BOTANICAL AND PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *THUNBERGIA LAURUFOLIA* LINDL.

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Abstract

Thunbergia laurifolia Lindl. is known as Nwe-nyo belong to the family Acanthaceae. The plant was collected from Hmawbi Township, Yangon Region, during the flowering and fruiting period from December to May, 2019-2020. In this research, morphological characters of leaves, inflorescence, flower, calyx, corolla, androecium and gynoecium are presented. The plant is perennial herbaceous climber; simple leaves, opposite and distichous; terminal inflorescence with racemose flowers are borne; bisexual, zygomorphic flower. In microscopical characters of leaves, the lamina epidermal cells of anticlinal walls were wavy, both surfaces; stomata were present on lower surface, in this type of diacytic (cross celled) stomata type; in the transverse section of lamina, two layers of palisade mesophyll cell and about 7-10 layers of spongy mesophyll cell were present. In midrib outline, both epidermal cells are barrael-shaped; adaxial side and abaxial side are convex. In petiole outline, both epidermal cells are rounded to barrel-shaped. In the diagnostic characters of leaves powdered samples were observed with sensory characters and microscopical characters. These characters of Nwe-nyo plant were identified by many literatures. Furthermore, the results of phytochemical analysis were various extracts of leaves showed the presence of alkaloid, Glycoside, Phenolic compound, flavonoid, steroid/terpenoid, tannin, saponin, α amino acid, protein, reducing sugar, starch and carbohydrate.

Keywords Botanical investigation of *Thunbergia laurifolia* Lindl. and phytochemical analysis

Introduction

Thunbergia laurifolia Lindl. is a genus of flowering plant of Acanthaceae family, including 200 species and native to tropical region of Africa, Madagascar, Australia and South Africa. *Thunbergia laurifolia* Lindl. species are annual, perennial and shrubs *Thunbergia laurifolia* Lindl. are placed in Acanthaceae for 14 genera are list in Myanmar (Kress,2003). The genus *Thunbergia laurifolia* is made up of about 200 species from warm areas of central and south Africa and Asia (Wagner *et al*., 1999). Flowers of *Thunbergia laurifolia* Lindl. can be divided into 3 types depending on flower color, including while, yellow and purple. The purple type is believed to possess health benefits (Sarawoot Palipoch *et al*., 2013)

The chemical composition of *Thunbergia laurifolia* Lindl., contains number of compounds such as apigenin, coffecic acid, glycosides, flavonoids and phenolic compounds. Many researches have evaluated that these phytochemical substances have the major on diabetes. (Sarawoot palipoch *et al*., 2013 and Piya Kosai *et al*., 2015). phenolic acids such as caffecic acid and gallic acid and alkaloids and flavonoids (Thongsuard *et al*., 2005). The effects of *Thunbergia laurifolia* Lindl., leaves extract significantly decrease the levels of blood glucose.

Thunbergia laurifolia Lindl., is traditional herb in Thailand that has a variety potential benefit such as addiction, detoxification and poisoning, hangovers, liver disease, muscle pain, stomach aches, and ulcers (Govermann *et al*, 2013). *Thunbergia laurifolia* Lindl., is traditionally used for anti-inflammation (Boonyarikpunchai *et al*., 2014), anticancer (Jetawanchaikasem *et al*., 2013). The aqueous leaves extract of *Thunbergia laurifolia* Lindl., showed that contain total phenolic content and total antioxidant capacity (Moe Pwint Phyu *et al*.,2013).

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Materials and Methods

Collection and Identification of *Thunbergia laurifolia* Lindl.

In this research, the species *Thunbergia laurifolia* Lindl. was collected from Hmawbi Township, Yangon Region. They were collected during the flowering and fruiting period from December to May in the year of 2019-2020

For morphological and taxonomical studies, the fresh specimens were identified by using with the help of available literature such Hooker, 1885; Burkill, 1935; Hundley and Chit Ko Ko, 1961; Backer, and Brink, 1963; Lawrence,1969; Dassanayake and Claylon.,1998; Croft, J., Cross, N., Hinchcliffe, S., Lughadha, E.N., Stevens, P.F., West, J.G. and Whitebread, G., 1999; Kress *et al*., W. J, R. A. Defilipps Ellen farr and Yin Yin Kyi., 2003. Herbarium specimens were also prepared and kept in the Botany Department, WYU.

