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Der Pharmacia Lettre, 2015, 7 (3):61-70
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ISSN 0975-5071
USA CODEN: DPLEB4

Pharmacognostic study of *Clerodendrum splendens* flower and stem

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ABSTRACT

To present detailed pharmacognostic study of Clerodendrum splendens flower and stem an important plant in Indian system of medicine. The macroscopy, microscopy, physiochemical analysis, preliminary testing of the Flower and Stem part for standardization was investigated. Morphological study of flower shows that it is pentamerous with 5 free sepals, 5 gamopetalous corolla and five free petal lobes. Microscopic study of flowers shows that sepals are thick with blunt margins and are concave on the abaxial side and glandular trichomes are frequently seen on the inner epidermis. There are five petal lobes which are imbricate and aestivation and these are thicker in the middle and gradually tapering towards margins. The anther is dithecous and four chambered and the anther dehisces longitudinally through the stomium. The pollen grains are circular and have slightly echinate exine and thin smooth infine. The basal part of the petal forms a tubular structure. Fairly prominent circular vascular strands are located along the median part of the corolla tube. Morphologically, stem is hollow cylindrical having dark greenish surface with characteristic mushy odor. Microscopic study shows that stem is a hollow cylinder with even outline. Calcium oxalate crystals of prismatic type are located in cortical sclerenchyma elements and pholem rays are the diagnostic feature of the stem. It can be concluded that pharmacognostic profile of Clerodendrum splendens flower and stem is helpful in developing standards for quality, purity and simple identification.

INTRODUCTION

The genus *Clerodendrum* [Family Laminaceae (Verbenaceae)] is widely spread in tropical and subtropical region of the world and it comprised of small trees, shrubs and herbs. Clerodendrum is very large and diverse genus and till now 580 species of the genus have been identified and widely distributed in Asia, Australia, Africa and America. *Clerodendrum splendens* (glory tree) is one of the important species of genus *Clerodendrum* native to tropical western Africa. It is a twining evergreen climber, growing to 3 meters (9.8 ft) or more, with panicles of brilliant scarlet flowers in summer. The plant exhibit a wide spectrum of folk and indigenous medical uses mainly for the treatment of asthma.

Standardization of crude drug is an integral part of establishing its correct identity. Pharmacognostic evaluation of plant parts assists in standardization of quality, purity and sample identification. Hence the objective of present study is to evaluate various pharmacognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical studies of the plant for establishing its standardization parameters.

MATERIALS AND METHODS

2.1. Chemicals and instruments

Formalin, acetic acid, ethyl alcohol, toluidine blue, glycerin, hydrochloric acid, potassium hydroxide and all other chemicals used in the study were of analytical grade.

2.2. Plant material

The plant specimens of *Clerodendrum splendens* plant for the proposed study were collected from Nashik district of Maharashtra in Feburary 2012 and authenticated by Dr. P.S.N. Rao, Botanical Survey of India, Pune, where herbarium voucher specimen No. (BSI/WC/Tech/2005/101) has been deposited. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58°-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.3. Macroscopic and microscopic analysis

The macroscopy and microscopy of the plant Flower and Stem were studied according to the methods of brain and Turner. For microscopic study paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure. The sections were stained with toluidine blue as per the previous published method. Since toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI (for Starch). [1-3]

2.4. Physiochemical analysis

Physiochemical values such as ash values and percentage of extractive values were studied according to the official methods and previously published methods. [4-6]

2.5. Preliminary phytochemical screenings

Preliminary phytochemical screenings was performed according to the previously published methods. [7-9]

RESULTS

3.1. Macroscopic characteristics of flower

The flower is pentamerous with 5 free sepals, 5 gamopetalous corolla and five free petal lobes. The stamens are five, incurved within the corolla tube and become exerted when the corolla tube is open (Figure 1).

