Investigation of Phytochemical and Antimicrobial Activities on the leaves of *Clerodendrum serratum* Linn. (Yin – bya – net)

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Abstract

Clerodendrum serratum Linn. (Yin – bya – net), belonging to the family Lamiaceae was selected as the plant materials because this plant is used as rheumatism, asthma, dyspepsia, febrifuge, cephalagia, bronchidilator, ophthalmia, menospause and metritis in Myanmar traditional medicine. This plant was collected from Kanni village, which is located in the Bago Township of Bago Region. In this paper, investigation of the morphological, anatomical, phytochemical and antimicrobial activities were conducted. Morphological characters of vegetative and reproductive parts were investigated to ascertain their correct identification. The fresh specimen of leaves were used to study the anatomical and the powdered of dried specimens were also used to preliminary phytochemical test and antimicrobial activities. The watery, ethanol, methanol, acetone,ethyl acetate, chloroform, and pet-ether extracts of leaf was tested with six different types of microorganisms by using paper disc diffusion methods. According to these results, leaf extracts of *Clerodendrum serratum* Linn. showed antimicrobial activities on *Bacillus subtilis.* Among them, ethyl acetate extract showed the highest inhibitory zone of 19.78 mm on *Bacillus subtilis*.

Introduction

Most of the drugs derived from plants were developed because of their use in traditional medicine. The Lamiaceae is closely related to the family Verbenaceae. Several recent, phylogenetic studies have shown that some genera classified in Verbenaceae belong instead in Lamiaceae for example, *Vitex* and *Clerodendrum* (www. plantzafrica.com.). According to Hundley and Chit KoKo (1987), the Verbenaceae family has 21 genera and 100 species and the Lamiaceae family has 41 genera and 133 species were attributed to Myanmar.

Anatomical characters of Lamiaceae family were described the present of diacytic type stomata on both surfaces of leaf. The collateral vascular bundle was located in the midrib and petiole (Metcalfe and Chalk, 1950).The major chemical constituents as present in the *Clerodendrum serratum* Linn. Genus contain carbohydrate, phenolic, flavonoid, terpenoid and steroid (Shrivastava N. *et al*., 2007).Traditionally, *Clerodendrum serratum* Linn has been used as rheumatic, anti-oxidant and antibacterial and antifungal. And also used in various indigenous system of medicines such as syphilis, typhoid, cancer, jaundice and hypertension. Besides, the antimicrobial values of this herbal plant have also been reported in its stems and leaves (Journal of Applied Pharmaceutical Science, 2012).The aims of the present

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study are to investigate the morphological, anatomical, phytochemical constituents and antimicrobial activity of *Clerodendrum serratum* Linn. The objectives are to identify the vegetative and reproductive parts of the plant and examine the anatomical characters and the preliminary phytochemical tests for the evaluation of major constituents present in this plant.

Materials and Methods

Botanical studies

The specimens of *Clerodendrum serratum* Linn used in this study were collected from Bago Township in Bago Region during flowering and fruiting periods extending from September to December. Fresh specimens of the vegetative and floral parts were used for identification in the Department of Botany, Taungoo University with the help of reference literature (Hooker, 1885; Kirtikar and Basu, 1935; Rendle, 1967).The samples were washed with water to remove impurities. After washing and cleaning, the sample was air dried and ground to get powdered and stored in air tight container. Anatomical characters of leaves and powdered samples were presented by using available literature (Wallis, 1967; Trease and Evans, 2002; Pandey, 1996).

Chemical Studies

Preliminary phytochemical investigation of the leaves of Yin-bya-net has been conducted with tested reagents in Botany Department of Taungoo University according to the methods of British Pharmacopoeia, 1965; Harbone 1973; Marini-Bettelo, 1981; Trease& Evans, 2002.About 30g of the leaf powdered of Yin-bya-net were extracted by using 100ml of watery, ethanol, methanol and pet-ether (60-80C^{o}). These extracts were screened to know the presence or absence of alkaloids, glycosides, reducing sugars, saponin, phenolic compounds, flavonoids, steroids and terpenoids, carbohydrates, α amino acids, starch, and tannins.

