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Pharmacognostic evaluation of *Clerodendron multiflorum* (Burmf).O Kuntze

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ABSTRACT

Clerodendron multiflorum (Burmf).O Kuntze, belonging to the family Verbenaceae is a common hedge plant occurring throughout India. Whole plant is medicinal. It shows antidiabetic activity. Plant parts are used in dyspepsia, stomachache, cholera, dysentery, fever and nervous disorders. Anatomical characters of root, stem, petiole and leaf were studied. Root shows presence of secondary vascular cylinder with calcium oxalate crystals and starch grains in the cortex. Stem also shows secondary vascular tissue with patches of sclerenchyma in pericycle. Vascular bundle in the petiole is in the form of an arc. Leaf is amphistomatic with anisocytic stomata with multicellular, uniseriate and non-glandular trichomes. Preliminary phytochemical investigations revealed the presence of carbohydrate, sugar, starch, proteins, phenolics, amino acids, glycosides, alkaloids and tannins. Secondary metabolites such as flavonoids, glycosides, alkaloids, tannins and phenolics are responsible for medicinal property of the plant.

Key words: *Clerodendron*, trichome, glycoside, alkaloid, starch grains.

INTRODUCTION

Clerodendron multiflorum (Burm f). O Kuntze belonging to family Verbenaceae occurs throughout India. Also planted as a field –hedge. It is a large scrambling shrub or small tree, 4-8 m tall. Bark light brown. Branches pubscent , lenticellate, often drooping. Leaves ovate deltoild upto 7cm long, crenate serrate, acute. Flowers creamy-white; in axillary and terminal cymose panicles. Drupes obovoid, wrinkled, black, enclosed by enlarged calyx. Seeds white, oblong. The plant shows antidiabetic activity. Plant parts used in dyspepsia, stomachache, colic, cholera, dysentery, postnatal fever, during convulscence from measles. Root and bark are bitter tonic, used in debility and nervous disorders. Roots are also used in dysuria and retention of urine. Decoction of root used as ademulcent in gonorrhea (Ambasta,1994).

The ethanolic extract of leaves exhibited hepatoprotective activity. The aqueous extract of leaves exhibited anthelmintic activity (Khare, 2007).

MATERIALS AND METHODS

Plants were collected and important parts like root, stem, petiole and leaves were preserved in 4% formalin. The ethno-medicinal information about the plant was obtained through interrogation and literature survey followed by thin section study of individual plant parts. All the sections were stained in safranin and dehydrated following the usual method of Johnson (1940) and mounted in D.P.X. for microscopic observation. To study the stomatal complex and hairs from leaves, epidermal peelings of fresh leaves were directly done mechanically by forcep. The peels were stained with safranin by mounting in glycerine. For phytochemical analysis, (Khandelwal, 2009; Kokate et al. 2003) the plant parts root. stem, leaves and flowers were dried in a shed under normal environmental conditions for about one week. These dried parts were broken into small pieces with the help of cutter and grinded to coarse powder. Coarsely grinded plant parts were extracted in Soxhlet Apparatus successively with solvents such as Acetone, Benzene, Chloroform, Ethyl alcohol, Petroleum ether and Distilled water. The extracts obtained were concentrated and dried. The plant extract was subjected to chemical tests for the presence of phytochemical classes like carbohydrates, proteins, amino acids, fats and oils, steroids, glycosides, alkaloids, tannins and phenolics.

RESULTS

T.S. of Root

Outline of young root is not circular. Thick cuticle is present. Epidermis single layered, cells parenchymatous, rectangular, compactly arranged without intercellular spaces measuring about 20.8 x 16.64 µm in size. Cortex multilayered, cells parenchymatous, oval, thin walled, with small intercellular spaces measuring about 33.28 x 37.44 µm in size .Calcium oxalate crystals and starch grains are present in cortex. Vascular bundles are radial. Xylem is triarch and exarch.

