MORPHOLOGICAL, HISTOLOGICAL STUDY OF LEAVES AND PHYTOCHEMICAL ANALYSIS OF LATEX OBTAINED FROM *CALOTROPIS GIGANTEA* (L.) R.BR AND *THEVETIA PERUVIANA* (PERS.) SCHUM.

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Abstract

Two latex producing plants were used in present study. The first plant,*Calotropis gigantea* (L.) R. Br is collected from Dagon University Campus and its local name is 'Mayoe-gyi' (oleander in English) and belongs to the family Asclepidaceae. The second one *Thevetia peruviana* (Pers.) Schum.is taken from the area of North Dagon Township. Myanmar people call it 'Set-hnit-ya-thi' (Madar in English) and belongs to the family Apocynaceae. The collected plants were subjected in the plantidentification using the available literatures at the Botany Department, Dagon University.In morphological study the *Calotropis gigantea* (L.) R. Br. was perennial shrub. Leaves were usually simple and milky latex present. *Thevetia peruviana* (Pers.) Schum.leaves were simple, spirally arranged and also produce milky latex. The histological study, free hand sections of fresh specimen of leaves were studied under the microscope. The upper epidermal cells of *Calotropis gigantea* (L.) R. Br. were polygonal in shaped, the stomata were paracytic type and found on both surfaces. In *Thevetia peruviana* (Pers.) Schum., the upper surface view of cells were wavy in shaped, the stomata were anomocytic type and found on the lower epidermis of the leaf. In transverse section, vascular bundles of the two plants were bicollateral type and crescent shaped. The preliminary phytochemical properties were also examined from the latex of these two plants by using the methods of Central Council for Research in Unani Medicine.The presence of glycoside was mostly found in phytochemcial examination. Latex in nature is a milky fluid found in 10% of all flowering plants. The latex of many species can be processed to produce many materials.

Kew words : latex, paracytic, anamocytic, biocollateral, crescent

Introduction

Medicinal herbs are the local heritage with global importance. Medicinal plants as herbs are the great importance to the health of individual

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and communities. Among these, plant latex has much more attention in the research area because of its dazzling features in plant defense mechanism.

The medicinal properties of *Calotropis gigantea* (L.) R. Br. are known in this country from the earliest timealso known as Mayoe-gyi in Myanmar, belongs to the family Asclepiadaceae. *Calotropis gigantea* (L.)R. Br. is an erect perennial shrub, growing chiefly in waste lands. It ascends to an altitude of 3,000 ft. on the Himalayas, and extends from the Pumjab to south India, *Calotropis gigantea* (L.) R.Br. plant is a shrub or small tree 8-10 ft, milky latex which is present in all parts of the plant (Backer, 1965 and Huber, 1983). The milky juice is also used for tanning and dyeing. *Calotropis* juice is caustic when applied to unbroken skin or mucous membranes. All parts of the plant are considered to have valuable alterative properties when taken in small doses. Madar juice is also given internally or applied locally to procure abortion. In some parts of India, it is also used as a cattle poison (Chopra, 1982).This plant is popularbecause it produces a large quantity of latex. The plant has potential pharmacological properties. The abundance of latex in the green parts of the plantreinforces the idea that it produced and accumulated latex as a defense strategy against organisms such as bacteria, fungi and insects (Kumar *et.al.,* 2012).

Thevetia peruviana (Pers.) Schum. is an evergreen, tropical shrub or small tree in the family Apocynaceae. It is a close relative of *Nerium oleander* L. giving it one common name as yellow oleander and is also called lucky nut in the West Indies.This plant is cultivated as an ornamental plants. The leaves are willow- like, linear- lanceolate, and glossy green in color. They are covered in waxy coating to reduce water loss. The stem is green turning sliver gray as it ages. Flowers bloom summer to fall. The long funnel-shaped sometimes fragrant yellow flowers are in few flowered terminal clusters. *Thevetia peruviana* (Pers.) Schum. contains a milky sap containing acompound called thevetin that is used as a heart stimulant but in its natural form is extremely poisonous, as are all parts of plants, especially the seeds (Backer, 1965 andDutta, 1987).