Microbiological studies

Extraction and testing of antimicrobial activity

Preparation for leaf extracts

Three grams of powdered samples (leaves) were dissolved in 30ml of watery, ethanol, methanol, acetone, ethylacetate, chloroform, and pet-ether for ten days respectively. Then the extracts were filtered. The filtrates were concentrated on water bath. The dried extracts were dissolved in 2-3 drops of each solvent to get the required concentration before testing. Preparations of test organisms were obtained from Department of Botany, University of Dagon. These test organisms were sub – culture inoculated in 5ml of nutrient broth and incubated for 5 hours of bacteria and 24 hours of fungi at room temperature.

Antimicrobial activity of leaf extracts by paper disc diffusion method

The paper discs were applied and 20µl extracts were placed on petridishes and dry at room temperature. Forty ml of sterilized nutrient agar cooled at 50Ċ and mixed to dry 10µl test organisms and then shaked and poured onto each petridish and allowed to solidity for 15 minutes. Paper discs with 20µl extract were gently placed on nutrient agar medium. Then the plates were incubated for 24-48 hours at room temperature. The size of paper disc having six millimeters in diameter was utilized for antimicrobial test. The clear zone (inhibitory zones) surrounding the paper disc were measured. (Cruickshank, 1975).

Results

Morphological characters of *Clerodendrum serratum* Linn.

Perennial erect shrubs, above 3-6 ft high, aromatic, stems and bracnches stout, quadrangular, with longitudinal groove; roots brown in colour. Leaves simple, opposite and decussate, petiolate, petiole very short, stout, about 0.2 – 0.5 cm long, flattened above and rounded below, exstipulate; lamina oblong or elliptic, about 7-22 cm long and 3-5 cm wide, acute at the base, the margin serrate with acute teeth, the tips acuminate. Inflorescences terminal dichasial cymes of many-flowered, 13-23 cm long and 4-8cm wide, erect or nutant. Flowers purplish blue, about 2.5 cm and 1.0 cm wide, bracteate, bracteolate, pedicellate, about 0.2-0.3 cm long, complete, bisexual, irregular, zygomorphic, penta-merous, cyclic, hypogynous, sepals (5), synsepalous, the tube campanulate, short, the lobes ovoid, valvate, pinkish, persistent, inferior; petals (5), synpetalous, bilabiate, valvate, petaloid (purplish blue), inferior; stamens 4, apostamenous, alternate to the petals, epipetalous, didynamous, filament unequal, the anther dithecous, introrse, dorsifixed, longitudinal dehiscence, inferior; carpels (2), bicarpellary, syncarpous, tetralocular (due to the formation of false septum), axile placentation, one ovule in each locule in T.S ; the style long and curved, about 2-5 cm long, stigma bi-fid , disc- absent, ovary superior. Fruits drupe, 2-4 lobed. Figure.1

(A)Plants in natural habit | (B) Dorsal View of leaves | (C) Ventral View of leaves | (D) Inflorescence | (E) Close up view of Flowers | (F)T.S of ovary

Figure 1. Morphological Characters of *Clerodendrum serratum* Linn.

Anatomical Characters of leaves of *Clerodendrum serratum* Linn.

Lamina

In surface view, the epidermal cells of both surfaces were parenchymatous cells, compact, thin wall, irregular in shape with wavy anticlinal wall. Stomata were present on both surfaces but more abundant on the lower surface. They are oval or oblong in shape, diacytic type with reniform shaped guard cells. Chloroplasts are abundant in the guard cells. Trichomes were not observed on both surfaces. In transverse section, the cuticle is thin on both surfaces. Both upper and lower epidermal cells are wavy anticlinal wall, barrel shaped and compactly arranged. Mesophyll cell consisted of palisade and spongy parenchyma. Palisade cells are found below the upper epidermis, only one layer compact, vertically elongated thin walled. The spongy mesophyll cells lie above lower epidermis and are oval or irregular in shape, 6-8 layer parenchymatous cells with a few numbers of intercellular spaces. Vascular bundles of lateral vein embedded in the mesophyll tissue. The arrangement is collateral and close type. Each bundle is surrounded by a compact layer of thin walled parenchymatous sheath, distinct from the neighbouring cells. Xylem is composed of annular and spirally thickened vessels, tracheids, fibres and parenchyma. Phloem composed of sieve tube, companion cells and phloem parenchyma.