T.S. of Root (Secondary Growth)

Outline circular. Cork yellowish brown, multilayered, cells thick walled, quadrangular, compactly arranged without intercellular spaces, measuring about 33.28 x 25 µm in size. Cortex multilayered, cells parenchymatous, polygonal, compactly arranged without intercellular spaces, measuring about 49.50 x 41.6 µm in size. Abundent starch grains are present. Vascular cylinder shows secondary phloem in outer continuous ring. Secondary xylem forms a continuous cylinder traversed by narrow rays. Vessels in radial rows and circular in outline. Calcium oxalate crystals and strach grains are present in xylem parenchyma and xylem rays. Pith small, cells parenchymatous, thin walled, oval enclosing small intercellular spaces.

T.S. of Stem

Trichomes multicellular, 2 to 3 cellled, unbranched, uniseriate nonglandular, measuring about 65.56 x 41.6 µm in size. Epidermis single layered, cells parenchymatous, rectangular, compactly arranged without intercellular spaces, measuring about 25 x 12.48 µm in size. Cortex multilayered, cells parenchymatous, oval , thin walled, isodiametric enclosing small intercellular spaces, measuring about 50 x 20.8 µm in size. Endodermis single layered, cells barrel shaped, compactly arranged without intercellular spaces. Pericycle multilayered, parenchymatous containing patches of sclerenchyma. Vascular tissue shows secondary phloem in form of outer continuous ring. Phloem elements are compactly arranged without intercellular spaces. Secondary xylem in continuous cylinder. Xylem vessels in radial rows measuring about 62.4 x 74.88 µm in size and xylem tracheids measuring about 16.64 x 20.8µm in size.Vascular cylinder is traversed by less elongated medullary rays. Pith wide, homogeneous, cells parenchymatous, thin walled. isodiametric polygonal, enclosing small intercellular spaces. measuring about 74.88 x 62.4µm in size.

T.S. of petiole

Outline more or less circular with a deep furrow on adaxial side . Trichomes many, multicellular, 2 to 3 celled, uniseriate, unbranched, nonglandular measuring about 332 x 25 µm in size. Epidermis single layered, cells parenchymatous, cuticularized. rectangular, thick walled, compactly arranged without intercellular spaces, measuring about 29 x 21 µm in size. Cortex multilayered, cell parenchymatous, thin walled, oval, enclosing small intercellular space. Smaller cells measuring about 83.2 x 75 µm in size towards the peripheri and larger cells measuring about 124 x 91 µm in size towards the centre. In the centre large, conjoint collateral open and endarch vascular bundle is present in the form of an arc. Xylem facing towards the centre

Table 1: Preliminary Phytochemical Investigations of *Clerodendron multiflorum*(Burm.f)

Sr. No	Test performed	Observation	Inference
1	CARBOHYDRATES		
a	**Molisch test** To the test tube, few drop of Molisch's reagent was added (Alcholic α- Napthol). 2ml of conc. Sulphuric acid was added slowly form the side of the test tube	Violet ring is formed at junction of two liquids	Carbohydrate present
b	**Fehling's test** 1ml Fehling's A and 1ml Fehling's B was mixed and boiled for 1 min. To this solution was added equal volume of test solution. And boiled for 5-10 min	First yellow , then brick red ppt is observed	Reducing sugars present
c	**Benedict's test** Equal volume of Benedict's reagent and test solution was mixed in the test tube and heated to boiling water bath for 5 min	Solution appeared green yellow or red	Reducing sugars present
d	**Barford's Test** Test solution was heated with Barford's reagent on water bath.	Red ppt is obtained	Monosaccharide present
e	**Aniline acetate test** Test solution was boiled in test tube. Filter paper soaked in aniline acetate was held in the vapour	Filter paper did not turned pink	Pentose sugars absent
f	**Cobalt- Chloride test** 3ml test solution was mixed with 2ml cobalt chloride. Boiled and cooled. Few drops of NaOH solution was added.	Solution appeared greenish blue and pink	Glucose and fructose present
g	**Iodine test** 3 ml test solution and few drops of dilute Iodine Solution was mixed	Appearance of blue colour	Starch present
2	**PROTEINS**		
a	**Heat test** The test solution was heated in boiling water bath	Coagulation occurred	Protein present
b	**Biuret test** Test solution was treated with biuret reagent (40% sodium hydroxide and dilute copper sulphate solution)	Violet or pink colour	Protein present
3	**AMINO ACIDS**		
a	**Million's test** Test solution was treated with Million's reagent and heated on water bath	Brick red ppt	Amino acid present
b	**Ninhydrin test** Test solution with Ninhydrin reagent was boiled	Purple or Bluish colour	Amino acid present
4	**FATS &OILS**		
a	**Filter Paper Test**	No change	Fats and oils absent
5	**GLYCOSIDES**		
a	**General test** 200mg of drug with 5ml of dilute sulphuric acid was extracted by warming on a water bath, filtered and neutralized the acid extract with 5% solution of sodium hydroxide. 0.1 ml of fehlings solution A and B was added until it became alkaline (Test pH - Paper) and heated on water bath for 2min	Formation of Red ppt.	Glycoside Present

