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Research Article

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Phytochemical and pharmacognostical investigation of Agnimantha (*Clerodendrum phlomidis* Linn. f.)-root

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ABSTRACT

Clerodendrum phlomidis Linn. f. (Family- Lamiaceae) is a large shrub about 2-3 m high with profuse branching, sometimes attaining a height of 6 m to become a small tree. Branches and twig hairy, lighter in weight when dried. Leaves simple, opposite, petiolate, flowering throughout the year and fruiting September to March in particular. The present communication provides a detailed account of the pharmacognostic evaluation carried out on Agnimantha root. The study includes macro and microscopic characters, preliminary phytochemical analysis, fluorescence study, physicochemical parameters and HPTLC fingerprinting aspects. Established parameters can be used as standards for quality control and identification of the plants in compound formulations and also preparation of a monograph of the plant.

Keywords: Agnimantha, HPTLC fingerprinting, Phyto-chemical investigation, Pharmacognostic evaluation, Microscopy

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INTRODUCTION

Clerodendrum phlomidis Linn. f. (Family- Lamiaceae) is a large shrub about 2-3 m high with profuse branching, sometimes attaining a height of 6 meter to become a small tree. Branches and twig hairy, lighter in weight when dried. Leaves simple, opposite, petiolate, flowering throughout the year and fruiting September to March in particular [1]. Nishteswar and Hemadri, 2010). Agnimantha is generally grown throughout India, by farmers as hedge plant around cultivated fields and orchards. Sometimes it is planted in gardens and backyards for its sweet-scented flower. The plant is distributed in Sri Lanka also. Various part of the Agnimantha have been used as traditional Ayurvedic medicine in India. Agnimantha leaves juice and decoction and root powder have been therapeutically used as folk medicine to control skin disorder, neuralgia, gonorrhoea, hyperacidity, cough, asthma, anaemia and constipation and also a general health promoter. It is use for the treatment of rheumatism, chronic syphilitic sores and urinary disorders. Warm poultice of leaf applied on hydrocele or swelling in testes, juice of leaves used in scrofula, veneral disease, black spots of face is removed by applying root paste or grind the root in goat milk and apply [2-5]

Despite the numerous medicinal uses attributed to this plant, there are no systematic pharmacognostical studies on the seed of this plant have so far been carried out. Hence, the present work deals with the morphological, anatomical evaluation, physicochemical tests, preliminary pytochemical screening and HPTLC fingerprint profile of

Clerodendrum phlomidis Linn. f. which could serve as a valuable source of information and provide suitable standards for the further identification of this plant.

EXPERIMENTAL SECTION

Collection and processing of plant material-
The root of *Clerodendrum phlomidis* were collected from village Bhaganpur District Chitrakoot (U.P.) India. The plant was identified and authenticated. The voucher specimen (AD/AS/118/2016) maintained in the herbarium of Department of Pharmacognosy, Ayurveda Sadan (Research Laboratory), Deendayal Research Institute Chitrakoot for further reference. Root were cleaned, dried and grind use in a mill grinder to make a fine powder. Finally the drug was stored at air tight container to prevent moisture and used for further experimentation.

Macroscopy
Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated.

Powder microscopy
The dried root were subjected to powdered and completely passes through 355 μm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 μm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerin, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, about 1 g of powder warmed over water bath with 50% con. Nitric acid till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope [6].

Physico-chemical parameters
Physico-chemical parameters such as moisture content (loss on drying at 1050C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were calculated [7].

Determination of moisture content (Loss on drying)
The moisture content of the drug is important to determine as it play role in prevention role of microbial growth in the drug powder. Procedure set from here forth determine the amount of volatile matter. A define amount of drug powder, without preliminary drying, was allowed to dried for 5 hours temperature at 1050 C, cooled and weighed; it was again dried for 30 minute cooled and weighed. Then the percentage of moisture content was calculate with respect to the air dried drug.

Determination of alcohol soluble extractive-
For alcohol soluble extractive, macerated the air dried drug with alcohol in a closed in a closed flask for 24 hours, shaking frequently during first 6 hours and allowed to stand for 18 hours. Then the solution was filtered and 25-30 ml of filterate was evaporated to dryness in a tetrad flat bottomed shallow dish and dried and weighed.

Determination of water soluble extractive-
Water soluble extractive was determined in the same way. Instead of alcohol, water was used as solvent . Then calculated the percentage of alcohol-soluble and water soluble extractive with respect to the air dried drug.

Determination of total ash-
Incinerated about 2g of accurately weighed drug in a crucible at a temperature up to 4500 C. Until free from carbon. Then it was cooled in a desiccator and weighed. Then the percentage of total ash was calculated with respect to air dried drug.

