

Pharmacognostical and Phytochemical Evaluation of Leaf of *Clerodendrum phlomidis* Linn. F.

Research Article

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Abstract

Clerodendrum phlomidis Linn.f. is a large bush or small tree belonging to the family Verbenaceae. The present study deals with the pharmacognostical and phytochemical study of leaf including chromatographic evaluation. *Clerodendrum phlomidis* Linn.f. leaf is rhomboid ovate, acute at apex crenate-dentate at margin, subcordate at base and velvety in texture. Leaf of the plant can be identified microscopically by the presence of hooked trichomes, glandular sessile trichomes, starch grains, oil globules, Anomocytic type of stomata and rhomboidal and prismatic crystal. Preliminary analysis revealed the presence of carbohydrates, steroid, alkaloids, tannin and phenol. HPTLC study of alkaloid showed the presence of two spots in short and three spots in long UV rays. The information generated by this study provides relevant Pharmacognostical and Physico-chemical data needed for proper identification and authentication of leaf of *Clerodendrum phlomidis* Linn.f.

Keywords: Alkaloid, Tannin, Trichome, Oil globule, Starch grain.

Introduction

Clerodendrum phlomidis Linn.f. is a large bush or small tree, reaching 9m high, with more or less pubescent branches. Leaves are 3.8-6.3 by 3.2-3.8cm, ovate or subrhomboid, obtuse or acute, coarsely crenate-dentate or subentire, undulate, glabrous above, more or less puberulous beneath, base truncate or subcordate. Petiole is 6-20mm long. Flower moderate sized, fragrant, in small dichotomous axillary cymes arranged so as to form a rounded terminal panicle. Bracts obovate or lanceolate, acute, leafy. Calyx 1cm long or more, divided about half way down, glabrous, not enlarged in fruit. Corolla white or pinkish. Drupe 6mm long, broadly obovoid, depressed, the top about level with the points of the persistent calyx-lobes, normally 4-lobed with 1 pyrene in each lobe(1). The study is conducted to identify the leaf of plant species with the help of pharmacognostical and phytochemical tests.

Materials and Methods

Collection and authentication:

For this work, the drug sample i.e leaf of *Clerodendrum phlomidis* Linn.f. was collected from Jamnagar, Gujarat, after proper identification and authentication through referring various floras and taking advise from taxonomist. Sample was then

subjected to various pharmacognostical and phytochemical parameters.

Micromorphological Study

Collected leaf was observed carefully for their morphological characters and micro measurements were noted. Morphological characters like colour, odour and size were noted down(2).

Microscopic study

Free hand sections of leaf i.e. petiole, mid-rib, upper and lower epidermis of the research drug was taken and cleared with chloral hydrate. They were first observed in distilled water and then stained with phloroglucinol and Concentrated Hydrochloric Acid. Microphotographs were taken by using Carl Zeiss Trinocular microscope, attached with camera(3). Fine powder was made of these leaves and powder microscopy was carried out of the sample(4).

Micrometric evaluation

Evaluation of the length and breadth of leaves, trichomes, stomata, prismatic and rhomboidal crystals was done with the help of Carl Zeiss Trinocular microscope attached with camera with preloaded micrometric analysis software(5) and mean value was taken into consideration.

Micro-chemical tests

Some micro-chemical tests were performed to detect presence of starch grains, crystals and lignified elements(6) in all samples.

(i) Test for starch grains

Sections and powder were treated with a drop of iodine solution. Starch grains turned blue when examined microscopically.

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(ii) Test for crystals of calcium oxalate

They are insoluble in glycerine and phenolic reagents but soluble in hydrochloric acid. On adding a drop of hydrochloric acid to the section and powder; calcium oxalate crystals were dissolved and effervescence was seen.

(iii) Test for fibres (Lignified elements)

Section and powder were treated with phloroglucinol and hydrochloric acid. Sclerenchymatous fibres turned pink in colour.

Chromatographic Analysis

Chloroform:Methanol (8.5 : 1.5 v/v) was used as solvent system for HPTLC. As per the standard procedure at 256nm and 366nm analysis was done(7).