A	**Test for Anthraquinone Glycosides**		
a	**Modified Borntrager's test** 200 mg of test material was boiled with 2ml of sulphuric acid and treated with 2ml of 5% aqueous ferric chloride solution (freshly prepared) for 5min. It was shaked with equal volume of chloroform. Lower layer of chloroform was separated and shaked with dilute ammonia (half of volume of chloroform).	Ammonical layer showed pink to red color	Anthraquinone glycoside present
B	**Test for cardiac glycosides** **i] Legal's test** Test solution was treated with pyridine made alkaline with sodium nitroprusside	Pink to red color	Cardiac glycosides present
C	**Test for saponin glycosides** 2 ml of solution of drug in water was placed in test tube and shaked	Foams are not formed	Saponin glycosides absent
	Test for flavonoid glycosides		
a	**Shinoda test -** Test solution was treated with fragment of magnesium ribbon and conc. HCl was added.	Appearance of Pink colour	Flavonoids present
6	**ALKALOIDS**		
a	**Dragendorffs test-** Test solution was treated with Dragendorffs reagent (potassium bismuth iodide)	Orange brown ppt	Alkaloids present
b	**Mayer's test** Test solution was treated with Mayer's reagent (Potassium mercuric iodide)	No ppt	Alkaloids absent
7	**TANNINS AND PHENOLICS**		
a	**Ferric chloride test** Test solution was treated with few drops of 5% ferric chloride solution	Deep blue colour appeared	Hydrolysable Tannins present
B	To the test solution few drops of potassium dichromate solution was added	Red ppt	Tannins and phenolic compound present

and phloem towards the outer side. Central vascular bundle is surrounded by a pericycle which consists of patches of sclerenchyma.

Leaf: Surface view

Two types of multicellular, uniseriate trichomes arise from the epidermis a) Non glandular trichomes are 2 to 3 celled, slight warty and measuring about 249 x 16.64 µm in size b) Glandular trichomes are sunken with one celled stalk and 7 to 8 celled head and measuring about 99 x 83 µm in size. Leaf is amphistomatic, stomata many 1 to 2 cells apart, anisocytic more in lower epidermis. Guard cells measuring about 8.32 x 16.64 µm in size. Pore is small, oval, measuring about 4.16 x 8.32µm in size. Epidermal cells are parenchymatous , polygonal, thin walled, compactly arranged without intercellular spaces and measuring about 41.6 x 20.8 µm in size.

T.S. of leaf

Cuticle is present. Epidermis single layered, cell parenchymatous, rectangular, compactly arranged without intercellular spaces, measuring about 16.64 x 20.8 µm in size. In the midrib below epidermis, thin walled, polygonal parenchyma cells measuring about 65.56 x 54 µm in size are present .Mesophyll is not differentiated into palisade and spongy parenchyma. Conjoint, collateral, endarch and open vascular bundle are present in the form of an arc. Pericyclic fibres are associated with central vascular bundles. Lamina dorsiventral. Mesophyll is differentiated into palisade

and spongy parenchyma. Palisade in one layer below upper epidermis. Cells are parenchymatous, columner, elongated, compactly arranged, with their long axis, at right angle to the leaf epidermis, without intercellular spaces measuring about 100 x 16.64µm in size. Spongy parenchyma is present above lower epidermis. Cells parenchymatous, oval, thin walled, enclosing large intercellular spaces, measuring about 33.28 x 30 µm in size. Conjoint, collateral and open vascular bundles run parallel in lamina.

