (4, 5, 6, 7, 8, 9, 10, 11, 12, and 13) show the chemical parameter distribution map of the research area.

Classification of water based on TDS Levels

Total Dissolved Solids (TDS) (Gorrell, H.A. (1958)) refers to the inorganic salts and small amounts of organic matter present in water, measured primarily in milligrams per liter (mg/L) or parts per million (ppm) shown in table (1). The classification and measurement of TDS are crucial for assessing water quality, especially in drinking water and environmental monitoring. Table (5) is the result of the TDS method.

Table 1. Water can be classified according to its TDS concentration

Classification	TDS Range (mg/L)
Fresh Water	Less than 1,000 mg/l
Brackish Water	1,000 to 10,000 mg/L
Saline Water	10,000 to 35,000 mg/L
Hypersaline	Greater than 35,000

Classification of Piper Diagram

This method is proposed by Piper (1944) and Hill (1940). The tri-linear diagram is widely used to depict chemical data and show the relative concentrations of the major cations (Ca⁺², Mg⁺², and K⁺) and anions (CO₃⁻, HCO₃⁻, Cl⁻ and SO₄⁻). Cations are plotted on the left triangle and anions on the right triangle. Piper diagrams are shown in the Figure (14). Kurlov's method and Piper's method compare the results of the research area in groundwater types, in Table (5).

Classification of Kurlov's Method

The Kurlov method (Kurlov, (1962)) utilizes the types of groundwater based on chemical composition. This method involves calculating the percentage of milliequivalents of cations and anions present in water samples. The classification is based on specific ion content, particularly when the concentration of any ion reaches or exceeds 25%. The primary ions analyzed include sodium, potassium, calcium, magnesium, chloride, sulphate, and bicarbonate. Table (5) is the result of the kurlov's method.

Pumping out the test in the study area

A pumping test (Cooper and Jacob's, (1946)) also known as a "well test" or "aquifer test," is a fundamental technique in hydrogeology used to assess the properties of an aquifer, such as its hydraulic conductivity, transmissivity, storativity, and the extent of its influence. The data collected during the test are analyzed using mathematical models to estimate aquifer parameters such as hydraulic conductivity, transmissivity, storativity, and the radius of influence of the well. Pumping tests provide valuable information about the behavior and characteristics of aquifers, which is crucial for designing and managing groundwater extraction systems, remediation efforts, and sustainable groundwater management. The pumping-out test is measured by the discharge rate and drawdown. It also measures the drawdown of the piezometers surrounding the pumped well at a certain time during the pumping of the well. This measurement indicates how much water stored in the aquifer can be released and the cone of depression has increased at a certain time during pumping. The test is pumped out for 24 hours, and a recovery test of 12 hours is measured. Figure (15) shows that Constant-rate pumping test data are plotted on a Semi-logarithmic scale.

Recovery test in the Study area

The recovery test ((Theis, (1935)) is an aquifer test used to assess the rate at which water levels in a well return to their original levels after pumping has stopped. It's an important tool for evaluating the hydraulic properties of an aquifer and the efficiency of a well. The objectives of the recovery test are to measure the residual drawdown to calculate the transmissivity of the aquifer and check the result of pumping test data. Residual drawdown is the difference between the original water level before the start of pumping and water level measurement at a time after the cessation of pumping in a well. Residual drawdown data is more reliable than pumping test data because recovery occurs at a constant rate. Figure (16) shows that Recovery test data are plotted on a Semi-logarithmic scale.

Sodium Adsorption Ratio (SAR)

If the water used for irrigation is high in Na and low in Ca the ion-exchange complex may become saturated with Na which destroys the soil structure, due to the dispersion of the clay particles and reduces the plant growth. Concentrations of ions are expressed in meq/L. The classification of the irrigation water types (SAR) (Richards L. A. (1954)) is shown in table (5). Figure (17) The Classification of the Sodium Adsorption Ratio (SAR) results on the Sodium vs. Electrical conductivity Chart. The SAR is computed using the following equation:

$$SAR = \frac{Na^{+}}{\frac{\sqrt{Ca^{2+} + Mg^{2+}}}{2}}$$

The concentration of ions is expressed in meq/L. The higher cumulative concentration of HCO_3^- than Ca^{2+} and Mg^{2+} concentration is an indication that residual carbonate will react with sodium, thereby resulting in sodium hazard.

Table 2. Classification and use of irrigation waters (Wilcox. L.V. (1955))

USSL Classification	Remarks	
$C_1 - S_1$	Excellent Water	More suitable for agriculture
$C_2 - S_1$	Good Water	Suitable for agriculture
$(C_1 - S_2), (C_2 - S_2), (C_3 - S_2) (C_3 - S_1)$	Moderate Water	Fairly suitable for agriculture
$(C_1 - S_3), (C_2 - S_3), (C_3 - S_3), (C_4 - S_3), (C_4 - S_2),$ $(C_4 - S_1), (C_1 - S_4), (C_2 - S_4), (C_3 - S_4),$ $(C_4 - S_4)$	Bad Water	Need some treatments for agriculture

Magnesium Adsorption Ratio (MAR)

Magnesium Adsorption Ratio (MAR) was calculated using the Ca^{2+} and Mg^{2+} content of the water samples by the equation proposed by Raghunath (1987):

$$\text{MAR} = \left(\frac{\text{Mg}^{2+}}{\text{Mg}^{2+} + \text{Ca}^{2+}} \right) \times 100\%$$

where the concentration of ions is expressed in meq/l. Table (3) shows of the water classified based on the MAR values. Table (5) shows the result of the MAR.

Table. (3) Water classification based on the MAR Values

Parameter	Range	Class
MAR	< 25	Suitable
	25 - 50	Permeability
	50 - 75	Doubtful
	> 75	Unsuitable

Results and Outcomes

The result of the chemical analysis parameter in the research area

Table 4. the result data of the chemical analysis parameter in the research area

No.	TDS	EC	pH	TH	TA	Na+	k+	Ca+2	Mg+2	Cl-	SO4-2	HCO3-
1	285	440	7	134	88	36	3	35	11	47	11	88
2	505	780	7	266	98	53	7	76	18	96	155	98
3	310	490	7	204	50	21	4	71	7	55	90	50
4	650	1000	8	124	90	102	3	42	4	73	123	90

No.	TDS	EC	pH	TH	TA	Na+	k+	Ca+2	Mg+2	Cl-	SO4-2	HCO3-
5	260	410	7	164	54	23	4	54	7	61	32	54
6	265	415	8	168	60	19	4	55	8	65	53	60
7	310	480	7	184	66	20	3	50	14	54	93	66
8	250	375	8	146	52	19	3	35	14	72	45	52
9	270	420	8	176	78	17	3	58	8	78	28	78
10	540	835	8	230	184	63	5	59	20	80	89	184
11	310	480	8	180	82	24	4	52	12	64	66	82
12	290	450	8	178	42	18	4	48	14	67	85	42
13	350	540	7	202	142	26	4	54	16	64	49	142
14	710	1000	8	338	178	126	5	107	17	89	181	178
15	300	465	8	168	90	27	20	54	8	64	31	90
16	140	220	8	58	20	0	0	21	1	64	4	20
17	455	700	7	310	100	56	6	97	16	74	99	100

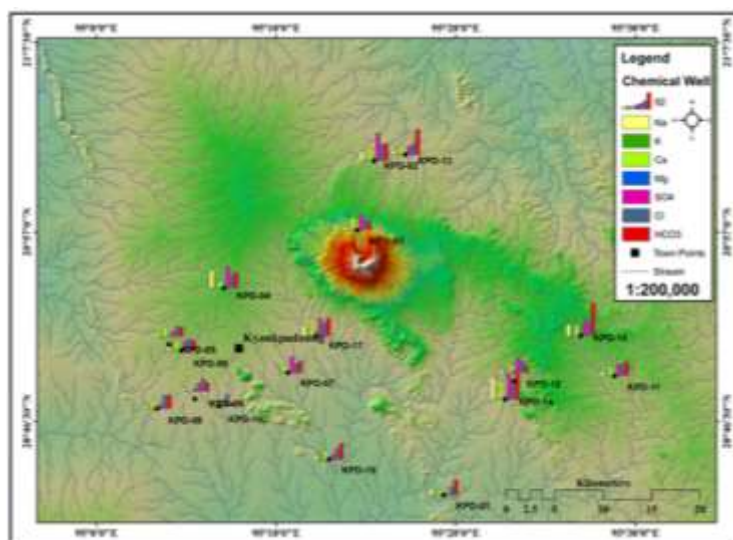
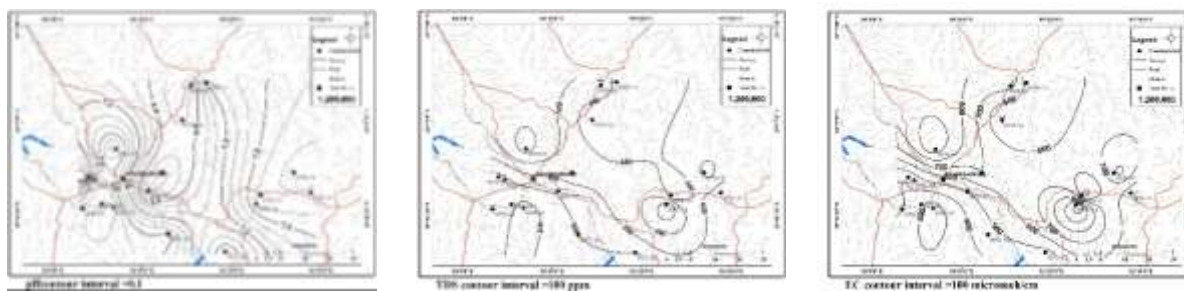


Figure 4. The chemical parameter distribution map of the research area

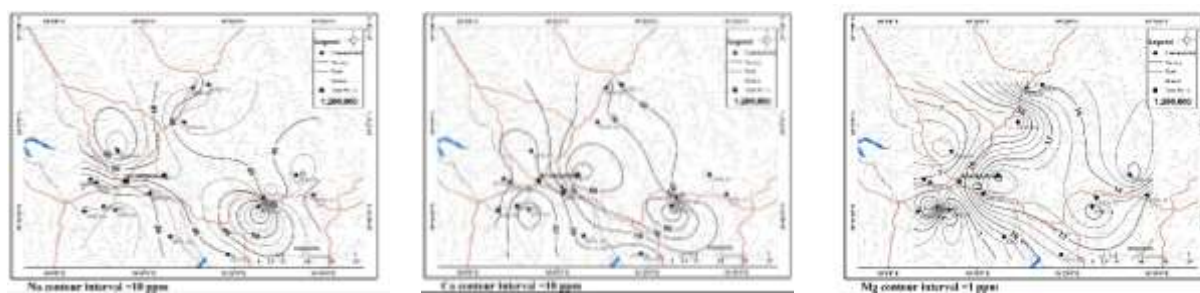
Chemical Analysis is important to specify the characteristics of groundwater. Determination of pH, total dissolved solids (T.D.S), and Electrical Conductivity (E.C) maps are shown in Figures (5,6, and 7) representing.



Figures 5,6, and 7. Contour distribution map of the pH, TDS, and E.C. in the research area.

Major Cations

Cations commonly contained in tube wells have been determined. Common cations are Sodium Na^+ , Calcium Ca^{+2} , and Magnesium Mg^{+2} are represented in Figure. (8,9, and 10).



Figures 8,9, and 10. Contour distribution map of the Na, Ca, and Mg in the research area

Major Anions

Anions play on the quality determination of groundwater. Only major anions of Bicarbonate (HCO_3^-), Sulphate (SO_4^-) and Chloride (Cl^-) ions should be taken into account are representing shown in Figures (11,12, and 13).



Figures 11,12, and 13. Contour distribution map of the HCO_3 , SO_4 , and Cl in the research area

The result of the classification of Piper Diagram (Piper, (1944))

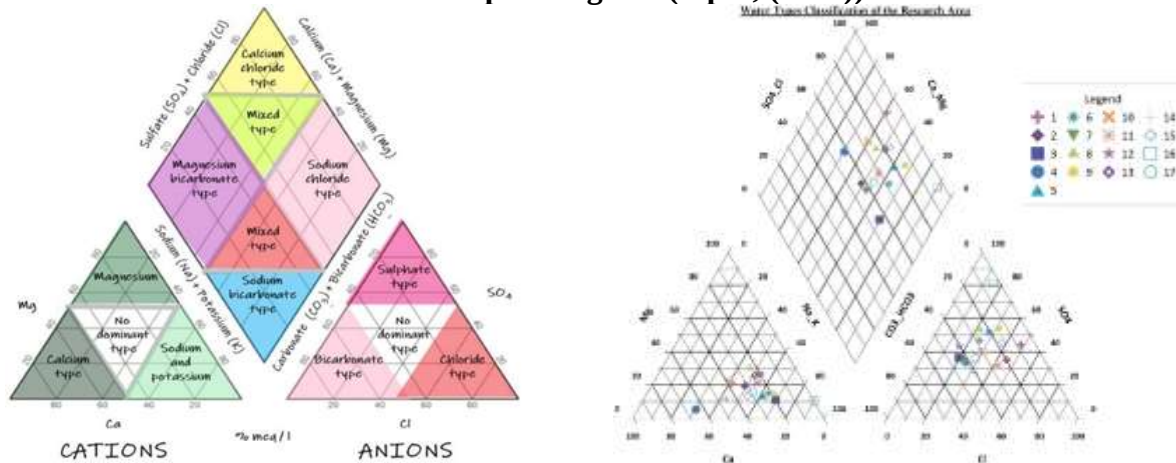


Figure 14. Tri-linear diagrams are shown the classification of the Piper method

Calculation of Pumping out test (Copper and Jacob's, 1946)

Pumping-out test data is calculated by using Jacob's Method. The Jacob's Method is based on the Theis' Equation.

$$KD = \frac{2.3Q}{4\pi\Delta S}$$

Where, KD = the transmissivity of the aquifer, m²/day

Q= the constant well discharge, m³/day

ΔS= the drawdown difference, m

Q= 288 m³/day

ΔS= 0.26 m

$$KD = \frac{2.3Q}{4\pi\Delta S} = \frac{2.3 \times 288}{4 \times 3.14 \times 0.26} = 202 \text{ m}^2/\text{day}$$

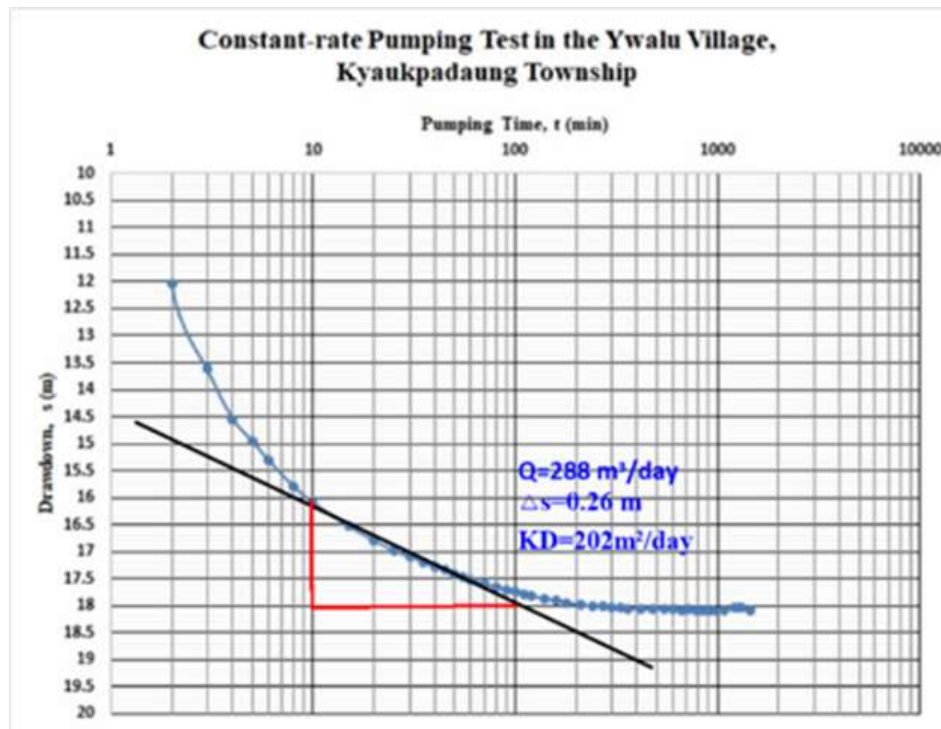


Figure 15. shows that Constant-rate pumping test data are plotted on a Semi-logarithmic scale.

Calculation of Recovery Test Data (Theis, 1935)

Theis' recovery method is widely used for the analysis of recovery tests. Theis' recovery equation (1935) is described as follows:

$$KD = \frac{2.3Q}{4\pi\Delta S'}$$

Where, KD = the transmissivity of the aquifer, m^2 / day

Q = the constant well discharge, m^3 / day

Δ = the residual drawdown, m

Theis Recovery Method

$$Q = 288 \text{ m}^3 / \text{day}$$

$$\Delta S' = 1.2 \text{ m}$$

$$KD = \frac{2.3Q}{4\pi\Delta S'} = \frac{2.3 \times 288}{4 \times 3.14 \times 1.2} = 43.94 \text{ m}^2 / \text{day}$$

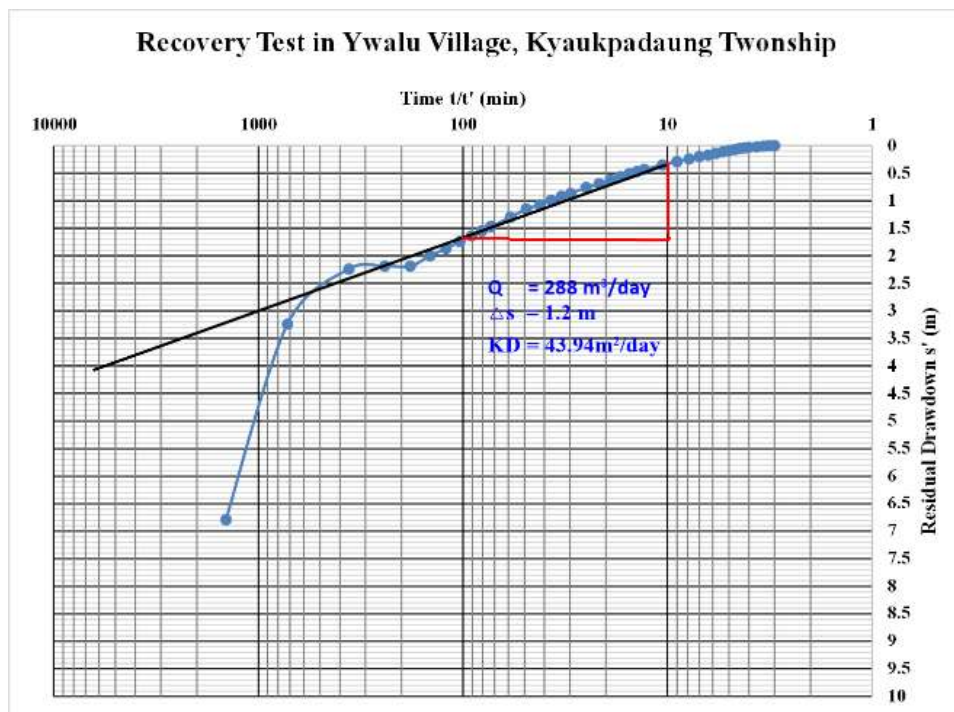


Figure 16. shows that Recovery test data are plotted on a Semi-logarithmic scale.

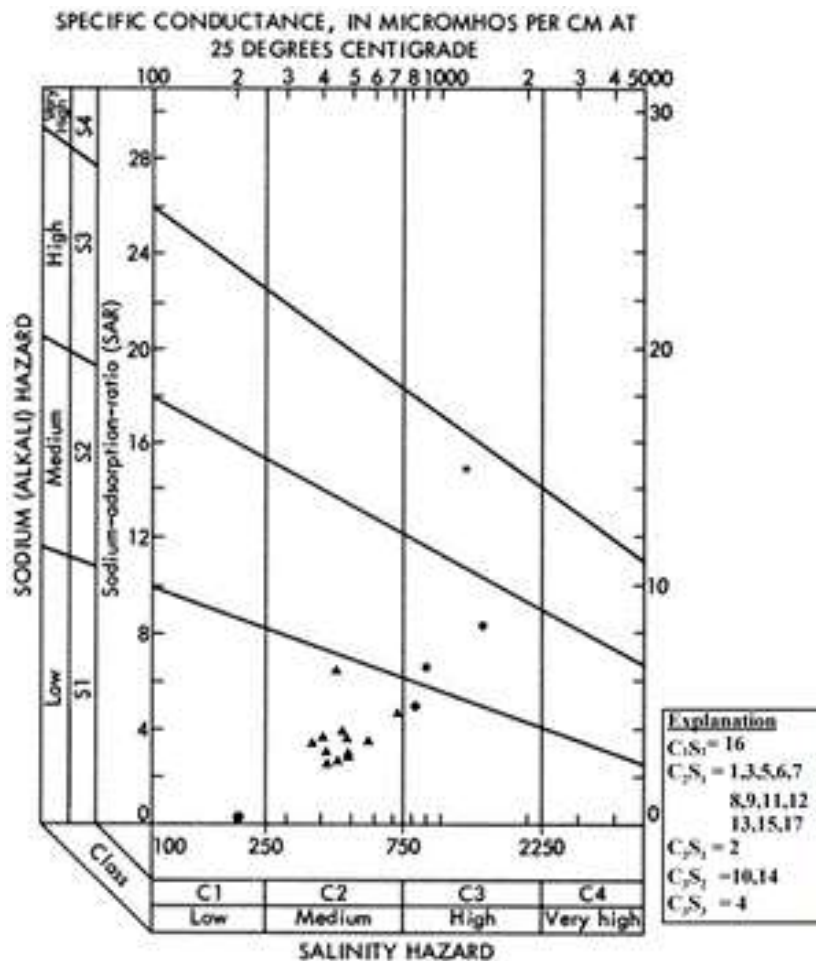


Figure 17. The Classification of the Sodium Adsorption Ratio (SAR) results in Sodium vs. Electrical Conductivity Chart

Table 5. Summarized the result of the chemical analysis data

No.	TDS	Piper	Kurlov's	SAR	MAR
1	Fresh	Mixed	HCO ₃ -ClCa-Na	C2S1	Permissible
2	Fresh	Mixed	SO ₄ -ClCa-Na	C3S1	Permissible
3	Fresh	NaCl	SO ₄ -ClCa	C2S1	Suitable
4	Fresh	Mixed	SO ₄ -ClNa-Ca	C3S3	Suitable
5	Fresh	NaCl	Cl-HCO ₃ Ca	C2S1	Suitable
6	Fresh	NaCl	Cl-HCO ₃ Ca	C2S1	Suitable
7	Fresh	NaCl	SO ₄ -ClCa-Mg	C2S1	Permissible
8	Fresh	Mixed	ClCa-Mg	C2S1	Permissible
9	Fresh	NaCl	Cl-HCO ₃ Ca	C2S1	Suitable
10	Fresh	Mixed	HCO ₃ -Cl-SO ₄ Ca-Na	C3S2	Permissible
11	Fresh	NaCl	Cl-SO ₄ -HCO ₃ Ca	C2S1	Permissible
12	Fresh	NaCl	Cl-SO ₄ Ca-Mg	C2S1	Permissible
13	Fresh	NaCl	HCO ₃ -ClCa-Mg	C2S1	Permissible
14	Fresh	Mixed	SO ₄ -HCO ₃ -ClNa-Mg	C3S2	Suitable
15	Fresh	NaCl	Cl-SO ₄ Ca	C2S1	Suitable
16	Fresh	NaCl	ClCa	C1S1	Suitable
17	Fresh	NaCl	Cl-SO ₄ -HCO ₃ Ca-Na	C2S1	Suitable

Discussion

The research area is the Fresh Water Type. According to the SAR method, the irrigation water types fall in C1S1, C2S2, C3S1, C3S2, and C3S3. SAR water types (C3S2) are a moderate sodium hazard, especially in fine-textured soils with low leaching capacity. Can be used in coarse-textured or well-drained soils. SAR water types (C3S3) are a Significant risk of sodium accumulation; careful management and possibly soil changes are required to mitigate impacts. MAR water types are permissible water types.

Summary and Conclusion

The research area is located in Kyaukpadaung Township, Nyaung-U District, Mandalay Region. The Irrawaddy aquifer is the main aquifer of the research area. Chemical Analysis is important to specify the characteristics of groundwater. The concentration of hydrogen ions (pH) is between 6.67 and 7.77. The total dissolved solids are a maximum of 650 ppm. Electrical conductivity (E.C) is not more than 1000 μ mho/cm. According to the TDS, Piper, and Kurlov's method, the research area is the freshwater types and the research area can be classified into 2 types and 4 types. The concentration of Bicarbonate ions is widely distributed in most of the water of the studied region and the amount present in groundwater is relatively higher than other

anions. The specific yield of the groundwater is 1000 to 2000 gallons per hour for 4-inch diameter wells and the depth of the water-bearing horizon ranges from 40 ft to 270 ft in the Irrawaddy aquifer. Calculated by the pumping test, the transmissivity of the aquifer (KD) is 202 m²/day. Calculated by the recovery test, the transmissivity of the aquifer (KD) is 43.94 m²/day. The irrigation water types were analyzed by the SAR method and MAR method. According to the SAR method, the irrigation water types fall in C1S1, C2S2, C3S1, C3S2, and C3S3. According to the MAR method, the water types are permissible. The groundwater of the research area is suitable for drinking water, domestic uses, and irrigation water.