Thus, the aim of this study is to examine medicinal plants scientific to know its medicinal values. The main objectives are to verify and confirm the morphology characteristics of vegetative and reproductive parts, to ascertain

thephytochemical test of latex from *Calotropis gigantea* R. Br and *Thevetia peruviana* (Pers.) Schum.

Materials and Methods

Botanical Studies

1. Collection and identification

The specimens of*Calotropis gigantea* (L.) R.Br., and *Thevetia peruviana*(Pers.) Schum.were collected from North DagonTownship, Yangon Region, especially during the flowering and fruiting periods from February, 2017 to June, 2017.After the collection, the specimens were identified with the help of available literatures Hooker,1885; Kirtikar and Basu,1935; Dutta,1979; Dassanayake,1983and Huber, 1983. Both the vegetative and reproductive parts of the specimens were used for the morphological studies.

2. Histological study

For histological studies, leaves and stem were examined by preparing free hand sections from the fresh specimens, according to the methods of Esau,1965; Trease and Evans, 1978; Pandey,1981 and Tandon,2011.

The samples were washed and dried at room temperature and then crushed into powder to study the powdered characteristic. The observation of the powdered drugs was made by using the powders of the vegetative plant parts. The powders were cleared in chloral hydrate solution on a glass slide and observed under the compound microscope.

Chloral hydrate solution was used as a clearing reagent. The presence of calcium oxalate crystals and prisms were tested by 80% sulphuric acid. Solution of phloroglucinol with concentrated hydrochloric acid was tested for lignin.

3. Chemical Studies

The preliminary phytochemical studies on the latex of *Calotropis gigantea* (L.) R.Br., and *Thevetia peruviana*(Pers.) Schum.had been undertaken. The experiment was carried out to determine the presence or absence of alkaloid, α -amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, steroids, terpenoids and

tannin, according to the method of Central Council of Research in Unani Medicine,1987.

Preliminary phytochemical test (Extraction)

For preliminary phytochemical investigation, the collected plant parts were washed repeatedly with tap water. Then, latex were taken from the leaves and stored in air tight container for chemical analysis.2.5 mLof latex was extracted with 50 mL of two different solvents like ethanol and distilled water respectively.

Results

Morphological Characters of *Calotropis gigantea* (L.)R.Br., and *Thevetia peruviana* (Pers.) Schum.

Morphological characters of *Calotropis gigantea* (L.)R.Br.

Scientific name : *Calotropis gigantea* (L.)R.Br.
Myanmar name : Mayoe-gyi
English name : Crown flower
Family : Asclepiadaceae
Flowering and fruiting period : Throughout the year

Perennial shrubs, milky latex present (Fig. 1.1). Leaves simple, opposite and decussate, exstipulate, short, petiolate, lamina broadly ovate, the bases cordate, the margins entire, the tips acuminate, the upper and lower surface silvery hairs (Fig. 1.2-1.3). Inflorescence axillary, umbelloid cymes (Fig. 1.4). Flowerpurple in colour, ebracteate, ebracteolate, pedicellate, complete, bisexuals, actinomorphic, 5 merous, cyclic, hypogynous (Fig. 1.5). Sepals 5, synsepalous, imbricate, petaloid (lightgreen). Petals 5, apopetalous, connate at the base, valvate, petaloid (purple), inferior. Stamens 5, adnate or adherent to the gynoecium to produce a gynostegium, anther 2-celled, dithecous, adnate fixation, corona of 5 fleshy organs adnate to a coloum with an upcurved spur and auriculate at the base (Fig. 1.6). Ovary oblong, 2 carpelled, 2 loculed, style slender, stigma apex pentagonal, marginal placentation, superior(Fig. 1.7). Fruit ovoid, oblong follicles, seeds with a tufted micropylar coma of long silky hairs, the embryo large, the endosperm thin and small.

Morphological characters of *Thevetia peruviana* (Pers.) Schum.

Scientific name : *Thevetia peruviana* (Pers.) Schum.

