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Assessment of quality control parameters of the leaves of *Clerodendrum phlomidis* Linn.f

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Abstract
Clerodendrum phlomidis is commonly known as *Agnimantha* in Ayurveda and it belongs to the family Verbenaceae. Owing to its multifarious biological activity, the plant has gained importance in the treatment of many diseases such as asthma, rheumatism, dysentery, tooth ache, leprosy, inflammatory diseases and is of great demand in the fast growing herbal market. The aim of the present study is to perform the pharmacognostical, phytochemical and HPTLC finger printing profile on the leaves of *Clerodendrum phlomidis.*
Free hand sections of leaves are taken using a rotary microtome and stained with toludene blue to carry out pharmacognostical studies. The aqueous and ethanolic leaf extracts are analysed for the presence of phyto constituents. HPTLC studies are carried out for the aqueous and ethanolic leaf extracts.
The leaves are identified by the presence of plano convex midrib, collateral vascular bundle, and occasionally the presence of umbrella shaped glandular trichomes. Powder microscopy showed the presence of fibre, non glandular trichomes, epidermal cells and cyclocytic stomata. The phytochemical analysis showed the presence of flavanoids, tannins, phytosterols, carbohydrates and proteins in aqueous extract and ethanol extract contained alkaloids, flavonoids, tannins, sterols, proteins and carbohydrates.
The information derived from the present study helps in the botanical identification of leaves of *Clerodendrum phlomidis* and also be used as a valuable information to ensure the quality of the drug as adulteration is becoming common these days. The study will help in the better understanding of the use of this plant leaf for biological activities and for dissemination of knowledge for the researchers who undertake this plant part for further study.

Keywords: *Agnimantha*, *Clerodendrum phlomidis* leaves, microscopy, phytochemical analysis

Introduction
Plants have been used as a source of medicine since ancient times in all cultures. The use of plants for alleviation of human is as old as human themselves. Traditional medicine is still in the mainstay of healthcare in developing countries. For over 2500 years, people in India are known to have used plants in organised health care regimes. *Clerodendrum phlomidis* Linn.f.suppl. commonly called as Agnimantha in Ayurveda belongs to the family Verbanaceae which comprises of 35 genera and 1200 species [1]. These are found mainly in the tropical regions of the world. Herbs, shrubs and small trees represents this family which are known for heads, spikes and clusters of flowers [2]. In folklore and traditional system of medicine, *Clerodendrum phlomidis* Linn.F.suppl. finds multifarious application in the treatment of many diseases such as asthma, rheumatism, dysentery, tooth ache, leprosy and other inflammatory diseases [3]. The plant is called by various names such as Jeyanthi, Vaijeyanthi, Arni in Sanskrit, Thalludhalai in Tamil, Arni in Malayalam, Taluki in Telugu, Taggi Beru in kannada, Aranimula in Marathi and in Guajarati.
As one among the ten important constituent of Dasamoola, the roots of the plant *Clerodendrum Phlomidis* is included in the list of 70 medicinal plant species of high trade procured from the tropical forests [4] [5]. Over exploitation of this plant roots may lead to the extinction of this species. Although the roots are considered as authentic drug, it is the leaf that finds application in folklore medicine [6]. The presence of chemical constituents namely the secondary metabolites contributes to the various biological activities such as anti oxidant, anti inflammatory, anti asthmatic, analgesic, psychopharmacological activity [7].
About 90% of the raw materials for the herbal medicinal product is procured from wild sources. It's really a great challenge to meet the demand of the huge population and it is difficult to provide a bench mark to procure genuine herbal drug in the market. As there is always a chance of adulteration or substitution of the raw material, proper identification of the plant or the plant part is essential.
As root of this plant is extensively studied, an attempt has been made in the present study to perform the pharmacognostical and phytochemical studies on leaves of this plant to identify the unexplored biological compounds. As flavonoids and their derivatives are the main bioactive components of these species, HPTLC finger printing profile of flavonoids in the leaves will give an insight to the researchers to discover the potentials of flavonoids.

Materials and Methods
Collection, Identification and Authentication of Plant Material
The leaves of *Clerodendrum phlomidis* were collected from Srivaikundam region of Thoothukudi district, Tamil Nadu in 2017. The plant was identified and authenticated by Prof. Jayaraman, Director, Plant Anatomy Research Center (PARC) Chennai, Tamilnadu. The leaves were cleaned, dried and pulverised into coarse powder using grinder. The

drug was stored in air tight container to prevent moisture and used for further studies.