The anatomical characters of fresh leaves were also examined by free hand sections according to the literature of Metcalfe and Chalk (1950) and Trease and Evans (2002). In anatomical studies, chloral hydrate solution was used as cleaning reagent to examine the section cutting. The specimens were recorded with photographs.

Preliminary phytochemical investigation was carried out on the powdered samples of leaves of *Thunbergia laurifolia* Lindl. About 2g of powdered leaves, of *Thunbergia laurifolia* was extracted with 20 ml of methanol on each sample and aqueous solution were subjected for qualitative chemical analysis to detect the presence or absence of alkaloid, glycosides, phenolic compounds, flavonoids, steroids and terpenoids, tannins, saponins, amino acids, protein, reducing sugar, starch and carbohydrates. The experimental procedure was prepared and tested by the methods mentioned in Marni Bettolo *et al*., (1981), Central Council for Research and Unani Medicine, (1987) and Trease and Evans, (2002). The results were shown in Table1.

Result

Morphological Characters of *Thunbergia laurifolia* Lindl., Gard. Chron.260. 1856.

Myanmar Name : Nwe-nyo
English Name : Laurel clock vine,Trumpets vine
Family : Acanthaceae

A perennial herbaceous climber, grow like a twining vine. Leaves usually opposite and distichous pairs along the stem, simple, ovate-oblong and tip acute, up to 1-16 cm long, 2.5 - 5.8 cm broad on leaves. Inflorescence grows on terminal and flowers are borne on hanging racemose, 3 to 23 cm long and 9 to 28 flowers can bear on each peduncle. Flowers are usually opened out into five petals and joined at the base to form tube (yellowish throat and petals are in pale purple color), bisexual, zygomorphic (one of which is longer than the other petals). Calyx 2 lanceolate, tipped with a pointed and it long about 1.2 to 3.9 cm. Corolla-5 lobes, synpetalous, valvate aestivation, begin as approximately 1.8 to 2.3 cm long and 1.5 to 2.5 cm wide. Androecium-stamen 4, didynamous, epipatelous, dithecous, stout, basifixed, longitudinal dehiscence, very hairy on anther and long from 1.2 to 1.5 cm. Gynoecium oval shaped, mostly 3-4 cm long, bicarpellary, axile placentation (disc present), style long about 3 cm and curve at tip, stigmas are flattened and folded form. Fruits elliptical shape or bird’s beak and long about 3.5 cm, consists of two to four seeds in each capsule.

Flowering and fruiting period : Throughout the year
Part Used : Leaves and stem
Uses : The drop of juice leaves is put into ear to treat deafness and it is applied as a poultice on cuts and boils. The leaves extract of this plant is reported as an antidote against poisonous and anti-inflammatory activity.
Specimen examined : Ye' Linn Maung, (2) Quarter, Hmawbi Township, Yangon Region, 17˚ 06' 08" North Latitude, 96˚ 03' 04" East longitude, 22.8 km.

Morphological Characters of *Thunbergia laurifolia* Lindl.