Determination of acid ash soluble-
To the crucible total ash, 25 ml of dilute HCL added and boiled. Then the solution was filtered through an ashless filter paper and the insoluble matter was collected on the filter paper. It was washed with hot distilled water until the filtrate is neutral. Then the filter paper containing the insoluble matter was dried, transferred to the original crucible and ignited to constant weight. Then the residue was cooled in a desiccators for 30 minutes and weight without delay. Then the percentage of acid-insoluble ash was calculated with respect to the air-dried drug

Preliminary phyto-chemical investigation
Preliminary phyto-chemical tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavanoids, tannins, carbohydrates, proteins and saponins [8-9].

Preparation of Extracts-
The fine powder, 2 g of powder was dissolved separately in 100 ml commercially available pure ethanol. The solution was kept at room temperature for 18 hours to allow the extraction of compounds from root with occasional shaking. The solution was filtered through Whatman filter paper no.1 and a yellowish colour was obtained. The solvent was evaporated and the substance was obtained was stored in the refrigerator prior to use.

1. Alkaloids-
Wagner's test- 1 ml of alcoholic extract was acidified with HCL (v/v) and few drop of Wagner's reagent was added to it. Presence of alkaloid is indicated by formation of yellow or brown coloured precipitate.

2. Tannin-
Lead acetate test- To the filtrate, a few drops of aqueous basic lead acetate solution were added. Reddish brown bulky precipitate indicates the presence of tannins.
Ferric chloride test- To the filtrate, a few drops of ferric chloride solution were added. A blackish precipitate indicates the presence of tannins.

3. Carbohydrate-
Fehling's test- Take 2 ml of aqueous extract of the drug add 1 ml of mixture of equal part of Fehling's solution "A" and "B" and boiled the contents of the test tube for few minutes A red or brick red precipitate is formed.
Molish test- In a test tube containing 2 ml of aqueous extract of the drugs add 2 drops of a freshly prepare 20% alcoholic solution of nepthal and mix pour 2 ml conc. Sulphuric acid so as to from a layer below the mixture. Carbohydrate it present produced a red violet ring which disappears on addition of an excess of alkali solution.
Protein test-
Biuret test- 1 ml of hot aqueous extract, 5-8 drops of 10% NaOH solution was added followed by 1-2 drop of 3% copper sulphate. A violet colour indicated the presence of proteins.
Millon's test- Dissolve small Quantity of aqueous extract of drug in 1 ml of distilled water and add 5-6 drops of millon's reagent. A white ppt. is formed which turns red on heating.

4. Saponins- 5 ml of aqueous extract, added a drop of sodium bicarbonate was added and shaken vigorously and left for few minutes. Formation of honey comb like froth indicates the presence of saponins.

5. Flavonoid
Shinoda's test- To 1 ml of alcoholic extract, 0.5g of magnesium ribbon or magnesium foil was simultaneously added with a few drops of conc. HCL. Change in colour (from red to pink) shows the presence of flavonoids.
Fluorescence Studies
The fluorescence response of powdered drugs exposed to UV radiation (254 nm and 366nm wavelength) was studies using the standard procedure [10].

High Performance Thin Layer Chromatography (HPTLC) fingerprint profile
For HPTLC, the powdered root 2 gm of sample was extracted with 50 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F254 (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of *Toluene* : *Ethyl acetate: (7:3* v\v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spots were made before and after derivatization (with 5% *Methanolic- sulphuric acid* reagent) at 254nm and 366nm with Win cat software and Rf values noted [11-14].

RESULTS AND DISCUSSION

Macroscopic characters

The *Agnimatha* root colour is brownish white, odour pleasant and appreciable taste.

Powder microscopic characters

Under microscope examined powder shows Starch grains and prismatic crystals of calcium oxalate, Reticulate thickening, Simple pitted vessel, Longitudinally cut fragment of medullary ray, Sclereids, Cork cells and fibres (Plate 1).

Table1. Physico chemical analysis of *Clerodendrum phlomidis*

S .No.	Tests	Result
1	Loss on drying (LOD	4.02%
2	Water Soluble Extractive Value (WSE	15.66%
3	Alcohol soluble extractive value	3.52%
4.	Total ash value	9.46%
5.	Acid insoluble ash value	3.14%

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in table 1.