Myanmar name : Set- hna- ya-thi

English name : Yellow oleander

Family : Apocynaceae

Flowering and fruiting period : throughout the year

Small tree, perennial, milky latex present (Fig. 2.1).Leaves simple, whorled, sub-sessile, exstipulate (Fig.2.2-2.3).Inflorescence axillary or terminal, dichasial cyme (Fig. 2.4).Flower bracteate, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, pentamerous, cyclic, hypogynous (Fig. 2.5). Sepal 5, aposepalous, quancuncial, sepaloid, persistent, inferior; Petal (5), synpetalous, fennel-shaped, twisted, petaloid (yellow),inferior; Anther (5), alternate, petalostomonous, filament very short, anther dithecous, introrse, basifixed, longitudinal dehiscence, inferior (Fig. 2.6). Carpel (2), bicarpelly, syncarpous, four-locular due to present of false septum, two ovules in each locule, axile placentation, style long, stigma bilobed, disc present, superior (Fig. 2.7). Fruit sub-globose.

Figure 1.1 Habit

Figure 1.2 Upper surface view of leaves

Figure 1.3 Lower surface view of leaves

Figure 1.4 Inflorescence

Figure 1.5 Flowers

Figure 1.6 L.S of flower

Figure 1.7 T.S of ovary

Figure 1. Morphological characters of *Calotropis gigantea* (L.) R.Br.

Figure 2.1 Habit

Figure 2.2 Upper surface view of leaves

Figure 2.3 Lower surface view of leaves

Figure 2.4 Inflorescences

Figure 2.5 Flower

Figure 2.6 L.S of flower

Figure 2.7 T.S of ovary

Figure 2. Morphological characters of *Thevetia peruviana* (Pers.) Schum.

Histological Characters of *Calotropis gigantea* (L.)R.Br., and *Thevetia peruviana* (Pers.) Schum.

Microscopical characters of leaves of *Calotropis gigantea* (L.)R.Br.

Lamina

In surface view, the upper and lower epidermal cells were parenchymatous, polygonal in shape, cell compact and anticlinal wall straight. Stomata were present on both surfaces and abundant in lower surfaces. The types of stomata were paracytic. Multicellular trichomes were present on both surfaces (Fig.3.1- 3.2).

In transverse section the lamina was dorsiventral and thick cuticle layer was present on both surfaces. The epidermal cells were one layer on both sides, cells were more or less rectangular in shaped. The mesophyll layer composed of palisade and spongy parenchyma. Palisade parenchyma cells are found on upper side and three-layered, the cells vertically erect, compact, spongy parenchyma cells lie internal the lower epidermis consisted of 5-7 layers of cells, irregular to isodiametric shaped and loosely arranged (Fig. 3.3).

Midrib

In surface view, the epidermal cells were parenchymatous and compactly arranged and irregular. Multicellular trichomes were present (Fig. 4.1).

In transverse section, convex at lower side and concave at upper sides covered with thin cuticle. Both epidermal cells were rounded or oval shaped. Below the epidermis, the cortex was differentiated into outer collenchyma and inner thin-walled parenchyma cells. The collenchymatous cells were 5-6 layers in thickness towards the upper surface and 4-5 layers in thickness towards the lower surface. They were polygonal to isodiametric in shaped. The parenchyma cells were 22-25 layers in thickness above the vascular bundle and 26-28 layers in thickness below the vascular bundle. They were thin-walled and rounded or oval in shaped. Intercellular spaces and crystals of calcium oxalate were present in parenchymatous cells. The vascular bundle was crescent-shaped in outline, bicollateral and closed type (Fig. 4.2- 4.6).

Petiole

In surface view, the epidermal cells were parenchymatous, thin-walled and mostly rectangular in shape and elongated along the length of the petiole(Fig. 5.1).

In transverse section, the petiole was semi circular shape in outline. The cuticle layer was thick. The epidermal cells were barrel-shaped. Muticellular trichomes (unicinate) were present. The cortex was made up of two different types of tissues, outer collenchymatous and inner parenchymatous tissues. The collenchymatous tissues below the epidermis 5-6 layers in thickness, oval shape, the parenchymatous tissues 11-14 layers in thickness, thin walled, rounded or oval in shape. Intercellular spaces and

calcium oxalate crystals (druses) were present. The vascular bundles were crescent shaped in outline and embedded in the parenchymatous tissues. Vascular bundles were bicollateral and closed type (Fig. 5.2- 5.4).

Microscopical characters of Leaves of*Thevetia peruviana* (Pers.) Schum.

Lamina

In surface view, the epidermal cells of both surfaces are parenchymatous cells. The upper epidermal cells are wavy in shaped and the lower epidermal cells are also slightly wavy walls. Stomata are numerous anomocytic types and present on lower surface (Fig. 6.1- 6.2).