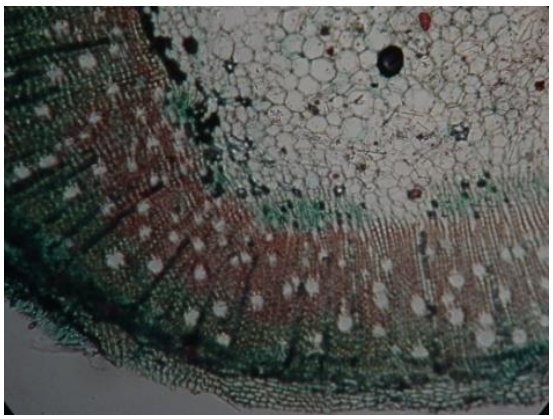
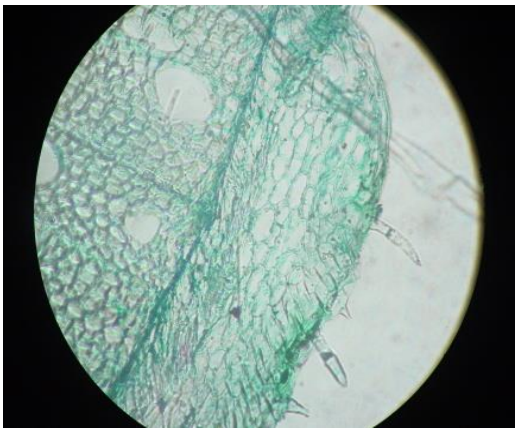
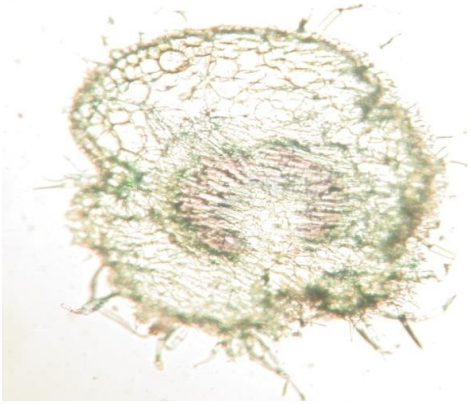



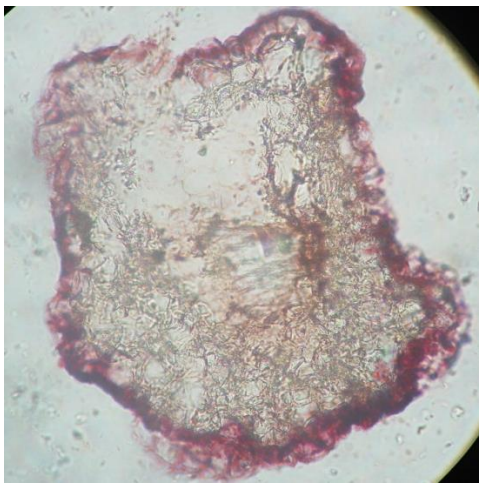
Fig. 1 : *Clerodendron multiflorum* (Burm.f.) T.S. of Stem x 160