Chromatographic Condition

Application mode	Camag Linomat V
Development Chamber	Camag Twin trough Chamber.
Plates	Precoated Silica Gel GF254 Plates.
Chamber Saturation	30 min.
Development Time	30 min.
Development distance	8 cm.
Scanner	Camag Scanner III.
Detection	Deuterium lamp, Tungstan Lamp
Data System	Win cats software.

Observations and Results

Table No. 1: External morphology of leaf of *Clerodendrum phlomidis* Linn.f.

Parameters	<i>Clerodendrum phlomidis</i> Linn.f.
Colour	Green
Size	8×5cm
Apex	Acute
Shape	Rhomboid ovate
Odour	Characteristic
Taste	Pungent, bitter and sweet
Margin	Crenate-dentate
Base	Sub-cordate
Texture	Velvety
Venation	Reticulate, 4-5 pairs

Microscopic characters of *Clerodendrum phlomidis* Linn.f. leaf

Transverse section of petiole

Diagrammatic sketch showed round to oval shaped section with presence of multicellular warty trichomes which covers the epidermis followed by hypodermis and central ground tissue.

Epidermis was single layered, cuticularised with compactly arranged cells. The epidermal cells had numbers of multicellular warty trichomes, hooked shaped trichomes and sessile glandular trichomes. Two types of sessile trichomes one filled with oil and other multicellular sessile trichomes were present.

Hypodermis was made up of 6-7 layers of collenchyma cells compactly arranged without any air space. Ground tissue was made up of parenchyma cells, some of which were filled with chlorophyll pigments. Parenchyma cells showed presence of prismatic crystals of calcium oxalate and rhomboidal crystals.

Vascular bundles were situated at the centre, having arc shape, open and collateral in arrangement. Phloem was situated beneath xylem and consisted of phloem fibres and sieve elements.

Transverse section through midrib

Epidermis –In both upper and lower epidermis presence of multicellular warty trichomes, hooked trichomes and glandular sessile trichomes was seen. Some of the epidermal cells were found interrupted by stomatal openings. Epidermis was covered with cuticle. Epidermal cells and trichomes showed presence of colouring matter in them.

Mesophyll- was differentiated into upper palisade and lower spongy parenchyma cells. Palisade parenchyma consisted of 2 layers, while spongy parenchyma consisted of 4-5 layers of parenchyma cells. Between spongy and palisade parenchyma many vascular strands were passing and both layers were filled with oil globules. Vascular bundle showed phloem towards lower epidermis and xylem towards upper epidermis. Phloem was made up of phloem fibers and some sieve plates and xylem consisted of xylem parenchyma and its fibers.

Surface study: Lower epidermis

Anomocytic type of stomata was present, measuring 0.7×0.4 mm, 0.6×0.3 mm, 0.6×0.3 mm at different angles with mean size of 0.6 × 0.3mm. Glandular sessile type of trichome was present, with radius of 5mm, 4mm and 5mm, having mean radius of 4.6mm. Multicellular warty trichomes with 6- celled structure were present. Number of stomata present at different angles was 7, 6, 8 respectively in one exposed area. Number of epidermal cells in that area was 36, 48, 42. Their ratio i.e. Stomatal Index was observed to be 14.193. Two glandular sessile trichomes were present in per square mm area.

Upper epidermis

Greenish chlorophyll pigments were more in upper epidermis as compared to lower epidermis. Anomocytic type of stomata were present measuring 0.9×0.4 mm, 0.9×0.3 mm, 0.9×0.4 mm at different angles with mean size of 0.9 × 0.4mm. Glandular sessile trichomes with radius of 5mm, 4mm, 5mm at various angles with average radius of 5mm were present. Number of stomata present at various angles in particular observed area was 5, 5, 6 respectively while number of epidermal cells in that area of observation were 36, 33, 33 respectively. Stomatal Index was found to be 9.7. Only one glandular trichome was present in per square mm area. Multicellular warty trichomes were also found present.

