INVESTIGATION OF MORPHOLOGICAL, HISTOLOGICAL CHARACTERS AND QUALITATIVE ANALYSIS OF *ROTHECA SERRATA* (L.) STEANE & MABB.

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Abstract

Rotheca serrata (L.) Steane & Mabb. (Family Lamiaceae) is an important medicinal plant growing in the tropical and warm temperate regions. This plant is locally known as Yin-bya in Myanmar. It was collected from Hpa-an University Campus, Hpa-an Township, Kayin State from March to September 2019. The flowering and fruiting period is from May to September. In morphological study, the plant is perennial shrub, stem bluntly quadrangular. Leaves opposite and decussate, sharply serrate margin. Flower numerous, showy, dichotomous cymes. Fruits black in mature, seeds pyrene. In histological characters, diacytic stomata, uniseriate glandular and non glandular trichomes were present. Calcium oxalate crystals were present in mesophyll tissue of lamina and acicular crystals in petiole and stem. The vascular bundles were collateral and close type in the midrib, collateral and open type in petiole and stem. The present of alkaloid, flavonoid, phenolic compound, starch, reducing sugar, glycoside, saponin, tannins, α-amino acid and carbohydrates were found in phytochemical investigation. In physicochemical properties, the solubility of powdered leaves was found to be most soluble in polar solvent.

Keywords: *Rotheca serrata* (L.) Steane & Mabb., morphological, histological characters, phytochemical and physicochemical analysis.

Introduction

According to World Health Organization (WHO), about 80% of individuals from developed countries use traditional medicine. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Baker *et. al.,* 1995 and Reddy *et. al.,* 2001).

Lamiaceae are comparatively a large family composed of about 200 genera and 3300 species (Trease and Evans, 2002). The largest genera are *Rotheca* (400) (Cronquist, 1981).

The genus *Rotheca* (L.) Steane & Mabb. [Family Lamiaceae (Verbenaceae)] is very widely distributed in tropical and subtropical regions of the world. Estimates of number of species *in Rotheca* vary widely, about 450 (Rahman *et.al.*, 2007).

Rotheca serrata (L.) Steane & Mabb. is a genus of flowering plants in the Verbenaceae family. The plant *Rotheca serrata* (L.) Steane & Mabb. is commonly known as Yin-bya-net in Myanmar and Blue glory in English.

According to traditional uses the roots and leaf extracts of *Rotheca serrata* (L.) Steane & Mabb. has been used for the treatment of rheumatism, asthma and other inflammatory diseases (Hazekamp *et. al.,* 2001). The roots of the plant have been claimed to be used in dyspepsia, seeds in dropsy and leaves as a febrifuge and in cephalalgia and ophthalmia (Anonymous, 1992). Aqueous extracts of leaves of *Rotheca serrata* (L.) Steane & Mabb. possess bronchodilator property (Kirtikar and Basu, 1935 and Steane *et. al.,* 1999).

The aims of the present study are to identify and confirm the morphological characters, to investigate the phytochemical constituents and to determine the physicochemical properties of this plant.

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Materials and Methods

Collection and identification of *Rotheca serrata* (L.) Steane & Mabb.

The specimens used in this research were collected from the surrounding area of Hpa-an University Campus, Hpa-an Township, Kayin State during flowering period from May to September in 2019. The vegetative and reproductive parts of the fresh specimens were identified by using available literatures such as Hooker, 1885; Kirtikar and Basu, 1935; Backer and Brink, 1965; Cronquist, 1981; Dassanayake, 1983; Hundley and Chit Ko Ko, 1983; Hu Qi-ming and Wu De-Lin, 2009)

Histological study of *Rotheca serrata* (L.) Steane & Mabb.

Free hand sections of fresh specimen from lamina, midrib, petiole, stem and root were made and studied under microscope. Chloral-hydrate solution B.P as clearing reagent, solution of phloroglucinol B.P followed by with concentrated hydrochloric acid for lignin, acetic acid and 80% sulphuric acid B.P for calcium oxalate crystals, iodine solution B.P for starch, sudan III and IV for oil and ethanol for mucilage were used to examine for free hand sections and the powdered samples.

Preliminary phytochemical test on leaves of *Rotheca serrata* (L.) Steane & Mabb.

In this qualitative analysis, the air dried powdered of the leaves were tested at Department of Botany, Hpa-an University by the method of Vogel, 1966; British Pharmacopoeia, 1968; Marini Bettolo *et. al*., 1981; Robinson, 1983; Central Council for Research in Unani Medicine, 1987; Harborne, 1993; Trease and Evans, 2002.