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COMPARATIVE HYDROCHEMICAL ASSESSMENT OF GROUNDWATER FROM DIFFERENT LIMESTONE AQUIFERS: A CASE STUDY FROM PYIN OO LWIN AREA, MANDALAY REGION

Zin Nwe Khaing¹, Zaw Myo Oo², Min Han Nyein³

Abstract

The study area is located Pyin Oo Lwin Township, Mandalay Region. It lies between north latitude 21°57 'N and 22° 05'N between east longitude 96° 23'E and 96° 33'E. The study area is composed of mostly ridge and valley terrain and slightly occurs narrow flat land, it located within western part of the Shan Plateau region. The present area is mainly composed of five lithostratigraphic units mainly composed limestone and dolomite ranging in age from middle Ordovician to Devonian. May Myo dolomite aquifer, the thickness of the fracture zone is at (85 ft to 275 ft). Zebingyi aquifer can be occurred at Zebingyi and Bantha village. Naungbaw aquifer, the thickness of the fracture zone is at (300 ft to 355 ft) depth. The use of water for any purpose is guided under standard set of the WHO and other related agencies. The water quality for irrigation is calculated by Total Dissolved Solid, Sodium Absorption Ratio, Magnesium Hazard, Residual Sodium Bicarbonate, Potential Salinity, Kelly's Ratio. Most of the TDS values of collected water samples from these areas do no exceed 1000ppm, but well no. (POL-3, 4, 7, and 17) contain higher than the 1000ppm. All of the water resource can be used for drinking purpose because of the low range of standard guideline value but some water samples contains high EC value.

Keywords: Pyin Oo Lwin, Groundwater, Total Dissolved Solid, Tube well, EC.

Introduction

Pyin Oo Lwin Township is located in Mandalay Region. It lies between north latitude 21°57 'N and 22° 05'N between east longitude 96° 23'E and 96° 33'E. The study area is located about 3600 ft above the sea level. This area refers to the one inch military Topographic map of 93 B/8, 93 B/5, 93 B/12, 93 C/9. The area of the town is (70 Sq. Km).

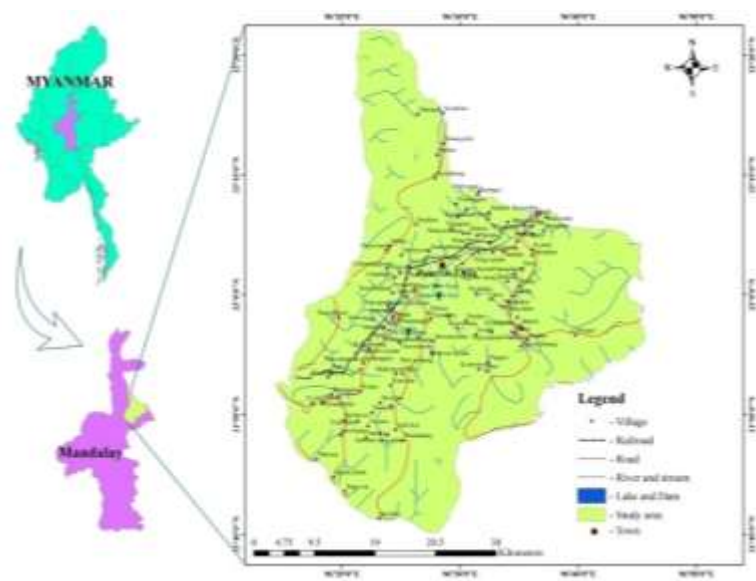


Figure 1.1 location Map of the Pyin Oo Lwin Area

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Drainage

The drainage system is controlled by topography and structure of rocks. Dendritic pattern is mainly developed and shows the reflection of homogeneous feature of bed rocks and underlying folded structure localized drainage patterns such as trellis, radial, and parallel drainage patterns are also observed in the area. The drainage map of the study area is shown in (Fig.1.2)

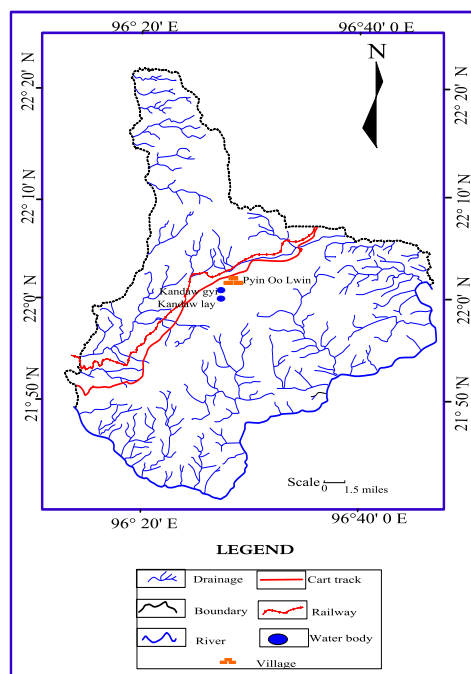


Figure 1.2. Drainage map of the study area

Purpose of Study

The main objectives of the present investigation are as follows;

1. To know type of groundwater aquifer.
2. To know the quality of groundwater.
3. To evaluate the suitability of groundwater.
4. To compare the WHO standard for drinking water purpose.

Methods of Study

The following works had been carried out during the study;

1. The water levels of tube wells are measured by using water level indicator and location of tube wells by GPS.
2. Collection of water sample from the field for chemical analysis and then brought back to the National Health laboratory.
3. Measurement of cations (main Ca^{++} , Mg^{++} , Na^{++} , Fe^{++} and K^{+}) and anions (mainly HCO_3^- , CO_3^- , SO_4^- and CL^-), TDS, TH, pH, and EC were determined and classified by Piper Diagram Method.
4. Water samples were evaluated using the guideline values from World Health Organization, 2011.

5. Calculations of Magnesium Absorption Ratio (MAR), Residual Sodium Bicarbonate (RSBC), Sodium Absorption Ratio (SAR) and Percentage Sodium were investigated to know irrigation water quality.

Regional Geologic Setting

The present area is mainly limestone unit of the five lithostratigraphic units ranging in age from middle Ordovician to Devonian. The Sitha Formation is exposed in the south eastern part of the study area. It is followed by the Kyaingtaung Formation (late Ordovician) which is exposed in the southern part of the present area. The phacoidal limestone Nyaungbaw Formation is mainly found in the eastern part of the study area. The Zebingyi Formation is exposed in the south-western part of the study area. The contact between the Zebingyi Formation and the Nyaungbaw Formation appears to be a conformable and gradational one. The Maymyo Dolomite Formation is widely distributed in the Northern part of the area. The stratigraphic contact between the Maymyo Dolomite Formation and the Zebingyi Formation is ascertained. See in the Figure (1.3).

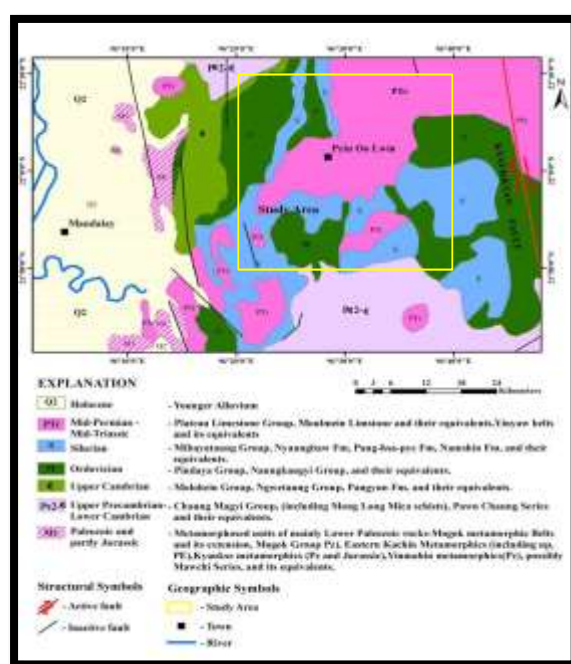


Figure 1.3 Regional Geological Map of the Study Area (From the Geological Map of the Myanmar, 2014)

STRATIGRAPHY

The study area mainly composed of Ordovician Unit, Silurian Unit and Devonian unit. These units are as follows.

Stratigraphic Succession

5. Maymyo Dolomite Formation
4. Zebingyi Formation
3. Nyaungbaw Formation
2. Kyaingtaung Formation
1. Sitha Formation

Geologic Age

- Middle to Late Devonian
- Early Devonian
- Silurian
- Late Ordovician
- Middle Ordovician

Chemical Composition of Groundwater in Study Area

General Statement

Geochemical properties and principals that govern the behavior of dissolved chemical constituents in groundwater are referred to as hydrogeochemistry. The chemical composition of groundwater is related to the solid product of rock, weathering and changes with respect to time and space. The present research area, mainly composed the limestone units of the Ordovician to Devonian; the Maymyo dolomite formation, Nyaungbaw formation and Zebingyi formation selected for aquifer assessment.

The use of water for any purpose is guided by standard set by the WHO and other related agencies. In this study, the results of analyzed chemical parameters in the study area were correlated with those of the WHO. (Research Journal of Environmental and Earth Sciences 3 (2): 131-141, 2011). In the present study, the following are analyzed; Total Dissolved Solids, Electrical Conductivity, Hydrogen Ion Concentration, Total Hardness, Total Alkalinity, Major Cations (Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Potassium (K^{+1}), Iron (Fe^{+2}), Sodium (Na^{+1})), Major Anions (Chloride (Cl^{-1}), Sulfate (SO_4^{-2}), Bicarbonate (HCO_3^{-1}), Carbonate (CO_3^{-2})).

Table 1.1 Comparative study of chemical composition for three different limestone formations

Formation	TDS (mg/L)	EC Micro mho/cm	PH	TH	Major Cations (mg/L)				Major Anions (mg/L)		
					Ca^{2+}	Mg^{+2}	Na^{+1}	K^{+1}	Cl^{-1}	SO_4^{-2}	HCO_3^{-1}
May Myo dolomite Formation	240-1150	370-1796	6.8-7.7	140-360	24-112	2-23	2-43	2-45	25-100	15-235	40-175
Nyaungbaw Formation	390-1000	600-1562	7.2-7.5	270-332	24-85	14-33	8-31	16.8-25	36-60	50-167	86-140
Zebingyi Formation	530-1010	530-1010	6.8-7.1	100-360	16-72	4.8-17	6.44	5.47	50	10-157	23-79

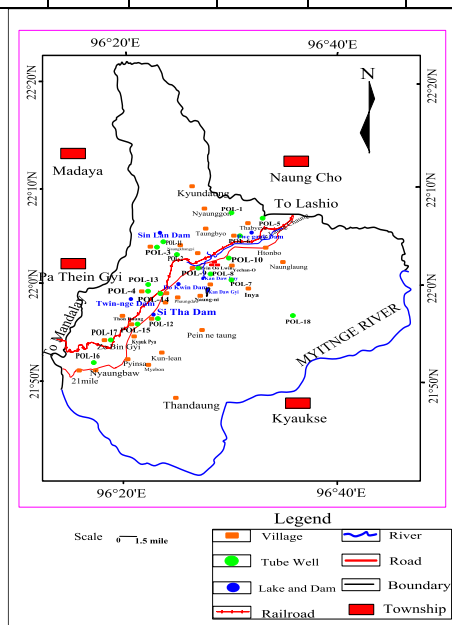


Figure 1.4 Location map of the tube well location from which water samples are taken

Chemical Distribution of Groundwater

The result of the chemical analysis of the various groundwater of the study area are expressed in parts per million (ppm), in equivalents per million (epm) and percentage of equivalents per million (% epm). Graphical presentation of the chemical composition of the groundwater are shown in bar graph using percentage of equivalents per million. (Figure 1.5).

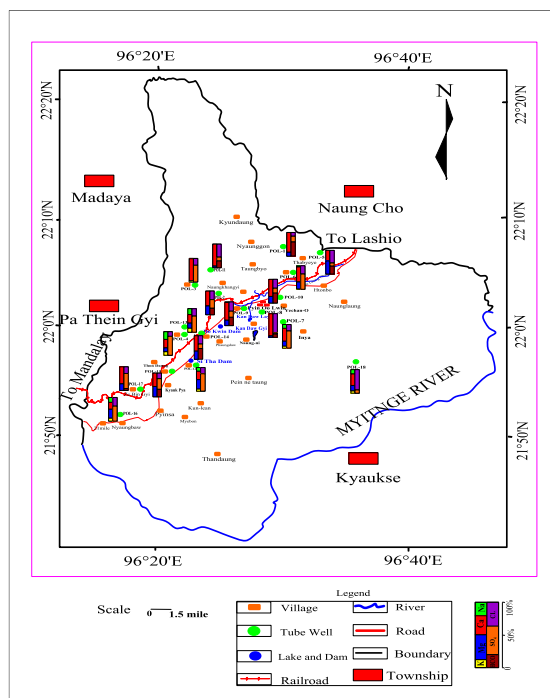


Figure 1.5 Chemical Composition of Groundwater in the Study Area

Classification of Type of Groundwater in the Study Area

1. Total Dissolved Solid
2. Piper Method

Based on the Concentration of Total Dissolved Solids. This classification is suggested by Freeze and Cherry (1979) as follow;

Water Type	Concentration of TDS (ppm)
Fresh water	0-1000
Brackish water	1000-10,000
Salty water	10,000-100,000
Brine water	More than 100,000.

According to Gorell's classification, all the water encountered in the study area is fresh water. Although most of TDS values of collected water samples from these areas do not exceed 1000 ppm, however, POL-3, POL-4, POL-7, POL-17 contain 1100 ppm, 1050 ppm, 1150 ppm, 1010 ppm respectively. Therefore, TDS of these wells are very slightly higher than the 1000 ppm. See in the table (1.2).

Table 1.2 Water Type of the Study Area (Suggested by Freeze and Cherry (1979))

(a) For May Myo Dolomite formation of the Pyin Oo Lwin area

Sample No	TDS (ppm)	Type of Water
POL-1	740	Fresh
POL-2	900	Fresh
POL-3	1100	Brackish
POL-4	1050	Brackish
POL-5	690	Fresh
POL-6	810	Fresh
POL-7	1150	Brackish
POL-8	240	Fresh
POL-9	300	Fresh
POL-10	320	Fresh
POL-11	680	Fresh
POL-12	950	Fresh

(b) For Naungbaw formation of the Pyin Oo Lwin area

Sample No	TDS (ppm)	Type of Water
POL-13	1000	Fresh
POL-14	400	Fresh
POL-15	390	Fresh
POL-16	810	Fresh

(c) For Zebingyi formation of the Pyin Oo Lwin area

Sample No	TDS (ppm)	Type of Water
POL-17	1010	Brackish
POL-18	530	Fresh

Piper Method

It is expressed as percentages of total cations in milliequivalents per liter, plot as a single point on the left triangle, while anions, similarly expressed as percentages of total anions, appear as a point in the right triangle. Piper diagram of water samples from the study area shown in Figure and Table.

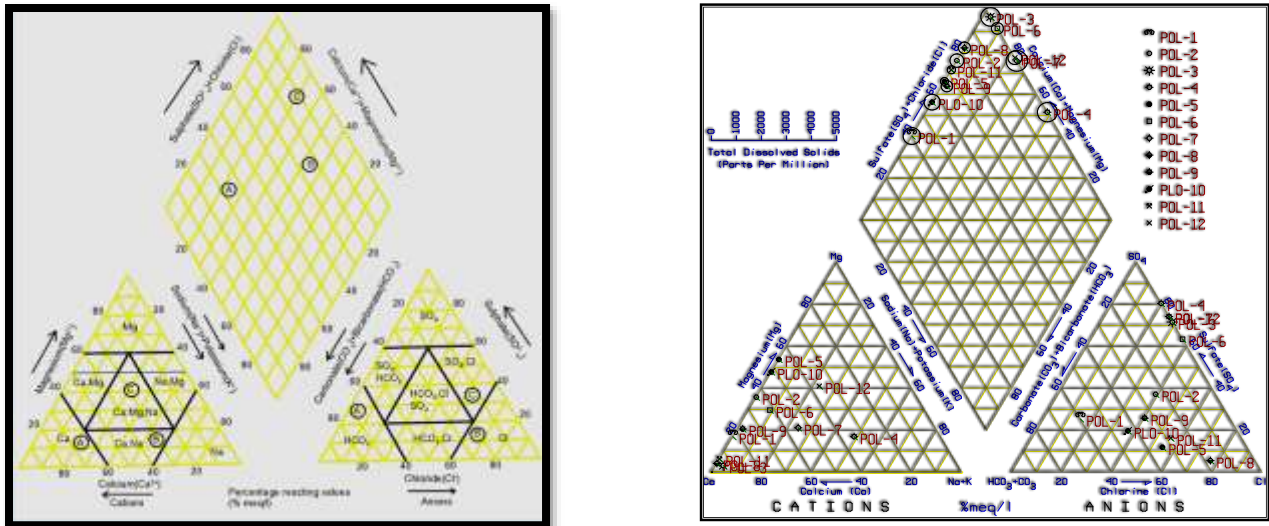


Figure 1.6 Piper Diagram for May Myo Dolomite formation in the Pyin Oo Lwin Area

Table 1.3 Groundwater Classification by Piper diagram

(a) For May Myo Dolomite formation of the Pyin Oo Lwin Area

No		Water Type by Piper Methods
POL-1	Ca SO ₄ -HCO ₃	Calcium Sulphate Bicarbonate water
POL-2	Ca HCO ₃ -CL-SO ₄	Calcium Bicarbonate Chloride Sulphate water
POL-3	Ca SO ₄	Calcium Sulphate water
POL-4	Ca-Na SO ₄	Calcium Sodium Sulphate water
POL-5	Ca-Mg HCO ₃ -Cl	Calcium Magnesium Bicarbonate Chloride water
POL-6	Ca SO ₄	Calcium Sulphate water
POL-7	Ca-Mg-Na SO ₄	Calcium Magnesium Sodium Sulphate water
POL-8	Ca Cl	Calcium Chloride water
POL-9	Ca HCO ₃ -CL-SO ₄	Calcium Bicarbonate Chloride Sulphate water
POL-10	Ca- Mg HCO ₃ -Cl-SO ₄	Calcium Magnesium Bicarbonate Chloride Sulphate water
POL-11	Ca HCO ₃ -CL	Calcium Bicarbonate Chloride water
POL-12	Ca-Mg-Na SO ₄	Calcium Magnesium Sodium Sulphate water

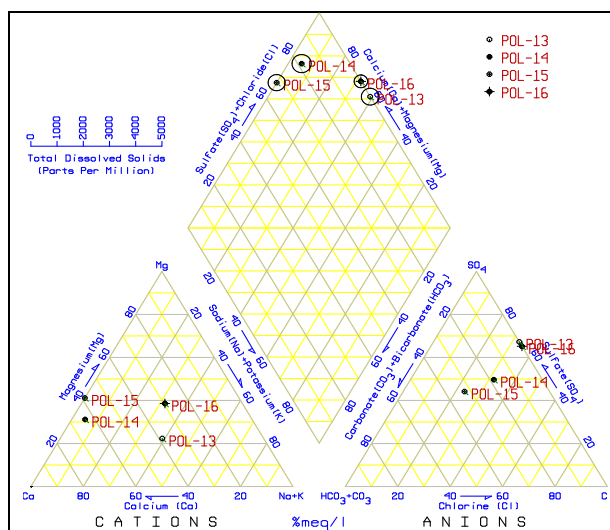


Figure 1.7 Piper Diagram for Naungbaw formation in the Pyin Oo Lwin Area

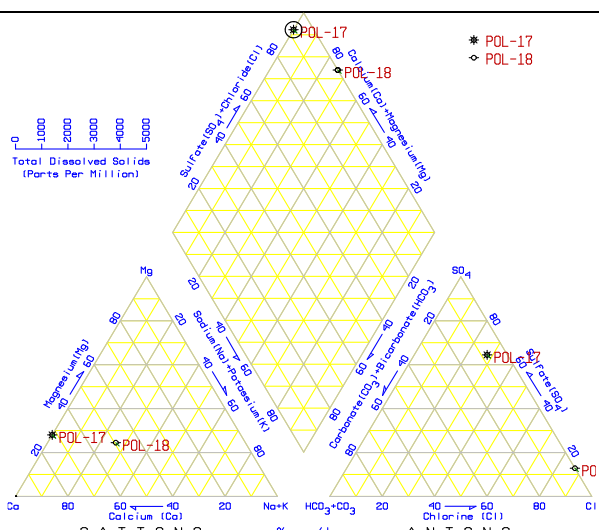


Figure 1.8 Piper Diagram for Zebingyi formation in the Pyin Oo Lwin Area

Table 1.3 Groundwater Classification by Piper diagram

(b) For Naungbaw formation of the Pyin Oo Lwin Area

No		Water Type by Piper Methods
POL-13	Ca-Mg-Na SO ₄	Calcium Magnesium Sodium Sulphate water
POL-14	Ca SO ₄ -CL	Calcium Sulphate Chloride water
POL-15	Ca-Mg HCO ₃ -CL-SO ₄	Calcium Magnesium Bicarbonate Chloride Sulphate water
POL-16	Ca-Mg-Na SO ₄	Calcium Magnesium Sodium Sulphate water

Table 1.3 Groundwater Classification by Piper diagram

(c) For Zebingyi formation of the Pyin Oo Lwin Area

No		Water Type by Piper Methods
POL-17	Ca SO ₄	Calcium Sulphate water
POL-18	Ca-Mg-Na CL	Calcium Magnesium Sodium Chloride water

Utilization of Groundwater in Pyin Oo Lwin Area for Irrigation

Irrigation water criteria are dependent on the type of plants, amount of irrigation water used, soil and climate, as some plants are highly sensitive to a certain concentration of an element in the irrigation waters. The amount of water available for flushing the irrigated soil is decisive for the appearance of side-effects such as increase of unwanted compounds in the top layer of the soil. Soil and Climate condition are ultimately determining the degree of danger for unfavourable possibilities. A high evaporation and a soil of low permeability give a poor drainage and a

dangerous built up of soil-moisture salinity. A high amount of water for flushing will then be required.

In evaluating the quality of water for irrigation sodium is significant factor. A high percentage of Na in water in relation to Ca+Mg tends to break down the soil structure and the soil becomes more plastic and this will restrict water movement.

Table 1.4 Comparison of water quality with the limits set out by FAO for irrigation water

Salinity	Spec. conduct at 25°C in micromhos	Na Class	S.A.R with spec .100	Conduct. of 5000	FAO Guideline
C1	<250	S1	<10	<2.5	Excellent
C2	250-750	S2	10-18	2.5-6.5	Good
C3	750-2250	S3	18-26	6.5-11	Fair
C4	>2250	S4	>26	>11	Poor

Electrical Conductivity (EC)

Electrical Conductivity in the study area show that most of samples are medium to high saline. This sample is safe under permeable soil and moderate leaching for irrigation. Well no. (POL-8,9,10,14,15) are moderately saline. (Table (1.5), (1.6 (a, b, c))).

Table 1.5 Quality of irrigation water in relation to EC after Richard (1954)

No	Electrical conductivity ($\mu\text{mhos/cm}$)	Type of water	Suitability for irrigation	Remarks
1	Below 250	Low saline water	Entirely safe	Nil
2	250-750	Moderately saline	Safe under practically all condition	POL-8,9,10,14,15 fall in this zone
3	750-2250	Medium to high saline	Safe under permeable Soil and moderate leaching	POL-1,2,3,4,5,6,7,11,12,13,16,17,18
4	i.Above 2250 ii.2250-4000 iii.4000-6000 iv.Above 6000	High salinity Very high salinity Excessive salinity class	Unfair for irrigation Unfair for irrigation Unfair for irrigation	Nil Nil Nil

(Source; Res.J. Environ. Earth Sci., 3 (2) 131-141, 2011)

Table 1.6 Physico-chemical characteristics and suitability for irrigation

(a) For May Myo Dolomite Formation of the Pyin Oo Lwin Area

Sample no	PH	EC	TDS	MH	SAR	Na%	PS	RSB C	KR	Remarks
POL-1	7.4	1156*	740	17.01	-	-	1.33	-1.12	-	Suitable
POL-2	6.8	1406*	900	0.36	-	-	2.5	-1.75	-	Suitable
POL-3	6.8	1718*	1100*	3.45	0.07	3.02	3.84	-	0.02	Suitable
POL-4	7.2	1640*	1050*	33.11	1.53	48.41	3.47	-	0.63	Suitable
POL-5	7.4	1078*	690	54.05	-	-	1.57	-0.31		Suitable
POL-6	7.1	1265*	810	32.49	0.17	8.53	2.66	-	0.06	Suitable
POL-7	7.2	1796*	1150*	28.06	0.66	24.02	4.14	-	0.21	Suitable
POL-8	7.6	370	240	4.28	-	-	2.93	-3.74	-	Suitable
POL-9	7.7	470	300	21.08	0.06	-	1.64	-2.47	0.02	Suitable
POL-10	7.5	510	320	29.42	-	-	2.44	-2.31	1.63*	Unsuitable
POL-11	7.4	1062*	680	6.27	-	-	1.62	-1.67	-	Suitable
POL-12	7.2	1484*	950	53.24	0.51	22.55	2.77	-	0.19	Suitable

(b) For Naungbaw Formation of the Pyin Oo Lwin Area

Sample no	PH	EC	TDS	MH	SAR	Na%	PS	RSBC	KR	Remarks
POL-13	7.2	1562*	1000	36.62	1.09	39.38	3.43	-	0.43	Suitable
POL-14	7.5	630	400	32.81	0.02	-	3.01	-3.28	0.005	Suitable
POL-15	7.3	600	390	41.12	-	-	1.97	-2.47	-	Suitable
POL-16	7.2	1265*	810	56.73*	0.49	31.93	2.73	-	0.31	Unsuitable

(c) For Zebingyi Formation of the Pyin Oo Lwin Area

Sample no	PH	EC	TDS	MH	SAR	Na%	PS	RSBC	KR	Remarks
POL-17	7.1	1578	1010*	28	-	-	3.01	-3.21	-	Suitable
POL-18	6.8	828	530	32.77	0.36	26.12	1.52	-	0.24	Suitable

Drinking Water

Most of the people obtain drinking water from private tube wells and W.R.U.D tube wells. The standard proposed by National Drinking Water Quality Standard, Myanmar, 1990 is shown in table and it is compared with the obtained value of groundwater analysis data from the study Area. According to the chemical analyses, some water samples contain high EC value.