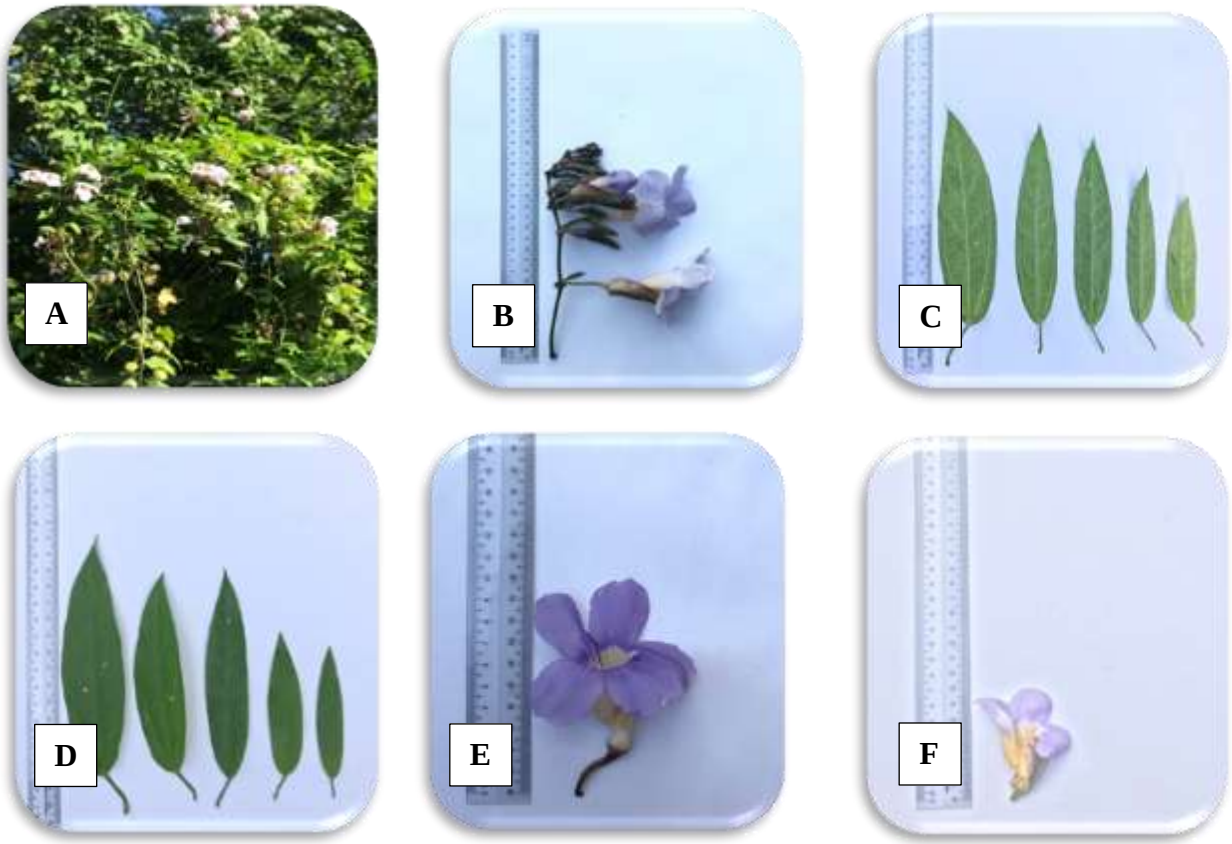

Figure 1 A. Plant in natural habit B. Inflorescence with floral buds C. Leaves (ventral view) D. Leaves (Dorsal view) E. Flower F. L.S of flower