Physicochemical properties on powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

The physicochemical characters such as moisture content, extractive values for the various solvents were determined according to British Pharmacopoeia, 1968 and WHO, 1998, at Department of Botany, Hpa-an University.

Results

Morphological characters of *Rotheca serrata* (L.) Steane & Mabb.

Habit; perennial shrub, scarcely woody, not much branched. Stem; bluntly quadrangular. Leaves; simple, opposite and decussate, apically clustered and variable in size, lamina oblong or elliptic about, acute, coarsely and sharply serrate margin, glabrous, base acute, petiolate, about. Inflorescence; terminal or axillary, green, quadrangular, in lax pubescent, dichotomous cymes, with a pair of acute bracts at each branching and a flower in the fork, each in the axil of a large leafy bract and collectively forming a long lax terminal usually pyramidal erect panicle about. Flower; showy, pale blue, bracteate, bracteolate, pedicellate, glabrous, complete, bisexual, irregular, zygomorphic, pentamerous, cyclic, hypogynous. Calyx; 5, synsepalous, puberulous, cup-shaped, connate at the base, enlarge at the middle, apex deeply five-lobe, lobe elliptic, inferior. Corolla; (1+4), synpetalous, the lower larger lobed, bluish purple, ovate to lanceolate, corolla tube slender, hairy within the tube, inferior. Stamen; 4, epipetalous, filament much more curved, densely hair at the base, didynamous, white, anther dark purple, dithecous, dorsifixed, longitudinal dehiscence, inferior. Ovary; 2, syncarpous, globose, false septum, each with two ovules, axile placentation. Style; filiform, white colour, glabrous, stigma bifid, purple, disc present, superior. Fruits; broadly obovoid, succulent, dark green in immature, black in matures, glabrous. Seeds; normally four lobed with one pyrene in each lobe, dark purple.