In transverse section, the arrangement of the lamina tissue is dorsiventral. Both the upper and lower epidermis are covered with thin layer of cuticle. Both the upper epidermal cells are made up of parenchymatous and rectangular cells.The mesophyll layer consists of palisade and spongy parenchyma cells. The palisade mesophyll are found below the upper epidermis and make up of one layers and vertically elongated at right angle to the surface. They are tightly packed with one another and contained numerous chloroplasts. The spongy mesophyll consists of 10-11 layers of cells which are irregular to more or less rounded cells in shape and loosely arranged. The vascular bundles of lateral vein are embedded in mesophyll cells(Fig. 6.3).

Midrib

In surface view, the epidermal cells were parenchymatous and compactly arranged and polygonal in shaped (Fig. 7.1).

In transverse section, the midrib is convex in the upper surface andconcave in the lower surface in outline. Both surfaces are covered with thin cuticle. The epidermal cells are one layer, barrel shaped and compactly arranged. The lower epidermal cells are similar in shape and size to the upper epidermal cells. The cortex is made up of collenchymatous and thin wall parenchymatous cells. The collenchymas cells are 5-6 layers in thickness toward the upper surface and 6-7 layers in thickness toward the lower surface. The parenchyma cells are 18-19 layers in thickness above the vascular bundle and 20-21 layers in thickness below the vascular bundle. Every portion of the parenchymatous cells are thin walled, irregular rounded in shaped and inner cellular spaces are numerous among them. The vascular bundle is crescent in shaped and bicollateral types (Fig. 7.2- 7.3).

Petiole

In surface view, the epidermal cells were parenchymatous and compactly arranged and rectangular in shaped (Fig. 8.1).

In transverse section, the petiole is crescent shape in outline. The cuticle layer is thin. The epidermal cells are barrel shaped of parenchymatous cells and compactly arranged on both surfaces.The cortex is made up of two different types of tissues and below the epidermis. The collenchymatous tissues are towards the peripheral regions and thin- walled parenchymatous cells are towards the inner regions. The outer collenchymatous cells below the epidermis consists of 2-3 layers in thickness on the upper sides and 3-4 layers in thickness on the lower sides. The parenchymatous cells consists of 26-27 layers in thickness above the vascular bundle and 20-21 layers of parenchymatous layers in thickness below the vascular bundle.Vascular bundle is crescent shaped present in the central region and bicollateral types. Xylem present between the inner and outer phloem of vascular bundles (Fig. 8.2- 8.3).

Diagnostic Characters of powdered leaves of *Calotropis gigantea* (L.)R.Br, and *Thevetia peruviana* (Pers.) Schum.

Diagnostic characters of powdered leaves of*Calotropis gigantea* (L.) R.Br

The epidermal cells were parenchymatous, thin walled and wavy in surface view. Paracytic types of stomata were present. The lignified vessels were found in the form of pitted. Tracheids, fibers and fiber- tracheids were also found. Solitary and prismatic crystals of the calcium oxalates were present(Fig. 9).

Diagnostic characters of powdered leaves of*Thevetia peruviana* (Pers.) Schum.

The epidermal cells were parenchymatous, thin walled and wavy in surface view. The lignified vessels were found in the form of annular and pitted. Tracheids, fibers and fiber- tracheids were also found. Calcium oxalates were present (Fig. 10).