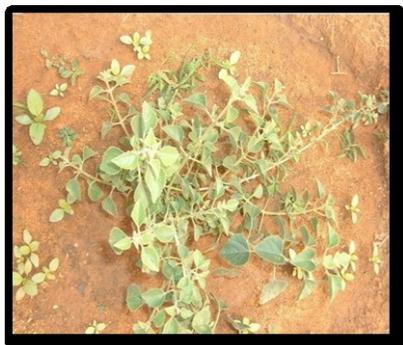
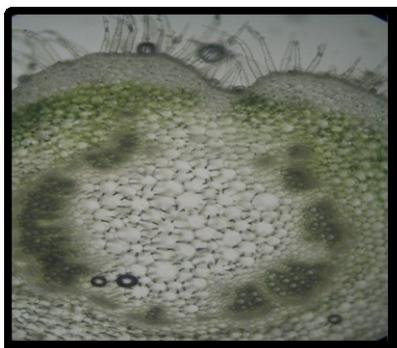
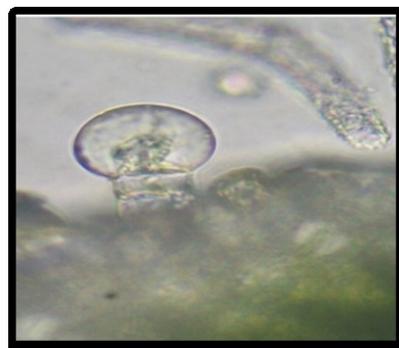
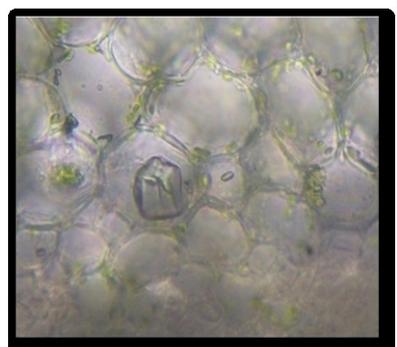
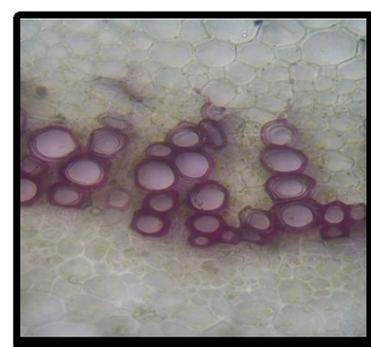
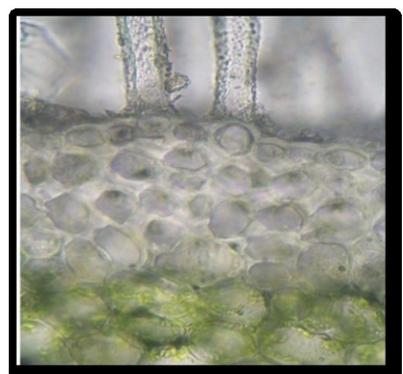
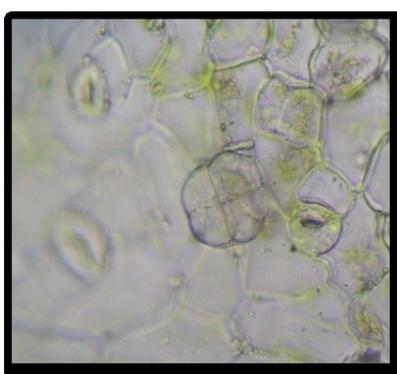
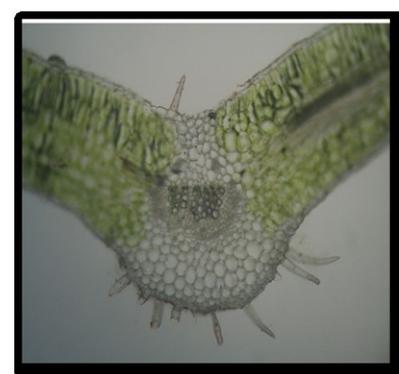
Fig. 1 *C. phlomidis* Linn Plant**Fig. 2** *C. Phlomidis* Inflorescence**Fig. 3** *C. phlomidis* Fruit**Fig. 4** *C. phlomidis* Leaf**Fig. 5** T S of Petiole**Fig. 6** Bicellular stalked Sessile Trichome**Fig. 7** Warty and hooked Trichome**Fig. 8** Rhomboidal Crystal**Fig. 9** Vascular Bundle**Fig. 10** Oil Globule**Fig. 11** Anamocytic stomata**Fig. 12** Leaf TS through mid-rib