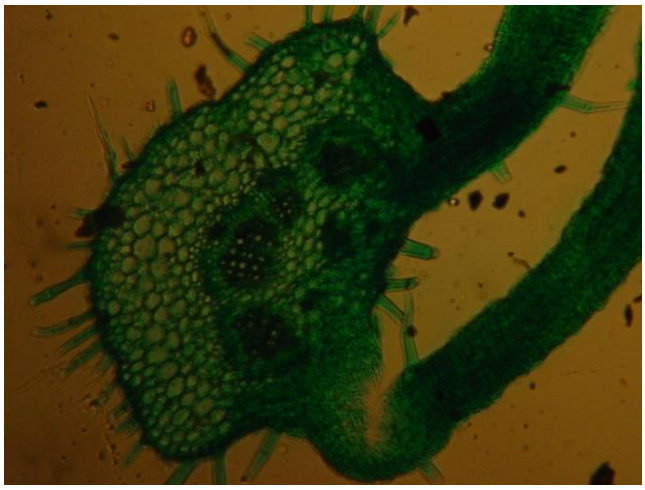
T.S. of root x 640



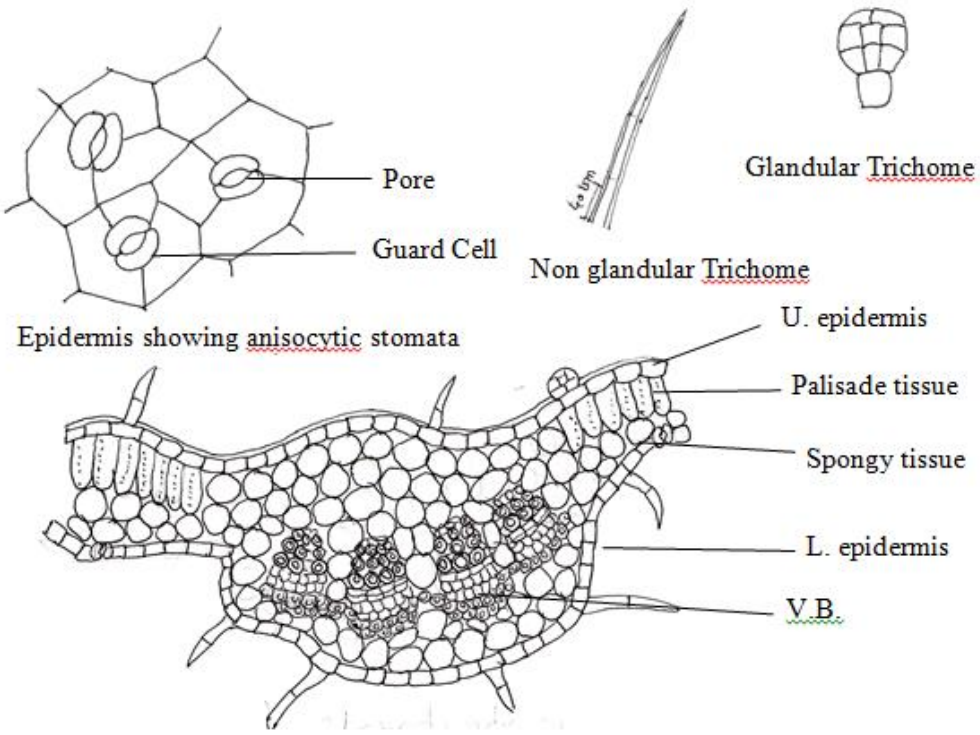
T.S. of Petiole x 160



T.S. of root x 80



T.S. of midrib of leaf x 160



DISCUSSION

Microscopic characters are useful in identification of drug. Trichomes, stomata, calcium oxalate crystals, starch grains, fibres vessels are important anatomical characters of the genus *Clerodendron multiflorum (*Burn f) kuntze

In this plant multicellular, uniseriate, nonglandular trichomes are present on the surface of stem, petiole and leaf. In addition to this glandular trichomes also arise from the epidermis of leaf. In the root calcium oxalate crystals and starch grains occur abundantly. This genus is also characterized by amphistomatic leaf with anisocytic stomata and pericyclic fibres associated with conjoint collateral and open vascular bundles of petiole and midrib of leaf.

Preliminary phytochemical investigations revealed the presence of carbohydrate, sugar starch. proteins, phenolics, amino acids, glycosides and tannins. Carbohydrate, sugar, starch, proteins amino acids form reserve food. Secondary metabolites such as flavonoid glycosides, alkaloids, tannins and phenolic are responsible for medicinal property of the plant.

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