Table 1.7 Comparison with National Drinking Water Quality Standard

Characteristics	Guideline Value		The range obtained value from ground water	Remarks
	Desirable	Max Permissible		
Calcium	75 mg/L	200 mg/L	16- 112 mg/L	Good
Magnesium	30 mg/L	150 mg/L	2- 3.3 mg/L	Good
Sodium	- mg/L	200 mg/L	2.22- 31.26 mg/L	Good
Potassium	- mg/L	200mg/L	2.35- 45.36 mg/L	Good
Sulphate	- mg/L	400 mg/L	10- 235 mg/L	Good
Chloride	200 mg/L	600 mg/L	19- 100 mg/L	Good
Iron	0.5 mg/L	1.5 mg/L		
TDS	- mg/L	1000 mg/L	240- 1150 mg/L	Good (Except POL-3,4,7,17)
pH	6.5	9.2	6.8- 7.7	Good
Hardness CaCO₃	- mg/L	500 mg/L	100- 360 mg/L	Good
Colour (Pt.Co.Scale)	5	50		
Turbidity (NTU)	5	20		
Aluminium	- mg/L	0.2 mg/L		
Copper	- mg/L	1 mg/L		
Manganese	- mg/L	0.3 mg/L		
Zinc	5 – mg/L	15 mg/L		
EC	- micromho/cm	1500 micormhos/cm	370-1796 micromhos/cm	Good (Except POL-3,4,7,13,17)

Summary and Conclusion

Pyin Oo Lwin Township is located in Mandalay Region. It lies between north latitude 21°50 'N and 22° 10'N between east longitude 96° 10'E and 96° 40'E. The study area is located about 3600 ft above the sea level. The study area is mainly composed of five lithostratigraphic units ranging in age from middle Ordovician to Devonian. TDS value is 240 ppm to 1150 ppm for May Myo dolomite aquifer and 390 ppm to 1000 ppm for Naungbaw formation. EC value is 370 micro mho/cm to 1796 micro mho/cm for May Myo dolomite formation and 600 micro mho/cm to 1562 micro mho/cm for Naungbaw formation and 530 micro mho/cm to 1010 micro mho/cm for Zebingyi formation. And then pH value fall 6.8 to 7.7 for May Myo dolomite formation and 7.2 to 7.5 for Naungbaw formation. The pH value is ranging from 6.8 to 7.1 for

Zebingyi formation. Robinove (1958) classification which is based on total dissolved solid (TDS) shows that almost all the water samples of the study area can be regarded as fresh water. All the water falls in the S1C2 and S1C3 types S1C1 means low Sodium water with Medium salinity water S1C3 means low Sodium water with High salinity water. So, all the water are suitable for irrigation water. Other methods used Sodium Absorption Ration, Residual Sodium Bicarbonate, Kellys Ration, Magnesium Hazard, Potential Salinity. All of the samples are suitable for irrigation water. The chemical content of the water is compared with WHO guideline and it was found that some water sample contain high EC value for drinking purpose.

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PETROGRAPHY, PETROCHEMISTRY OF LAMPROPHYRES AND ECONOMIC ASPECTS IN WINPYAN QUARRY, BILIN TOWNSHIP, MON STATE

Mya Moe Khaing¹, Nway Cherry Win², Kyu Kyu Hlaing³, Aung Min Thu⁴

Abstract

The research area is situated about 170 km north-east of Yangon and about 96 km north-west of Mawlamyine. Lamprophyres are melanocratic, porphyritic and hypabyssal rocks. Lamprophyre is mainly composed of hornblende, plagioclase, orthoclase and opaques. Under the microscopic study, this lamprophyre may be classified as spessartite; it has medium-grained porphyritic and panidiomorphic texture, dominantly comprising euhedral to subhedral forms of hornblende phenocrysts, groundmass of plagioclase and minor amount of K-feldspar and quartz. According to the chemical analysis data, $\text{MgO-K}_2\text{O-Al}_2\text{O}_3$ and $\text{Fe}_2\text{O}_3\text{-Al}_2\text{O}_3\text{-MgO}$ ternary diagrams show that the samples fall in the lamprophyre field. $\text{MgO-Al}_2\text{O}_3\text{-CaO}$ triangular diagram indicates that the samples fall in the field of alkaline lamprophyre. These lamprophyres have high K_2O and Na_2O concentration and show alkaline characters. Due to the alkaline characters, lamprophyres are porphyritic, volatile-rich igneous rocks, which are emplaced as dykes. Lamprophyre is observed as dyke intruding into the pyroxenite at Winpyan quarry. This dyke is a very late phase of large igneous intrusion. By the field investigation and petrographic analysis, the petrogenesis of lamprophyre may be considered as magmatic origin. Lamprophyre in the research area are generally thought to be derived from low degree partial melting of a lithospheric mantle that has been previously metasomatised by subduction-related fluids. Absolute age of the lamprophyre is still unknown, due to lack of radiometric dating of the rock. In the research area, the age of the biotite granite is 51.9 ± 0.7 Ma (Eocene) in age. Therefore, the age of lamprophyre may be younger than Eocene age. Specular hematite ore minerals found near the lamprophyre dyke. Specular hematite has been used as jewellery; such as necklaces, rings and bracelets. By wearing specular hematite, it makes to treat disorders of the blood, to heal broken bones, to help the effects of electromagnetic fields and to protect toxic energy from computers.

Keywords; lamprophyre, alkaline lamprophyre, magmatic origin, specular hematite

Introduction

Location and size

The research area is situated about 170 km north-east of Yangon and about 96 km north-west of Mawlamyine. It is lying between Latitude $17^\circ 12' 45''\text{N}$ to $17^\circ 21' 45''\text{N}$ and Longitude $97^\circ 09' 00''\text{E}$ to $97^\circ 14' 45''\text{E}$ in one-inch topographic map No. 94G/3 and G/4. It extends about 8 km from east to west and 14.4 km from north to south. The total areal coverage is about 116.5 km. The location map of the research area is shown in Figure 1.

Physiography and Drainage

The northern, north-western and south-eastern parts of the study area are mountainous terrain and southern and western parts have rolling hills and central and southern parts have flat-lying topography, Figure 2. In the study area, the longest Bilin River flow from north to south, in the eastern boundary of the study area. Gonnyinnwe Chaung (flowing north to south) is found in the western part of the area. In the eastern part of the area, Yinon Chaung flow from east to west and Paingdawe Chaung is located in the central part and flow from east to west. The drainage pattern of the research area is medium to coarse dendritic, radial and sub-parallel drainage pattern. The drainage pattern of the research area is shown in Figure 3.

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Aim and Objectives

- To carry out the petrography and petrochemistry of the lamprophyre exposed in the research area
- To investigate possible origin and age of lamprophyre
- To know economic aspects in the research area

Research Methodology

A detailed geological field investigation was carried out by using available simple equipments. The geological data in the field were plotted on the enlarged map from one-inch to one-mile topographic map. Detailed microscopic examination, 10 thin sections from lamprophyres were studied under polarizing microscope. As a laboratory method, XRF analyses were done to estimate the percentage of chemical composition in the research area.

Literature Background

Lamprophyres are named after the Spessart region in southern Germany. *Lamprophyres* are uncommon; small-volume ultrapotassic igneous rocks primarily occur as dykes. Lamprophyres are melanocratic, porphyritic, fine to medium-grained igneous rock usually formed at a moderate distance below the surface. Lamprophyres have to be derived from deep, volatile-driven melting in a subduction zone setting, (after Rock, 1991).

Results and Finding Petrography of Lamprophyre

Field description

Lamprophyre (spessartite) has medium-grained texture, it is observed as dyke which is injected into the country rock of pyroxenite at Winpyan quarry, Figure 5. A. Trend is $40^{\circ}/220^{\circ}$. Fresh surface are greenish gray and gray in colour and weathered colour are dark gray and yellowish gray, it is compact and well jointed. Phenocrysts of hornblende crystals are observed on fresh surface of lamprophyre. Good exposures occur in Winpyan old quarry, Figure 5. B. and the thickness of lamprophyre (spessartite) dyke is about 2 m to 4 m.

Microscopic description

Under the microscopic study, this lamprophyre may be classified as spessartite; spessartite is mainly composed of hornblende, plagioclase, orthoclase and opaques. It has medium-grained porphyritic and panidiomorphic texture, dominantly comprising euhedral to subhedral forms of hornblende phenocrysts, groundmass of plagioclase and minor amount of K-feldspar and quartz. Hornblendes occur as principal mafic minerals with simple twin, Figure 5. C. Pale green and yellowish brown colour of fractured and skeletal hornblende is observed, Figure 5. D. Hornblende crystals are highly corroded and penetrated each other, Figure 5. E. Phenocrysts of hornblende are common. Basal section of hornblende crystals is also found in spessartite, Figure 5. F. Plagioclase occurs as fine-grained in the groundmass. Sometimes, it shows as lath-shaped, Figure 5. G. Zoning pattern is well developed in plagioclase feldspar. Alkalifeldspar occurs as minor amount with subdral forms and shows simple twin. Quartz shows corroded and surrounded by fine sericite. Simple contact twin of hornblende is present, Figure 5. H. Accessory minerals are apatite, sphene and opaque minerals.

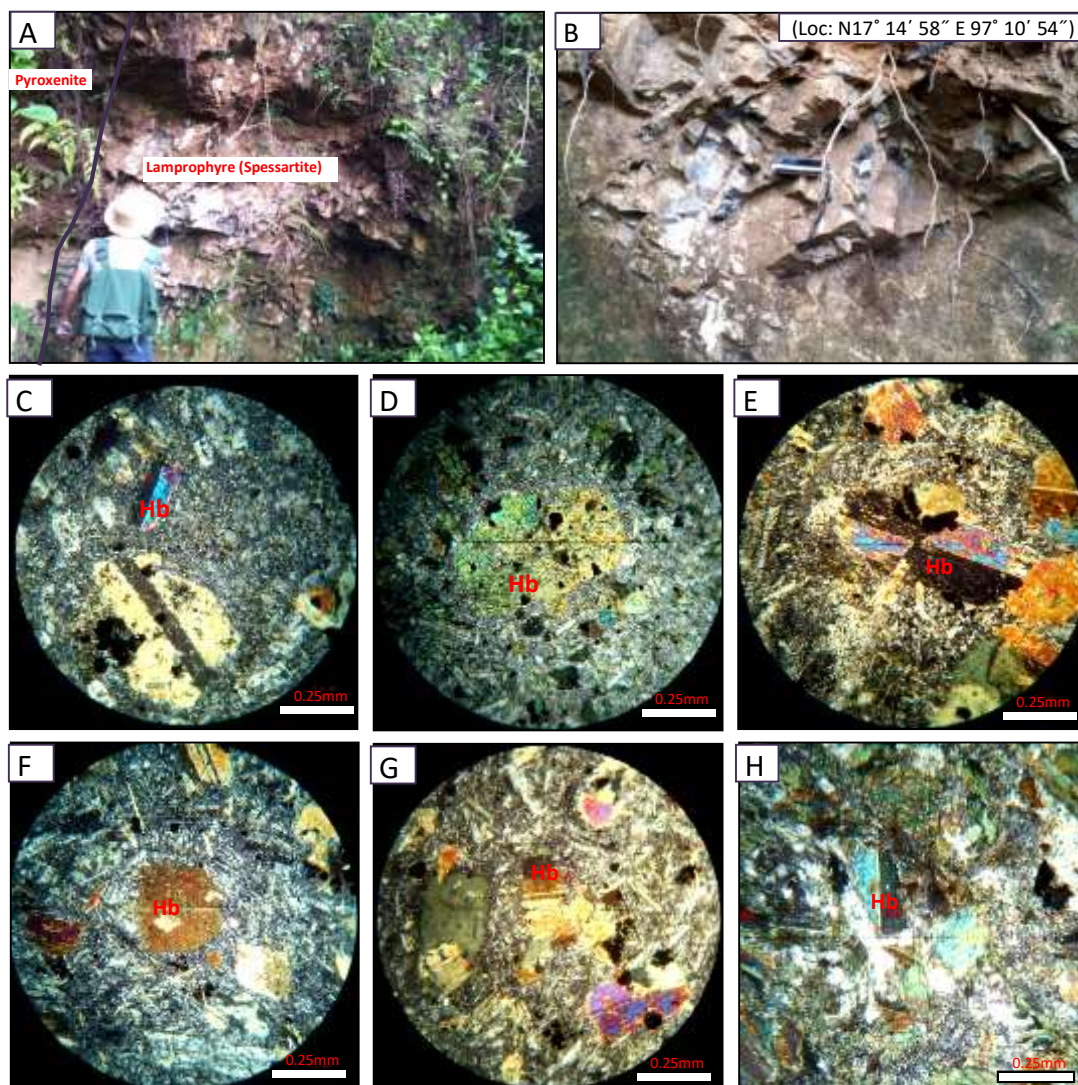


Figure 5. (A) Lamprophyre (spessartite) is intruded into the pyroxenite body at Winpyan quarry, facing 60°
 (B) Exposure of lamprophyre (spessartite) at Winpyan old quarry, facing 90°
 (C) Simple twin of hornblendes (Hb) in spessartite (between X.N, 10X)
 (D) Fractured and skeletal hornblende (Hb) in spessartite (between X.N, 10X)
 (E) Simple twin nature of hornblende (Hb) in spessartite (between X.N, 10X)
 (F) Basal section of hornblende crystal (Hb), magnetite and feldspar laths in spessartite (between X.N, 10X)
 (G) Phenocrysts of hornblende (Hb) and groundmass of feldspar laths
 (H) Simple contact twin of hornblende (Hb) in spessartite (between X.N, 10X)

Classification of Lamprophyre

According to the (Le Maitre *et al.* 1989), lamprophyre can be classified nine types, Table 1. In the research area, lamprophyre is composed of plagioclase feldspar > K-feldspar and hornblende phenocrysts are the dominant mafic minerals and plagioclase feldspar laths are groundmass. So, it can be defined as spessartite.

Table 1. Classification and nomenclature of lamprophyres based on their mineralogy (after Le Maitre and Rock, 1991)

Light-coloured constituents		Predominant mafic minerals			
Feldspar	Foid	Biotite, diopsidic augite, (±olivine)	Hornblende, diopsidic augite, (±olivine)	Amphibole, (barkevikite, kaersutite), Ti-augite, olivine, biotite	Melilite, biotite, ±Ti-augite, ±olivine, ±calcite
or > pl	—	Minette	Vogesite	—	—
pl > or	—	Kersantite	Spessartite	—	—
or > pl	Feld > foid	—	—	Sannaite	—
pl > or	Feld > foid	—	—	Camptonite	—
—	Glass or foid	—	—	Monchiquite	Polzenite
—	—	—	—	—	Alnöite

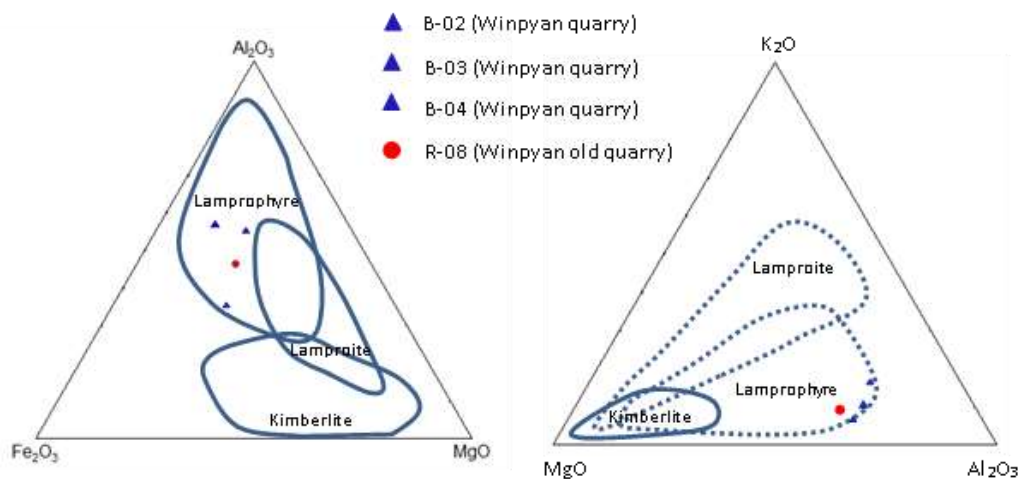
Or, alkali feldspar; pl, plagioclase; feld, feldspar; foid, feldspathoid.

Petrochemistry of Lamprophyre

The analysis of lamprophyre samples was determined by DSSTRC (Defence Service Science and Technology Research Centre) in Pyin-Oo-Lwin Township. Major element chemistry of lamprophyres is given in Table 2. Compositionally, the samples are basic to ultrabasic; the silicon dioxide percentage varies from 47.28% to 51.93%. Al_2O_3 content is high, varying from 13.1% to 20.26%. The concentration of Fe_2O_3 varies from 9.09% to 13.06% and that of MgO varies from 4.39% to 8.8%. The amount of CaO varies from 7.49 % to 9.34%. The analyses of these rocks are plotted in ternary discrimination diagrams. In the Fe_2O_3 - Al_2O_3 - MgO triangular diagram (after Cornelissen and Verwoerd, 1975; Kornprobst, 1984), the samples are predominantly in the field of lamprophyre, Figure 6. A. MgO - K_2O - Al_2O_3 ternary diagram (after Bergman, 1987) shows the samples fall in the lamprophyre field, Figure 6. B. MgO - Al_2O_3 - CaO triangular diagram (after Rock, 1987) indicates that the samples fall in the field of alkaline lamprophyre (AL), Figure 7. SiO_2 vs $\text{Na}_2\text{O} + \text{K}_2\text{O}$ diagram (after Irvine and Baragar, 1971) shows alkaline nature of lamprophyre, Figure 8. The lamprophyres belong to alkaline suite; they are enriched in alkalis. The lamprophyres have high K_2O and Na_2O concentration and show alkaline characters. Sodium and Potassium are known to be relatively more mobile (after Pearce and Cann, 1973; Floyd and Winchester, 1975), this suggests feldspar crystallization to have been relatively late and fractionation dominated by olivine and clinopyroxene crystallization. Alkaline lamprophyres are porphyritic, volatile-rich igneous rocks, which are typically emplaced as small-volume intrusions, dykes. Lamprophyre in the research area are generally thought to be derived from low degree partial melting of a lithospheric mantle that has been previously metasomatised by subduction-related fluids (after Rock, 1991).

Table 2. Major oxide (wt %) and trace elements (ppm) abundances of the lamprophyre from the research area

Sample No	B-04	R-08	B-02	B-03	Sample No	B-04	R-08	B-02	B-03
SiO ₂	48.8	51.93	47.84	47.28	Mo	0.06	0.06	0.12	0.11
TiO ₂	0.67	0.63	0.56	0.506	Nb	0.05	0.06	0.05	0.08
Al ₂ O ₃	19.4	14.5	13.1	20.26	Pb	6.23	2.15	3.33	3.454
Fe ₂ O ₃	10.5	9.69	13.06	9.097	Ga	-	0.01	1.295	1.341
MnO	0.237	0.15	0.251	0.086	Zn	6.27	11.08	9.657	6.573
MgO	4.39	7.05	8.8	6.263	Cu	5.74	3.88	5.817	8.012
CaO	7.49	7.54	8.694	9.34	Co	10.3	9.58	9.459	7.996
Na ₂ O	4.29	3.03	3.26	4.01	Si	19.19	19.31	21.77	19.85
K ₂ O	3.56	2.91	2.48	1.331	Fe	919	897	918.7	898.8
P ₂ O ₅	0.366	0.52	0.421	0.087	Mn	17.55	21.7	20.34	19.12
Total	99.7	97.9	98.47	98.26	Ti	14.14	19.83	14.5	19.69
A/CNK	1.264	1.07	0.907	1.380	Nd	0.73	0.84	0.707	0.656
A/NK	2.471	2.44	2.28	3.29	Pr	0.51	0.534	0.537	0.541
Cl	29.15	25.29	22.744	26.316	Ce	29.8	37.3	28.66	29.33
Ca	38.12	58.51	49.19	38.4	La	0.29	0.311	0.274	0.233
Cd	2.814	1.58	4.299	7.321	Ba	0.39	0.311	0.388	0.623
Ag	3.87	3.29	1.34	4.708	Sb	0.07	0.212	0.051	0.055
Pd	3.89	2.68	0.29	0.966	Zr	36.96	63.69	25.72	63.9
Rh	1.36	-	0.524	2.092	Y	1.72	2.15	0.50	0.69
K	11.46	11.47	10.45	11.87	Sr	306.7	675.4	565.9	405.58
Rb	28.18	27.33	3.5	9.84					

**Figure 6. (A)** Fe₂O₃-Al₂O₃-MgO diagram shows the samples fall in the Lamprophyre field

(B) MgO-K₂O-Al₂O₃ diagram showing the fields of Classical Kimberlite, Lamproite and Lamprophyre, this diagram shows the samples fall in the field of Lamprophyre (after Bergman, 1987)

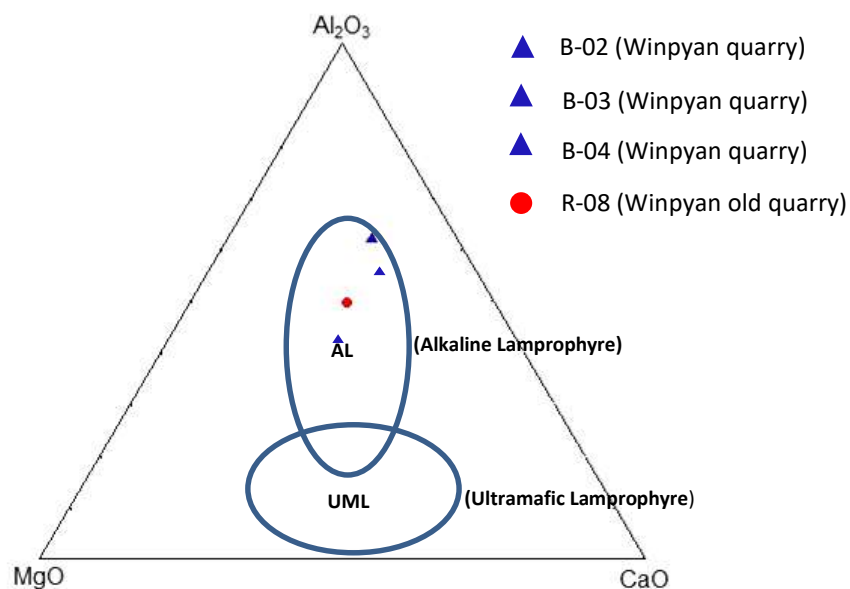


Figure 7. MgO-Al₂O₃-CaO diagram showing the field of AL (Alkaline Lamprophyre) (after Rock, 1987)

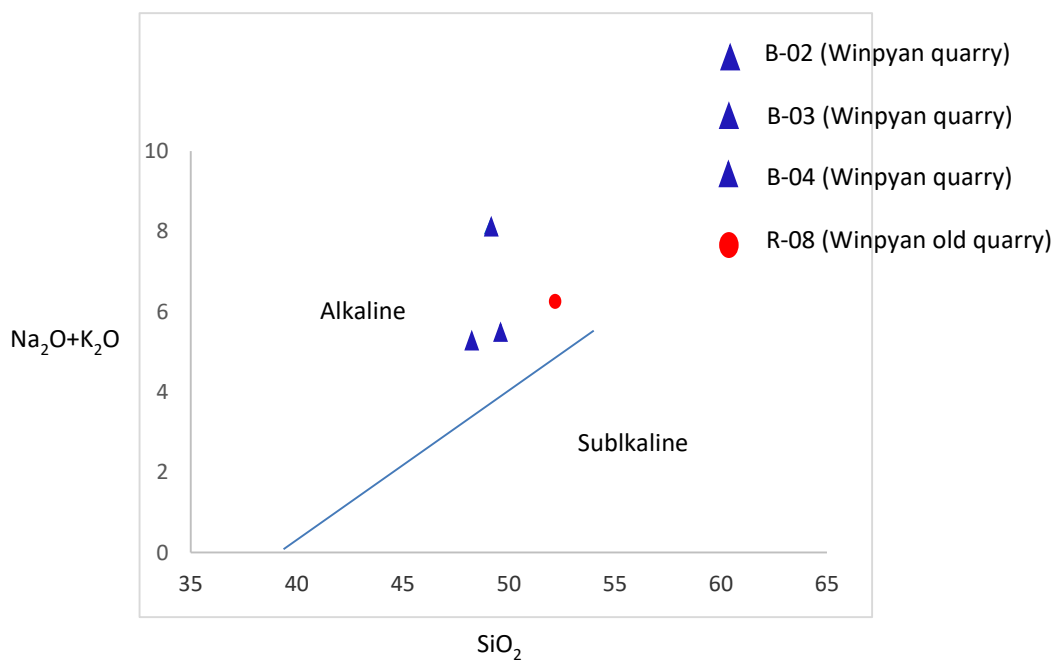


Figure 8. SiO₂ vs Na₂O + K₂O diagram showing alkaline nature of lamprophyre (after Irvine and Baragar, 1971)

Possible origin of Lamprophyre

By the field investigation and petrographic analysis, the petrogenesis of lamprophyre may be considered as magmatic origin. Mineral composition occurs as two generations; phenocrysts and groundmass, i.e., plagioclase feldspar and hornblende in spasserite. It strongly shows zone of plagioclase and hornblende abundantly. Sometimes, it has characterized by granitic composition and aplitic texture; it is possible that basic magma intruded into the granite consolidated to fracture. It displays sharp contact with the country rocks. Moorehouse, 1959 describes that basic materials derived from the mantle and siliceous magma are assimilation involved. Lamprophyric magma formed by remelting crystal of ferromagnesium minerals accumulation at the base of differentiating magma. So, the lamprophyric magma responsible for these dykes is a very late phase of large igneous intrusion.

Possible age of Lamprophyre

Absolute age of the lamprophyre dyke is still unknown, due to lack of radiometric dating of the rock. Pyroxenite xenolith is observed in the biotite granite at the south-eastern part of Alugale village, Figure. 9. It indicates that biotite granite is younger than pyroxenite. The lamprophyre is observed as dyke intruding into the pyroxenite at Winpyan old quarry and which from field relationships is the youngest intrusion at Bilin area. On the result of U-Pb zircon dating methods, the zircon age of the biotite granite from the northern part of Laykhin pagoda in Bilin area is 51.9 ± 0.7 Ma (Eocene), Figure. 10. Therefore, the age of lamprophyre may be younger than Eocene age.