Pharmacognostical studies

Microscopy

The small quantity of leaves were fixed in FAA (Formalin-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.

The 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 done [8] and the sections were stained with Toluidine blue [9]. 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).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [10] were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.

Extraction of Plant Material

The leaf powder of the plant is extracted with 95 % ethanol and water by using Soxhlet apparatus and maceration technique respectively. After extraction the extracts were concentrated under reduced temperature and the percentage yield were calculated with respect to air dried material.

Qualitative Phytochemical Analysis

Ethanol and aqueous extracts of *Clerodendrum phlomidis* leaves were subjected to preliminary phytochemical screening for the presence or absence of various bio active components like alkaloids, flavonoids, terpenoids, saponin, Tannin, carbohydrates using standard methods [11].

Hptlc Studies

HPTLC analysis of aqueous and ethanolic leaf extracts of Clerodendrum phlomidis was carried out using CAMAG HPTLC system with Linomat V sample applicator and a TLC scanner equipped with WINCATS -4. Software for the interpretation of chromatogram. Silica gel 60 F254 HPTLC plate of uniform thickness 0.2mm was used as a stationary phase and Toluene, ethyl acetate, formic acid (5:4:1) was used as a mobile phase. The plate was developed using CAMAG twin trough chamber. About 10µl of aqueous and ethanolic extracts were prepared and applied on the silica gel using sample applicator. The plate was developed in the solvent system to a distance of 8cm.The plate is observed under UV light at 254nm using CAMAG REPROSTAR [12].

Results

Pharmacognostical studies

Morphology: Leaves are simple, petiolate, opposite, exstipulate, sub rhomboid, obtuse or acute, dentate or entire (fig 1.2)

Microscopy

The anatomical studies of the leaf showed the presence of thick midrib and lamina. The midrib is planoconvex with flat adaxial side and convex semi circular abaxial side (Fig.2.1.) The midrib is 650 µm thick. The adaxial epidermis of the midrib consists of small, less prominent squarish cells. The abaxial epidermis is thick walled, squarish and more distinct (Fig 2.3). The ground tissue in the adaxial part includes about four layers of thick walled cells, the abaxial part has angular thin walled parenchyma cells (Fig 2.3).

The vascular system of the midrib consists of a large, bowl shaped main vascular bundle and on the adaxial part are three, small clusters of vascular strands. (Fig 2.4). The larger bundle consists of large clusters of circular, highly thick walled liquefied xylem elements. The protoxylem are directed adaxially (Fig 2.4). The phloem is situated on the lower part of the xylem. It includes prominent sieve elements and companion cells. The adaxial smaller bundles consists of a small cluster xylem elements with an arc of phloem elements.

The lateral vein is smaller in size measuring 100 µm thick. It consists of a small less prominent adaxial cone and wide and thick abaxial part. The adaxial epidermis has larger, squarish thin walled cells and the small thick walled abaxial epidermal cells. (Fig.2.1). The vascular bundle is triangular in outline; it is simple and prominent. This colateral bundle has upper vertical rows of small xylem elements and lower are of phloem elements.

Lamina (Fig 2.5) is smooth on both adaxial and abaxial surfaces. The lamina is 230 µm thick. The adaxial epidermal cells are large squarish cells with thick cuticle. The abaxial epidermal cells are smaller, rectangular or squarish with thick cuticle. The mesophyll tissue is differentiated into adaxial, two layers of wide short palisade cells and abaxial zone of angular thick walled compact spongy parenchyma cells.

Occasionally glandular trichomes are seen on the epidermis. The trichome is umbrella shaped (Fig.2.5). The gland is embedded within a shallow cavity of the epidermis. It has a short wide stalked cells and semi circular glandular head. The glandular trichome is 30 µm in height. The stomata are on the adaxial epidermis. They are at the level of the epidermis (Fig 2.6).