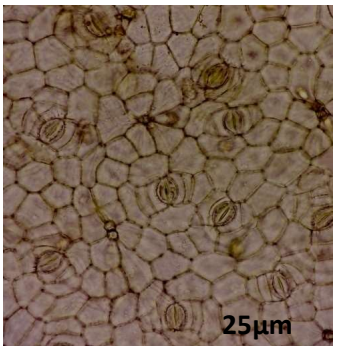

Figure 3.1 Surface view of upper epidermis showing epidermal cells and stomata

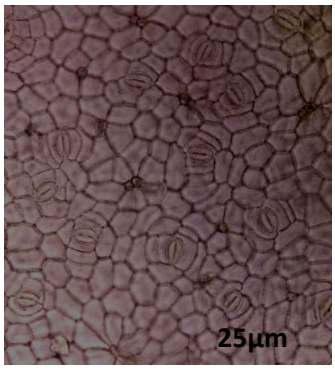

Figure 3.2 Surface view of lower epidermis showing epidermal cells and stomata

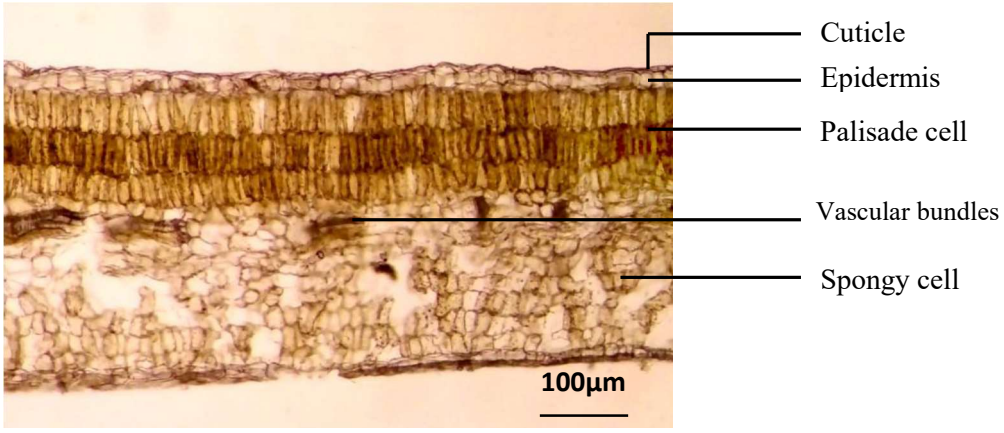

Figure 3.3 T.S of lamina

Figure 3. Microscopical characters of lamina of *Calotropis gigantea* (L.)R.Br.

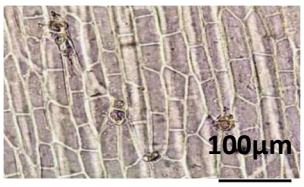

Figure 4.1 Surface view of midrib

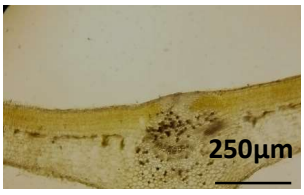

Figure 4.2 T.S of midrib (tip)

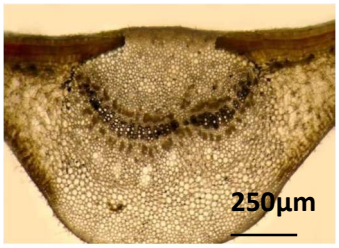

Figure 4.3 T.S of midrib (middle)

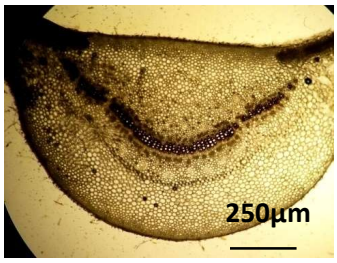

Figure 4.4 T.S of midrib (basal)

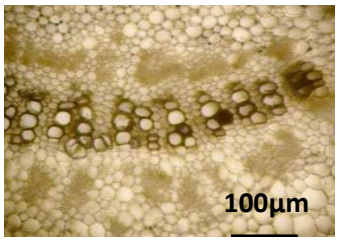

Figure 4.5 Closed up view of vascular bundles

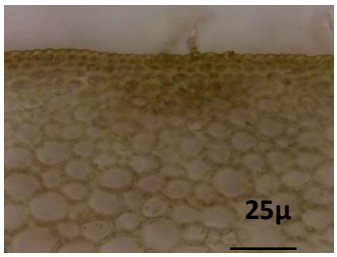

Figure 4.6 Epidermal cells with trichome and cortical region

Figure 4. Microscopical characters of midrib of *Calotropis gigantea* (L.)R.Br.