Figure 9. Pyroxenite xenolith found in biotite granite at the south-eastern part of Alugale village, facing 20° (Loc: N 17° 16' 00" E 97° 11' 10")

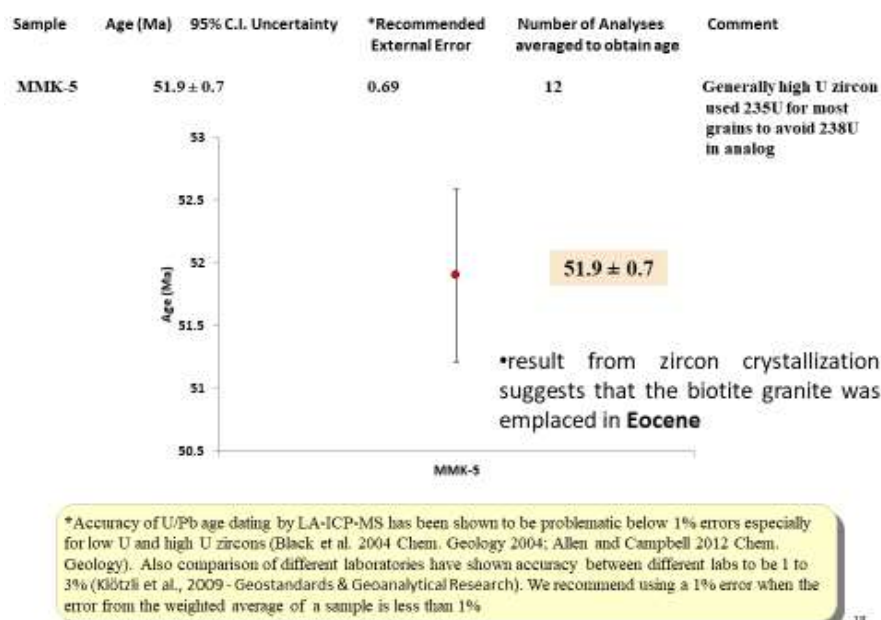


Figure 10. Biotite granite (MMK-5) from the northern part of Laykhin pagoda (Loc: N 17°14'16", E 97°12'46") gives the zircons age of 51.9 ± 0.7 Ma (after Mya Moe Khaing, 2017)

Economic Aspects

Specular hematite occurs from the south-eastern part of Kyaukseing Taung, shown in Figure 11. A, B, C, D. Specular hematite observed near the lamprophyre dyke. The lamprophyre rocks are being used as construction and dimension stones. This specular hematite crystal is a beautiful variety of hematite that has a rich metallic luster on a sparkling silvery polished surface. Specular hematite has been used as jewellery; such as necklaces, rings and bracelets, Figure 12. By wearing specular hematite, it makes to treat disorders of the blood and to heal broken bones. Specular hematite crystal can help regulate the effects of electromagnetic fields and is excellent to have on hand when exposed to toxic energy from computers or other electrical systems.

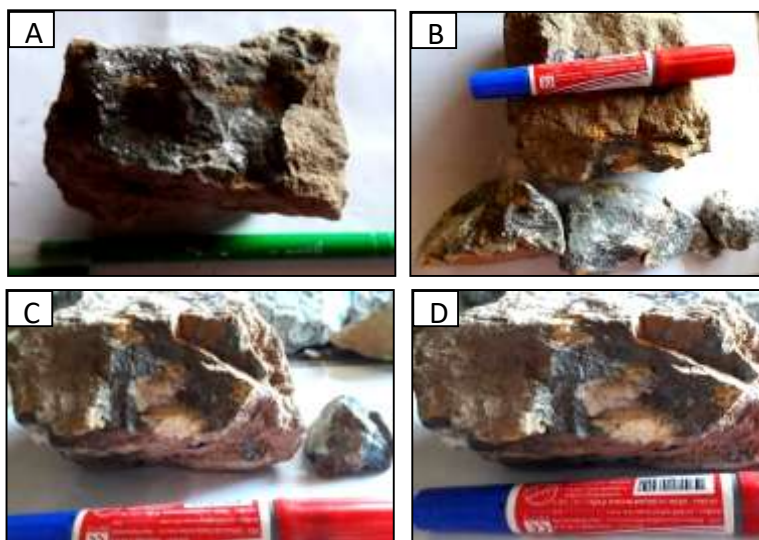


Figure 11. (A, B, C, D) Specular hematite samples occurs at the south-eastern part of Kyaukseing Taung (Loc: N 17° 15' 01" E 97°10' 27")



Figure 12. Jewellery samples of specular hematite

Conclusion and Discussion

Lamprophyre is mainly composed of hornblende, plagioclase, orthoclase and opaques. It has medium-grained porphyritic and panidiomorphic texture. Mineral composition occurs as two generations; phenocrysts and groundmass, euhedral to subhedral forms of hornblende phenocrysts, groundmass of plagioclase and minor amount of K-feldspar. It strongly shows zone of plagioclase and hornblende abundantly. By the field investigation and petrographic analysis, the petrogenesis of lamprophyre may be considered as magmatic origin. Lamprophyres are porphyritic, volatile-rich igneous rocks, which are typically emplaced as small-volume intrusions, dykes. Lamprophyre is observed as dyke intruding into the pyroxenite at Winpyan old quarry. So, dyke is a very late phase of large igneous intrusion. Lamprophyre in the research area are generally thought to be derived from low degree partial melting of a lithospheric mantle that has been previously metasomatised by subduction-related fluids. Absolute age of the lamprophyre dyke is still unknown, due to lack of radiometric dating of the rock. Pyroxenite xenolith is observed in the biotite granite at the south-eastern part of Alugale village. It indicates that biotite granite is younger than pyroxenite. On the result of U-Pb zircon dating method, the zircon age of the biotite granite from the northern part of Laykhin pagoda in Bilin area is 51.9 ± 0.7 Ma (Eocene). Therefore, the age of lamprophyre may be younger than Eocene age. The lamprophyre rocks are being used as construction and dimension stones. Specular hematite found near the lamprophyre dyke. Specular hematite crystal is a beautiful variety of hematite that has a rich metallic luster on a sparkling silvery polished surface. By wearing the specular hematite crystal, it can help regulate the effects of electromagnetic fields and is excellent to have on hand when exposed to toxic energy from computers or other electrical systems.

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INVESTIGATING THE PETROGRAPHY AND DIAGENESIS STUDY OF THE OKHMINTAUNG FORMATION: A CASE STUDY FROM CHAUNG ZON AREA, PWINTBYU TOWNSHIP, MAGWAY REGION

Than Zaw¹, Thin Thin Han²

Abstract

The reserach area is located in Chaung Zon Village, Pwintbyu Township, Magway Region. Okhmintaung Formation is characterized by light grey, medium to coarse grained, thick bedded to massive type sandstones intercalated with thin-bedded mudstones and bluish grey shales are also occurred. The sandstones of the Okhmintaung Formation may be termed as “Sublithicarenite”. Alteration of biotite to muscovite and oxidized biotite are more common in the Okhmintaung sandstone in the study area. The cementing material in the Okhmintaung sandstones are calcite and iron cements, glauconite, rutile and clay matrix. The common characteristics of late burial or phyllomorphic stage are the alteration of clay mineral into micas and the development of well crystallized phyllosilicate minerals.

Keywords: Stratigraphy, Petrography, Nomenclature of sandstone, Diagenesis, Diagenesis stage.

Introduction

Location and size

The study area is located between north latitude 20° 12' 30" - 20° 16' 30" and east longitude 94° 25' 00" - 94° 28' 30". It is situated about 16 miles (25.75 km) west of the Pwintbyu Township. It falls in 2094/7 (84 L/7) and 2094/8 (84 L/8) referring to UTM topographic maps. Pathein-Monywa Highway is passing through the eastern part of the study area. The location map is shown in Figure (1-A).

Literature survey

The Tertiary rocks of Myanmar had been studied by geologists since 1869, but geological investigation has not yet completed. Theobald (1873) introduced the term “Pegu Group” and subdivided into two parts by an important unconformity which approximated the Oligocene-Miocene boundary and non-marine Plio-Pleistocene “Fossil Wood Group of the Irrawaddian”. The Minbu Basin of Central Cenozoic Belt, which includes the study area, was paid much attention by many geologists for primarily due to hydrocarbon prospects. As a result, the Tertiary rock units of Minbu Basin had been mapped and discussed by various geologists since 1800s and studied the stratigraphy, structure and oil prospects.

Aim and Objectives

The aim of the this stud is to investigate the petrographic characteristics and diagenetic processes of the Okhmintaung Formation exposed in the Chaung Zon area, Pwintbyu Township, Magway Region, with the objective of understanding the formations sedimentary history, mineralogical composition and the impact of diagenetic alteration on its geological properties.

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- To prepare thin section for clastic rocks.
- To identify petrogenesis of sediments exposed in the study area by using the suitable advanced techniques possibly
- To take photograph all thin-sections and micro fossils
- To identify the all thin-section under the polarizing microscope
- To investigate the diagenetic processes and alternation processes

Material and Methods

Suitable sandstone samples collected during field works are three dimensionally cut into thin sections and studied under a polarizing microscope. The detrital grains are identified by point counting on a slide and the modal composition is roughly estimated by comparing with percentage estimation charts of Folk *et al.*, 1970 in Tucker, 2001

Tectonic Framework of study area in the Minbu Basin

The study area is situated in the Central Cenozoic Belt of Myanmar (Chibber, 1934; Tainsh, 1950; Maung Thein 1976, 2010, 2014) which constitutes one of the four geotectonic belts of Myanmar. The study area is located in northwestern part of the Minbu Basins.

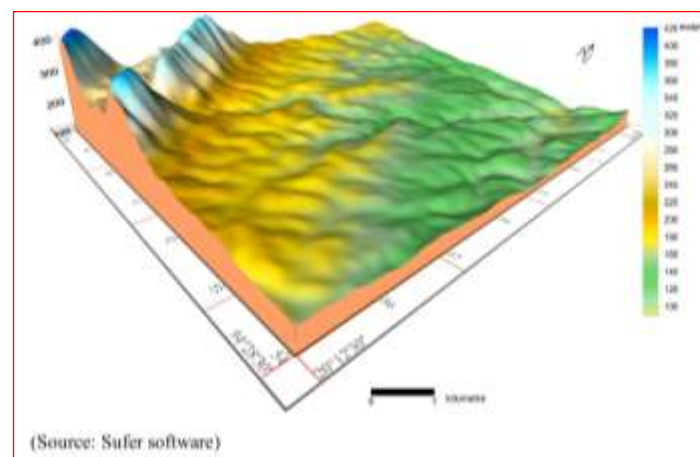
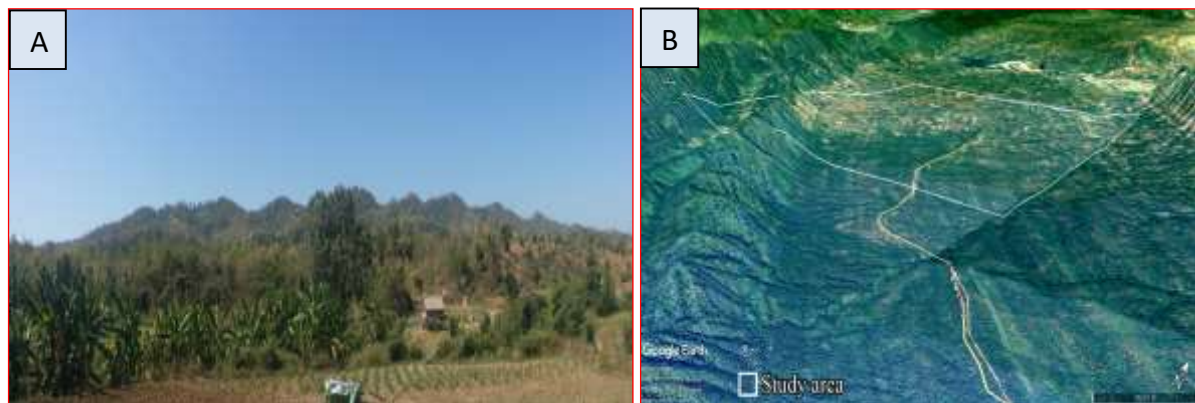
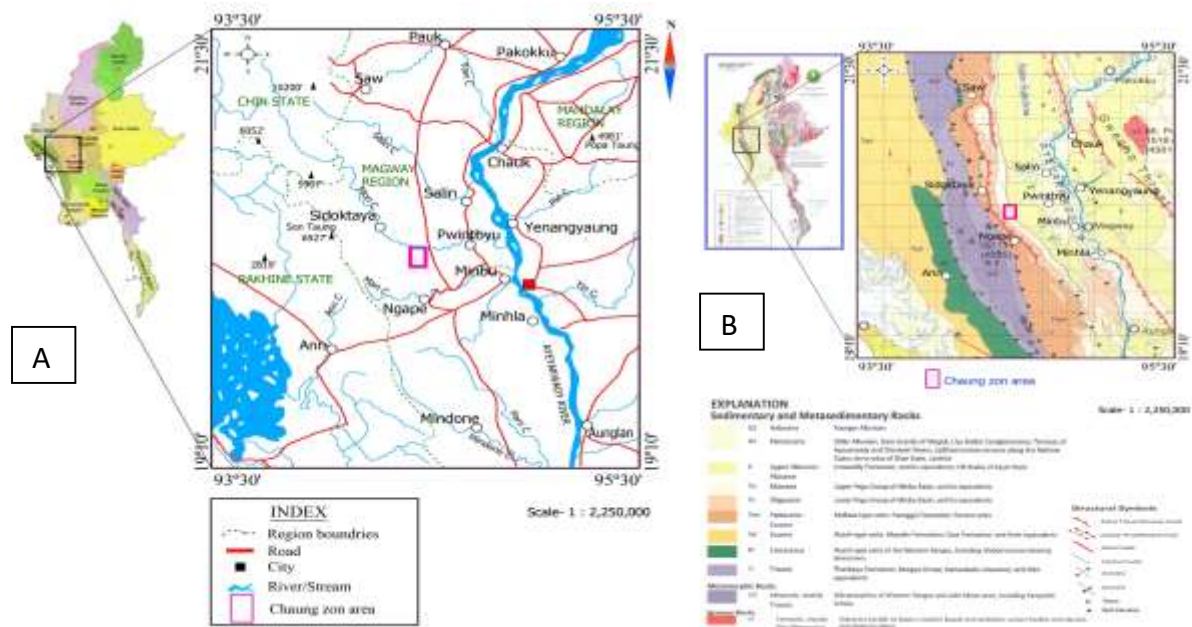
According to the tectonic model of Maung Thein (1983), Minbu Basin, a part of Central Myanmar Belt is associated to be a fore-arc related basin. The study area generally encompasses a small segment of the Minbu Basin. It is composed mainly of Oligocene and Miocene sediments.

The study area comprising is surrounded by Central Volcanic Line and Pegu Yoma in the east, by the Western Ranges in the West, by the Chindwin Basin in the north and, by the Thayetmyo Syncline in the south respectively.

The eastern part is composed of Miocene stratigraphic sequence units and western part is Oligocene units. The study area is mainly composed of stratigraphic units from east to west; Irrawaddy Formation, Obogon Formation, Kyaukkok Formation, Pyawbye Formation, Okhmintaung Formation, Padaung Formation, and Shwezetaw Formation.

In the area, the Oligocene-Miocene succession is molassic in nature and generally dipping towards the east. The Miocene strata are constituted mainly of buff- coloured, fine- to medium-grained, thinly bedded sandstone intercalated with light gray to bluish gray shale, light gray to brown, medium- to coarse-grained, medium to thick bedded sandstone with minor gray shale and clay, bluish gray sandy clays with dispersed gypsum plates; concretionary bluish gray clays; intraformational conglomerate and subordinate sandstone.

The Oligocene rocks are buff to light greenish gray and compact, fine- to medium-grained, massive sandstone, dark bluish gray clay and mudstone with fine-grained, hard and compact sandstone, greenish gray, fine to medium-grained, massive sandstone, sandy shale and silty sand. The regional geologic setting of Chaung Zon area is shown in Figure (1-B).



Stratigraphy

General Statement

The study area, occupying a small segment of the western margin of the Inner-Myanmar Tertiary Basin, comprises mainly Oligocene-Miocene sediments deposited in marine to deltaic environments. The rocks sequence of the area in descending order are; Shwezetaw Formation (Early Oligocene), Padaung Formation (Early Oligocene), Okhmintaung Formation (Late Oligocene), Pyawbwe Formation (Early Miocene), Kyaukkok Formation (Middle Miocene), Obogon Formation (Late Miocene) and Irrawaddy Formation (Late Miocene to Pliocene). The author specialized the study of Okhmintaung Formation.

Okhmintaung Formation

The lower part of the Okhmintaung Formation is characterized by light grey, medium-to coarse-grained, thick-bedded to massive type sandstones intercalated with thin-bedded mudstones and bluish grey shales are also occurred. In the middle portion, dark grey-coloured, medium-to coarse-grained, thick-bedded sandstone intercalated with thinly mudstone is occurred. In the upper part, light grey, fine-to medium-grained, medium-to thick-bedded sandstones are found. Sand-balls or concretions and polypertthite concretions are also occurred in this upper part of lower portion. Shale partings are present in this sandstones bed.



Figure. (4) The outcrop nature showing the Okhmintaung Formation, a) Light grey colored, medium-grained, thin to medium-bedded sandstones intercalated with 2-4 cm thick mud layer in the middle part (20° 15' 51" N - 94° 24' 38"E), b) Dark brown to yellowish brown colored, medium- to coarse-grained, thick- to massive sandstones exposed in the middle part (20° 15' 51" N - 94° 24' 38" E) and c) Hard and competent sandstones exposure with tabular and planar type cross bedding structures in the lower part (20° 15' 51" N - 94° 24' 38" E)

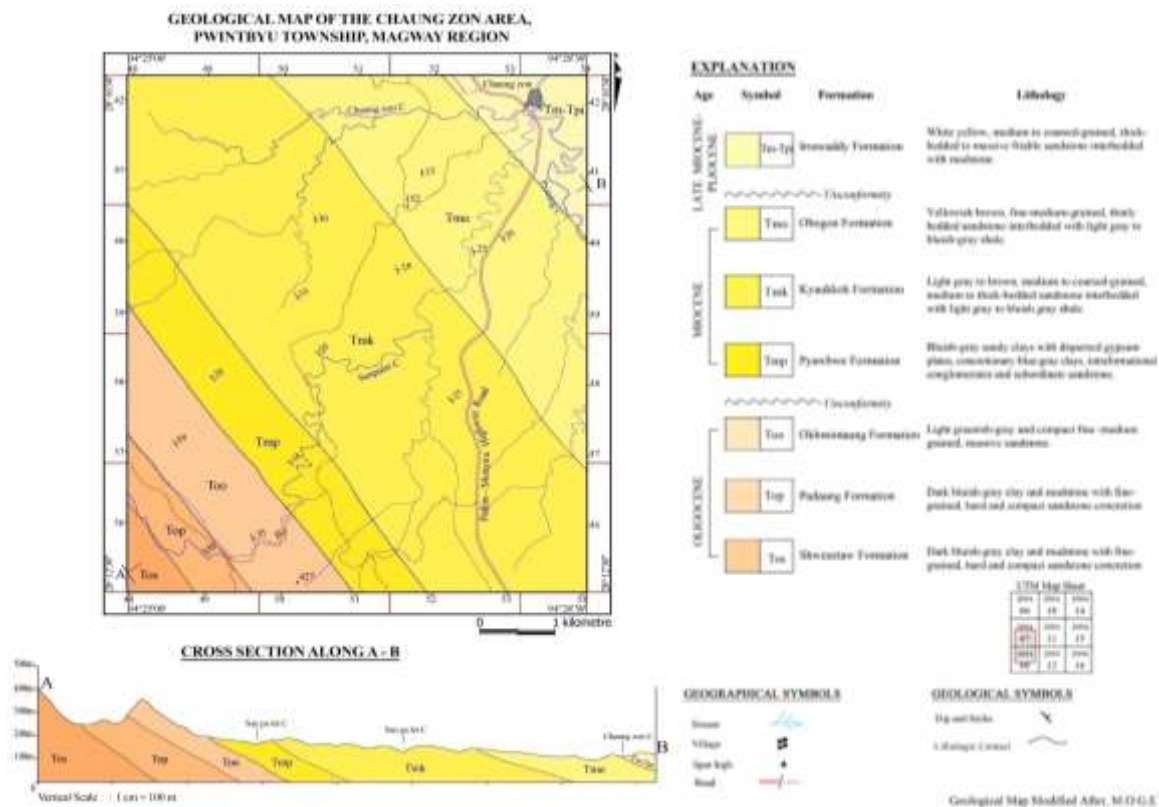


Figure 5. Geological map of the research area (Modified after MOGE)

Petrography

The percentage estimation of sandstones was shown in charts of Folk *et al.*, 1970 in Tucker, 2001 (Table 1). The sandstones are classified according to the simple classification of sandstone (after Pettijohn *et al.*, 1987 in Tucker, 2001). The sandstones of Okhmintaung Formation are composed of 55% to 72% detrital grains, 28% to 45% chemical cements and 2% to 5% of matrix. Detrital grains in these sandstones are generally moderately sorted and subangular to subrounded. The diameters of detrital grains are 0.1 mm to 2 mm.

Table 1. The detrital composition means of sandstones (in volume %) of Okhmintaung Formation of the study area. Quantitative models are recalculated from the data of thin slide.

Simple No.	Quartz	Feldspar	Rock Fragment	Total
Okh-1	60	18	22	100
Okh-2	66	14	20	100
Okh-3	60	20	20	100
Okh-4	72	14	14	100
Okh-5	70	10	20	100
Okh-6	72	10	18	100

Table 2. The detrital and cement composition of sandstones (in volume %) of Okhmintaung Formation.

Slide no	Qtz	Fel	Mica	Rx fg	Hev ml	Calcite cement	Iron cement	Mtx	Other detrital	Total
Okh-1	31	5	3	8	3	30	12	3	5	100
Okh-2	30	10	4	12	2	27	10	2	3	100
Okh-3	18	8	4	8	3	40	10	4	5	100
Okh-4	31	6	3	6	2	36	9	3	4	100
Okh-5	30	5	4	10	3	29	10	5	2	100
Okh-6	31	5	3	9	4	25	11	6	5	100

Quartz

Detrital quartz grains comprise 55% to 72% of total volume of the rock. Most are monocrystalline quartz grains which form subangular-to subrounded. Most of the monocrystalline quartz grains possess unit extinction where the whole crystal is extinguished uniformly under crossed polar. Some of the grain show undulated extinction where extinction is not uniform but sweeps across the crystal as it is rotated from 6° to 15°. Undulated extinction usually is a reflection of a stain in crystal lithic (Fig 5. a & c). Some crystals incorporated with mineral inclusion (Fig 6. a & b). They may be volcanic origin (Fig 5. a, c & 6. a). Polycrystalline quartz grains are straight boundaries represent up to 10% of total rock volume.

Most of quartz grains are monocrystalline represent up to 40% of total rock volume. When the boundaries between the crystals are sutured this is a characteristic of quartz from a metamorphic source. Composite quartz from igneous sources have straighter crystal boundaries. Some quartz grains are irregularly fractured.

Feldspar

Feldspar occupies 10% to 20% of the total detrital population. They are generally fresh and some are moderately weathered. Most of them are show angular, subangular to subrounded, and subsequent to elongated shape. Alkali feldspar are more common than calcic plagioclase, partly because they are more resistant to chemical weathering and partly because the ultimate source of many terrigenous rock is granite or gneiss, in which the feldspars are mainly the alkali variety. Orthoclase crystals usually break along the twin planes so that the simple Carlsbad twinning is rarely seen. Plagioclase can be identified by its polysynthetic and multiple twinning. Feldspars with overgrowth are found (Fig 7 b & d).

Rock fragments

The various rock fragments dominate a significant portion of the detrital grains averaging about 18% to 25% of the total volume. The various types of rock fragments are chert, clay,

volcanic rock fragments are also observed in the sandstone of Okhmintaung Formation. The sedimentary rock fragments other than chert are relatively uncommon because they usually break down fairly easily into their component grains. Some large sandstone fragments, in which the component particles are all quartz, are clearly distinguishable even with PPL. Some metamorphic fragments formed elongated grains (Fig 5.d) which are classified as the slate fragments. The contrast with the composite quartz grains of igneous and metamorphic sources, where individual crystals are also found (Fig 6. b & 7. b). They are angular, subangular to subrounded and range in grain size from 0.5 mm to 1.5 mm and some are coated by iron cement.

Mica

Both biotite and muscovite comprise 4% to 10% of the detrital framework. Biotite and muscovite are well observed in Okhmintaung sandstones. Biotite flakes dominant. They show preferred orientation. Bifurcation of biotite mica and some biotite alter to glauconite in Okhmintaung sandstones (Fig 5.5 b). Biotite range in grain size about 0.15 mm to 0.2 mm in diameter. Some biotites are partly altered to chlorite, glauconite (Fig 5.4 b) and muscovite and iron oxide.

Heavy mineral

Heavy minerals comprise about 1% to 2% of the total rock volume. Heavy minerals grains composed of glauconite, magnetite, rutile, zircon, tourmaline and other opaque minerals. Glauconite grains are rounded to subrounded and range in size from 0.1mm to 0.3mm and most of heavy minerals are coated by calcite cement and iron cement.

Bioclasts

Biogenic fragments, both micro- and macro-fossils are present in the fossiliferous sandstones, especially, some samples no.5 and 6 of the Okhmintaung sandstones. They constituted more than 15 % the total rock volume of in this slide and include foraminifera, gastropods, bivalves and enclaves (Fig 7.c).

Cement and Matrix

In the Okhmintaung Formation, calcite cement constitutes about 25% to 40% and 3% to 10% iron cement of the total rock volume. The detrital fragments are firmly welded by calcite cement (Fig 5. c & d and 6. a, b, c & d and 7.a). Generally, the cement is filling of the pore spaces and some voids remain. The boundaries of the calcite cement are showing sharp and straight characters, while some show curve to slightly sutured boundaries. Replacement of some silicate minerals by calcite cement is a common feature in this sandstone. Some detrital grain such as quartz, feldspar, and mica are markedly corroded and replaced by calcite. In this formation, iron oxide, hematite and clay matrix are also observed. The occurrence of the hematite cement as inclusion in the calcite cements and as scattered grains along the detrital grain boundaries. A few sandstones have a matrix of carbonate mud.