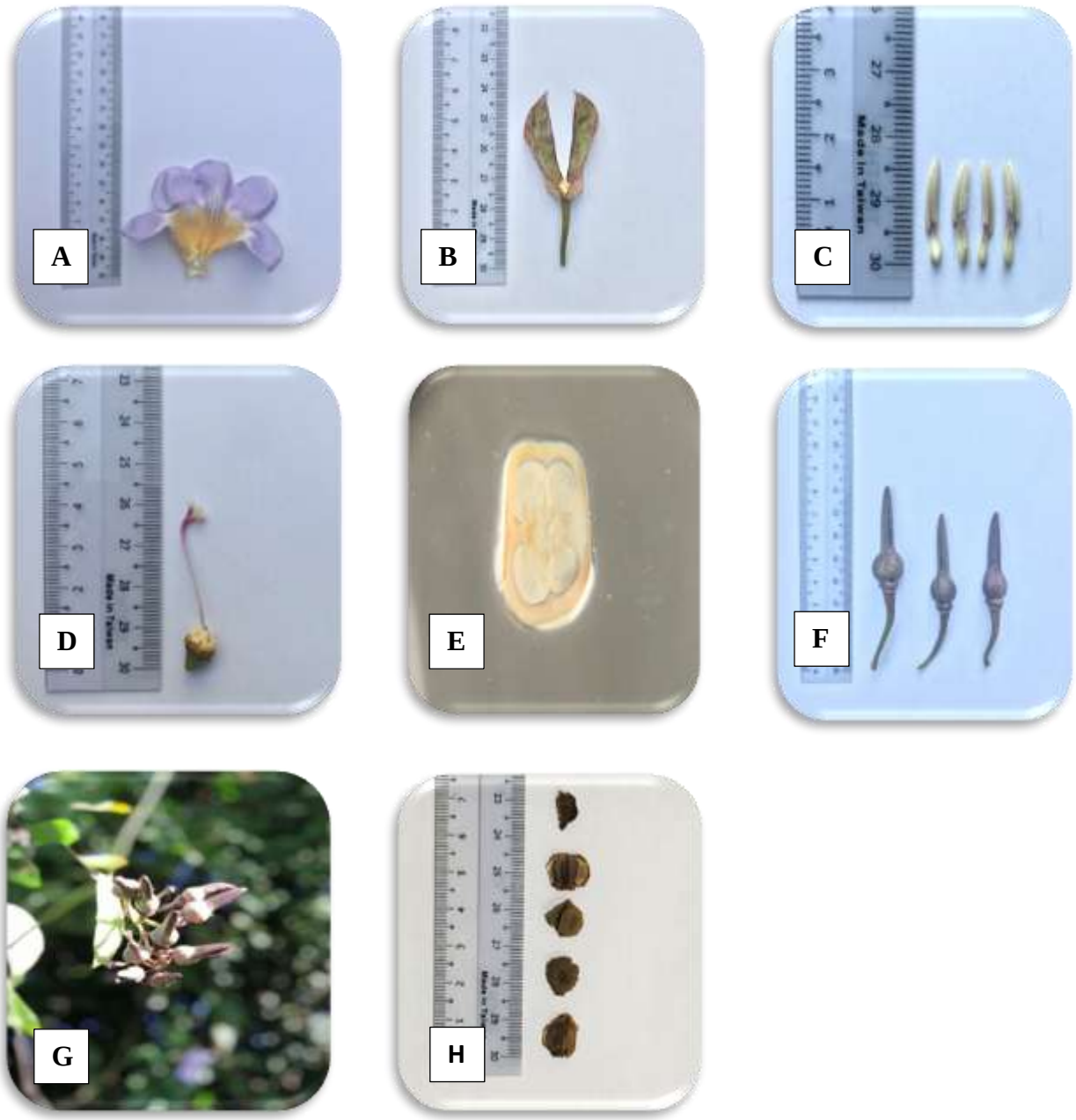

Figure (2) A. Corolla B. Calyx C. Stamens and filament
D. Ovary with disk E. T.S of ovary F. Various sizes of fruits
G. Fruits H. Various sizes of Seeds

Microscopical characters of *Thunbergia laurifolia* Lindl.

Lamina

The lamina of *Thunbergia laurifolia* Lindl. studied is dorsiventral.

In surface view, both upper and lower epidermal cells one-layered of parenchymatous cell walls straight. Stomata present on lower surface, diacytic type. There is no stoma on upper surface.

In transverse section, epidermis one-layered on both sides, compactly barrel-shaped, outer and inner wall straight, thin, smooth, cuticle present on both surface. Epidermis one-layered on both sides, compactly barrel-shaped, outer and inner wall straight. The mesophyll composed of palisade parenchyma at adaxial side and spongy parenchyma at abaxial side. The palisade mesophyll 2 layered, cell vertically elongated, compactly arranged numerous chloroplasts present. Spongy mesophyll 2 layered, irregular to isodiametric in shape, loosely arranged, intercellular spaces large. In mesophyll cells small vascular bundles embedded, rounded. It is not well developed.

Midrib

In surface view, epidermal cells parenchymatous, rectangular walled, cell walls thin and straight, stomata absent.