Figure 1: Macroscopic characters of *Clerodendrum splendens* flowers

3.2. Macroscopic characteristics of stem

Stem of *Clerodendrum splendens* is hollow cylindrical having dark greenish surface with characteristic mushy odor. The stem is about 2 mm thick.

Figure 2: Macroscopic characters of *Clerodendrum splendens* stem

3.3. Microscopic characteristics of flower

Sepals

The sepals are thick with blunt margins and are concave on the abaxial side (Figure 3). It is 150 µm thick. The sepal has their epidermal layers of narrow rectangular cells. The ground mesophyll tissues include undifferentiated polygonal, thin walled compact parenchyma cells. Small vascular strands are located in the median part of the leaf blade. Glandular trichomes are frequently seen on the inner epidermis (Figure 3).

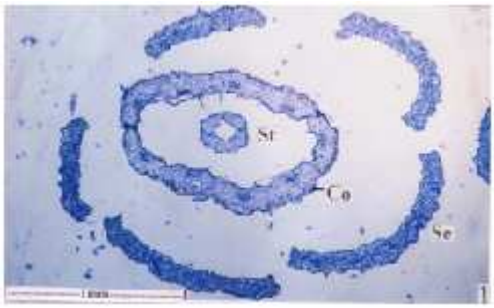

Figure 3: Transverse section of flower through sepals and corolla tube
Where, Co: Corolla tube, Se : Sepal, St : Style

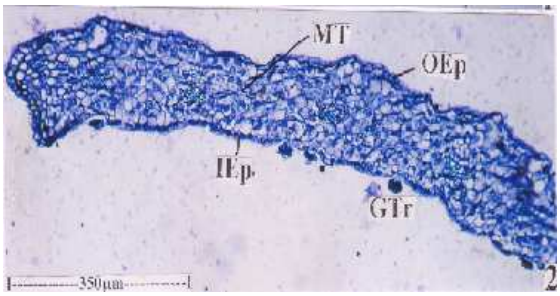

Figure 3: Transverse section of sepals showing glandular trichomes
Where, GTr : Glandular Trichomes, IEp : Inner Epidermis, MT: Mesophyll Tissue, and OEp : Outer Epidermis.

Petals

There are five petal lobes which are imbricate and aestivation. The petals are 120 µm thick. There is no distinct midrib; it is uniform in thickness. The outer epidermal cells of the petal are semicircular or slightly papillate. The inner epidermal cells are also papillate with beak-like outer tangential walls. The mesophyll tissue consists of homogenous, parenchymatous circular compact cells (Figure 4). Small collateral vascular bundles are located in the mesophyll tissue. The petals are thicker in the middle and gradually tapering towards margins (Figure 5).

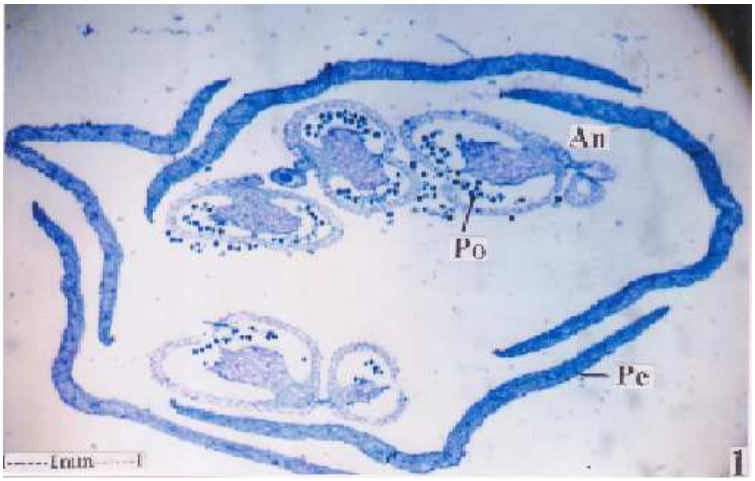

Figure 4: Transverse section of flower through petal lobes and anthers
Where, An: Anther, Pe : Petal; Po : Pollen