Nomenclature

Okhmintaung sandstone have less than 75% of quartz grains, feldspar comprise 10% to 20% and rock fragments 14% to 20% of the total detrital grains. When the sandstone composition is plotted in the triangular diagram of the Pettijohn *et al.*, (1987), all the sandstones of the Okhmintaung Formation may be termed as “Sublithicarenite” seen in Figure (8).

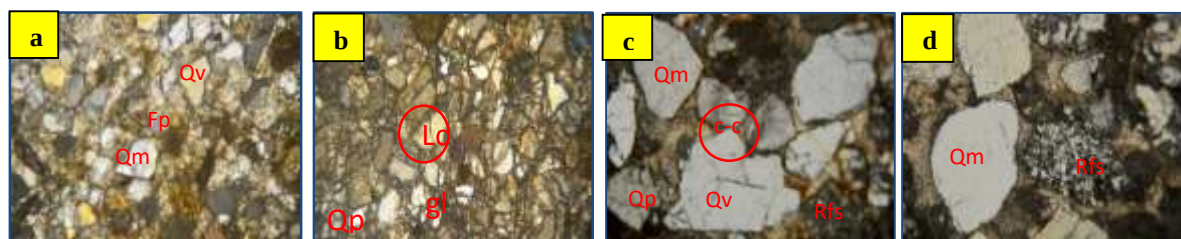


Figure 5. Photomicrographs of sandstones from Okhmintaung Formation. a) and b) Subangular to subrounded quartz grains (Qm, Qp & Qv), plagioclase feldspar (Fp), chert (cht) rock fragment (Rf) and glauconite (gl) which are single crystals, taken with plane-polarized and the matrix between sand grains contain iron oxide and calcite, c) and d) Quartz crystal may sometimes incorporate minerals inclusion and some volcanic quartz (Qv) show uniform or straight extinction and rounded by light brown matrix. Sedimentary rock fragments (Rfs) are found. Quartz grains are found in concave-convex contact, point contact and suture contact. Polycrystalline quartz (Qp) grains are large and rounded boundaries. Concave-convex (c-c) contact also occurred.

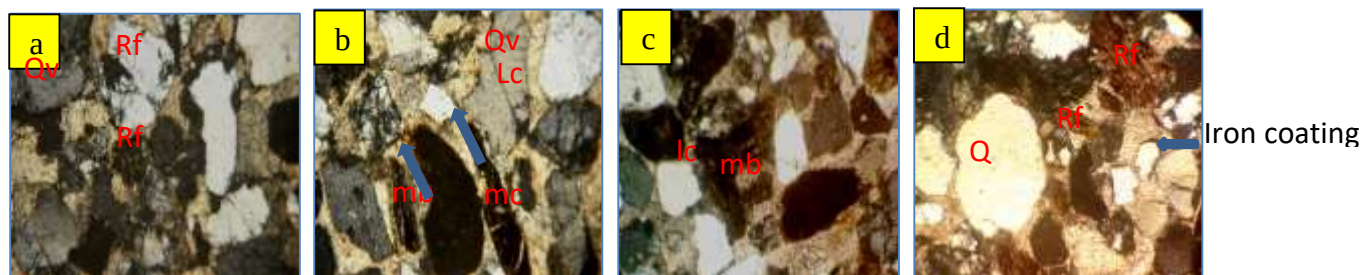


Figure 6 Photomicrographs of sandstones from Okhmintaung Formation. a) and b) Some metamorphic quartz (mQ) show cross-hatch fracture and straight boundaries and volcanic quartz grains (Qv) are long contact and tangential contact. Biotite micas (mb) are long and fracture features in this sandstones. The detrital grains in this sandstone are parallel orientation. c) and d) Quartz overgrowth (Qm) thin oxide and or thin oxide or clay coating between the grains and the overgrowth is visible (PPL). A single-crystal quartz grain with strongly undulate extinction; grain extinguishes completely with more than 5° of stage rotation (XN); volcanic rock fragments with small laths of plagioclase feldspar and a very finely crystalline matrix. Biotite micas (mb) and rock fragments (Rf) are coarse-grained metamorphic rocks often schistose.

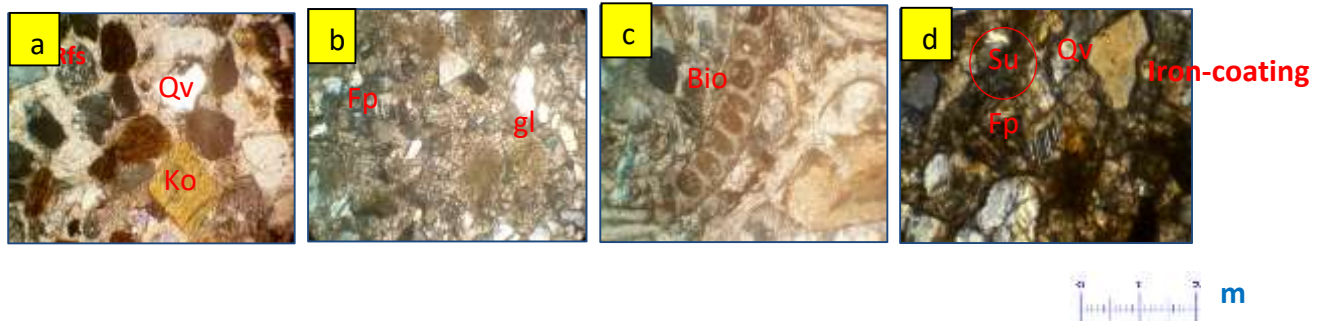


Figure 7. Photomicrographs of sandstones of Okhmintaung Formation. a) Quartz overgrowth (Qv) thin oxide and or clay coating between the grains and the overgrowth is visible (PPL). Sedimentary rock fragments (Rfs) source from sandstones. Orthoclase feldspars (Ko) are cloudy appearance due to alteration. b) & d) Partially dissolved feldspar grains and glauconite (gl) are occurred and overgrowth quartz grain crystal (Qv) also present. Fractured quartz with embayment indicating igneous derivatives. Plagioclase feldspars (Fp) are polysynthetic and multiple twinning. c) Foraminiferal occur in fossiliferous sandstones.

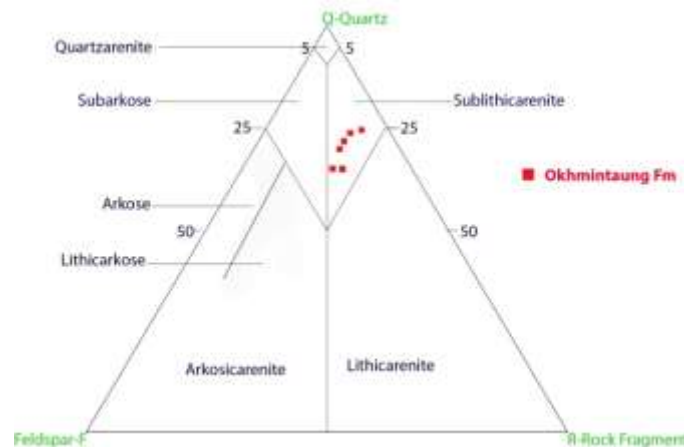


Figure 8 Compositional diagram of sandstone of Okhmintaung Formation (After Pettijohn et al., 1987)

Diagenesis of Okhmintaung Formation

Compaction: The first stage of diagenesis is the compaction of the sediment. It increases the density of the sediments. It occurs in all loose aggregate as the clasts rearrange themselves under moderate pressure. Pore-water in the voids between grains is expelled in the processes and compaction by repacking may reduce the volume of a body of sand by around 10% (Nichol, 2009). Before cementation compaction take place dominantly by mechanical processes, resulting slippage between individual grains reorientation and subsequences fractures of some grains.

Cementation: Quartz cement often occurs as overgrowth on the original quartz grains. Calcite cement is composed of 16% to 51% of the total rock volume. Calcite is soluble in surface water and therefore cemented sandstones often have their cement partially dissolved. Dissolution of calcite cement results in the secondary porosity. Iron oxide cement comprises 5% to 11% of total rock volume. Some iron oxides are the next common and may be cementing material in the sandstone.

Replacement: The process of practically simultaneous capillary solution and deposition by which as new mineral of partly or wholly differing chemical composition may grow in the body of an old mineral or mineral aggregates. In the study area, calcite cement is corroded and wedge apart to the detrital quartz and feldspar.

Authigenesis: Authigenesis is formed when new minerals are crystallized in the sediment or rock during diagenesis. These new minerals may be produced by reaction involving phase already present in the sediment, through precipitation of material introduced in the fluid phase, or a chemical reaction between primary sedimentary minerals and ions is introduced by the fluids. This process overlaps with weathering and cementation, usually involves recrystallization and may result in replacement.

Result and Discussions

1. Okhmintaung sandstone has less than 75% of quartz grains, feldspar comprise 10% to 20% and rock fragments 14% to 20% of the total detrital grains. When the sandstone composition is plotted in the triangular diagram of the Pettijohn *et al.*, (1987), all the sandstones of the Okhmintaung Formation may be termed as “Sublithicarenite”.
2. **Redoxomorphic Stage:** This stage is characterized by all the reflection due to oxidation and reducing in the sediments during and immediately after burial. The reaction in this stage occurs along the sediment in which total iron content exceeds 3%. Alteration of biotite to muscovite and oxidized biotite are more common in the Okhmintaung sandstone in the study area.
3. **Locomorphic Stage:** During this stage, precipitation of mineral matter fill in the pore spaces and replacement of detrital mineral grains are significant. The cementing material in the Okhmintaung sandstones are calcite and iron cements, glauconite, rutile and clay matrix. Pore spaces between the detrital grains are also filled by silica cement.
4. **Phyllomorphic Stage:** This stage is the last stage of diagenesis which involves authigenesis of some minerals. The common characteristics of late burial or phyllomorphic stage are the alteration of clay mineral into micas and the development of well crystallized phyllosilicate minerals. Biogenic fragments, both micro- and macro-fossils are present in the fossiliferous sandstones, especially, some samples no.5 and 6 of the Okhmintaung sandstones.

Summary and Conclusion

The study area is located between north latitude 20° 12' 30" - 20° 16' 30" and east longitude 94° 25' 00" - 94° 28' 30". It is situated about 16 miles (25.75 km) west of the Pwintbyu Township. It falls in 2094/7 (84 L/7) and 2094/8 (84 L/8) referring to UTM topographic maps. The lower part of the Okhmintaung Formation is characterized by light grey, medium-to coarse-grained, thick-bedded to massive type sandstones intercalated with thin-bedded mudstones and bluish grey shales are also occurred. In the middle portion, dark grey-coloured, medium-to coarse-grained, thick-bedded sandstone intercalated with thinly mudstone is occurred. In the upper part, light grey, fine-to medium-grained, medium-to thick-bedded sandstones are found. Sand-balls or concretions and polyperthite concretions are also occurred in this upper part of lower portion, Shale partings are present in this sandstones bed.

Detrital quartz grains comprise 55% to 72% of total volume of the rock. Most are monocrystalline quartz grains which form subangular-to subrounded. Feldspar occupies 10% to 20% of the total detrital population. They are generally fresh and some are moderately weathered. The various rock fragments dominate a significant portion of the detrital grains averaging about 18% to 25% of the total volume. Both biotite and muscovite comprise 4% to 10% of the detrital framework. Biotite and muscovite are well observed in Okhmintaung sandstones. Heavy minerals grains composed of glauconite, magnetite, rutile, zircon, tourmaline and other opaque minerals. Calcite cement constitutes about 25% to 40% and 3% to 10% iron cement of the total rock volume. When the sandstone composition is plotted in the triangular diagram of the Pettijohn *et al.*, (1987), all the sandstones of the Okhmintaung Formation may be termed as “Sublithicarenite”.

The first stage of diagenesis is the compaction of the sediment. It increases the density of the sediments. It occurs in all loose aggregate as the clasts rearrange themselves under moderate pressure. Calcite is soluble in surface water and therefore cemented sandstones often have their cement partially dissolved. Dissolution of calcite cement results in the secondary porosity. The process of practically simultaneous capillary solution and deposition by which a new mineral of partly or wholly differing chemical composition may grow in the body of an old mineral or mineral aggregates. Authigenesis is formed when new minerals are crystallized in the sediment or rock during diagenesis. These new minerals may be produced by reaction involving phase already present in the sediment, through precipitation of material introduced in the fluid phase, or a chemical reaction between primary sedimentary minerals and ions is introduced by the fluids.

Redoxomorphic Stage is characterized by all the reflection due to oxidation and reducing in the sediments during and immediately after burial. The reaction in this stage occurs along the sediment in which total iron content exceeds 3%. During the **Locomorphic Stage**, precipitation of mineral matter fill in the pore spaces and replacement of detrital mineral grains are significant. **Phylломorphic Stage** is the last stage of diagenesis which involves authigenesis of some minerals. The common characteristics of late burial or phylломorphic stage are the alteration of clay mineral into micas and the development of well crystallized phyllosilicate minerals.

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PETROGRAPHIC AND GEOCHEMICAL STUDIES OF THE SPINEL-BEARING PERIDOTITES EXPOSED IN SOUTHWEST OF MINDON TOWNSHIP, MAGWAY REGION

Ye Kyaw Thu¹, Than Zaw², Khin Myo Si³, Soe Soe Aye⁴

Abstract

The ophiolite of the study area is a remnant of the Tethyan ophiolites preserved at the eastern margin of the Indo-Myanmar Orogenic Belt. Mineralogical features, textural relations and relative abundance of constituent minerals reveal that the samples are dominated by spinel lherzolite and spinel harzburgite with minor dunite, and characterized by olivine (>40 % volume) with varying amount of orthopyroxene and clinopyroxene. The samples display the preferred orientation of olivine, strain shadow, undulose extinction, kink bands of cleavage traces in pyroxenes, and are essentially spinel bearing suggesting derivation from deeper source in the mantle beyond the stability limit of plagioclase. Magnetite forms along fractures and rims of the spinel grains due to the result of cation exchange between spinels and coexisting silicates forming low pressure and low temperature metamorphic alteration. The samples have relatively homogeneous whole-rock compositions with Mg# values (90–92), and SiO₂ (43.6 – 44.8 wt%), Al₂O₃ (2.5–2.9 wt%) and CaO (2.3–2.5 wt%) contents and enriched in transition metals. The samples have negative correlations between SiO₂, Fe₂O₃, and CaO with MgO and are more similar to mid-oceanic ridge type. Correlating with the northern segment of the Western Ophiolite Belt it is likely suggest that the peridotite rock exposed in the northern and southern segment were formed in the mid-oceanic ridge setting and were accreted to the Indo-Myanmar Ranges from the downgoing Tethyan plate as part of subduction-accretion processes.

Keywords: The Western Ophiolite Belt, peridotite tectonite, spinel, deeper source, the mid-oceanic ridge setting

Introduction

Ophiolites are generally considered as the fragments of remnant oceanic lithosphere formed by seafloor spreading, which are of importance to plate reconstruction and understanding of orogeny (Dilek and Furnes, 2011). Ophiolites are widely distributed within Myanmar such as the Eastern Ophiolite Belt, the Central Ophiolite Belt and the Western Ophiolite Belt (Hla Htay et al., 2017). Among these belts, the Western Ophiolite belt was regarded as the remnants of the Neo-Tethyan oceanic lithosphere (Sengupta et al., 1990; Mitchell, 1993) and as the southern continuation of the Neo-Tethyan ophiolites occurring along the Yarlung Zangbo suture zone in southern Tibet farther northwest (Mitchell, 1993; Fareeduddin and Dilek, 2015). This belt roughly follows the trend of the eastern Indo-Burma Range and geographically continues northward to ophiolites in the Naga Hills, e.g., the Manipur (Ghosh et al., 2014; Singh et al., 2013), and extends southward to the Andaman–Nicobar Island. The geological works in the Western Ranges and its environs were carried out by many authors, and the Kalaymyo ophiolite from the northern part of Western Belt was suggested to be formed during the Early Cretaceous (~125 Ma), and represents relics of the Neo-Tethys Ocean (Liu et al., 2016).

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The ophiolites along the Indo-Myanmar Ranges are poorly constrained. Some have interpreted them as segments of an island-arc system that were emplaced as nappes during the India-Burma collision (Sengupta et al., 1990; Mitchell, 1993). The origin and tectonic setting of the Manipur ophiolites, located in the northern part of the Indo-Myanmar Ranges suggested as an island-arc origin (Bhattacharjee, 1991) and as a mid-oceanic ridge origin (Singh, 2013; Khogenkumar et al., 2016), which underwent high-pressure metamorphism and deformation in a subduction channel and then was incorporated into an accretionary prism in the upper plate (Fareeduddin and Dilek, 2015). In the middle segment of the belt is the mantle peridotites of the Kalaymyo ophiolite which was also suggested to be mantle residues originated in a mid-oceanic ridge setting with no subduction zone influence in their magmatic evolution (Liu et al., 2016; Niu et al., 2017). In the present study we document the mineralogical features, occurrence of spinel and preliminary studies on the major and trace element geochemistry of the peridotites in the southwest of Mindon area in the central part of the Western Ophiolite Belt. The present study indicate that the spinel-bearing peridotite rocks are discontinuously exposed along the eastern margin of the Indo-Myanmar Ranges and comparing with the northern segment a mid-oceanic ridge origin are dominated in the northern and middle segment.

Regional Geologic Setting

Ophiolitic rock associations occur in three belts trending nearly north-south parallel with each other, and from west to east these belts are the Western Ophiolite Belt (WOB), the Central Ophiolite Belt (COB) and the Eastern Ophiolite Belt (EOB) (Hla Htay et al., 2017). The narrow Western Ophiolite Belt (WOB) forms the longest belt, extending from north to southern part of Myanmar. The Western Ophiolite Belt lying along the Indo-Myanmar Ranges extends from the Naga hills in the north through the Chin hills to the Rakhine Yoma and to Andaman and Nicobar islands in the south. It forms a narrow linear belt about 1300 km long and about 50 km wide. Throughout the belt dismembered ophiolitic rocks are represented by ultramafic and mafic rocks associated with oceanic pelagic sediments.

The study area which occupies as a part of the Western Ophiolite Belt is sited at the the eastern part of the Indo-Myanmar Ranges, and appear between Tertiary molasses sediments of the Inner-Myanmar Tertiary Basin and the Indo-Myanmar Flysch sediments (Figure. 1). The ophiolitic rocks occur along large Kabaw fault zone, which dissects the Indo-Myanmar Ranges and are restricted to the eastern parts of the Indo-Myanmar Ranges in association with metamorphic rocks (Figure. 1). The ophiolitic rocks mostly occur as small and elongated tectonized serpentinite bodies within low-grade metamorphic rocks or slightly tectonized serpentinite occurring within volcanic rocks of mafic to intermediate composition (Hla Htay et al., 2017). The present area is placed in the southwestern part of Mindon area and consists mainly of Kanpelet schists, ophiolitic rocks and Paleocene to Middle Eocene clastic sediments (Figure. 1).

The peridotite exposures in the study area vary in mineralogical composition from harzburgite, lherzolite to minor wherlites and dunite. They occur as tectonized and cumulate types, and form as massive, foliated, fractured and highly jointed nature near their contact with the overlying sediments at Hlwar Chaung near Ywathit area (Figure. 2a and d). They also occur as isolated blocks (Figure. 2b) associated with low-grade metamorphic rocks and Thanbaya Formation, and show grayish green to dark green-colored. Olivine and pyroxene grains in most of the exposures show slightly to strong serpentinitization (Figure. 2a, c and d). Veinlets of

serpentine are commonly present in these rocks (Figure. 2c). Some exposures display lenses or disseminations of chromite. Dolerite dike are exposed as large sheeted blocks forming hill at Shwe daung maung at the eastern margin of Rakhine Yoma. Dark grey vesicular basalts occur as a float at associated with peridotites.

Among the spinel-bearing peridotite samples, YU301 and YU03 samples are plotted in harzburgite field and YU01, YU06 and SU15 samples in lherzolite field based on their relative mineral proportion, and are shown in Figureure 3. Abbreviations for minerals and end-members used in text and figures are after Whitney and Evans (2010).

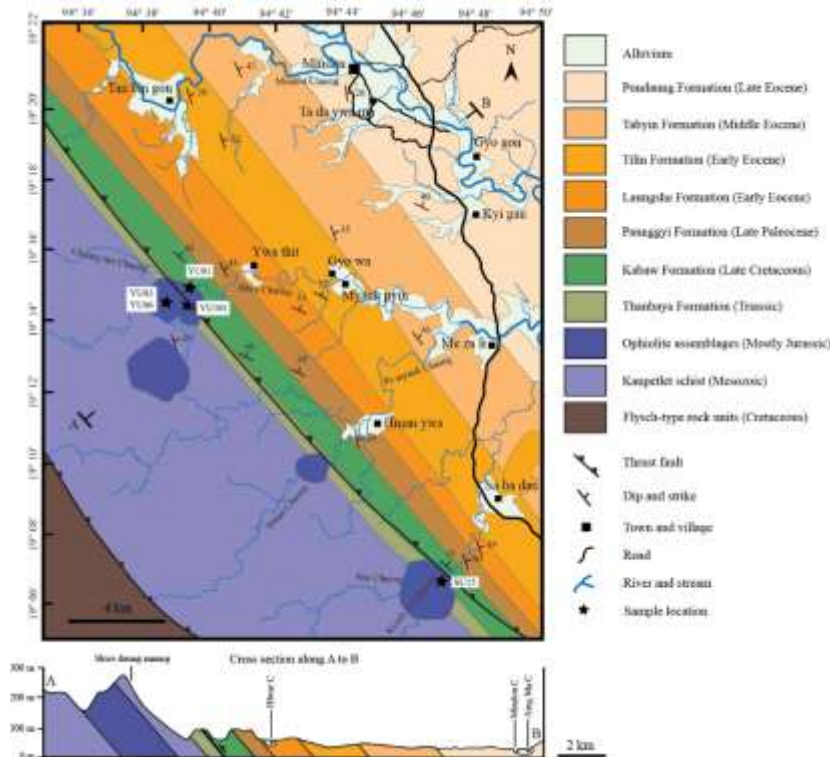


Figure 1. Geological map of the sampling area (combined with the data of thrust faults and flysch-type rock units from Geological Map of Myanmar of Myanmar of Geosciences Society, 2014 and with structural data of the northern part from Htet Aung Thu, 2015).



Figure 2 (a) peridotite exposure in contact with Thanbaya Formation, (b) isolate harzburgite block at Hlwar Chaung southwest of Ywathit area, (c) serpentinization in peridotite and (d) massive serpentinized peridotite at Kyaukkyi Chanung southwest of Sabadan area.

Petrographic Features

Spinel-bearing lherzolites were collected from longitude 94° 39' 26" E and latitude 19° 14' 55" N for YU01 sample, 94° 38' 47" E and 19° 14' 38" N for YU06 sample and 94° 38' 47" E and 19° 14' 38" N for SU15 sample. Spinel-bearing harzburgite samples YU03 and YU301 were collected from 94° 38' 47" E, 19° 14' 38" N and 94° 39' 07" E, 19° 14' 26" N, respectively (Figure 1).

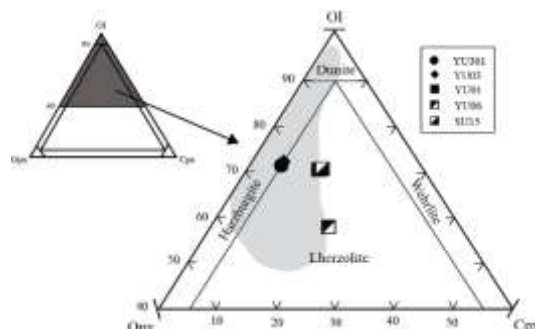


Figure 3. Classification diagram for spinel-bearing peridotite samples plotted based on primary mineral phases. The light grey shaded area denotes the most common types of peridotites in the upper part of the mantle (Bodinier and Godard, 2007).

These samples display mainly porphyroclastic texture. Their primary mineral assemblages of lherzolite samples are characterized by olivine (55–67 vol. %), orthopyroxene (16–21 vol. %), clinopyroxene (11–19 vol. %) and spinel and opaque minerals (5–9 vol. %), those of harzburgite are olivine (66–67 vol. %), orthopyroxene (20–22 vol. %), clinopyroxene (about 5 vol. %) and spinel and opaque minerals (up to 7 vol. %). Olivine and orthopyroxene show strain shadow, kink bands of cleavage traces, undulose extinction, shearing and recrystallization suggesting plastic deformation of upper mantle peridotites. In these samples, SU15 sample is fairly serpentinized and most silicate minerals are obliterated. Olivine grains display large porphyroclastic grains (>3 mm) (Figure. 4) and fine-grained neoblasts (<3 mm) (Figure. 4b). The olivine porphyroclasts are mostly elongated (Figure. 4g) and curved or tapered, and some grains show equigranular (Figure. 4d). The neoblasts show uniform extinction. Brecciated grains occur traversed by secondary serpentine (Figures. 4b and c). Smaller olivine grains (0.1–0.2 mm) occur as inclusion in orthopyroxene (Figure. 4b) and spinel, implying that these mineral form after olivine. Some olivine grains are altered to serpentine. Orthopyroxene shows porphyroclastic and subhedral to anhedral grains with an average of 0.2–1.5 mm in diameter (Figures. 4a–f and h) and some grains exhibit exsolution lamellae of clinopyroxene. Some orthopyroxene grain are surrounded and partly replaced by neoblastic olivine (Figure. 4b). Clinopyroxene grains occur as porphyroclastic, subhedral to anhedral grains and range in size of 0.3–1.7 mm (Figure. 4) and some occur as exsolution phase in orthopyroxene. Bent cleavage traces and strain shadow are observed in some grains (Figure. 4e). Spinel grains occur as subhedral to anhedral, blocky, skeletal, and irregularly rounded grains and range in size up to 0.7 mm (Figures. 4). Most of the crack and rim of spinel grain are replaced by secondary magnetite (Figures. 4c and h). Secondary phases include serpentine, chlorite, chromite and magnetite. Magnetite mostly occurs as alteration product after spinel grain along the crack and marginal rim (Figures. 4c and h) and are associated with secondary serpentine.