Preliminary phyto-chemical investigation

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The result are given in Table 2

Table 2. Phytochemical Analysis Of *Clerodendrum phlomidis* (root)

S. No.	Name of experiment	Result
1	Alkaloids	Present
2	Carbohydrates	Absent
3	Protein	Present
4	Saponin	Present
5	Flavonoids	Present
6.	Starch	Absent

Fluorescence Study of Roots of *Clerodendrum phlomidis*

Fluorescence result are given in table 3

Table 3. Fluorescence Study of Roots Of *Clerodendrum phlomidis*

S.No.	Drug powder+Chemical	Observation in day light	Observation in 366nm
1.	*Drug powder*	*Light yellow*	*Creamish white*
2.	*Powder+Acetic acid*	*Light yellow*	*Cream color*
3.	*Poder+50% KOH*	*Turmeric yellow*	*Greenish white*
4.	*Powder+1N HCL*	*Carrot redish*	*Brown*
5.	*Powder+1N NaOH water*	*Pale yellow*	*Greenish yellow*
6.	*Powder+ H2SO4*	*Black*	*Dark green*
7.	*Powder+Iodine water*	*Cream color*	*Greenish yellow*
8.	*Powder+1N NaOH methyl*	*Yellow*	*Yellowish green*
9.	*Powder+50%H2SO4*	*Light yellow*	*Greenish brown*
10.	*Powder+50%HNO3*	*Dark yellow*	*Dark brown*

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the TLC plate. Major spots Rf values with colour were recorded under, 254nm, 366nm, after derivatization 366nm. Chromatogram profile and Rf values are given (Plate 2 & Table 4).

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify *Clerodendrum phlomidis* root. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate's important phyto-constituents. These finding could be helpful in identification and authentication.

Table 4. Rf Values of HPTLC fingerprints profile of *Clerodendrum phlomidis*

Rf values	Before derivatization		After derivatization	
	At 254nm	At 366nm	**At 366nm**	**UV light**
Rf1	0.29(black)	0.29(sky blue)	0.30(brown)	0.51(blue)
Rf2	-	0.49(sky blue)	0.49(brown)	0.78(blue)
Rf3	-	0.66(sky blue)	0.78(brown)	-
Rf4	-	0.79(red)	0.88(light blue)	-