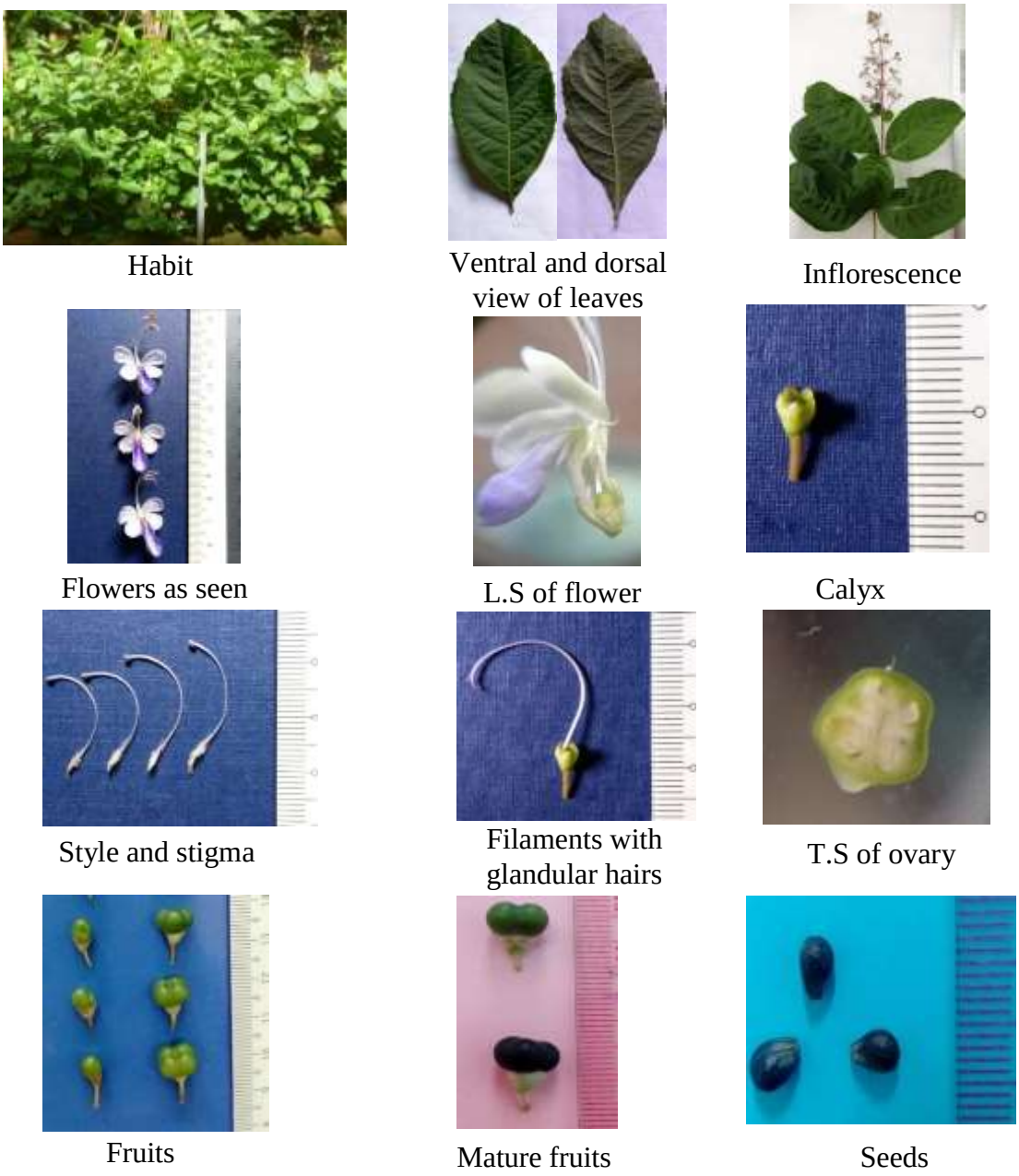


Figure 1 Morphological characters of *Rotheca serrata* (L.) Steane & Mabb.

Histological characters of *Rotheca serrata* (L.) Steane & Mabb.

Lamina

In surface view, stomata were present on both surfaces but abundant on the lower surfaces. They were diacytic type, elliptic shape in outline.

In transverse section, both the upper and lower epidermis is one layer thick and covered with thin cuticle. The epidermal cells are parenchymatous, barrel shaped and compactly arranged. In mesophyll cells are composed of palisade and spongy mesophyll cells. The palisade mesophyll cells were made up of 2 to 3 layers of vertically elongated cylindrical cells which are closely packed with one another. The spongy mesophyll layers are composed of 4 to 5 layers of parenchymatous

cells, which are irregular to oval in shaped and loosely arranged. Abundant calcium oxalate crystals are found in this region. The vascular bundles were embedded in mesophylls cells.

Midrib

In surface view, the epidermal cells are thin-walled parenchymatous and rectangular to polygonal in shape, elongated along the length of the midrib. Anticlinal walls are straight.

In transverse section of midrib, the apical portion was convex on both surfaces; the epidermis was covered with smooth cuticle. The upper epidermal cells were barrel shaped and lower epidermal cells were oval shaped, compactly arranged. The cortex was made up of two different types of cells, lamellar collenchymatous cells and thin walled parenchymatous cells. The lamellar collenchymatous cells are 3 to 4 layers' thickness towards the upper surfaces and 1 to 2 layers in thickness towards the lower surfaces. The parenchyma cells were absent towards the upper surfaces and 2 to 3 layers in thickness toward the lower surfaces. The vascular bundles are in the form of ring and collateral type. In apical regions of the leaf only one vascular bundle is left surrounded by prominent bundle sheath.

Petiole

In surface view, the epidermal cells are thin walled and rectangular to polygonal in shape with straight wall.

In transverse section, the petiole was slightly convex on the upper side and prominently rounded on the lower side. The cuticle layer was thin. The epidermal cells were barrel shaped and compactly arranged. The cortex was made up of two different types of tissue. The lamellar collenchymatous cells 3 to 4 layers and parenchymatous cells 4 to 5 layers towards the upper region and 6 to 8 layers towards the lower region and rounded to polygonal, thin walled parenchymatous cells 6 to 15 layers in thickness at the center. Acicular crystals (raphides and styloid) were scattered in the parenchymatous cells. The vascular bundles were circular in arrangement, bundles were collateral and open type.

Stem

In surface view, the epidermal cells were rectangular to polygonal-shape parenchymatous cells, thin walled, compactly arranged, anticlinal walls straight. Glandular and non-glandular uniseriate trichomes were present.

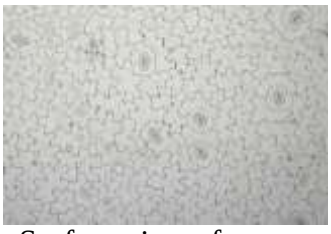
In transverse section, the young stem was quadrangular in outline. The cuticle layer was thin. The epidermal cells were thin walled, barrel shaped parenchymatous cells and one layer thick. The cortex region was made up of collenchymatous tissue and parenchymatous tissue. The collenchymatous tissues were lamellar types and consisted of 3 to 5 layers in thickness below the epidermis. The parenchymatous tissue consists of 4 to 5 layers, thin-walled, isodiametric to rounded in shape, beneath the collenchymatous tissues and the vascular bundles were surrounded the pith region, which composed of thin-walled parenchymatous cells. The vascular bundles were collateral and open type.