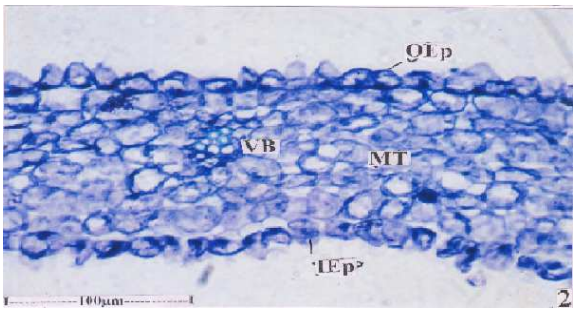

Figure 5: Transverse section of petals- enlarged
Where, IEP : Inner Epidermis, OEp : Outer Epidermis; MT : Mesophyll Tissue

Anther

The anther is dithecous and four chambered (Figure 6). The wall of the mature dehisced anther is 80 µm thick. It consists of the outer prominently papillate epidermal layer and wide, thick radially oblong enthelial cells (Figure 7). The inner layer of endothelial cells has several annular thickenings. The endodermal layer is disintegrated. The anther dehisces longitudinally through the stomium.

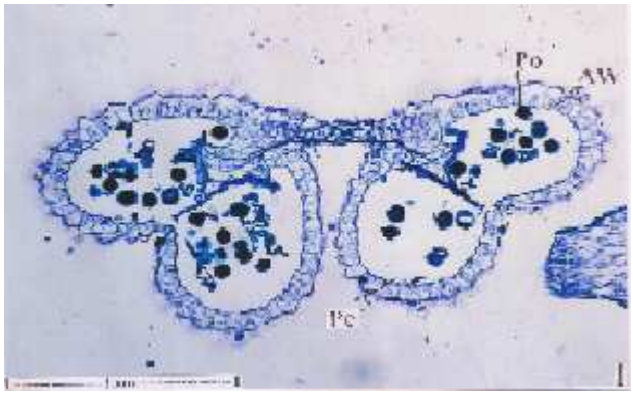

Figure 6: Transverse section of mature anther with pollen
Where, Pc: Pollen chamber; Po: Pollen

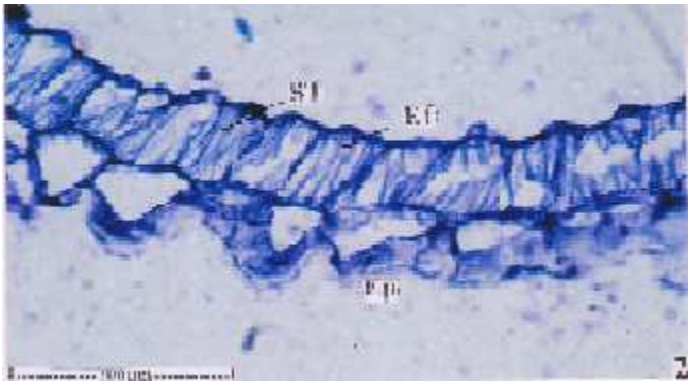

Figure 7: Transverse section of anther wall enlarged
Where, Ep: Epidermis, Et: Endothecium; ST: Spiral thickenings

Pollen grains

The pollen grains are circular and they are 40 µm in diameter. They have slightly echinate exine and thin smooth infine (Figure 8 and 9).