Midrib

In surface view, both upper and lower epidermis thin walled parenchymatous cells, rectangular shaped, compactly arranged, anticlinal wall straight. Trichomes were present.In transverse section, both lower and upper surfaces were convex and covered with thick cuticle. Both epidermal cells 1- layered, rounded shaped. The lower epidermal cells were similar in shaped and size to the upper epidermal cells. The cuticle layers of both upper and lower surfaces were the same.The cortex was made up of thick walled collenchyma and thin walled parenchyma cells. Below the upper epidermis, collenchymatous cells 4-6 layered and above the lower epidermis, 5-8 layered, oval or polygonal in shaped. Above the vascular bundle, parenchymatous cells 4-8 layered and below 5-8 layered, rounded to isodiametric in shaped. The vascular bundle was rounded shaped in outline, collateral and close type. Phloem cells were thin-walled and mainly composed of sieve tubes, companion cells and phloem parenchyma. Xylem cells were rounded to polygonal in shape and consist of vessels, tracheids, fibre and xylem parenchyma.

Petiole

In transverse section, the petiole was crescentic strand with incurved ends in outline, slightly convex in upper and deeply convex in lower. The epidermal cells were parenchymatous, rounded to oval in shaped, compactly arranged and 1-layer thick. The cortical cell was made up of two different type of tissue, collenchymatous and parenchymatous tissues. Below the upper epidermis collenchymatous cells 5-7 layered and above the lower epidermis 5-6 layered oval or polygonal in shape. Above the vascular bundle, parenchymatous cells 5-8 layered and below 6-9 layered, rounded to isodiametric in shape. Vascular bundle was rounded or oval in shaped, the bundles were collateral, close type. Phloem lies towards the outside and composed of

sieve tubes, companion cells and phloem parenchyma. Xylem lies towards the inside and composed of vessel, tracheids, fibre and xylem parenchyma.Figure. 2