In transverse section, the adaxial side and the abaxial side convex. The epidermis one-layered, cells collenchymatous, barrel-shaped or rounded. The adaxial collenchymatous cell 5 to 7 layered adaxial, 3 to 12 layered at the abaxial side, oval and polygonal in shape, thicked at the corner. Parenchymatous cells and endodermis are above the vascular bundle. Vascular bundle is collateral type and crescent-shaped Trace of small bundle are present.

Petiole

In surface view, the epidermal cells were thin-walled parenchymatous cells, irregular polygonal in shape. Stomata are present in petiole.

In transverse section, petiole semi-circular in outline with concave surface at the upper side with two wings and convex at the lower side. The cuticle layer was thin. The epidermis one-layered, the cell parenchymatous barrel-shaped.The collenchymatous cell 5 to 12 layered, supporting tissue, the layer at the adaxial and abaxial sides, intercellular spaces present. The sclerenchymatous cells one-layered, the cell barrel-shaped above the vascular bundle. The vascular buldles were collateral type and a large vascular cylinder surrounds the parenchymatous pith, where both the xylem and phloem are discontinuous.

Diagnostic characters of dried leaves from *Thunbergia laurifolia* Lindl.

The fragments of the leaves composed of fragmented reticulate vessel, spiral vessel, tracheids, trichome, pitted vessel and fragment of epidermal cell.

Microscopical characters of *Thunbergia laurifolia* Lindl.