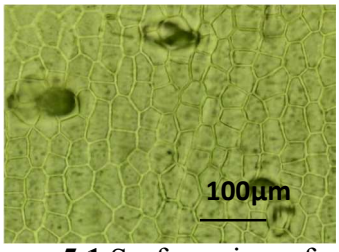

Figure 5.1 Surface view of petiole

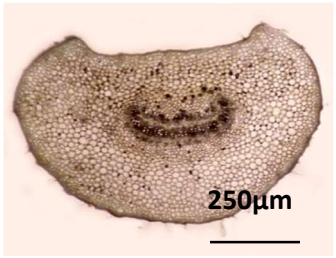

Figure 5.2 T.S of petiole

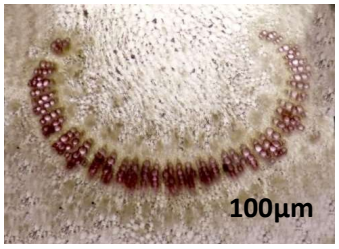

Figure 5.3 Closed up view of vascular bandles

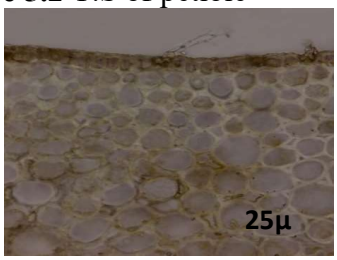

Figure 5.4 Epidermal cells with trichome and cortical region

Figure 5. Microscopical characters of petiole of *Calotropis gigantea* (L.)R.Br.

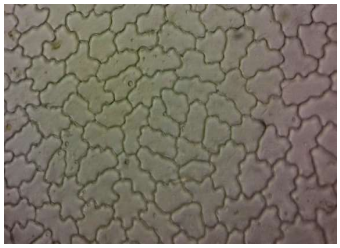

Figure 6.1 Surface view of upper epidermis showing epidermal cells

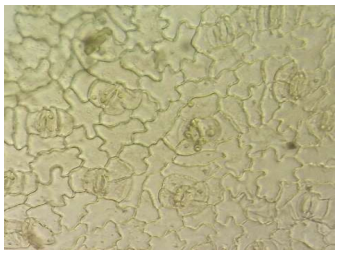

Figure6.2S Surface view of lower showing epidermal cells and stomata

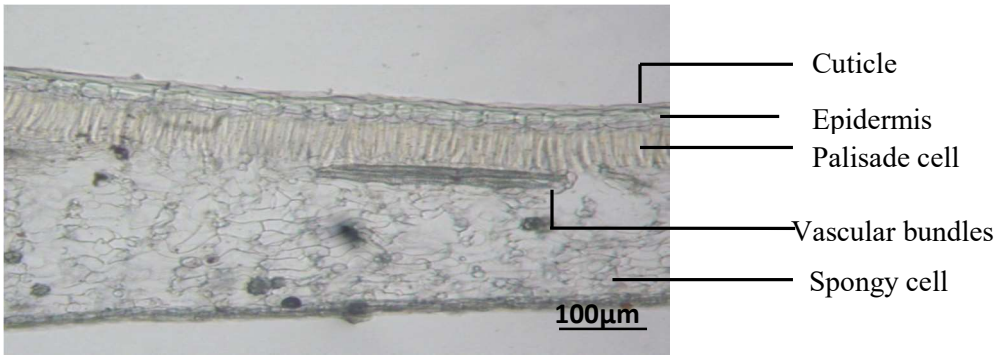

Figure 6.3 T.S of lamina

Figure 6. Microscopical characters of lamina of *Thevetia peruviana* (Pers.) Schum.

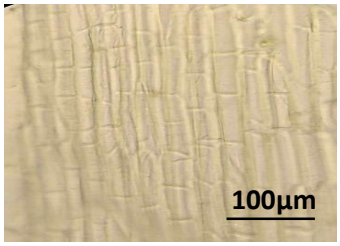

Figure 7.1 Surface view of midrib

Figure 7.2 T.S of midrib (tip)

100µm

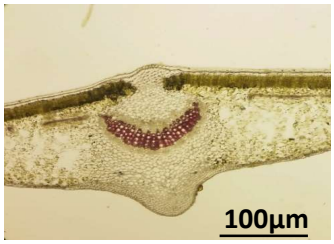

Figure 7.3 T.S of midrib (middle)

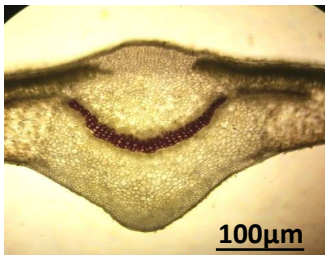

Figure 7.4 T.S of midrib (basal)

Figure 7. Microscopical characters of midrib of *Thevetia peruviana* (Pers.) Schum.