Metamorphic Condition of Spinel-bearing Peridotites Samples

The spinel composition is characterized by solid solution between chromite ($\text{Fe}^{2+}\text{Cr}_2\text{O}_4$) and spinel sensu stricto (MgAl_2O_4). The compositional heterogeneity of the Cr-spinel is a function of the fluid-rock interaction and metamorphic grade (Barnes, 2000; Farahat, 2008). Along grain boundaries and through cracks, magnetite was formed at lower temperature because of the readily exchange of Fe from host peridotite silicates e.g., olivine during serpentinisation (Gahlan et al., 2006). During metamorphism Cr-spinel changes to magnetite and/or ferritchromite along grain margins and fractures, as these are the only sites for metamorphic fluid interaction. Due to the lack of chemical data obtained from electron probe micro analyzer (EPMA), it is difficult to distinguish among opaque minerals and chemical composition of spinel and coexisting silicate minerals. Interpretation was made based on textural relationship, and implication on previous published works. In studied samples ferritchromite could not be identified, and instead magnetite forms along fractures and rims (Figures. 4c and h).

Based on the schematic illustration of chemical changes in primary Cr-spinel grains during metamorphism with element exchange (Bhat et al., 2019), the chemical modifications in primary Cr-spinel grains of the peridotite samples during secondary alteration and metamorphism are employed here and shown in figure 5. Olivine and pyroxene minerals are magnesian-bearing minerals, and magnetite were formed along cracks and grain boundaries due to the result of Mg^{2+} and Fe^{2+} cation exchange between spinel and coexisting silicates with limited amounts of Fe on their hydration (Figure. 5). Fe with minor Mn is incorporated into the Cr-spinel substituting for Al, Mg and Cr of the Cr-spinel, which show outward diffusion during metamorphism. This results in a decrease in $\text{Mg}/(\text{Mg} + \text{Fe}^{2+})$ and an increase in $\text{Cr}/(\text{Cr} + \text{Al})$ of the magnetite compared to the unaltered Cr-spinel core (Bhat et al., 2019). The ionic potential of Mn is relatively low and hence it is more liable to leakage by weakly acidic solutions (Economou-Elipoulos, 2003).

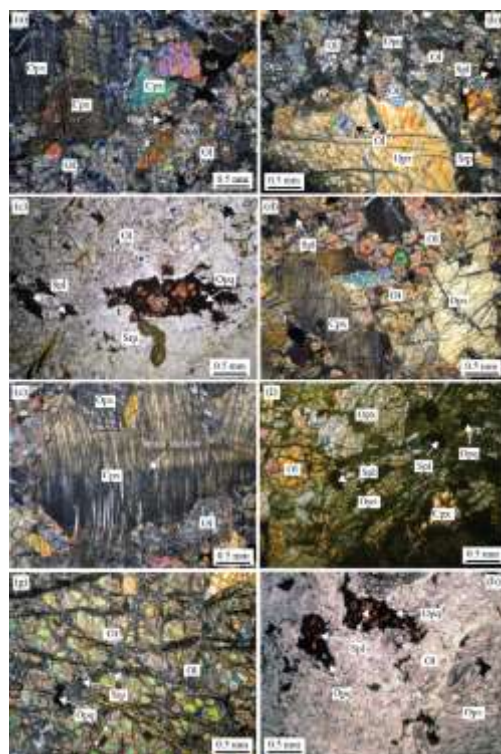


Figure 4. spinel-bearing peridotite samples showing (a) assemblages of olivine, orthopyroxene and clinopyroxene (YU01 sample), (b) orthopyroxene with inclusions of olivine grains and olivine neoblasts (YU01 sample), (c) reddish brown spinel grains replaced by magnetite along crack and rim (YU01 sample), (d) equigranular olivine with orthopyroxene and clinopyroxene (YU06 sample), (e) clinopyroxene showing strain shadow (YU06 sample), (f) assemblages of olivine, orthopyroxene and clinopyroxene, (g) preferentially oriented olivine porphyroclasts traversed by serpentine and opaque minerals (YU301 sample) and (h) spinel grains replaced by magnetite along crack and rim. All photomicrographs are under crossed polars except (c) and (h), which are under plane polarized light.

Based on the magnetite formation around the Cr-spinel this textural relationship suggests the host peridotite experienced at least greenschist facies metamorphism. Cr-spinel in studied samples was not completely replaced by magnetite composition (Figure. 5) suggesting these samples have not been achieved the higher metamorphic conditions (Farahat, 2008). Magnetite growth with limited Cr solubility form well below 500 °C in mantle peridotites (Barnes, 2000) and the studied samples have likely undergone low temperature metamorphism.

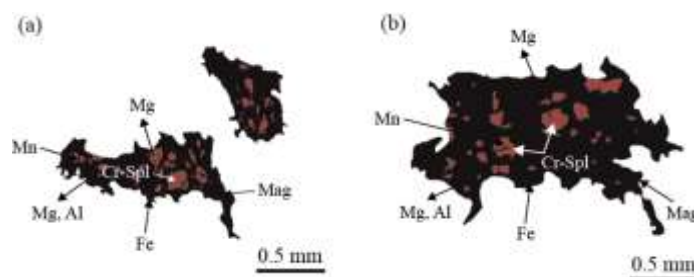


Figure 5. (a) and (b) Cr-spinel with arrow showing possible cation exchange with constituent silicate minerals using illustration for chemical variation of Bhat et al., 2019.

Geochemistry

Analytical Procedures

Samples were made for the detailed study of the constituent mineral assemblages under polarizing microscope. Samples were selected for geochemical analysis and made fine powder using tungsten bolt in jablab pulverizer and dried in the oven to dry out the water in the sample. Dried samples were weighed and placed in the microfurnance setting 950 °C for 5 hours. Whole-rock compositions used for the geochemical analysis were determined by an X-ray fluorescence spectrometer (WDXRF; Rigaku supermini 200) installed in Chemistry Laboratory, Department of Chemistry at Yadanabon University. Whole rock chemical analyses of studied samples are listed in table 1.

Whole rock major and trace Elements

Whole rock major element compositions of the peridotites are listed in table 1. The peridotites in the study area are variably altered to serpentine. The analyzed sample shows a range of SiO₂ (43.6 – 44.8 wt%), Al₂O₃ (2.5–2.9 wt%), MgO (38.7–41.3 wt%), Fe₂O₃ (8.3–9.6 wt%), CaO (2.3–2.5 wt%), Na₂O (0.1–0.2 wt%) and K₂O (< 0.1 wt%) (Table 1). The samples are rich in silica, titanium, aluminum and iron contents compared with the Kalaymyo peridotite, but poor in those compared with mantle peridotite. Most of the peridotites are enriched in the transition elements that are compatible in ultramafic rocks (Ni = 2040–3120 ppm, Cr = 2350–3660 ppm) compared to the Kalaymyo peridotite (Ni = 1860 ppm, Cr = 2520 ppm; Niu et al., 2017) and the primitive mantle (Ni = 1860 ppm, Cr = 2520 ppm; Palme and O'Neill, 2003). The samples have similar whole-rock Mg# [=100 x Mg/(Mg + Fe)] values (90–92), although the spinel bearing harzburgites contain higher concentrations of MgO (39.30–41.30 wt%) than the spinel bearing lherzolite (38.70–39.80 wt%). These concentrations can be used as an indicator of the degree of depletion. An increased depletion can be correlated with an increase in MgO value, which in turn increase in olivine abundance. The most applicable approach to evaluate the chemical changes in the studied samples is to compare the concentrations of elements of interest with MgO concentrations. The samples have negative correlations between SiO₂, Fe₂O₃, and CaO with MgO (Figure. 6).

Table 1 Analysis of the major and some trace elements of the peridotite samples from Ywathit and Sabadan areas.

Sample	YU301	YU03	YU01	YU06	SU15
Major elements (Wt%)					
SiO ₂	43.60	44.1	44.60	44.30	44.08
TiO ₂	0.11	0.11	0.10	0.11	0.09
Al ₂ O ₃	2.50	2.71	2.89	2.71	2.60
Fe ₂ O ₃ *	8.30	9.40	9.60	8.90	9.30
MnO	0.17	0.21	0.21	0.18	0.26
MgO	41.30	39.30	38.80	39.80	38.70
CaO	2.33	2.57	2.43	2.31	2.53
Na ₂ O	0.12	0.15	0.12	0.16	0.17
K ₂ O	0.08	0.08	0.08	0.09	0.08

Sample	YU301	YU03	YU01	YU06	SU15
P ₂ O ₅	0.03	0.02	0.03	0.03	0.03
Total	98.54	98.65	98.86	98.59	98.56
Trace elements (ppm)					
Cr	2400	2350	3320	2610	3660
Ni	2040	2790	2460	2750	3120
Zn	118	110	122	132	104
Co	247	210	230	220	235

* Fe₂O₃ as total Fe

Figure 6 compares the peridotite samples with abyssal (or mid-oceanic ridge type) of Niu et al. (1997) and suprasubduction zone (SSZ) type peridotites of Parkinson and Pearce (1998). The studied peridotites are more similar to mid-oceanic ridge type than SSZ type, similar to the compositions of peridotites from the Kalaymyo peridotite (Niu et al., 2017) in the northern segment of the Western Ophiolite belt. ACM (Al₂O₃–CaO–MgO) and AFM ((Na₂O + K₂O)–FeO–MgO) ternary diagrams show that the peridotite samples correspond to metamorphic peridotites (Fig. 7). The samples represent the basal parts of ophiolite sequences, which are depleted or residual mantle rocks (Coleman, 1971). The samples are also enriched in transition metals such as (Ni = 2040–3120 ppm, Cr = 2350–3660 ppm, Co (210–247 ppm), reflecting their mantle origin.

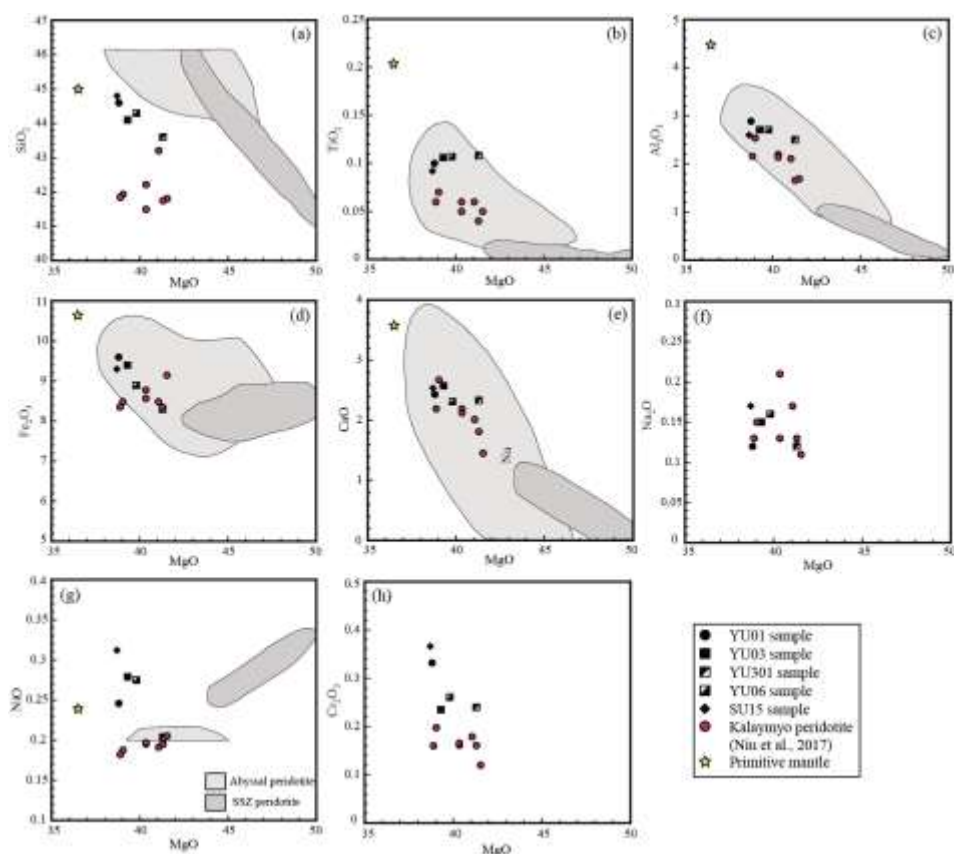


Figure 6. Variations diagrams of major element concentrations relative to MgO. The abyssal and suprasubduction zone (SSZ) peridotite fields are from Niu et al. (1997) and Parkinson and Pearce (1998), respectively, and data for other Kalaymyo peridotites are from Niu et al., 2017.

Comparison with the northern segment of the Western Ophiolite Belt

Ophiolitic rocks were emplaced along the eastern margins of the Indian plate during the northward movement of the Indian plate and the collision of the Indian continental block with the Asian continental block (Mitchell, 1993). The Western Ophiolite Belt roughly follows the trend of the eastern Indo-Burma Range (Figure. 8), and continues northward to ophiolites in the Chin Hill including Kalaymyo peridotite and Naga Hills including the Manipur (Ghosh et al., 2014; Singh, 2013; Singh et al., 2013). The peridotites from the Manipur ophiolite occurring in the northern part of the Indo-Myanmar Ranges mainly consist of lherzolites and clinopyroxene-bearing harzburgites (Ningthoujam et al., 2012; Singh, 2013), and are compositionally similar to abyssal peridotites, with negligible subduction-related modification (Ningthoujam et al., 2012; Singh, 2013), suggesting that the Manipur ophiolite was formed in the mid-ocean ridge (MOR) setting.

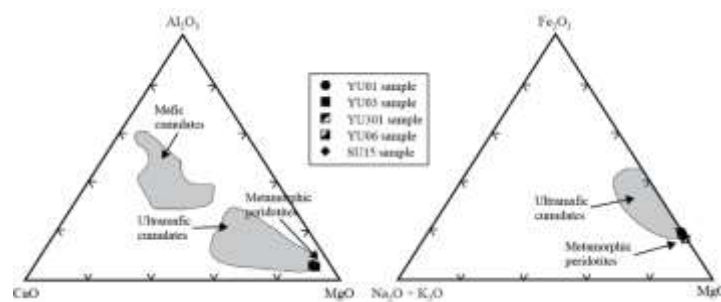


Figure 7. ACM (Al_2O_3 – CaO – MgO) and AFM ($(\text{Na}_2\text{O} + \text{K}_2\text{O})$ – FeO – MgO) ternary diagrams. Fields of mafic cumulates, ultramafic cumulates, and metamorphic peridotites are after Coleman (1971).

In addition, the Kalaymyo peridotites are characterized by harzburgite with the mineral assemblage of olivine, spinel, orthopyroxene, and clinopyroxene. They display fertile compositions and have been subjected to anhydrous melting and represent residual upper mantle peridotites after low to moderate degrees (Liu et al., 2016; Niu et al., 2017). Whole-rock chemical compositions resemble those of abyssal peridotites.

The present study area occurs in the southern part of the Indo-Myanmar Ranges (Figure. 8) and mainly comprises of spinel-bearing lherzolites and spinel-bearing harzburgites. Compared with the Kalaymyo peridotite the samples show higher SiO_2 , TiO_2 , Fe_2O_3 , NiO and Cr_2O_3 content. Most major elements compositionally fall within the field of the abyssal peridotite (Figs. 6a–e and g) and suggest that the peridotite rock exposed in the northern and southern segment (Figure. 8) were formed in the mid-oceanic ridge setting. The peridotites and their crustal rocks were accreted to the Indo-Myanmar Ranges from the downgoing Tethyan plate as part of subduction-accretion processes (Fareeduddin and Dilek, 2015). Further studies are still needed to understand the melt evolution, melt-rock interaction and tectonic characterization.

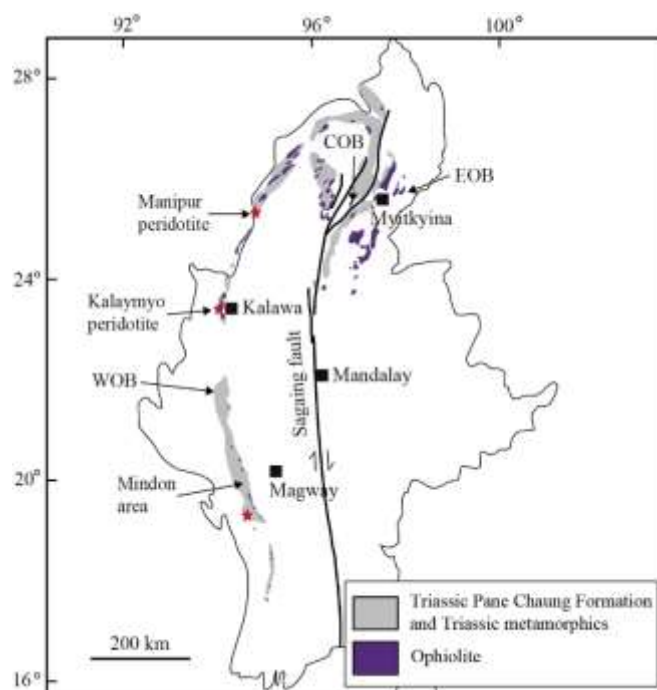


Figure 8. Map showing the main ophiolite occurrences in Myanmar and Triassic-age rocks association (after Searle et al., 2017). Red stars represent sampling areas.

Conclusion

The ophiolite of the study area is a remnant of the Tethyan ophiolites preserved at the eastern margin of the NNE–SSW trending Indo-Myanmar Orogenic Belt. The exposed ophiolite rocks are characterized by the occurrence of big massifs of peridotite tectonite, serpentinite, peridotite cumulate, dolerite dyke, basalt, pyroclastic rock, and pelagic sediment. Mineralogical features, textural relations and relative abundance of constituent minerals reveal that the samples are dominated by spinel lherzolite and spinel harzburgite with minor dunite, and characterized by olivine (>40 volume %) with varying amount of orthopyroxene and clinopyroxene. The samples display the preferred orientation of olivine, strain shadow, undulose extinction, kink bands of cleavage traces in pyroxenes, and are essentially spinel bearing suggesting derivation from deeper source in the mantle beyond the stability limit of plagioclase. Magnetite forms along fractures and rims of the spinel grains in studied samples due to the result of cation exchange between spinels and coexisting silicates, and suggests the peridotites have experienced low pressure and temperature metamorphic alteration. The samples have relatively homogeneous whole-rock compositions with Mg# values (90–92), and SiO₂ (43.6 – 44.8 wt%), Al₂O₃ (2.5–2.9 wt%) and CaO (2.3–2.5 wt%) contents and enriched in transition metals. The samples have negative correlations between SiO₂, Fe₂O₃, and CaO with MgO and are more similar to mid-oceanic ridge type than SSZ type. Correlating with the northern segment of the Western Ophiolite Belt it is likely suggest that the peridotite rock exposed in the northern and southern segment were formed in the mid-oceanic ridge setting and were accreted to the Indo-Myanmar Ranges from the downgoing Tethyan plate as part of subduction-accretion processes.

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SEISMOTECTONICS INVESTIGATION IN YANGON REGION, MYANMAR

Sandy Chit Ko¹, Tint Lwin Swe²

Abstract

The Yangon city is located on the convergence of the Yangon and the Bago rivers where the north-south trending ridge in the middle is made up of Miocene rocks and associated river terrace alluviums. Several major earthquakes visited this region frequently, some of which caused major damage to the built environment including ancient monuments and religious structures. There are a few noticeable events in the 20th century with their epicenters fell in and near the city such as the 1927 Rangoon Earthquake, the 1930 Bago earthquake, and the 1978 Dedaye Earthquake of which Yangon felt intensity from VI to VIII. Recently, slight to moderate earthquakes happened in the northern Yangon area (M5.1), causing damage to buildings and structures. Furthermore, several small-magnitude earthquakes were recorded in the city of Yangon itself. Therefore, an attempt has been made to understand the mechanism and sources of these events. Knowing the background geology of the area, field surveys were carried out to identify the regional structural trends and seismically active elements. Survey works include outcrop mapping of geological elements from which structural trends, sense of shear, and stress orientation can be interpreted. Generally, the observed faults have strikes of approximately N-S, NNW-SSE, NE-SW, and WNW-ESE directions. Field structural data also show right lateral and oblique reverse motion with the direction of maximum principle stress in NNE-SSW to NE-SW directions. In addition to field geological surveys, moment tensor solutions of significant earthquakes published by seismological centers are used to identify the nature of movement when the respective earthquakes happened. The recent events at the northern Yangon areas are found to be related to the WNW-ESE structural trend with an agreement of fault motion between seismic moment tensor and geological structural results. Several major geological lineaments are observed by distinct geomorphological expressions: some of them are often associated with earthquakes such as the Sagaing Fault in the east, the Dedaye Fault and faults near the Ma-u-bin area in the west, the Bago Yoma Fault, the Htan-ta-bin and Satthwartaw in the north and some cross faults in the Bago Yoma. Some concealed faults in the Ayeyawady Delta and off-coast of Pyapon and some faults of the Yangon are also highlighted in this paper as seismically active. In terms of fault size and seismicity of each area, the potential hazard that each tectonic element can give rise to is also discussed.

Keywords: Yangon, earthquake, fault, motion, seismically active, hazard

Introduction

Myanmar is situated within the Alpide-Himalayan earthquake Belt where devastating earthquakes have occurred from time to time. Yangon is the former capital and most populated city of Myanmar with > 5.2 million residents and is located at the southern part of Bago Yoma, as well as the southeastern part of the Ayeyarwady Delta and at the northern of the Andaman spreading center. The Sagaing Fault, which is located about 35 km east of the Shwedagon Pagoda, is a regional right lateral strike-slip fault trending in a north-south direction. There are several historical earthquakes in this region and the seismic sources for such events are from known and unknown structures. The location and regional seismicity of the area is shown in Figure (1). Study of seismicity and geomorphology and geological structures is carried out to identify active sources in the region. This research suggests earthquake potential concerning those identified seismic sources.

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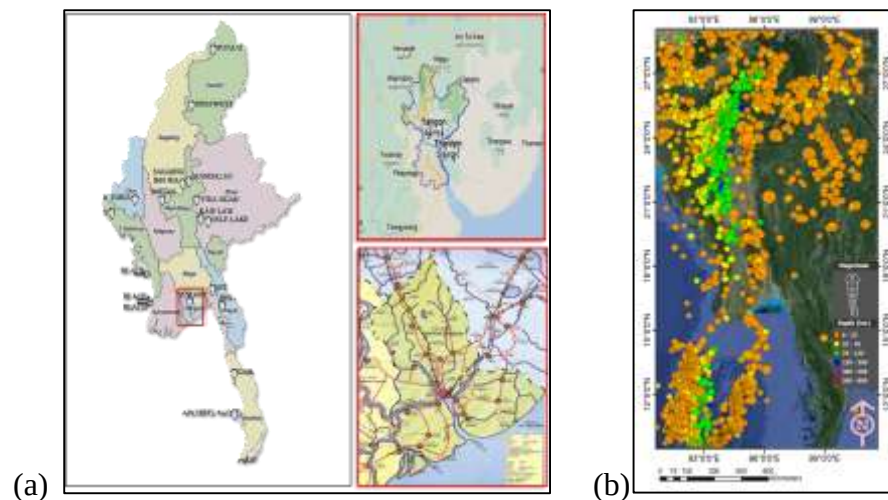


Figure 1. (a) Location Map of the Study Area and (b) Seismicity of Myanmar Source from ANSS earthquake catalog (Evidence between 1900 -2024)

Tectonic Setting

Myanmar is one of the earthquake-prone countries in the world as well as in ASEAN region. Regionally, three major tectonics provinces are hosting significant earthquakes within Myanmar territory: namely, the Western Fold Belt, the ranges resulted from subduction and collision between the India Plate in the west and the Burma Plate in the east, the Central Basin and the Eastern Highland as part of Sibumasu. As a tectonically active region, significant earthquakes often visit in and surrounding of Myanmar Territory.

Yangon region is located on the southern spur of the NNW–SSE trending Bago anticlinal ridge; both geological and geomorphological prominence ridge with 600 kilometers long and 65 kilometers wide. The Sagaing Fault, a major right lateral strike-fault of Myanmar is located approximately 35km in the east of Yangon. The Bago Yoma anticlinorium, the Sagaing fault and the Andaman spreading center are the most significant tectonic elements in this area. Figure (2) shows regional tectonic setting in Yangon and neighbouring areas.

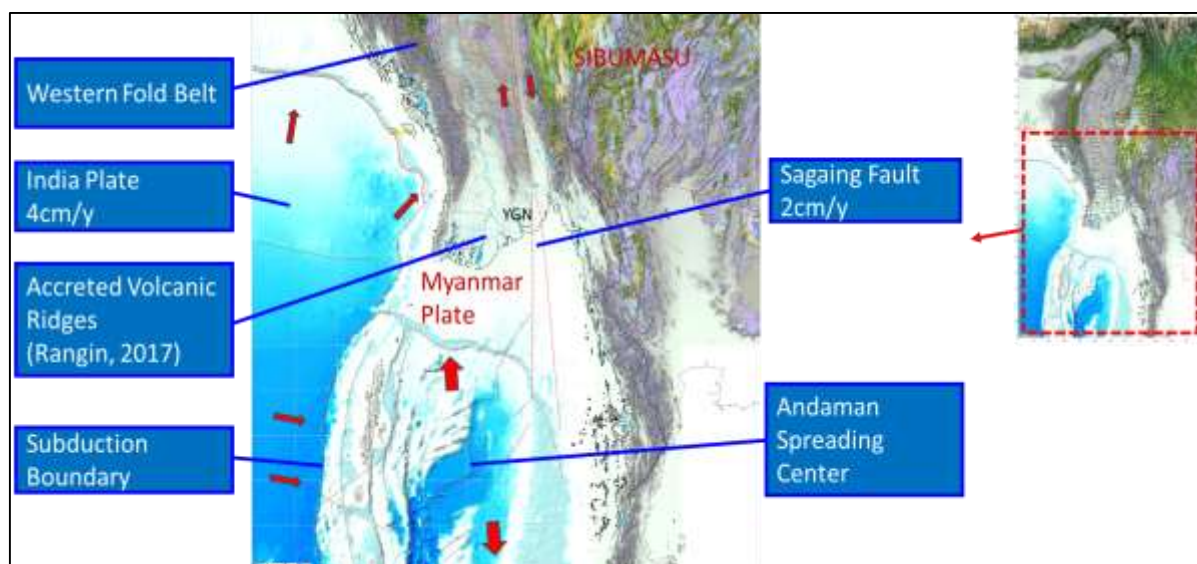


Figure 2. Regional Tectonic Setting of Yangon Region

General Geology

Regional geology of the Yangon Region comprises the rocks of Upper Pegu Group, Irrawaddy Formation and Alluvium (MGS, 2014). A previous study on the geology of the Greater Yangon (Win Naing, 1972) pointed out that the southern part of the Shwedagon-Hlawgar Ridge is composed of sand rocks of the Irrawaddy Group (Arzarnigon Sand rocks); it shows fairly consolidated nature by its exposed surfaces. Hlawga Shales (Win Naing, 1972) (equivalent to Pyawbwe Formation of upper Pegu Group) occupied the lowland areas in the west of Hlawgalake. Although the exposures are rare, some outcrops are found along the west bank of Hlawgalake. Shales and laminated clays of this formation are considered to be the core of the Hlawga anticline (Kyaw Tun, 1996). Thadugan Sandstone (Win Naing, 1972, Kyaw Tun, 1996) (equivalent to Kyaukkok Formation of upper Pegu Group) is mainly exposed around Thadugan Pagoda and also exposed in the southern western of Shwedagon Pagoda. Besapat Alternations (Win Naing, 1972) (equivalent to Obogon Formation of upper Pegu Group) which is exposed in the vicinity of Besapet Lake, Hlawga Park, and Kyauktan Township. Arzarnigon sand rocks (equivalent to Irrawaddy Formation) are the equivalent of the Irrawaddy Formation. The Danyingon clays (Win Naing, 1972) (equivalent to Irrawaddy Formation) mainly consist of blue clays, yellow clays, and siltstones interbedded with sand rocks. The younger alluvium is mostly exposed along the low-lying flat plains. The geology and locations of the outcrop are shown in Figure (3).