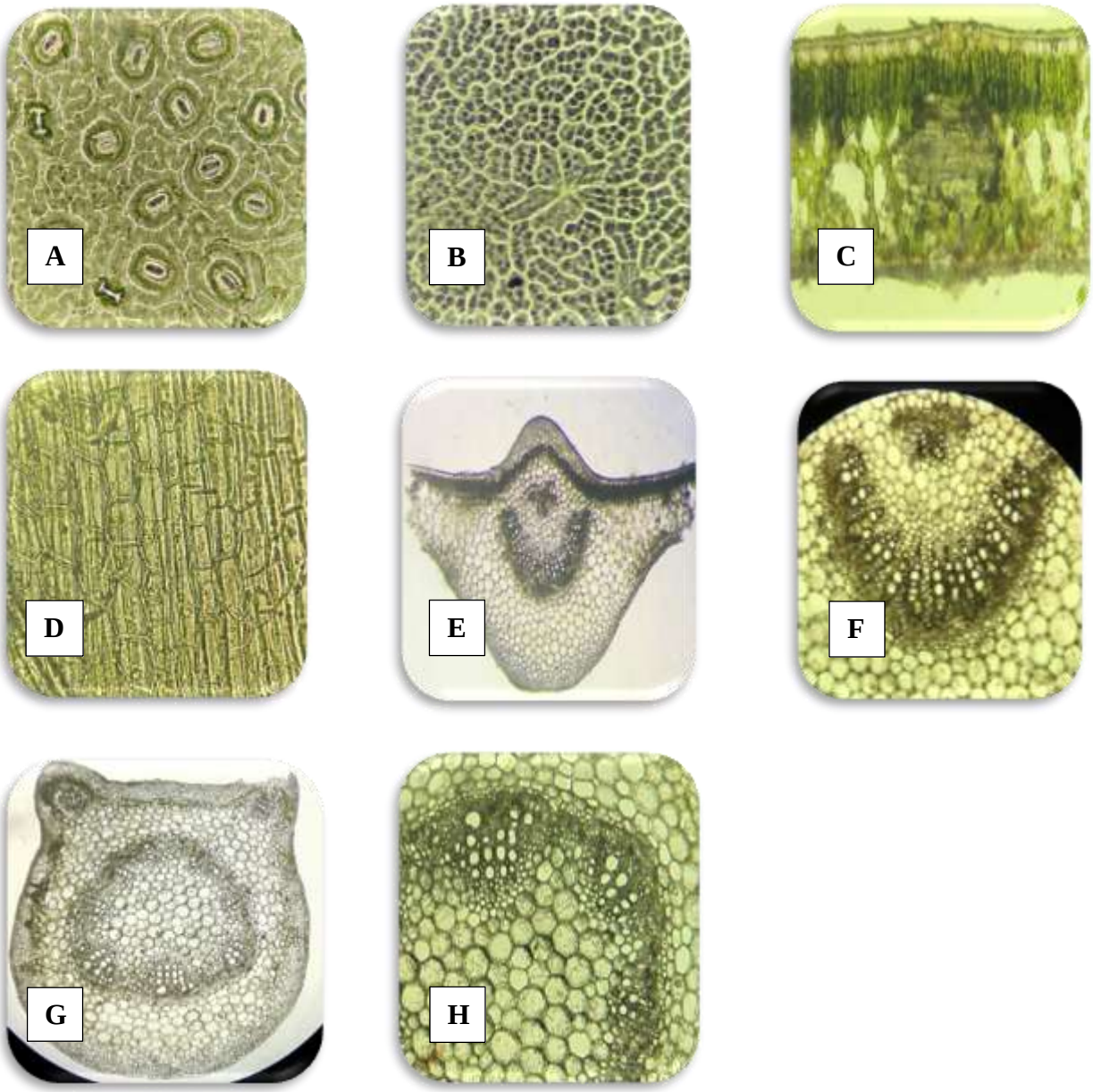

Figure (3)
A. Surface view of lower epidermis with stomata (400X)
B. Surface view of upper epidermal cells (400X)
C. T.S of lamina showing palisade and spongy mesophyll (400X)
D. Surface view of midrib showing epidermal cell and trichome (400X)
E. T.S of midrib showing collenchyma, parenchyma cells and vascular bundle (100X)
F. T.S of midrib showing vascular bundle (400X)
G. T.S of petiole showing collenchyma, parenchyma and vascular bundles (100X)
H. T.S of petiole showing close up view of vascular bundle (400X)

Diagnostic characters of powder samples of leaves, stems and roots of *Thunbergia laurifolia* Lindl.