Fig. 13 Coloring matter in Trichome & Epidermal cells



Fig. 14 Sessile Trichome

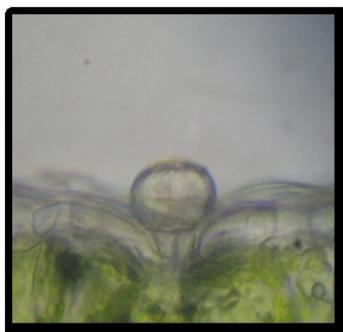


Fig. 15 Mesophyll with Vascular strands

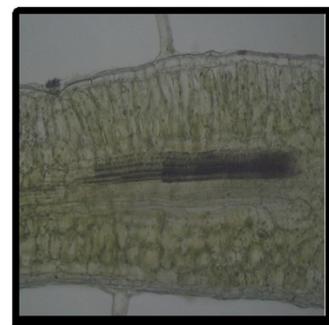


Fig. 16 Brown Content

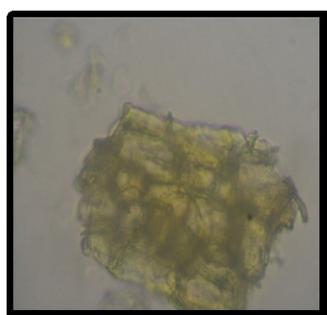


Fig. 17 Vascular Bundle with Xylem & Phloem



Fig. 18 Powder Microscopy Multicellular warty Trichome



Fig. 19 Fragment of Spiral vessel



Fig. 20 Prismatic Crystal of calcium oxalate

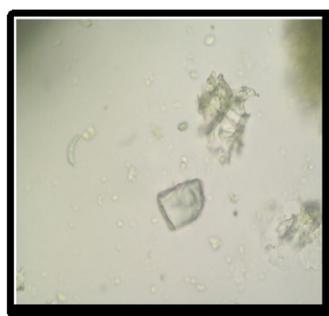
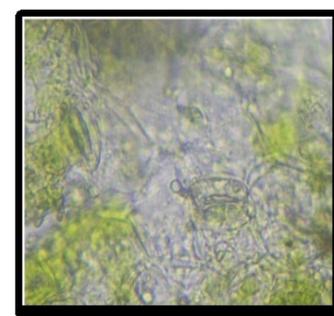


Fig. 21 Fragment of Stomata



Leaf powder microscopy:

Leaf powder was observed pale green in colour, smooth in touch with characteristic irritant smell and pungent-bitter and somewhat sweet taste.

Microscopic examination showed presence of multicellular warty trichome, spongy parenchyma cell, simple fibre, oil globule, spiral vessel, prismatic crystal, brown content, epidermal cell, glandular trichome fragments and presence of rhomboidal crystal fragments in addition.

Table No. 2 Micrometric evaluation of *C.phlomidis* Linn.f. leaf powder

S.N	Character	<i>Clerodendrum phlomidis</i> Linn.f.	Measurements
1	Crystal – Rhomboidal Prismatic	Present Present	0.4×0.2 0.5×0.3
2	Trichomes – Warty Multicellular Hook shaped Glandular- oil filled Glandular-multi head	Present Present Present Present Present	5.5×0.6 3.0×0.6 1.6×0.3 1.2×0.3 1.0×0.3

Shreedevi A et.al., *Clerodendrum phlomidis* Linn.F. Leaf – Phytopharmacognostical Profile