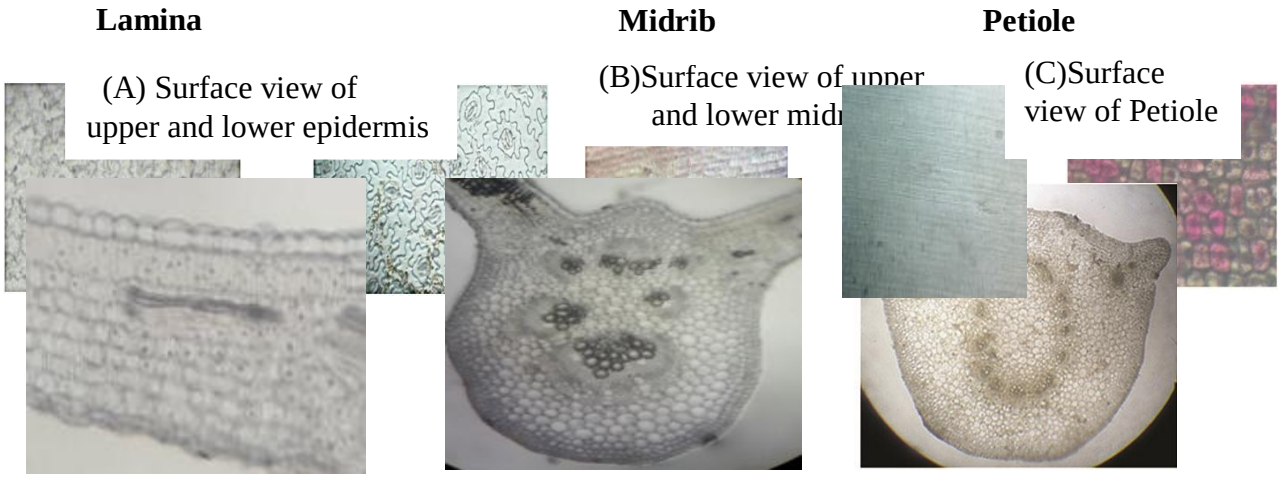


Figure 2. Anatomical Characters of leaf of *Clerodendrum serratum* Linn

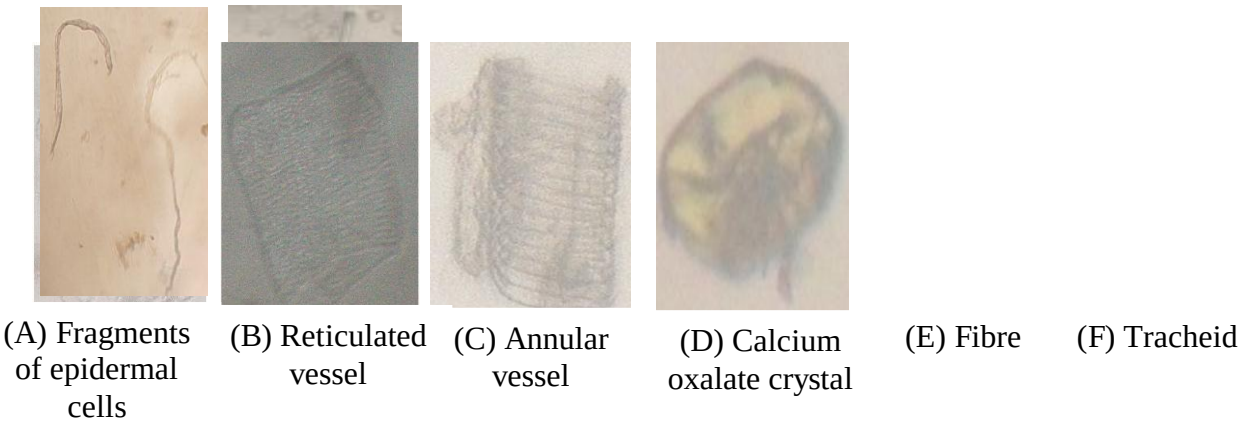


Figure.3 Microscopical Characters of Leaf Powdered of *Clerodendrum serratum* Linn

Table 1. Preliminary phytochemical investigation of the leaves of *Clerodendrum*

***serratum* Linn.**

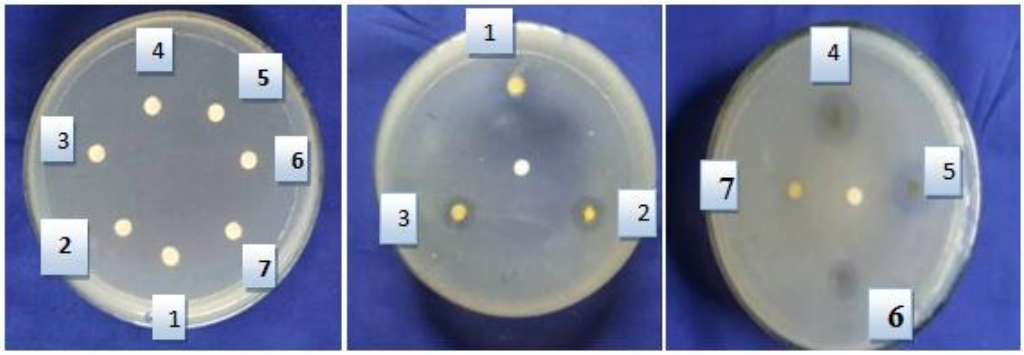
No	Chemical constituents	Reagent	Extract	Observation	Result
1.	Alkaloid	1.Dragendorff's Reagent	DW EtOH MeOH Pet-ether	Reddish ppt. Reddish ppt. Reddish ppt. No ppt.	+ + + _
		2.Mayer's Reagent	DW EtOH MeOH Pet-ether	White ppt. White ppt. White ppt. No ppt.	+ + + _
2.	Glycoside	10% lead acetate	DW EtOH MeOH Pet-ether	White ppt. White ppt. White ppt. No ppt.	+ + + _
3.	Reducing sugars	Benedict	DW EtOH MeOH Pet-ether	Reddish ppt. No ppt. No ppt. No ppt.	+ _ _ _
4.	Saponin	Distilled water	DW EtOH MeOH Pet-ether	Frothing No frothing No frothing No frothing	+ _ _ _
5.	Phenolic Compound	$FeCl_3$	DW EtOH MeOH Pet-ether	Deep blue color Deep blue color Deep blue color No color change	+ + + _
6.	Flavonoid Compound	Mg ribbon and Conc.:HCl	DW EtOH MeOH Pet-ether	Pink color Pink color Pink color No color change	+ + + _
7.	Steroid Terpcroid	Conc. H_2SO_4 acid	DW EtOH MeOH Pet-ether	No color change Reddish brown color Reddish brown color Pale blue color	_ + + +
8.	Carbohydrate	10% of α-naphthol, Conc.- H_2SO_4	DW EtOH MeOH Pet-ether	Red ring No ring No ring No ring	+ _ _ _
9,	Amino acid	Ninhydrin reagent on filter paper	DW EtOH MeOH Pet-ether	Purplish violet Purplish violet Purplish violet No color change	+ + + _
10.	Starch	Iodine	DW EtOH MeOH Pet-ether	Blue color No blue color No blue color No blue color	+ _ _ _
11.	Tannin	$FeCl_3$	DW EtOH MeOH Pet-ether	Blue green Blue green Blue green No ppts	+ + + _