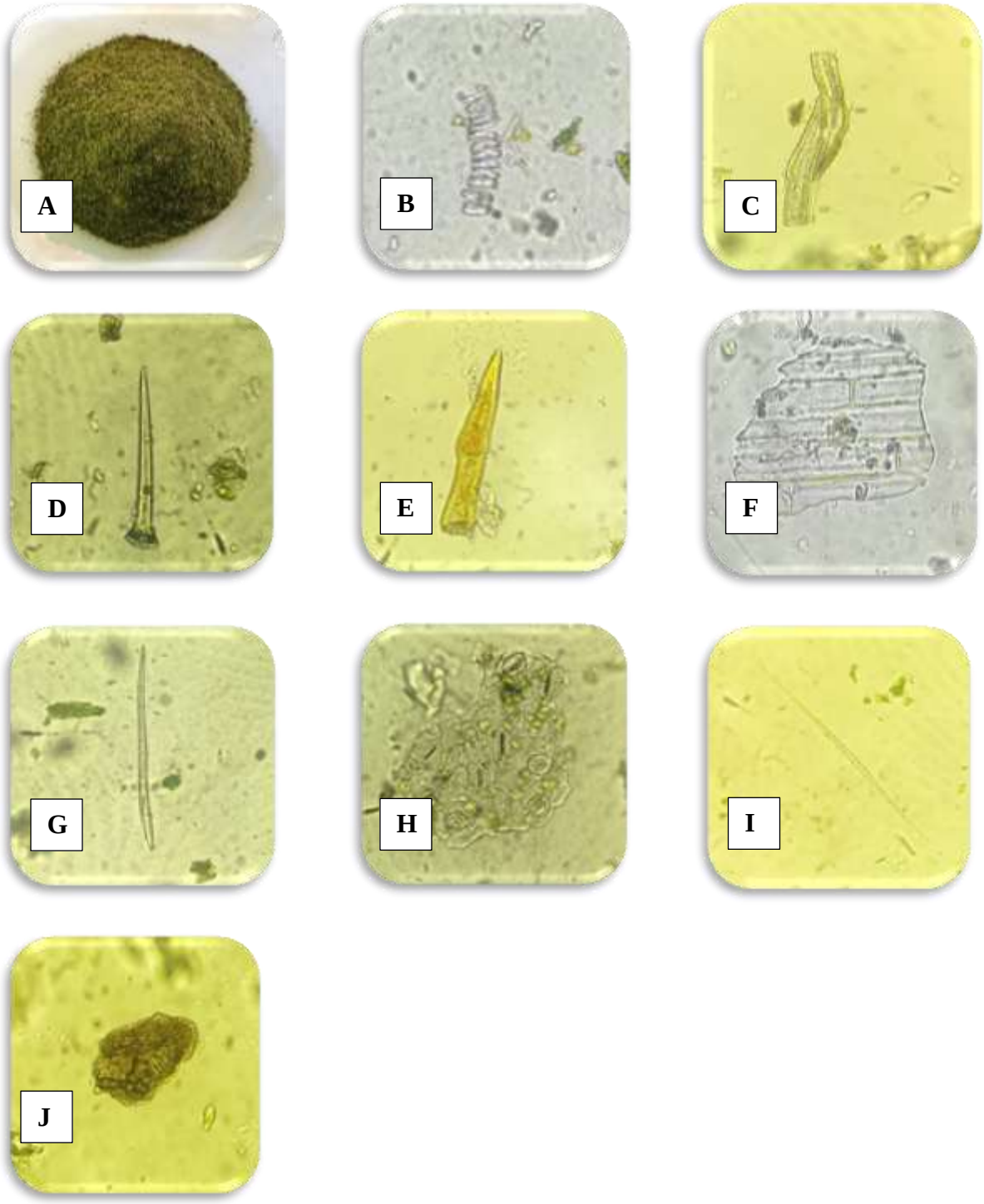

Figure (4) Diagnostic characters of powder samples of leaves

A. Powdered leaves. B. Spiral vessel. (400X)
C. Reticulate vessel element. (400X) D. Unicellular trichome. (400X)
E. Multicellular trichome. (400X) F. Fragment of epidermal cell. (400X)
G. Tracheid (400X) H. Fragment of stomata (400X)
I. Fibre (400X) J. Stone cell

Phytochemical Investigation of leaves of *Thunbergia laurifolia* Lindl.

Preliminary phytochemical tests were carried out on the powdered samples of leaves, of plant ***Thunbergia laurifolia*** Lindl., Nwe-nyo, to determine the presence and absence of chemical compounds, according to standard methods of Central council for research in Unani Medicine (1987) and Trease & Evans (2002).

Preliminary phytochemical tests of leaves indicated that alkaloids, glycosides, phenolic compounds, flavonoids, steroids and terpenoids, tannis, saponin, α-amino acids, proteins, reducing sugar, starch and carbohydrates were present. The result of phytochemical test of leaves were shown in Table 1.

Table 1 Phytochemical Investigation of the leaves *Thunbergia laurifolia* Lindl.

No.	Test	Solvent Extract	Test Reagents	Leaves	
				Observation	Result
1.	Alkaloids	1%HCl	Mayer's reagent	White ppt	+
			Wagner's reagent	White ppt.	+
			Hager's reagent	Yellowish green	+
2.	Glycosides	MeOH	1ml H2O + NaOH	Yellow green	+
3.	Phenolic compound	MeOH	2ml H2O + 10% FeCl3	Blackish	+++
4.	Flavonoids	MeOH	Mg coil + HCl (dil) H2SO4	Crimson	+
5.	Steroids/ Terpenoids	MeOH	CHCl3+ H2SO4(conc)	Green/Reddish brown	+
6.	Tannins	Water	5%FeCl3+ H2SO4(dil)	Yellow brown ppt.	+
7.	Saponin	Water	Shaken with 2ml H2O	Forthing	+
8.	α-amino acids	Water	Ninhydrin reagent	Purple spot	+
9.	Proteins	Water	Millon's ragent (Heated)	Purple ppt.	++
10.	Reducing sugar	Water	1ml H2O and mixture equal part fehling's A and B	Brick red ppt.(Heated)	++
11.	Starch	Water	Iodine	Brown ppt.	+
12.	Carbohydrates	Water	5%α- napthol sol; + H2SO4	Purple ring	++