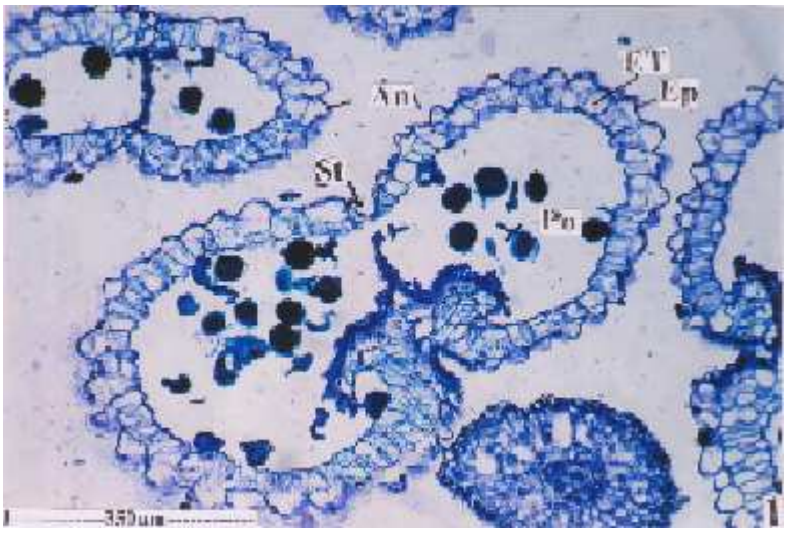

Figure 8: Transverse section of anther liberating the pollen
Where, An: Anther, Ep: Epidermis, Et: Endothecium; Po: Pollen; St: stomium

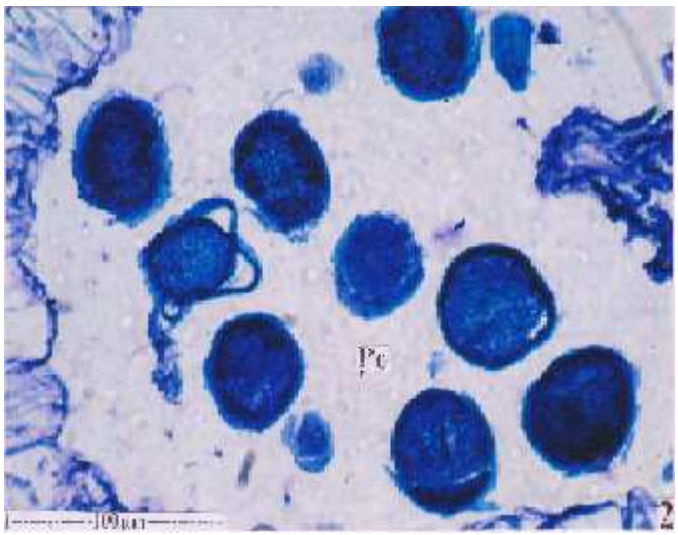

Figure 9: Transverse section of Pollen grains enlarged
Where, Po: Pollen

Corolla tube

The basal part of the petal forms a tubular structure. The corolla is 200 µm thick (Figure 10). It consists of the outer epidermis compromising large thick walled circular cells. The inner epidermal layer also consists of large papillate thick outer tangential walls. The ground tissue consists of 7-9 layers of large, thin walled compact parenchyma cells. Fairly prominent circular vascular strands are located along the median part of the corolla tube. The vascular strands have large groups of thick walled xylem elements and a thin layer of darkly stained pholem elements (Figure 11).

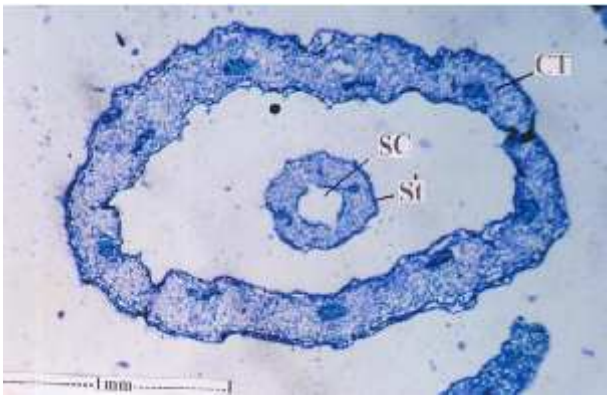

Figure 10: Transverse section of corolla tube and style
Where, CT: Corolla tube; SC: Stylar canal; St: style.