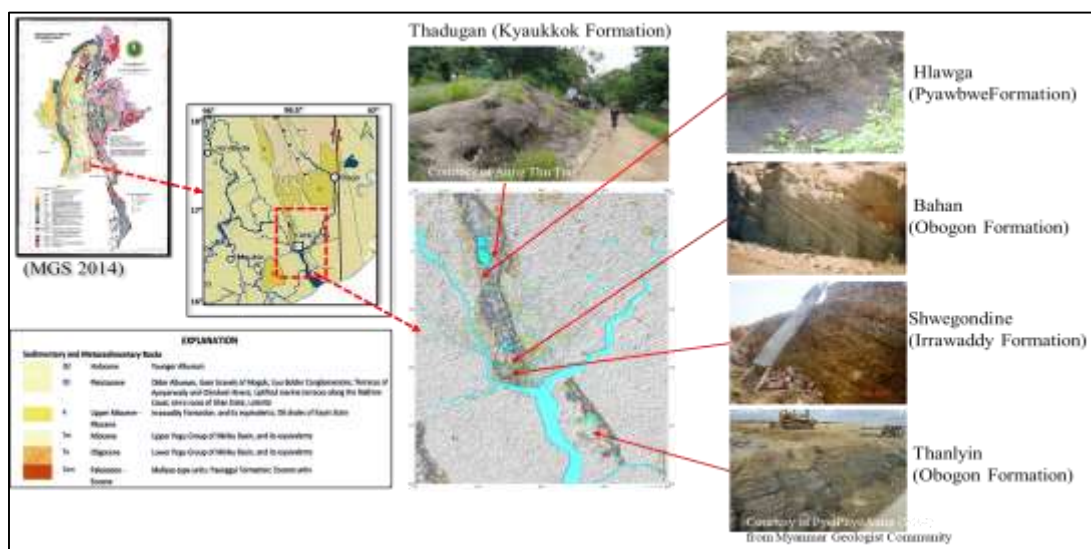


Figure 3. Geology and Some location of outcrop in the Yangon Region

Earthquakes of Yangon Region

The old settlements and ancient civilization of Myanmar had experienced the effect of strong earthquakes. Such historical records are available from some religious buildings, such as stupas and temples, in the form of stone inscriptions, remains, monuments, history documentation (Tint Lwin Swe, 2015). Some examples are the Shwedagon Pagoda of Yangon, the Shwemawdaw Pagoda of Bago and the Kyaikkauk Pagoda of Thanlyin, etc. These structures had long records of renovation works; some of them claim damage due to strong earthquakes. Summary of some significant historical earthquakes in this area are shown in (Fig-4). On 17 December 1927, a seven-grade earthquake hit Yangon and caused certain amount of damages. It was felt 15,000 sq.km from Kyangin to Dedaye along the western slope of Bago Yoma. In May

1930, Bago earthquake with magnitude 7.3M affected Yangon, vibration spread caused damage to the buildings and 500 persons and 50 persons were killed in Bago and Yangon, respectively. In 1978, magnitude 5.1M Dedaye earthquake with more than 20 aftershocks caused slight damage in that region and felt Yangon. Recent earthquakes in Yangon Region have happened on 17th March 2017, a shallow (33km depth) earthquake with magnitude of 5.1M in Taik Kyi area, about 40 km north of Yangon, the earthquake has injured 26 people. It damaged at least 40 buildings including religious structures, government and private offices and the bridges. On 23rd September 2023, a shallow (10 km depth) earthquake with magnitude 5.1M, near Hlegu shook Yangon, Hlegu , Bago and some buildings in the Mawbi Township, which are near the epicenter, were also affected (Fig-5). Some significant earthquakes in and around Yangon area since 1930 are summarized and shown in Figure 6.

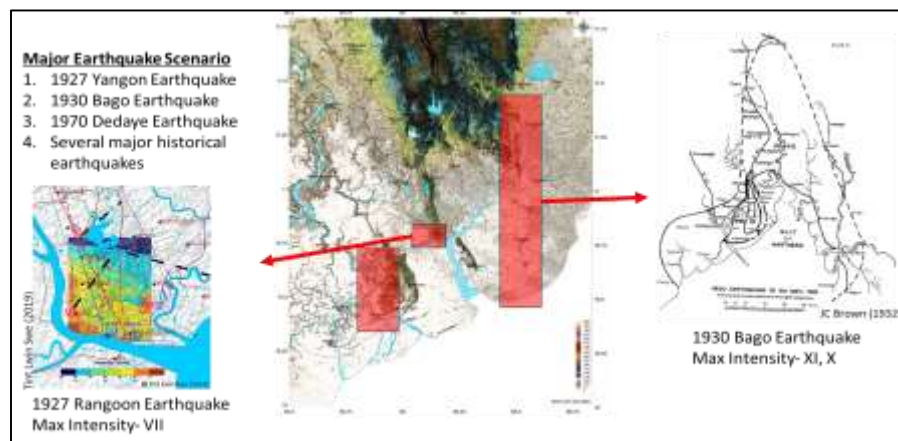


Figure 4. Earthquakes of Yangon Region- Historical Events

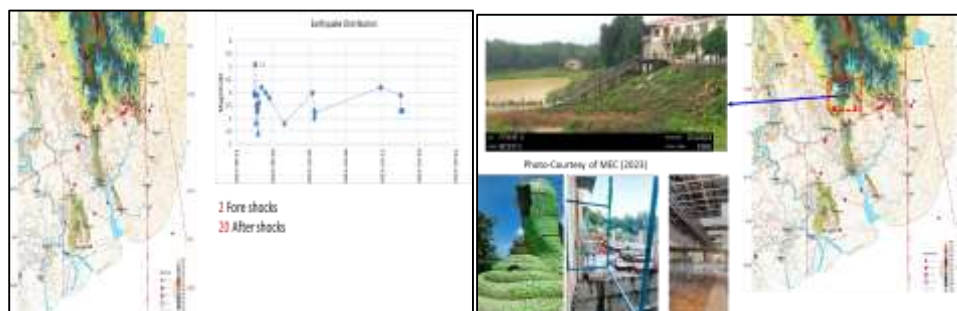


Figure 5. Earthquake events of 5.1 M (Hlegu) and Some damage at Mawbi

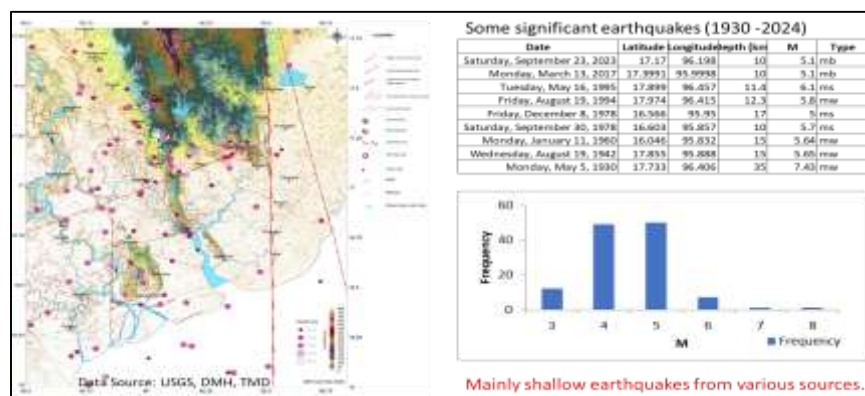
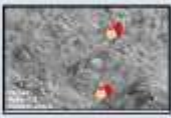
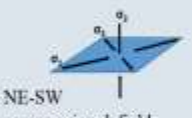


Figure 6. Significant earthquakes of Yangon Region (1930-2024)

Structural Analysis

Regional structural trends are generally in NNW-SSE to N-S, NW-SE and NE-SW directions. They may include older and younger fracture systems and regional stratigraphic trends including the West Bago Yoma and Thanlyin ridges. Lineament analysis suggests the maximum regional principle stress field varying from NNW-SSE to NNE-SSW direction. Boudin features are found in sandstone and shale interbedded near Thadugan Pagoda north of Yangon. Fault-related structural are parallel to the NNW-SSE regional stratigraphic trend; some show a dextral sense of shear and dextral oblique shear under NNE-SSW to NE-SW compression. The younger structural feature cut across the regional stratigraphic trend and older structural elements. Generally, the dipping of the beds and the structural dips are in the same eastward direction. At the northern part of the study area (N 16° 58' 12.51'', E 96° 6' 12.11'') east of Insein Township, it shows a dextral sense of shear in beds of the Besapet alternations. Sense of shear is vertical sigma σ_2 and orthogonal horizontal position of sigma σ_1 and sigma σ_3 under NE-SW compression. Near Thadugan Pagoda (N16° 59' 13.03'' E 96° 6' 1.04''), a dextral sense of shear is vertical sigma σ_2 and orthogonal horizontal position of sigma σ_1 and sigma σ_3 under NNE-SSW compression. At the south, beside the Thanlyin-Kyauktan car road (N16°39'45.40", E 96°19'15.72"), dextral sense of shear is observed on Thadugan sandstone showing vertical sigma σ_2 and orthogonal horizontal position of sigma σ_1 and sigma σ_3 under NNE-SSW compression. At southern part of Kyauktan Township (N16° 37' 48"; E 96° 19' 24"), a lateral oblique sense of shear on the NW trending nodal plane is obtained from of fault plane solution. The amount of maximum principle stress ($\sigma_1 = 45^\circ$) is close to normal principle stress ($\sigma_2 = 60^\circ$), which leads to the lateral sense of shear is followed by the oblique sense of shear and it is vertical sigma σ_2 and orthogonal horizontal position of sigma σ_1 and sigma σ_3 under NE-SW compression (Table -1).

Table 1. Interpretation of field survey and fault plane solution

No	Location	Rock type	Lower-hemisphere stereographic projection Shear sense interpretation	Principal Stresses orientation	Description
1.	N 16° 58' 12.51" E 96° 6' 12.11" near No.6 police station, Shwepyithar Tsp	 Besapet Alternation	 Dextral sense of shear	 NE-SW compressional field	Dextral (right lateral) shear sense under NE-SW direction σ_1 stress
2	N16° 59' 13.03" E 96° 6' 1.04" Thadugan, near Nat Nan	 Thadugan Sandstone	 Dextral sense of shear	 NNE-SSW compressional field	Dextral (right lateral) shear sense under NNE-SSW direction σ_1 stress
3.	N16° 37' 48" E 96° 19' 24" At Kyauktan Yay-lal Pagoda	 Obogone Formation equivalent	 Conjugate joint data show oblique slip shear sense	 NE-SW compressional field	Oblique shear sense under NE-SW direction σ_1 stress
4.	N16° 39' 45.40" E 96° 19' 15.72" Beside the Thanlyin-Kyauktan car road	 Obogone equivalent unit	 Dextral Sense of Shear	 NNE-SSW Compressional field	Dextral (right lateral) shear sense under NNE-SSW direction σ_1 stress

Generally, the strikes of faults mentioned in this area are approximately trending in N-S, NNW-SSE, NE-SW, and WNW-ESE directions. Field structural data also show right lateral and oblique reverse motion with the direction of maximum principle stress in NNE-SSW to NE-SW directions. In addition to field geological surveys, moment tensor solutions of significant earthquakes published by seismological centers are used to identify the nature of movement when the respective earthquakes happened. The recent events at the northern Yangon areas are found to be related to the WNW-ESE structural trend with an agreement of fault motion between seismic moment tensor and geological structural results (Fig-7). Several major geological lineaments are observed by distinct geomorphological expressions: some of them are often associated with earthquakes such as the Sagaing Fault in the east, the Dedaye Fault and faults near the Ma-u-bin area in the west, the Bago Yoma Fault, the Htan-ta-bin and Sathwartaw in the north and some cross faults in the Bago Yoma.

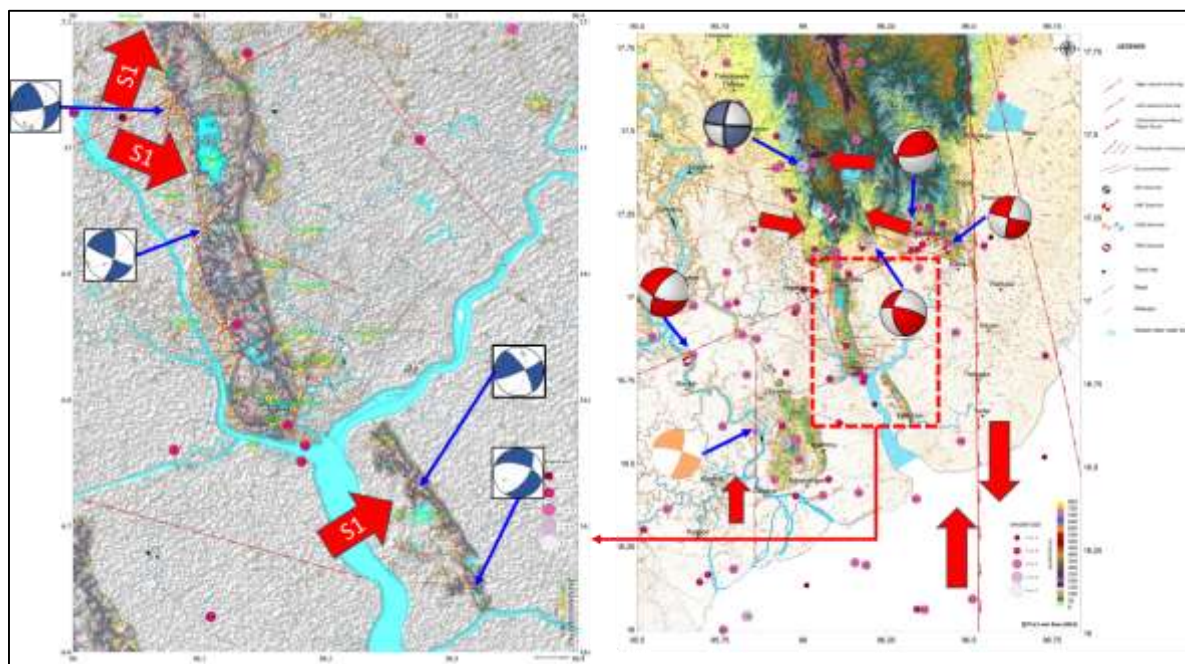


Figure 7. Fault Plane Solution and their related Fault Source in Research Area

Seismicity of Yangon Region

As a tectonically active region, significant earthquakes often occur in and surrounding Myanmar Territory. The distribution of magnitude, focal mechanism of earthquakes from 1923 to 2023, based on data available in the USGS earthquake catalog, DMH, TMD, GFZ earthquake data and related seismic sources are summarized in Figure (8).

Generally, an earthquake with equals or greater than 6 magnitude scale trembles in this region every year including the Andaman-Nicobar Islands. High seismic activities are related to mechanism of subduction and collision between the India plate and the Burma Plate, especially in the north, west and north-western parts of Myanmar as well as along the Sagaing Fault in the central Myanmar (Win Swe, 1981). Further south, the Sagaing Fault and its associated structures play crucial role for seismic hazard for the Yangon Region.

Focal mechanism interpretation indicates that majority are related to strike-slip motion especially related to NW-SE, NE-SW and N-S trending faults, and some oblique-slip (lateral shear together with reverse or normal) motion along NNW-SSE trending faults (Table-2).

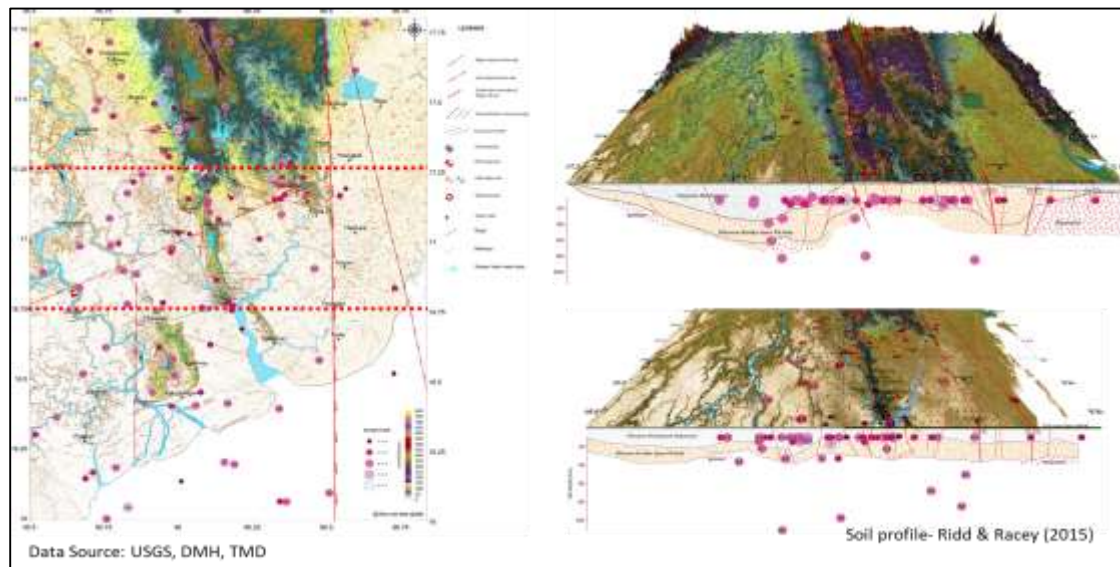


Figure 8. Generalized cross-section together with seismicity and their source structures

Table 2. Focal- mechanism solution of significant earthquakes in Yangon Region

Date	latitude	longitude	depth	M	Source	Picture	str1	dip1	rake1	str2	dip2	rake2	f1	f2
9/30/1978	16.603	95.857	10	5.7	USGS		17	76	-168	284	78	-14	strike-slip	left lateral strike-slip
3/13/2017	17.45	95.91	15	5.1	GFZ		95	87	-12	185	77	-176	left lateral strike-slip	strike-slip
6/7/2023	16.648	95.462	13	4.9	TMD		108	64	-29	211	64	-151	normal left-lateral oblique	normal right-lateral oblique
8/21/2023	20.462	94.454	20	4.8	TMD		128	44	-90	308	46	-90	pure dip-slip normal	pure dip-slip normal
9/25/2023	17.199	96.343	10	4	TMD		83	72	110	214	27	45	right lateral strike-slip	reverse left-lateral oblique
9/23/2023	17.17	96.198	10	5.1	TMD		283	74	44	178	48	158	reverse left-lateral oblique	reverse right-lateral oblique
10/29/2023	20.021	94.251	20	4.5	TMD		104	90	-155	14	65	0	normal right-lateral oblique	pure strike-slip
11/23/2023	17.168	96.435	10	4.1	TMD		103	88	-6	193	84	-178	left lateral strike-slip	strike-slip
Data Source: USGS, TMD, GFZ														Majority are in strike-slip motion

Seismic Source of Yangon Region

There are many possible seismic sources around the Yangon Region (Tint Lwin Swe, 2018) which are summarized below;

- The Bago segment of the Sagaing Fault- the important seismic source in the region which has sufficient size to generate a large magnitude earthquake and it has well-established

long earthquake history. Earthquakes in 1917 and 1930 Bago earthquakes were reported severe damage of Bago and Yangon cities.

- The Thanlyin Fault- the northwest trending structure seems a subsidiary of the Sagaing Fault. Some small magnitude earthquakes were recorded occasionally in this area, but the mode of faulting is not clear.
- The Dedaye Fault- located in the west of Yangon. The earthquakes with magnitude 5.7, 6.0 and 5.7 in 1970, 1991 and 2004 respectively were related to this strike-slip source.
- The West Bago Yoma Fault- Long, persistent and prominent structure at the north of Yangon. Recently, an earthquake with magnitude 5.1M jiggled near Taikkyi, giving minor damages.
- Sathwartaw Fault -located in the north of Yangon. The earthquakes with magnitude 5.1 and 20 aftershocks respectively were related to this strike-slip source.

Further to the above summary, some concealed faults in the Ayeyawady Delta and off-coast of Pyapon and some faults of the Yangon shall be included in account of geomorphological studies and recently recorded seismicity.

Based on the size of each active lineaments and areas and seismicity pattern, the potential of earthquake from each seismic source can be indicated in Figure 10, from which the Sagaing Fault may cause the major earthquake potential to this region.

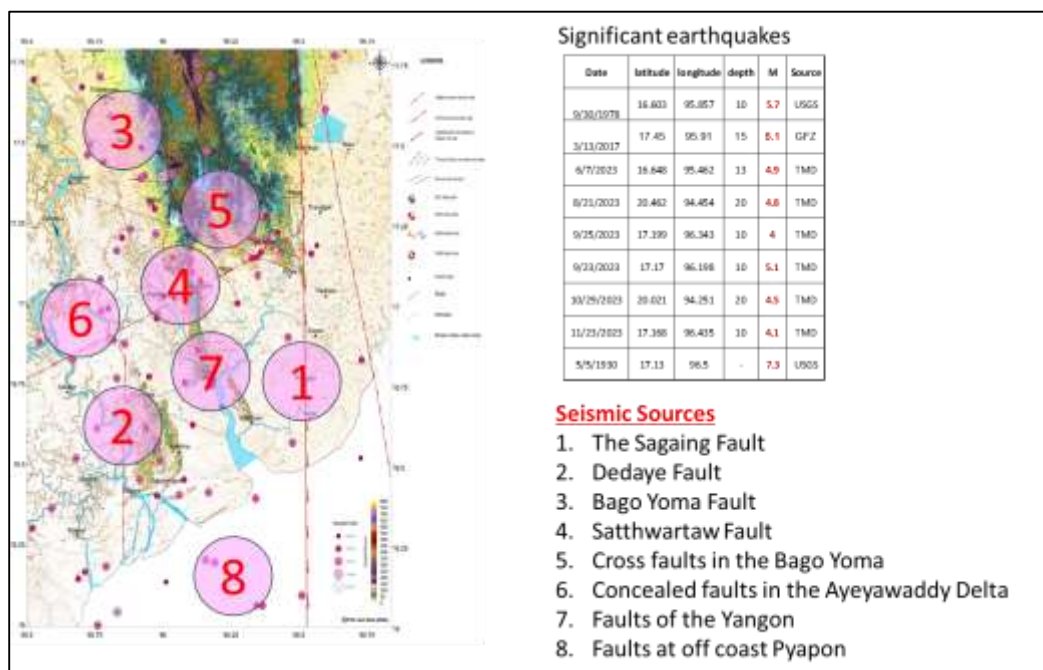


Figure 9. Significant earthquakes and Seismic Sources of Research Area

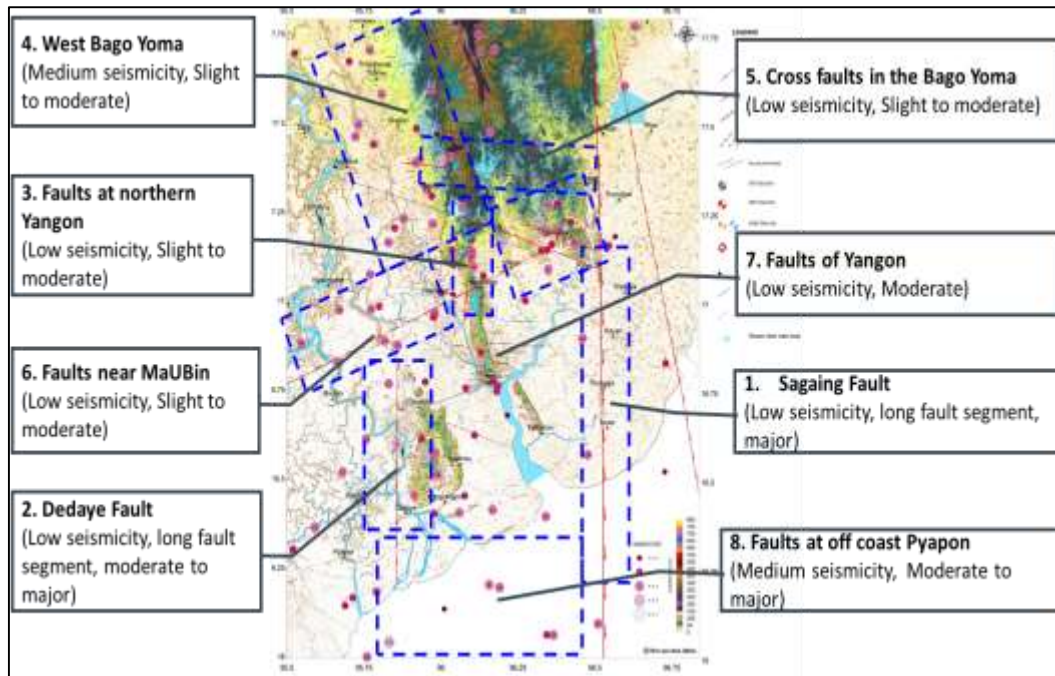


Figure 10. Earthquake Source and Potential of Research Area

Summary and Conclusion

Geology and field survey indicate geological structures including the well-known Sagaing Fault in the east, east dipping Bago Yoma Fault; apparently, extends to the Yangon Ridge. Generally, the observed faults have strikes of approximately N-S, NNW-SSE, NE-SW, and WNW-ESE directions. Field structural data show right lateral and oblique reverse motion with the direction of maximum principle stress in NNE-SSW to NE-SW directions. The cross faults are also observed from geomorphological and field survey; some of them are associated with minor earthquakes. Majority of recorded earthquakes are shallow and often associated with strike-slip and minor oblique reverse fault movements. It is due to east-west convergence and northward movement of the shallow igneous basement below the Bago Yoma and Ayeyawaddy Delta along the Sagaing Fault. There are total 8 earthquake sources identified in this area and each of them can give slight to major earthquake potential in this region.