Powder microscopy

The powder preparation was examined under the microscope and the following inclusions were observed. Long, narrow fibres are occasionally seen in the powder

(Fig.3.1). The fibres are thick walled and lignified. The cell lumen is narrow. Pits on the lateral walls not evident. The fibre is gradually tapering at the ends. The fibres are up to 610 µm, thickness is 15 µm. Non glandular trichomes (Fig.3.2) were fairly common in the powder and were found to be multicellular, uniseriate unbranched. The trichomes are three celled. The basal cell is broad and funnel shaped. The terminal cell is tapering into pointed end. The cell wall is thick and cell lumen is broad. No cell inclusions are seen. The trichomes are 140 µm to 170 µm long. The basal cell is 30-40 µm wide. In case of epidermal cells, the epidermal peelings are frequently seen in the powder. The adaxial epidermal cells are polyhedral in outline and have thick straight anticlinal walls. The cells have dense cell contents. (Fig.3.3). Abaxial epidermal peelings are also common in the powder. The epidermal cells are comparatively larger with walls. The anticlinal walls are highly waxy and the cells appear lobed (Fig.3.4). The epidermal layer is densely stomatiferous. The stomata are broadly elliptical measuring 20×30 µm in size (Fig.3.4, 3.5, 3.6). The guard cells are surrounded by five lobed subsidiary cells (Fig.3.6). The stomatal type is cyclocytic type.

Preliminary phytochemical screening

The phytochemical analysis of leaves revealed the presence of flavonoids, tannins, sterols, proteins, saponins and carbohydrates in aqueous extracts and the presence of alkaloids, flavonoids, tannins, sterols, proteins and carbohydrates in ethanolic extract.

Chromatographic studies

The HPTLC finger printing of aqueous extract revealed the presence of 11 phytoconstituents with R_f values of 0.12, 0.24, 0.34, 0.42, 0.47, 0.50, 0.56, 0.60, 0.70, 0.87, 0.98 at 254 nm (fig 4). The HPTLC finger printing of ethanolic extract revealed the presence of nine phytoconstituents having R_f values of 0.06, 0.014, 0.19, 0.29, 0.45, 0.61, 0.74, 0.83, 0.96 at 254nm in (fig 5).

Figures and tables

Fig 1.1 *Clerodendrum phlomidis* Fig 1.2 a twig with leaves

Fig 1: Leaves of *Clerodendrum phlomidis*

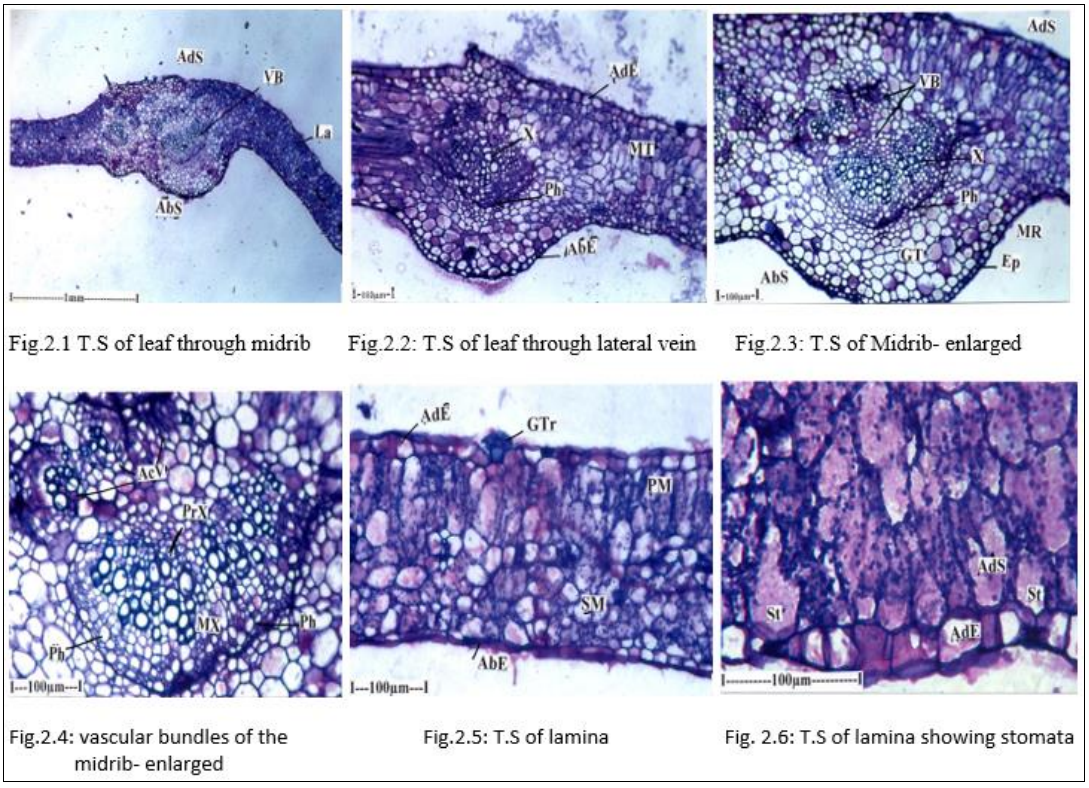


Fig.2.1 T.S of leaf through midrib Fig.2.2: T.S of leaf through lateral vein Fig.2.3: T.S of Midrib- enlarged