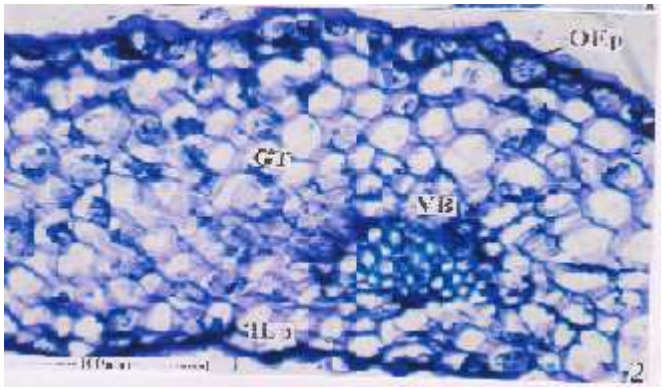

Figure 11: Transverse section of a sector of the corolla tube enlarged
Where, GT: Ground Tissue; IEp: Inner Epidermis; OEp : outer Epidermis; VB : Vascular Bundle.

3.4. Microscopic Characteristics of Stem

The stem is a hollow cylinder with even outline. The central part is occupied by a wide hollow canal which is formed by lysis of the pith cells (Figure 12), The stem is about 2 mm thick. It consists of a thin intact epidermal layer of darkly stained spindle shaped cells. There is a narrow cylinder of superficial periderm compromising two to four layers of periderm derivatives. Inner to the periderm, there is a narrow region cortex; the cortex includes about five layers of compact parenchymal cells. The inner boundary of the cortex is a thin, broken cylinder of sclerenchyma cells. The vascular cylinder consists of wide, continuous cylinder of secondary pholem, compromising radial files of sieve elements and parenchyma cells (Figure 13 & 14). The hollow secondary xylem cylinder includes diffusely distributed, solitary elliptic or circular, thin walled vessels and thick walled, lignified xylem fibres (Figure 14) Calcium oxalate crystals of prismatic type are located in cortical sclerenchyma elements and pholem rays (Figure 16).