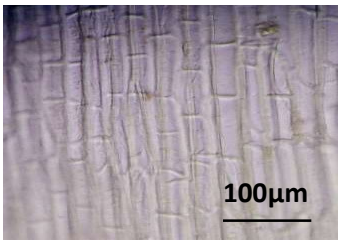

Figure 8.1 Surface view of petiole

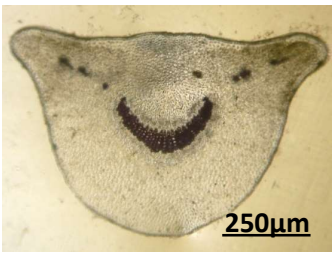

Figure 8.2 T.S of petiole

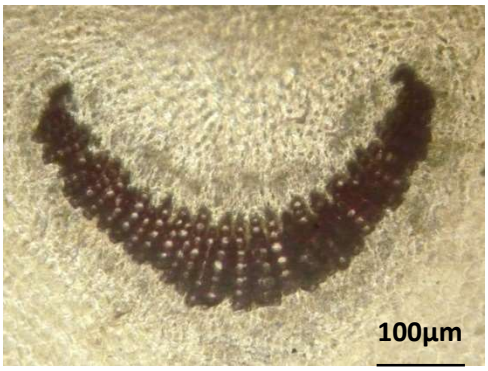

Figure 8.3 Closed up view of vascular bundles

Figure 8. Microscopicl characters of petiole of *Thevetia peruviana* (Pers.) Schum.

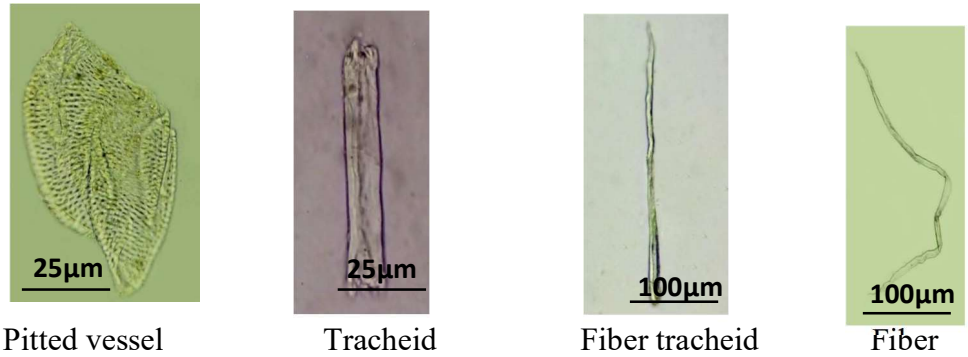

Figure 9. Diagnostic characters of powdered leaves of *Calotropis gigantean*(L.) R.Br.

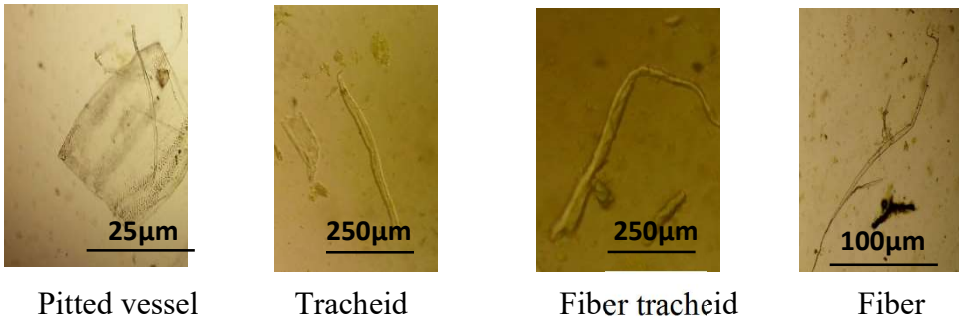

Figure 10. Diagnostic characters of powdered leaves of *Thevetia peruviana* (Pers.) Schum.

Chemical Studies

Preliminary phytochemical test of latex from *Calotropis gigantea* (L.)R.Brand *Thevetia peruviana* (Pers.) Schum.