+ = Present - = Absent ppt. = precipitate

Discussion and Conclusion

In the present study, the species ***Thunbergia laurifolia*** Lindl., is belong to the family Acanthaceae carried out according to Lawrence, (1969), Hundley and Chit Ko Ko (1969).***Thunbergia laurifolia*** is native to India, Burma, and Malaysia. In Myanmar, this plant is known as New-nyo or Kyi-Kan-Hnok-Thi. It is widely distributed, commonly grows in Kachin State, Mandalay and Yangon Region or planted throughout Myanmar. In Myanmar, the plant of ***Thunbergia laurifolia*** Lindl. haven't still been traditionally used for the treatment of disease. In this paper, the collected specimens are identified by using available literature.

In morphological study, these plants are perennial, herbaceous climber and it grows. Leaves are opposite ovate-oblong shaped and tip acute. It grows up to 8 - 16 cm long, 2.5 - 5.8 cm broad on leaves. These characters were in aggrement with those stated by Backer (1963) and Sarawoot (2013).

Inflorescence are on terminal peduncle and flowers are borne on hanging. It can grow from 3 - 23 cm long and 9 - 28 flowers can bear on each infloresence.Flowers are bisexual, zygomorphic and it opens out into five petals and joined at the base to form tube (yellowish throat and petals are in pale purple colour. Calyx are 2, lanceolate, tipped with a pointed and it persistent till long after the fall at the corolla. Corolla are five lobes, synpetalous and valvate aestivation form. Stamens usually 4 and didynamous or rarely 5, epipetalous dithecous, basifixed, longitudinal dehiscence. Ovary oval-shaped, mostly 3 - 4 cm long, bicarpellary, axile placentation (disc present). Styles are long about 3 cm and curved at tip and stigma are flattened and folded form fruit it grows like elliptical shaped or large capsules and consist two to four seeds in each capsule. Seeds flat and winged. These characters are in aggrement with those mentioned by Hooker (1885), Lawrence (1964), Dassanayake (1981) and Flora of Hong Kong (2009).

In microscopical study, the leaf is usually dorsiventral and presence of simple unicellular and multicellular trichomes. Stomata is diacytic type. Leaf is dorsiventral and the mesophyll layer consisting of 2 cell layers thick of regularly-shaped palisade parenchyma and two layer of spongy parenchyma reaching downward to the abaxial epidermis. Lower epidermis consists of one cell layers that covered by cutin, but less than that of upper epidermis and the amount of diacytic stoma in this part are more than that of upper epidermis which are agreed with Jackson (1990), Somporn Putiyanan *et al* (2008) and Cassandra (2010)

In the phytochemical investigation, the species of ***Thunbergia laurifolia*** **Lindl.** of leaves are showed that that the presence of flavonoids, alkaloids, tannins, steroid/terpenoid, saponins, glycosides, carbohydrates, protein, α-amino acids, proteins and phenolic compounds. Among them, abundant phenolic compound compound is observed in leaves. These characters are in agreement with those whose mentioned by Somporn Putiyanan *et al*., (2008) and Moe Pwint Phyu *et al.,* (2013).

In conclusion, the preliminary phytochemical test of leaves extracts showed that the presence of flavonoids, alkaloids, tannins, steroid/terpenoid, saponins, glycosides, carbohydrates, protein, α-amino acid, reducing sugar and phenolic compounds. Among them, phenolic compound presented abundantly in leaves, it plays an essential role not only in the treatment of diseases like diabetes but also in the commercial products as herbal tea. Then, to find out more active components of medicinal values from the leaves of ***Thunbergia laurifolia*** Lindl., it needs further studies for physicochemical properties and antimicrobial activity tests of the leaves.

Acknowledgements

Firstly, I sincerely thank to Dr. Ko Tin, Professor and Head, Department of Botany, West Yangon University, for his assistance and kindly letting me to do this research and for his precious advice. Secondly, I would like to thank to Dr, Yadana, Professor, Department of Botany, West Yangon University, for her generous support. Last but not least, I am exceedingly grateful to my colleagues who gave me encouragement.

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