Root

In surface view, the epidermal cells were thin walled, rectangular in shape and compactly arranged

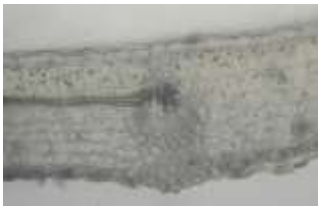
In transverse section, the roots were circular in outline. The epidermal cells were disorganized and displaced by periderm which consists of phloem or cork, the phellogen or cork cambium and phelloderm or secondary cortex. Phellem or cork cells consist of 1-2 layered,

irregular to round in shape. Phellogen or cork cambium was 2-3 layered and polygonal to round in shaped. Phelloderm or secondary cortex made up of 6-10 layered thickened, the parenchymatous cells oval to rounded, secretory ducts between them. Cortex, endodermis, pericycles and vascular bundle were clearly differentiated. Endodermis was a single layered, thin-walled parenchymatous cells and barrel shaped. Pericycle was lying internal to the endodermis thin-walled parenchymatous cells and barrel shaped. The vascular bundles were arranged in concentric ring. Xylem towards the inner and phloem outside the xylem. The xylem is endarch.



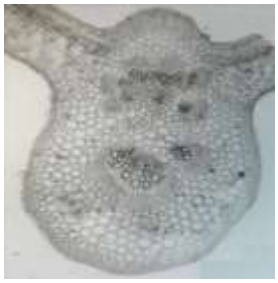
Surface view of upper epidermis (X 200)

Surface view of lower epidermis (X 400)



T.S of lamina (X 100)

Surface view of midrib (X 400)



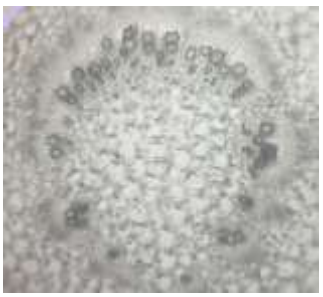
T.S of midrib (X 40)

Closed up view of cortical layer and vascular bundle of midrib(X 200)

Surface view of petiole (X 400)

T.S of petiole (X 40)

Closed up view of cortical layer of prtiole (X 400)



Closed up view of vascular bundle and crystals of petiole (X 200)

Surface view of stem (X 400)

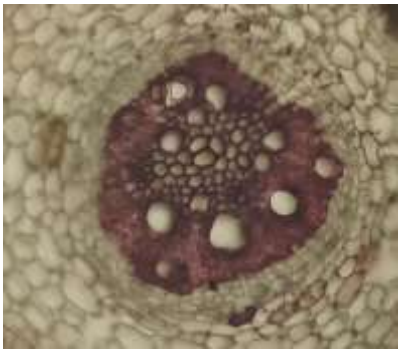
T.S of stem (X 40)

Closed up view of cortex layer, vascular bundle and crystals of stem (X 200)

Surface view of root (X 400)

T.S of the root (X 40)

Closed up view of cortex region and vascular bundle (X 200)



Closed up view of vascular bundle (X 200)

Figure 2 Microscopical characters of *Rotheca serrata* (L.) Steane & Mabb.

Preliminary phytochemical test of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

The phytochemical tests of powdered leaves of *Clerodendrum serratum* (L.) Moon indicated that the presence of alkaloid, flavonoids, phenolic compound, starch, reducing sugar, glycoside, saponins, tannins, α-amino acid and carbohydrates. The results were shown in Table (1).

Table 1 Phytochemical test of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

No.	Test	Extracts	Test reagents	Observation	Results
1.	Alkaloid	1% HCL	Dragendorff's Mayer's Wagner's Hager's	Orange ppt White ppt Brown solution Yellow ppt	+ + + +
2.	Flavonoid	EtOH	HCL / Mg	Pink	+
3.	Phenolic compound	H_2O	5% $FeCl_3$ solution	Brownish ppt	+
4.	Starch	H_2O	10% Iodine solution	Bluish black	+
5.	Reducing sugar	H_2O	Benedict solution	Orangr red	+
6.	Glycoside	H_2O	10% Lead acetate	White ppt	+
7.	Saponin	H_2O	Distilled water	Frothing	+
8.	Tannins	H_2O	5% $FeCl_3$	Brownish black ppt	+
9.	α-amino acid	H_2O	Ninhydrin reagent	Purple	+
10.	Carbohydrates	H_2O	10% α-napthol and concentrated H_2SO_4	Red ring	+

Present = (+)

Physicochemical properties of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

In physicochemical properties, the moisture content was usually determined by drying to constant weight and taking the loss in weight as moisture. The solubility of powdered leave was investigated to determine the amount of total solids soluble in various solvents. The solubility of powdered leaves was found to be mostly soluble in distilled water, moderately soluble in ethanol soluble and methanol soluble, least soluble in petroleum ether. The results were shown in Table (2).