S.N	Character	<i>Clerodendrum phlomidis</i> Linn.f.	Measurements
3	Stomata – (fragments) Anomocytic Diacytic	Present Absent	0.6×0.4
4	Fragments of fibre	Present	8.5×0.2
5	Fragments of spongy parenchyma	Present	-
6	Fragments of epidermal cells	Present	-
7	Fragments of annular vessels	Present	-
8	Oil globules	Present	-
9	Starch grains	Present	-

 Table No. 3 Histochemical evaluation of *Clerodendrum phlomidis* Linn.f. Leaf

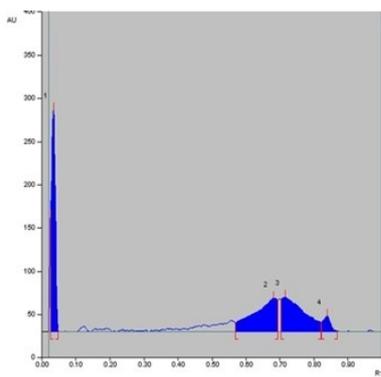
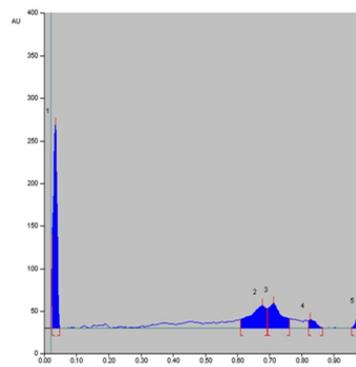
S. No	Reagent	Observation	Characteristics
1	Iodine solution–Starch	Macroscopic- it become blackish Microscopic- found round shape particles	+
2	Ferric chloride–Tannin	Samples become blackish	+
3	HCl–Calcium	It produced bubbles	+
4	HCl–Lignin	Lignified elements become red in colour	+
5	Sudan III solution–Oil globules	It becomes red in colour	+

 Table No. 4 Phytochemical evaluation of *Clerodendrum phlomidis* Linn.f.

Sr No	Tests	<i>C.phlomidis</i> Leaf
1.	Proteins	
a.	Biuret test	–ve
b.	Ninhydrin test	–ve
2.	Carbohydrate test for starch	
a.	Iodine test	+ve
b.	Fehling's test	+ve
c.	Benedict's test	–ve
d.	Test for non reducing sugar such as sucrose	–ve
3.	Tannins	
a.	Gelatin test	+ve
4.	Anthocyanins	
a.	Aqueous NaOH test	+ve
b.	Conc. H ₂ SO ₄ test	–ve
5.	Glycosides	
a.	Molisch's test	+ve
b.	Conc. H ₂ SO ₄ test	+ve
6.	Saponin	
a.	Foam test	-ve
7.	Flavonoids	
a.	Flavonoid test	–ve
b.	Shinoda test	–ve
c.	Conc. H ₂ SO ₄ test	+ve
8.	Phenols	
a.	Phenol test	+ve
9.	Steroids	
a.	Salkowski's test	+ve
10	Alkaloids	
a.	Dragendorff's test	+ve

Table No:5 HPTLC of Leaf of *Clerodendrum phlomidis* Linn.f. at 254nm and 366nm

Samples	Solvent System (V/V)	Short UV 254 nm		Long UV 366 nm	
		No. of spots	Rf value	No. of spots	Rf value
<i>Clerodendrum phlomidis</i> Linn.f.	Chloroform : Methanol (8.5 : 1.5 v/v)	2	0.03, 0.90	3	0.03, 0.86, 0.91

Fig. 22 Densitogram of *C. phlomidis* at 254nm

Fig. 23 Densitogram of *C. phlomidis* at 366nm


Conclusion

Pharmacognostical findings i.e. glandular sessile trichomes, starch grains, oil globules, Anomocytic type of stomata and rhomboidal and prismatic crystal were important characters to identify the leaf of *Clerodendrum phlomidis* Linn.f. Phytochemical evaluation of leaf of *Clerodendrum phlomidis* Linn.f. showed presence of starch, tannin, phenol and alkaloid. Thus with the help of pharmacognostical and phytochemical tests the leaf of *Clerodendrum phlomidis* Linn.f. was identified.

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