Fig.2.4: vascular bundles of the midrib- enlarged Fig.2.5: T.S of lamina Fig. 2.6: T.S of lamina showing stomata

Fig 2: Microscopical studies of *Clerodendrum phlomidis Linn.F.suppl.*

Legend: Abs-Abaxial side; Ads-Adaxial side; L.a – lamina; VB-vascular bundle, AbE-Abaxial epidermis; AdE-Adaxial epidermis; MT-Mesophylic tissue; Ph-Phloem;; x-xylem., AV- Accessary vascular bundles; Ads- Adaxial side; Ep-Epidermis; GT-Growth tissue; MR-Midrib; Mx-Meta xylem; Prx-Protoxylem; VB-vascular bundle; x-xylem; GTr-Glandular trichome; PM-palisade mesophyll; SM-spongy mesophyll; St- stoma.

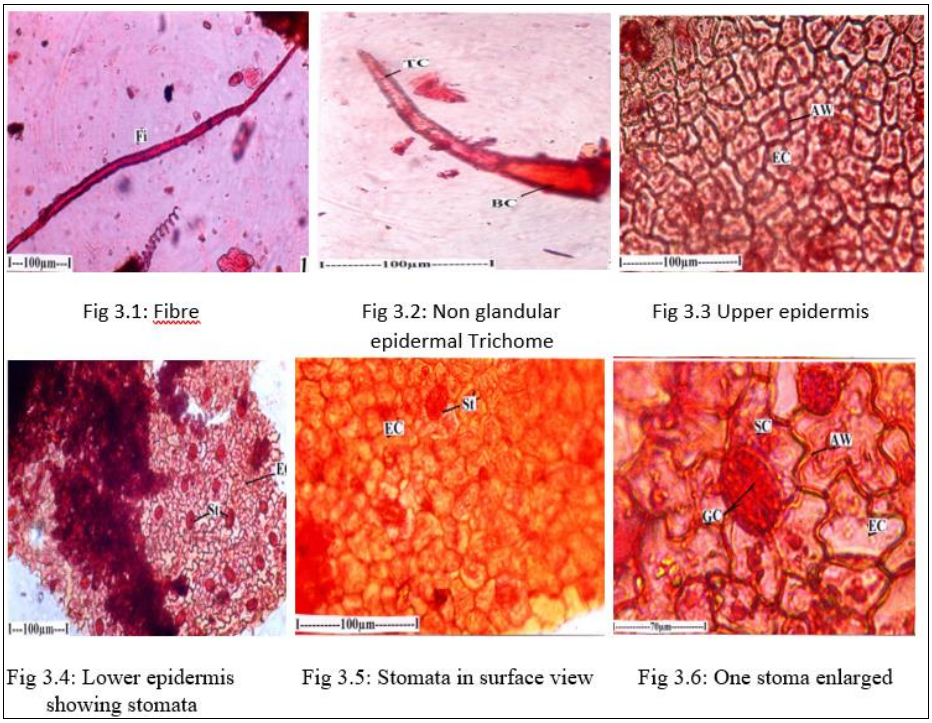


Fig 3.1: Fibre | Fig 3.2: Non glandular epidermal Trichome | Fig 3.3 Upper epidermis

Fig 3.4: Lower epidermis showing stomata | Fig 3.5: Stomata in surface view | Fig 3.6: One stoma enlarged