Table 2 Physicochemical properties of powdered leaves of *Rotheca serrata* (L.) Steane & Mabb.

No.	Physicochemical properties	Content (%)
1	Moisture content	16.6
2	Total ash	14.6
3	Distilled water soluble content	6.67
4	Acetone soluble content	3.3
5	Chloroform soluble content	3.3
6	Ethanol soluble content	5.0
7	Ethyl-acetate soluble content	2.67
8	Methanol soluble content	5.3
9	Petroleum ether soluble content	2.0

Discussion

In this research, the morphological studies on vegetative and reproductive parts, histological characters as well as phytochemical, physicochemical properties the leaves have been studied and described.

The plant of *Rotheca serrata* (L.) Steane & Mabb. are perennial shrubs, bluntly quadrangular. The leaves are simple, opposite and decussate, apically clustered, sharply serrate margins and glabrous. The inflorescences are both axillary and terminal cymes, solitary flower, in lax pubescent, dichotomous cymes with a pair of acute bracts at each branching **(**Kirtikar and Basu, 1935; Backer and Brink, 1965).

The flowers of these plants are pale blue, showy, bisexual, irregular, zygomorphic and hypogynous (Backer and Brink, 1965; Dassanayake, 1983; Hu Qi-ming and WU De-Lin, 2009).

The calyx is (5), synsepalous. The corolla consists of (1+4) petals, synpetalous, the lower larger lobed, bluish purple, hairy within the tube, inferior. The ovary is superior, each with two ovules, axile placentation, disc present. The fruits are broadly obovoid, succulent, dark green in immature, black in matures, glabrous. The seeds are dark purple. These characters are in agreement with those reported by (Hooker, 1885; Kirtikar and Basu, 1935; Backer and Brink, 1965; Dassanayake, 1983; Hu Qi-ming and WU De-Lin, 2009).

In histological studies, the surface views of upper and lower epidermis of the leaves have the epidermal cells with wavy anticlinal walls. The stomata are distributed on both surfaces of the leave and diacytic type. Calcium oxalate crystals are found in mesophyll cells. The vascular bundles were embedded in mesophylls cells.

In transverse section of petiole, the epidermal cells were barrel shaped. The cortex was made up of lamellar collenchymatous and polygonal thin walled parenchymatous cells. Acicular crystals (raphides and styloid) were scattered in the parenchymatous cells. The vascular bundles were collateral and open type. Patches of phloem fibers surrounded the collateral vascular bundles.

In transverse section of stem, the young stem was quadrangular in outline. The vascular bundles were collateral and open type. Acicular crystals were found in the parenchymatous cells.

In transverse section of roots were circular in outline. The epidermal cells were disorganized and displaced by periderm. The vascular bundles were arranged in concentric ring. The xylem is endarch. The histological characters of leaves; petiole, stem and root are in agreement with Esau, 1965; Pandey, 1978; Metcalfe and Chalk, 1979 and 1989; Trease and Evans, 2002.

According to the result of phytochemical studies, chemical constituents such as alkaloid, flavonoid, phenolic compound, starch, reducing sugar, glycoside, saponin, tannins, α-amino acid and carbohydrates were isolated from the leaves of *Rotheca serrata* (L.) Steane & Mabb. These researches were agreed with those reported by Prasad, 2012. The main properties of flavonoid include antioxidant activity (Belinha *et al*., 2007).

In physicochemical studies, showed that the powdered leaves were mostly soluble in polar solvent. These solubility properties are considered for the preparation of crude drugs in British pharmacopoeia (1968).

Conclusion

Rotheca serrata (L.) Steane & Mabb. has played an important role in Indian system of medicine. In addition to the common local use in respiratory diseases, other ethnomedicinal uses include treatment of pain, inflammation, rheumatism and fever especially malarial fever.

Therefore, the present research deals to provide a comprehensive overview of the traditional and ethnomedicinal uses, phytochemistry of *Rotheca serrata* (L.) Steane & Mabb. So, this plant should be investigation of effective pharmacological research in the coming future.

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