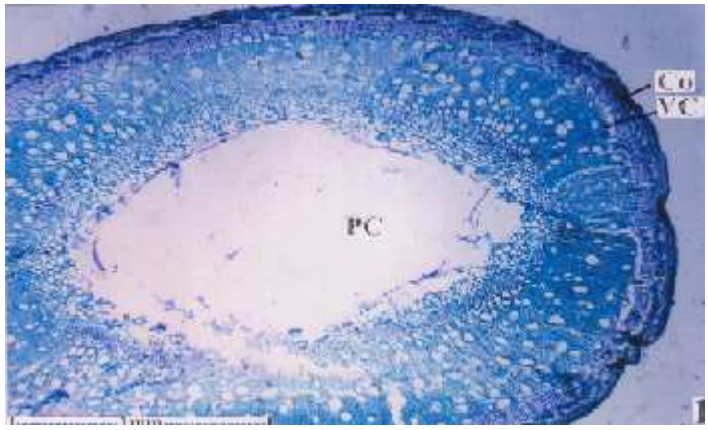

Figure 12: Transverse section of stem- ground plan

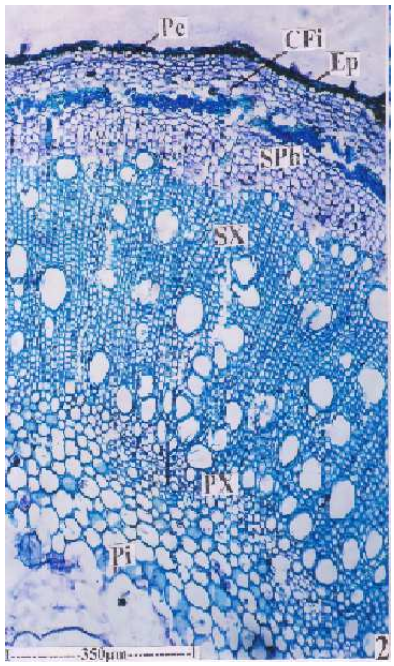

Figure 13: T.S of Stem- Sector enlarged

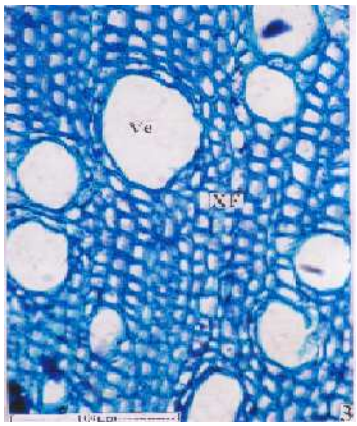

Figure 14: Secondary Xylem element enlarged

(Co: Cortex; CFi: Cortical fibres;Ep: Epidermis; Pe: Periderm, Pi: Pith, Pc: Pith Canal; SPh: Secondary Pholem; Sx: Secondary xylem)

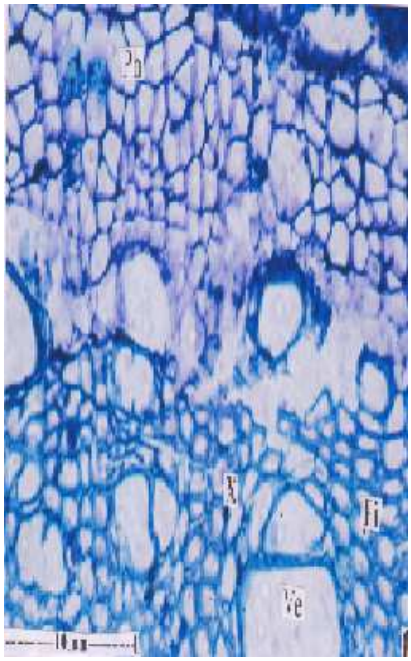

Figure 15: T.S of Stem showing secondary Pholem

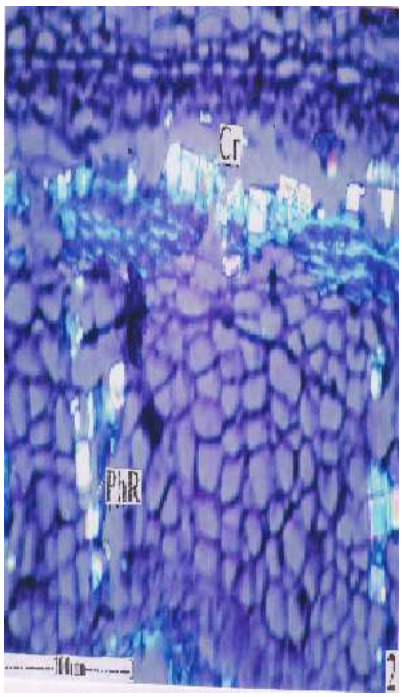

Figure16: Occurrence of calcium oxalate crystal in the cortical fibres and phloem rays
(Cr: Crystals; Fi: fibres; AdB; Ph: Pholem; X: Xylem, Ve: Vessel)

Physicochemical analysis

An ash value is criteria to judge about earthy matter or inorganic composition and other impurities present along with the drug. Various physicochemical parameters such as total ash, water soluble ash and acid insoluble ash of *Clerodendrum splendens* flower was found to be 5.83, 0.94 and 0.20 % w/w and for stem was 7.28, 0.40 and 0.47 % w/w, respectively. Moisture content in the flower and stem was found to be 4.90 % w/w and 6.81 % w/w, respectively. The extractive values are primarily useful for the determination of the exhausted or adulterated drug. Various extractive values such as petroleum ether soluble extract, chloroform soluble extract, methanol soluble extract of *Clerodendrum splendens* stem was found to be 8.86, 3.78, 4.1% w/w and petroleum ether soluble extract and methanol soluble extract of flower was found to be 6.86, 5.7% w/w respectively.