The preliminary phytochemical test of latex indicated the presence of alkaloid, glycoside, reducing sugars and tannin.

Table (1) Preliminary phytochemical test of water extract of latex from *Calotropis gigantea* (L.) R.Br.and *Thevetia peruviana* (Pers.) Schum.

No.	Chemical constituents	Reagent	Observation	Results	
				Calotropis gigantea (L.) R.Br.	*Thevetia peruviana* (Pers.) Schum.
1.	Alkaloids	Mayer's reagent Dragendroff's reagent	White ppt Orange ppt	+ +	- +
2.	α-amino acid	Ninhydrin reagent	Pale orange ppt	-	-
3.	Carbohydrates	10 %, α-napthol +H_2SO_4(conc:)	Brown ppt	-	-
4.	Phenolic compounds	4 % $FeCl_3$, solution	Green ppt	-	-
5.	Reducing sugars	Benedict's solution	Blue green ppt	+	+
6.	Starch	I_2 solution	Bluish pp	+	-
7.	Steroids/ Terpenoids	H_2SO_4	White ppt	-	-
8.	Flavonoids	Mg/HCl(conc:)	Pale brown ppt	-	-
9.	Glycosides	10 % lead acetate solution	White ppt	+	+
10.	Saponins	Distilled water	Foaming	+	-
11.	Tannins	1% $FeCl_3$ solution	Brown yellow ppt	+	+

(+) present; (-) absent

Discussion and Conclusion

In the present research, taxonomical studies on both vegetative and reproductive parts and the histological studies of *Calotropis gigantea* (L.)R.Br., and*Thevetia peruviana* (Pers.) Schum. had been undertaken.

In the morphological study, the plant of *Calotropis gigantea* (L.) R.Br. was perennial shrubs, milky latex which was present in all parts of plant. The

leaves were simple, opposite and decussate, exstipulate. The inflorescences were cymes. The flowers were peduncled, often many- flowered cymes. The stamens 5, mostly inserted at the base of the corolla, corona-scales 5, inserted in the staminal tube, filament separate or connate in the tube. The ovary 2 carples, style slender. These characters were in agreement with those mentioned by Backer, 1965; Chropa, 1982; Huber, 1983;Kirtika & Basu,1935; Kumar *et al*, 2012. In the histological study, multicellular, uniseriate trichomes were present on both surfaces of the leaves. The stomata were distributed on both surfaces of the leaves and paracytic types. Vascular bundles of midrib and petiole were bicollateral and crescent in shaped. These characters were in agreement with those mentioned by Eusa, 1965 and Metcalfe and Chalk, 1950.

The plant of *Thevetia peruviana* (Pers.) Schum. was erect shrubs or small tree and milky latex present. The leaves are whorl, simple and exstipulate. The inflorescences are cymose and flowers are bisexual. These characters are agreements with those reported by Backer, 1965 and Datta, 1987.The epidermal cells of upper surface are wavy in shaped and lower surface are slightly wavy. The stomata are anomocytic types and occur in the part of lower epidermis. In transverse section, vascular bundles of midrib and petiole are crescent shaped. These characters were agreements with those mentioned by Metcalfe & chalk, 1950 and Eusa, 1965.

In preliminary phytochemical tests of distilled water extracts of the two latex plants indicated that the latex of *Calotropis gigantea* (L.) R. Br. and *Thevetia peruviana* (Pers.) Schum.contained alkaloids, glycosides, reducing sugars and tannins.These characters were agreed with those mentioned bySarkar*et al*., 2013.

In conclusion, the collected samples were verified by using available literature as family Asclepiadaceae and Apocynaceae respectively. The morphological, histological characters and phytochemical studies of latex can give valuable information of *Calotropis gigantea* (L.) R.Br., and of *Thevetia peruviana* (Pers.) Schum. Therefore, some activities of these two plants for physicochemical test and antimicrobial test should be needed to investigate for the future research work.

Acknowledgements

I would like to express my sincere gratitude to Professor Dr Myat Myat Moe, Head of the Botany Department, Dagon University, for providing all departmental facilities and valuable suggestions. I am grateful to Dr Khin Lat Lat Mon, Professor, Botany Department,

Dagon University for providing all kindly necessary instructions. I want to indebted their sincere thanks to Myanmar Academy of Arts and Science for their allowing submitting it this article.

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