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MICROFACIES ANALYSIS OF THE MAYMYO FORMATION IN PADAUKPIN AREA, PYIN OO LWIN TOWNSHIP, MANDALAY REGION

Nilar Shein¹, Zin Maung Maung Thein², Maung Maung³, Khin Thazin Kyaw Gyi⁴

Abstract

Maymyo Formation is widely exposed in the Padaukpin area in Pyin Oo Lwin Township, Mandalay Region. The present study focuses on microfacies analysis and facies association based on petrographic aspect and evidences for interpretation of depositional environment. Field method and laboratory method were used to determine the depositional environment of Maymyo Formation. The present study of microfacies analysis is based on the proportion of skeletal and non-skeletal component. Based on detailed petrographic analysis, Maymyo Formation may be divided into six microfacies. The nature and distribution of the fauna and the observed microfacies types, suggest the deposition of Maymyo Formation is reef facies under shallow marine conditions with low to moderate energy.

Keywords: Microfacies analysis, Maymyo Formation, Depositional environment

Introduction

The Padaukpin area is located about 12 miles northeast of Pyin Oo Lwin in Mandalay Region. It lies between the latitude 22° 5' N to 22° 7' N and longitudes 96° 35' E to 96° 38' E in UTM topographic map sheet No. 2296-12 and one-inch topographic map sheet 93B/12 and cover about 20 square miles in area. This area is located near the fantastically developed large limestone cave, so-called Peikchinmyaung cave (Fig.1).

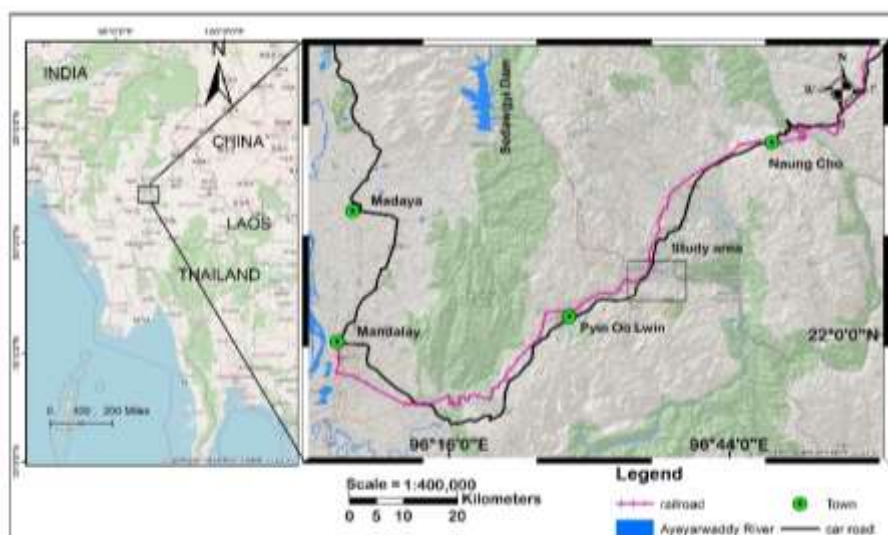


Figure 1. Location map of the Padaukpin area, Pyin Oo Lwin Township

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Regional Geologic Setting

Myanmar is made up of composite terranes including Sibumasu Block (Metcalf, 1984) and West Myanmar Block (Hutchison, 1989) that were derived from the Gondwana origin. The two blocks rifted northward across the Tethys oceans in different time. Collision of the Sibumasu Block with the Sukhothai Island Arc in the Late Triassic after the closing of Palaeo-Tethys Ocean produces the Changning-Menglian-Inthanon Suture Zone. The study area lies in the Shan Plateau, which occupies on the western part of the Sibumasu Block.

Thick-bedded carbonate rocks of Ordovician, Silurian, Devonian, Permian and early Mesozoic age cover large area of the Shan Massif, which are deposited in the Myanmar Malaysian Geosynclines. The whole area consists of Paleozoic rocks ranging in age from Ordovician to Permian. The Shan Burma Boundary Fault runs along the western margin of the Shan Plateau and separates the Central Lowlands to the west from the Shan Plateau to the east. In the Padaukpin areas, carbonates sequences of Middle Devonian have been recognized in the “Maymyo Formation”. The study area lies in the Shan Plateau, which occupies on the western part of the Sibumasu Block (Figure.2).

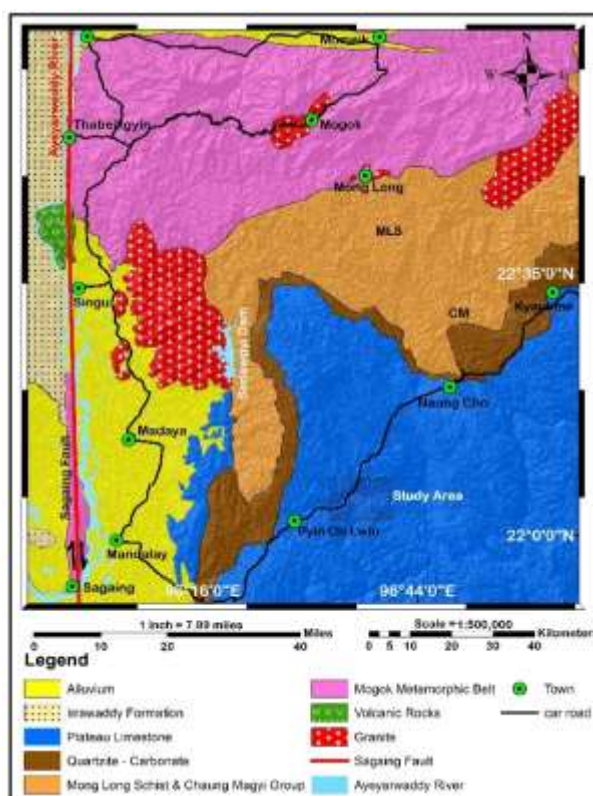


Figure 2. Regional Geological map of the study area and its environ (Source: after Mitchell, 2012, MGS, 2014)

Material and Methods

The present investigation focuses on the sedimentological studies of Maymyo Formation (Padaukpin area). Detailed stratigraphic measurements were made in appropriate section for stratigraphic units by using tape and compass methods. Samples have been collected by layer-by-layer basis for microfacies analysis. All collected samples and fossils were intended to various

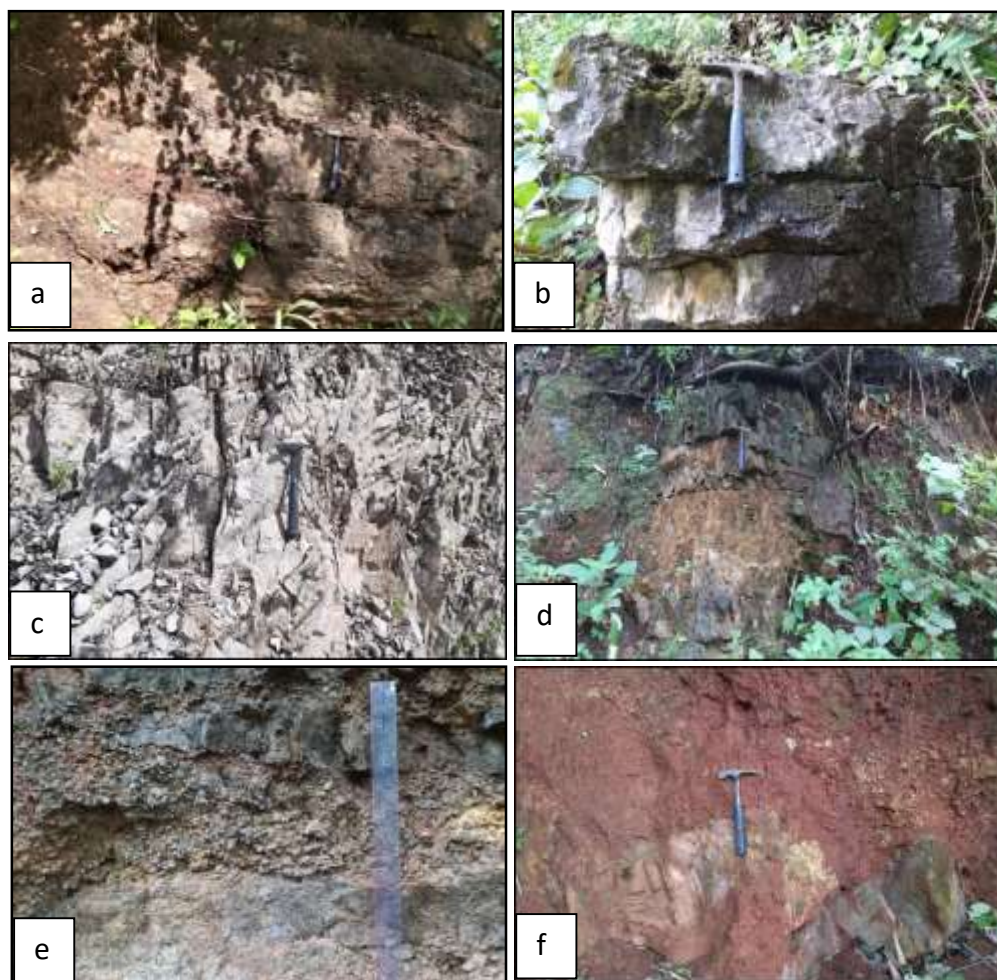


Figure 4 Outcrop nature of Maymyo Formation; (a) Light to dark grey, medium-bedded, relic fossil-bearing dolomitic limestone of the Maymyo Formation, (b) Light to dark grey color limestone with thickening upward and dolomitic nature in Maymyo Formation near two-step cave, (c) Closely jointed and intense brecciation of Maymyo Dolomite Formation in the north of Wetwin village, (d) Thick-bedded to massive, sandy limestone with bryozoans in the Maymyo Formation along Gaelaung Chaung and card-road of the Wetwin hydropower station, (e) Coral- bearing siltstone are interbedded with thin- to medium- bedded, grey to bluish grey limestone of the Maymyo Formation, (f) Carbonate unit overlain by hematite- bearing mudstone and siltstone forming deep red brown colored soil of the Maymyo Formation

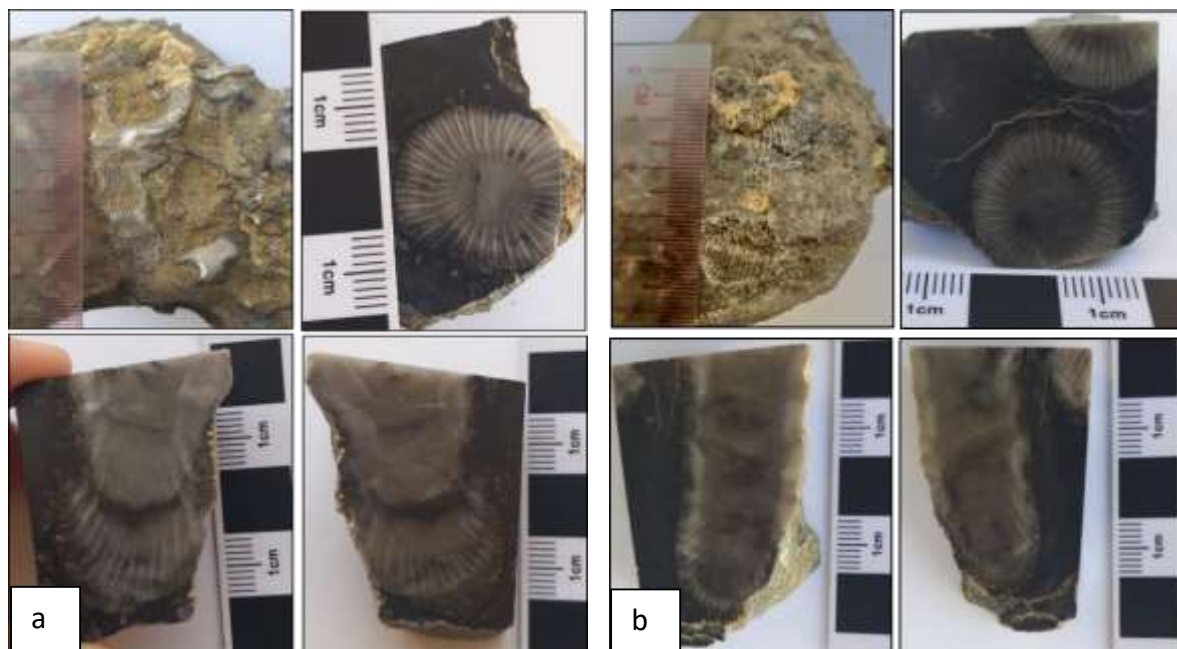


Figure (5.a) Polished section of *Macgeea birmanicum* species corals collected near Padaukpin village (N 22° 10' 54" - E 96° 59' 52")

Figure (5.b) Polished section of *Temnophyllum pyinoolwinwinensis* species collected near Padaukpin village (N 22° 10' 54" - E 96° 59' 52")

Result

Microfacies Analysis

In the study area, carbonate rocks are widely distributed. The rock specimens are collected from all units of the area for the petrographic studies and about 60 thin sections were made and examined. Descriptive petrography for each stratigraphic unit is attempted in this chapter. For the nomenclatures and depositional environment of the carbonate rocks, classification of the limestone by Wilson (1975), Reed (1981), Folk (1959) and Dunham (1962) are applied.

(1) Dolomitized Mudstone-Wackestone

The rock is medium- to thick-bedded, dark grey in weathered colour and light gray to milky white in fresh colour dolomitic limestone and highly brecciated. This rock shows choppy surface, strongly foetid odour and is extensively cropped out at the north of Wetwin village.

It is mainly composed of dolomite rhombs 90% and micrite 10%. Fine-grained calcite is composing more than half of the total volume and no transformation into neospar. Subhedral to euhedral dolomite rhombs are scattered in the micrite. These dolomite crystals are ranging in size from 0.05mm to 0.03mm and are formed by later diagenetic process: neomorphism. The dolomite aggregates are mosaics of anhedral or subhedral grains and tightly interlocked (Fig. 6.a). According to the classification of Dunham (1962), the rock can be named as **dolomitized mudstone-wackestone**. On the basis of the aforementioned petrographic data, the depositional environment of this limestone can be interpreted as restricted circulation shelf with water of Mg-rich situation (deep shelf margin/ Reef front).

(2) Packstone-Grainstone

This rock is medium- to thick- bedded, bluish grey to dark grey calcitic limestone, which is locally dolomitized and exposed near the Padaukpin village.

The limestone is mainly composed of sparry calcite 90%, neospar 7% and pyrite 3%. Most of the calcite crystals are twinned and the twin planes can be seen to be slightly bent. They elongated in one direction and oriented parallel to one another. Euhedral calcite with distinct twin planes indicates the later neomorphism in (Fig.6.b). Equigranular calcite crystals with different extinctions are also noted. Inter-granular pore spaces between calcite crystals indicate the secondary void-filling processes. According to the classification of Dunham (1962), the rock can be name as **packstone-grainstone**.

Occurrence of homogeneous sparite component and scarcity of biota in this limestone may be deposited in low to moderate energy bottom current in a relatively deeper water condition (fore Reef).

(3) Dolomitized Packstone – Grainstone

Megascopically, these limestones are medium-bedded, grey to dark gery, fine- to medium-grained and highly brecciated.

This facies is composed of (60%) of calcite and (35%) micrite, plus accessory minor allochems (5%). Spars are common with distinct cleavage-planes. Due to selective dolomitization, euhedral dolomite rhombs are also found in the area of sparite (Fig.6.c). Some dolomite rhombs range from 0.05mm to 0.3mm in size and are subhedral. Patches of sparry calcite are irregularly developed within the micrite. According to the classification of Dunham (1962), the rock can be name as **dolomitic packstone – grainstone**.

On the basic of the aforementioned petrographic data, this limestone may be deposited in an open sea shelf (Reef Crest) and later dolomitization by metasomatic processes.

(4) Ferruginous Bioclastic-Wackestone

Megascopically, this rock is medium-bedded, purple to reddish brown, ferruginous calcareous limestone with abundant fossils such as corals, brachiopods, bryozoans and crinoids.

Microscopically, it is composed of ferruginous micrite (60%), bioclasts (30%), and silt size quartz (10%). All layered structures are composed essentially of iron materials. Various bioclasts possibly solitary corals and brachiopods' shells are embedded in the ferruginous micritic groundmass (Fig.6.d). Minute quartz-silt grains in the micrite are also noted. Microcrystalline calcite, ferruginous materials and quartz-silt dominantly occupy the micritic groundmass. Minute brachiopod with pedicle view (pedicle opening filled with cements) is also present. According to the classification of Dunham (1962), the rock can be named as **ferruginous bioclastic-wackestone**.

Deeper water carbonates may be red, pink, or purple through preservation of Fe and Mn oxide pigments. The observed petrofabric features and pisoids may probably be indicated the shoal environment in agitated water (Reef front).

(5) Bioclastic Mudstone-Wackestone

Megascopically, this rock is medium-bedded, purple to reddish brown, ferruginous and calcareous limestone containing abundant fossils such as corals, brachiopods, bryozoans and crinoids.

This facies is composed of 45% allochems (mainly brachiopods) and micrite with iron materials 50 %, and quartz silt 5%. Thin-shell bivalves and minute bioclasts are embedded in micrite. Large shell (? Fenestrate bryozoan) embedded in micrite is highly crenulated on the outer wall whereas inner wall built with thin laminated layers (Fig.6.e). Outer wall is marked by neospars. Parallel orientation of quartz-silt and minute argillaceous materials are also observed.

According to the classification of Dunham (1962), the rock can be named as **bioclastic mudstone-wackestone**. Deeper water carbonates may be red, pink, or purple through preservation of Fe and Mn oxide pigments. The most probable depositional environment is postulated as “Fore Reef” with relatively deep-water condition.

(6) Dolomitic Bioclastic Wackestone-Packstone

This rock type is thin- to medium-bedded, grey to dark grey, highly jointed and brecciated dolomitic limestone with relict fossils.

This rock is composed abundantly of branching type bryozoan (70%) that are frond-like fenestrate types, micrite (20%) and dolomite (10%). It is also resembles with the part of a fern or palm tree. Outer margins constituted with minute spines and inner parts are composed of calcite crystals (Fig.6.f). Area between stipes showing selective dolomitization and non-dolomitized micrite still exit. According to the classification of Dunham (1962), the rock can be named as **dolomitic bioclastic wackestone-packstone**.

This type of rock may be deposited in an open marine environment. The associated fauna in this rock strongly suggests the good current circulation and normal marine condition. Later dolomitization is caused by metasomatic processes. On the basis of faunal content (Branching Bryozoans) and petrofabric characters, this facies is probably deposited on the “Reef Front” with moderately deep water condition.

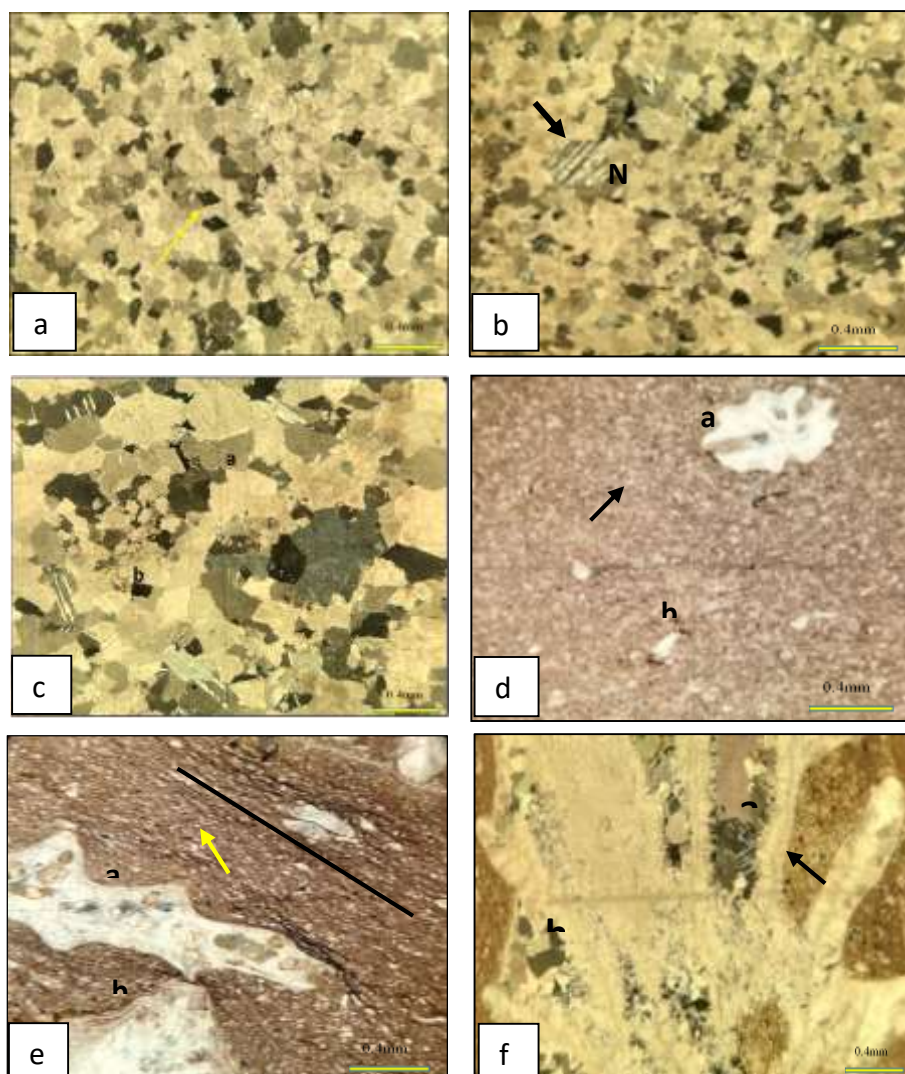


Figure (6) Thin section of photomicrograph showing the microfacies of the Maymyo Formation (Padaukpin area). **(a)** dolomitized mudstone-wackestone, euhedral dolomite crystal (arrow); **(b)** packstone-grainstone with neospar (N), and equigranular calcite (arrow); **(c)** dolomitized packstone – grainstone with irregular sparry calcite (a), and subhedral dolomite (b); **(d)** ferruginous bioclastic-wackestone with a small echinoid spine (a), bioclasts (b) with strongly foliated body whorl, and minute quartz-silt grains (arrow); **(e)** Bioclastic Mudstone-Wackestone containing thin-shell bivalves (a), large bioclast (? Coral) (b), minute bioclasts (c) and parallel orientation of bioclasts and grains (black line) ; **(f)** Dolomitic Bioclastic Wackestone-Packstone with selective dolomitization (a), dolomite rhombs (b) and frond-like fenestrate type's bryozoan (arrow)

Depositional Environments

Based on the analysis of the above microfacies Maymyo Formation (Padaukpin area) was deposited under shallowing marine conditions with low to moderate energy. Proposed schematic depositional environmental model of the Padaukpin-Wetwin area is shown in figure (7).

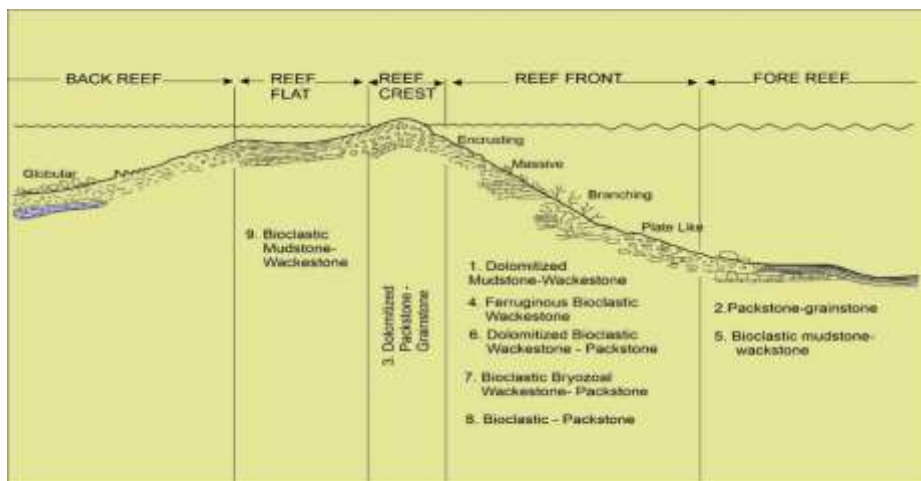


Figure 7. Proposed schematic depositional environmental model of the Padaukpin-Wetwin area (Tucker, 2001)

Conclusion

Maymyo Formation (Padaukpin area) is widely distributed around the environ of Pyin Oo Lwin Township. The Padaukpin area is covered by the Paleozoic strata, which are generally trending NE-SW direction. The “Maymyo Formaiton” consists predominantly of light to dark grey, medium-bedded, relic fossil-bearing dolomitic limestone. Maymyo Formation (Padaukpin area) may be divided into six microfacies. According to my field evidences, nature and distribution of the fauna, microscopic analysis of the current study combination with previous worker’s field research evidences, the study area Maymyo Formation (Padaukpin area) was deposited as reef facies under shallowing marine conditions with low to moderate energy environment.

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