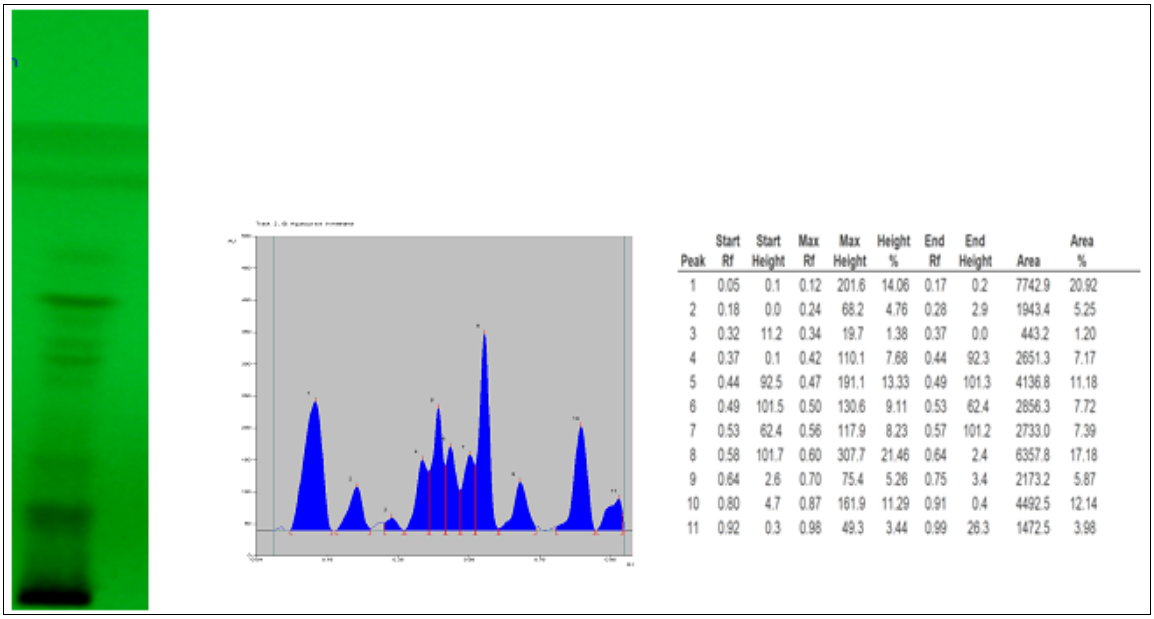
Fig 3: Powder Microscopy of *Clerodendrum phlomidis* leaves

Legend: GTr-Glandular trichome; PM- palisade mesophyll; SM- spongy mesophyll; St-stoma, BC-Basal cell, F-Fibre, TC-terminal cell, AW- Anticlinal wall, EC- epidermal cell, ST-stoma, GC-Guard cell, SC-Subsidiary cells.

Table 1: Preliminary Phytochemical analysis of *Clerodendrum phlomidis*

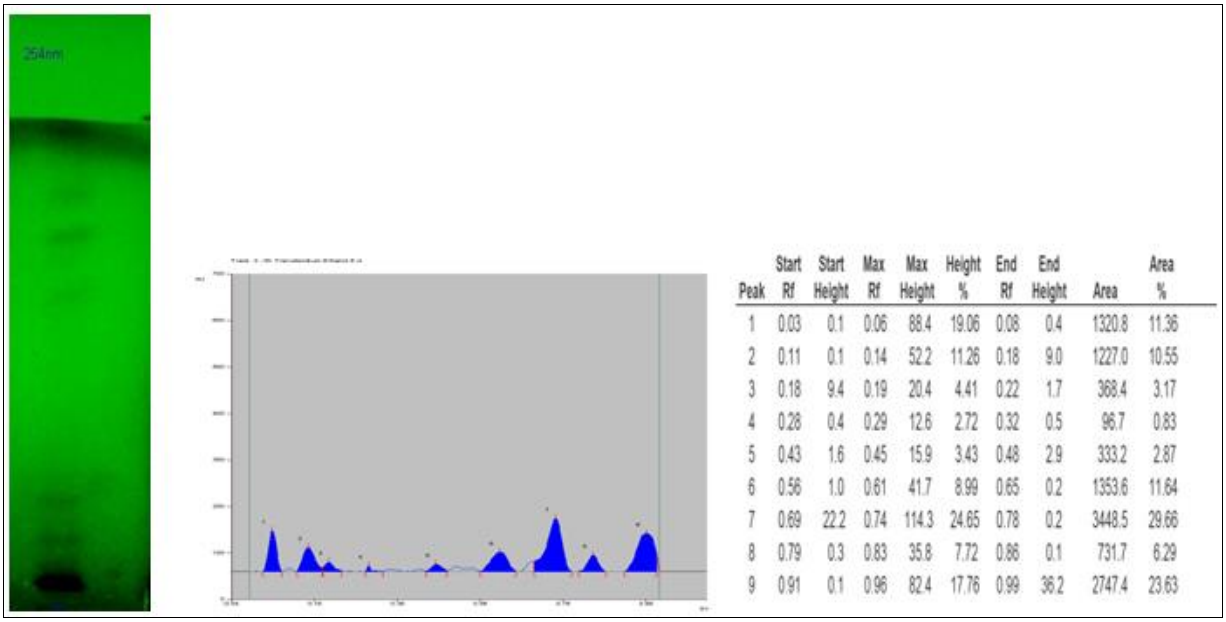
S.No	Phyto constituents	Aqueous extract	Ethanol extract
1	Alkaloids	-	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Sterols	+	+
5	Terpenoids	-	-
6	Glycosides	-	-
7	Starch	-	-
8	Proteins	+	+
9	Saponins	+	-
10	Carbohydrates	+	+