Key to table, (+) = Present (-) = Absent (ppt.) = precipitate

Microbiological studies

Antimicrobial activities of leaves extracts of *Clerodendrum serratum* Linn

In this experiment, six different types of test microorganisms, *Aspergillus nigra, Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Xanthomonas oryzae*. It show that their effect against *Bacillus subtilis*. Ethyl acetate extract showed the highest inhibitory zone of 19.78mm and ethanol extracts showed the lowest inhibitory zone of 10.17mm on *Bacillus subtilis*.



Control *Bacillus subtilis* *Bacillus subtilis*

Paper disc-6mm

Table2. Antimicrobial activity of Leaves extracts of *Clerodendrum serratum* Linn.

Solvents	Microorganisms					
	Aspergillus nigra	***Bacillus subtilis***	***Candida albicans***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Xanthomonasoryzae***
1.Warery	-	17.42	-	-	-	-
2. Ethanol	-	10.16	-	-	-	-
3. Methanol	-	10.17	-	-	-	-
4. Acetone	-	13.32	-	-	-	-
5. Ethyl acetate	-	19.78	-	-	-	-
6. Chloroform	-	13.29	-	-	-	-
7. Pet-ether	-	-	-	-	-	-

Discussion and Conclusion

The plant *Clerodendrum serratum* Linn involved in this research were collected from Bago Township in Bago Region during the flowering and fruiting period from September to December. In this investigation, the morphological, anatomical, phytochemical and antimicrobial activities of leaves have been undertaken. This plant belongs to the family Lamiaceae. It is shrubs or large shrubs usually aromatic, stem quadrangular. Leaves simple, opposite and decussate, without stipules. Inflorescences are dichasial cymes. Flowers are usually pentamerous, bisexual, zygomorphic. The corolla is bilabiate and the stamens didynamous. The carpel (2), bicarpellary, syncarpous, tetralocular, one ovule in each locule. Fruit a drupe or indehiscent capsule these characters were in agreement with those describe by Hooker, (1885), Kirtikar and Basu, (1933); Pandey, (2004); Hong Kong, (2009).

In anatomical study, the leaves were dorsiventral stomata commonly diacytic type. Stomata were present on both surfaces and but more abundant on lower surface. The mesophyll cell includes 1 layer palisade cells and spongy tissue layers with intercellular spaces. The collateral vascular bundle was located in the midrib and petiole. Petiole in transverse section, the petiole was crescentic strand with incurved end in outline and 2-3 small vascular bundle at each corners of petiole. The anatomical features of leaves are in agreement with those (Metcalfe and Chalk, 1960, Pandey, 1996 and Wallis, 1955).

The plant has an important place in the folkloric ailments such as asthma, body ache, bronchitis, cholera, dropsy, eye diseases, fever, inflammation, malaria, rheumatism, snakebite, tuberculosis, ulcers and wounds. The leaves are also useful as an external application for cephalgia and ophthalmic (Indo American journal of Pharmaceutical Research, 2013).

The phytochemical tests were determined on the aqueous, ethanol, methanol and pet-ether extracts of leaves of *Clerodendrum serratum* Linn. Alkaloid, glycoside, phenolic compound, flavonoid, α-amino acid and tannin were present in aqueous, ethanol and methanol extracts. Pet-ether extracts were not present. Reducing sugar, saponin, carbohydrate and starch were presence only in aqueous extracts. Ethanol, methanol and pet-ether extract were absence. Steroid /terpenoid was found ethanol, methanol and pet-ether extract and aqueous extract was not found. These plants contain carbohydrate, flavonoid, phenolic compound, terpenoid and steriod were in agreement with those mentioned by Shrivastava N.*et.al*, 2007.

In this study, antimicrobial activities of various extracts were tested on six different types of test microorganisms by using paper disc diffusion methods. From these data, the leaves extracts of *Clerodendrum serratum* Linn. showed antimicrobial activity against only the test organism on *Bacillus subtilis*.

The study focused on the morphological, anatomical, phytochemical and antimicrobial investigation of the plant. The plant was used in anti-inflammatory, anti-cancer, hepatoprotective, and anti-diarrhea and anti-microbial. The above data would be helpful in further study of the plant parts and research and development in the field of medicine.

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