Preliminary phytochemical screening

Preliminary phytochemical screening of *Clerodendrum splendens* flower extract mainly revealed the presence of steroids and triterpenes in petroleum ether and flavonoids and tannins in methanol extract. Preliminary phytochemical screening of *Clerodendrum splendens* stem extract mainly revealed the presence of steroids and triterpenes in petroleum ether and chloroform extract, alkaloids, flavonoids and tannins in methanol extract (Table 1).

Table 1: Preliminary phytochemical screening of *Clerodendrum splendens* flower and stem extracts

Chemical constituent	Chemical test	*Clerodendrum splendens* Flower		*Clerodendrum splendens* Stem		
		PEE	ETE	PEE	CLE	ETE
Alkaloid	Dragendorff's test	-	+	-	-	+
	Mayer's test	-	+	-	-	+
Steroids	Salkowaski test	+	-	+	+	-
	Liebermann-burchard test	+	-	+	+	-
Triterpene	Vanillin-sulphuric acid test	+	-	+	+	-
Tannin	Ferric chloride test	-	+	-	-	+
	Dilute nitric acid test	-	+	-	-	+
Glycoside	Keller-killani test	-	-	-	-	-
Carbohydrate	Molish test	-	-	-	-	-
	Fehling's test	-	-	-	-	-
Flavonoid	Shinoda test	-	+	-	-	+
	Lead acetate test	-	+	-	-	+
Saponins	Foam formation test	-	-	-	-	-
Proteins	Biuret test	-	-	-	-	-
	Millon's test	-	-	-	-	-
Amino acids	Ninhydrin test	-	-	-	-	-

PEE - Petroleum ether extract, CLE - Chloroform extract, ETE - Methanol extract '+' indicates present and '-'indicates absent.

DISCUSSION

The evaluation of a crude drug is an important diagnostic character useful in determining authenticity and identifying adulteration. As there is no pharmacognostic work recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Pharmacognostic parameters like macroscopic and microscopic features of flower and stem have been studied. Preliminary phytochemical screening reveals the useful findings about chemical nature of drugs. Total ash values and extractive values are useful in identification and authentication of the plant material [10-11]. Extractive values are useful to evaluate the chemical constituents of crude drug [12]. Preliminary phytochemical screening ascertains presence of steroids and triterpenes in petroleum ether and chloroform extract, alkaloid and flavonoids in ethyl acetate extract, alkaloids, flavonoids and tannins in ethanol extract and tannins, glycosides, carbohydrates and flavonoids in aqueous extract of the plant flower and stem extracts. Morphological study of flower shows that it is pentamerous with 5 free sepals, 5 gamopetalous corolla and five free petal lobes. Microscopic study of flowers shows that sepals are thick with blunt margins and are concave on the abaxial side and glandular trichomes are frequently seen on the inner epidermis. There are five petal lobes which are imbricate and aestivation and these are thicker in the middle and gradually tapering towards margins. The anther is dithecous and four chambered and the anther dehisces longitudinally through the stomium. The pollen grains are circular and have slightly echinate exine and thin smooth infine. The basal part of the petal forms a tubular structure. Fairly prominent circular vascular strands are located along the median part of the corolla tube. Morphologically, stem is hollow cylindrical having dark greenish surface with characteristic mushy odor. Microscopic study shows that stem is a hollow cylinder with even outline. Calcium oxalate crystals of prismatic type are located in cortical sclerenchyma elements and pholem rays are the diagnostic feature of the stem.

In conclusion, the pharmacognostic standards for flower and stem of *C. splendens* are set in present work. Set standards could be used tool for standardization of this medicinally useful plant.

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