(+) present (-) Negative



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.05	0.1	0.12	201.6	14.06	0.17	0.2	7742.9	20.92
2	0.18	0.0	0.24	68.2	4.76	0.28	2.9	1943.4	5.25
3	0.32	11.2	0.34	19.7	1.38	0.37	0.0	443.2	1.20
4	0.37	0.1	0.42	110.1	7.68	0.44	92.3	2651.3	7.17
5	0.44	92.5	0.47	191.1	13.33	0.49	101.3	4136.8	11.18
6	0.49	101.5	0.50	130.6	9.11	0.53	62.4	2856.3	7.72
7	0.53	62.4	0.56	117.9	8.23	0.57	101.2	2733.0	7.39
8	0.58	101.7	0.60	307.7	21.46	0.64	2.4	6357.8	17.18
9	0.64	2.6	0.70	75.4	5.26	0.75	3.4	2173.2	5.87
10	0.80	4.7	0.87	161.9	11.29	0.91	0.4	4492.5	12.14
11	0.92	0.3	0.98	49.3	3.44	0.99	26.3	1472.5	3.98

Fig 4: HPTLC Fingerprinting Profile of aqueous extract for flavonoids with the densitogram and corresponding Rf values at 254 nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.03	0.1	0.06	88.4	19.06	0.08	0.4	1320.8	11.36
2	0.11	0.1	0.14	52.2	11.26	0.18	9.0	1227.0	10.55
3	0.18	9.4	0.19	20.4	4.41	0.22	1.7	368.4	3.17
4	0.26	0.4	0.29	12.6	2.72	0.32	0.5	96.7	0.83
5	0.43	1.6	0.45	15.9	3.43	0.48	2.9	333.2	2.87
6	0.56	1.0	0.61	41.7	8.99	0.65	0.2	1353.6	11.64
7	0.69	22.2	0.74	114.3	24.65	0.78	0.2	3448.5	29.66
8	0.79	0.3	0.83	35.8	7.72	0.86	0.1	731.7	6.29
9	0.91	0.1	0.96	82.4	17.76	0.99	36.2	2747.4	23.63

Fig 5: HPTLC Fingerprinting Profile of ethanolic extract for flavonoids with the densitogram and corresponding Rf values at 254 nm

Discussion

Under anatomical studies the transverse section of *Clerodendrum phlomidis* leaves clearly depicted the presence of planoconvex midrib, smooth lamina, collateral vascular bundles, umbrella shaped glandular trichomes and the powder microscopy revealed the presence of fibres, non glandular trichomes and cyclocytic stomata which helps in the identity of the leaves. The phytochemical analysis showed the presence of Flavanoids, Tannins, Sterols, carbohydrates and proteins in both aqueous and ethanol extracts and Terpenoids, Glycosides, starch were found to be absent in both the extracts. The alkaloids were present in ethanol extract but absent in aqueous extract and in the case of saponins, it was present in aqueous extract but absent in ethanol extracts. These results were found to be similar with those of other researcher in similar plants [13, 14, 15, 16, 17, 18]. Flavonoids are the most important secondary metabolites present in these plant species [19]. The bands eluted in the finger printing profile represents the presence of different types of flavonoids. HPTLC is the best method to check the quality, stability, purity and identity of the plant derived drug, the finger printing profile will help to identify the subclasses of flavonoids which are of great interest in the alleviation of diseases. Various classes of flavonoids are found to have biological activities such as anti oxidant, anti inflammatory, antibacterial, anti-viral, immune modulatory activity etc [20].

Conclusion

Standardisation of crude drugs is a complex task, as the plant products are heterogenous in their composition. Appropriate Quality control of the starting material is vital in ayurvedic preparation. The pharmacognostical and phytochemical analysis of the leaves of *Clerodendrum phlomidis* is the basic etiquette for assessing the quality of the drug and future studies should focus on the fractionation of chemical constituents of leaves and look out for unexplored traditional uses of *Clerodendrum phlomidis* leaves.

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