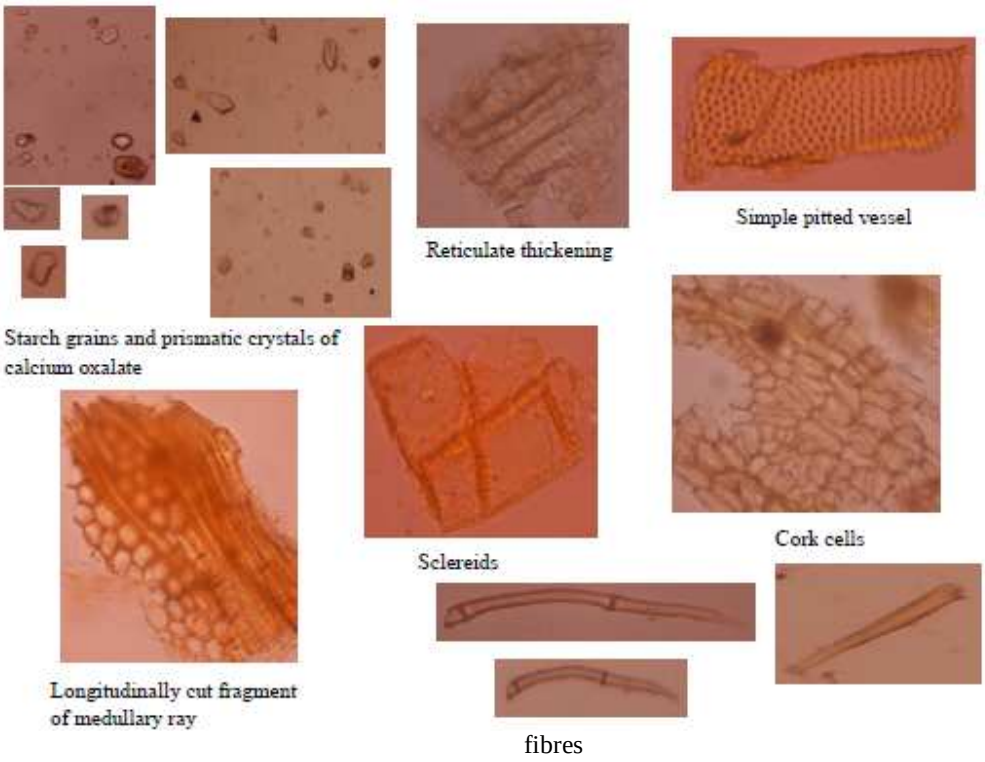
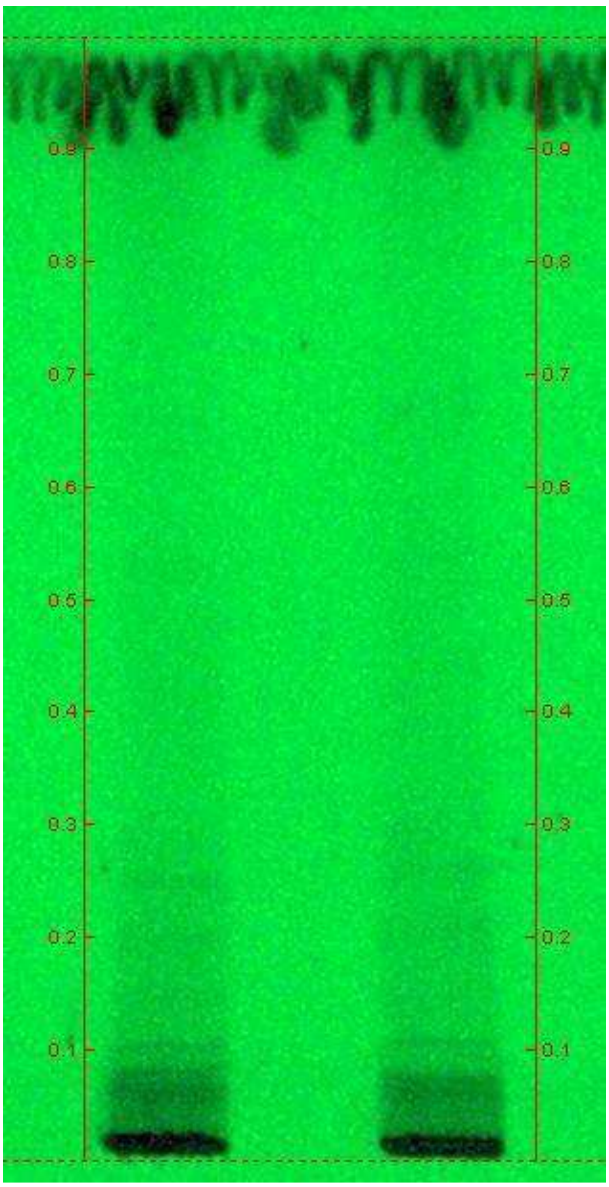
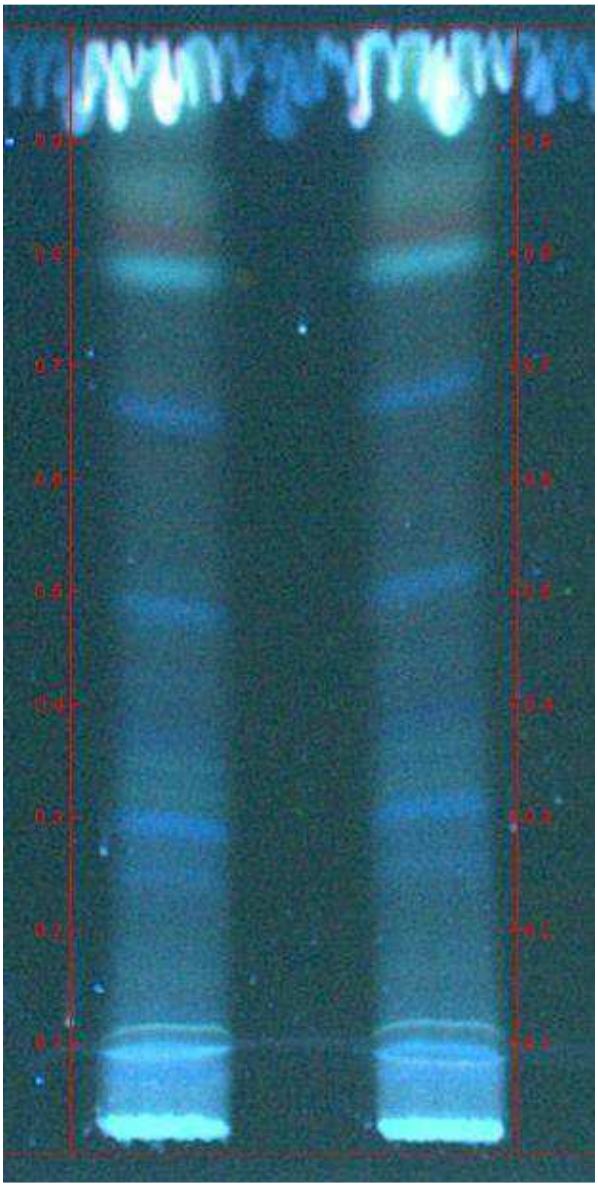


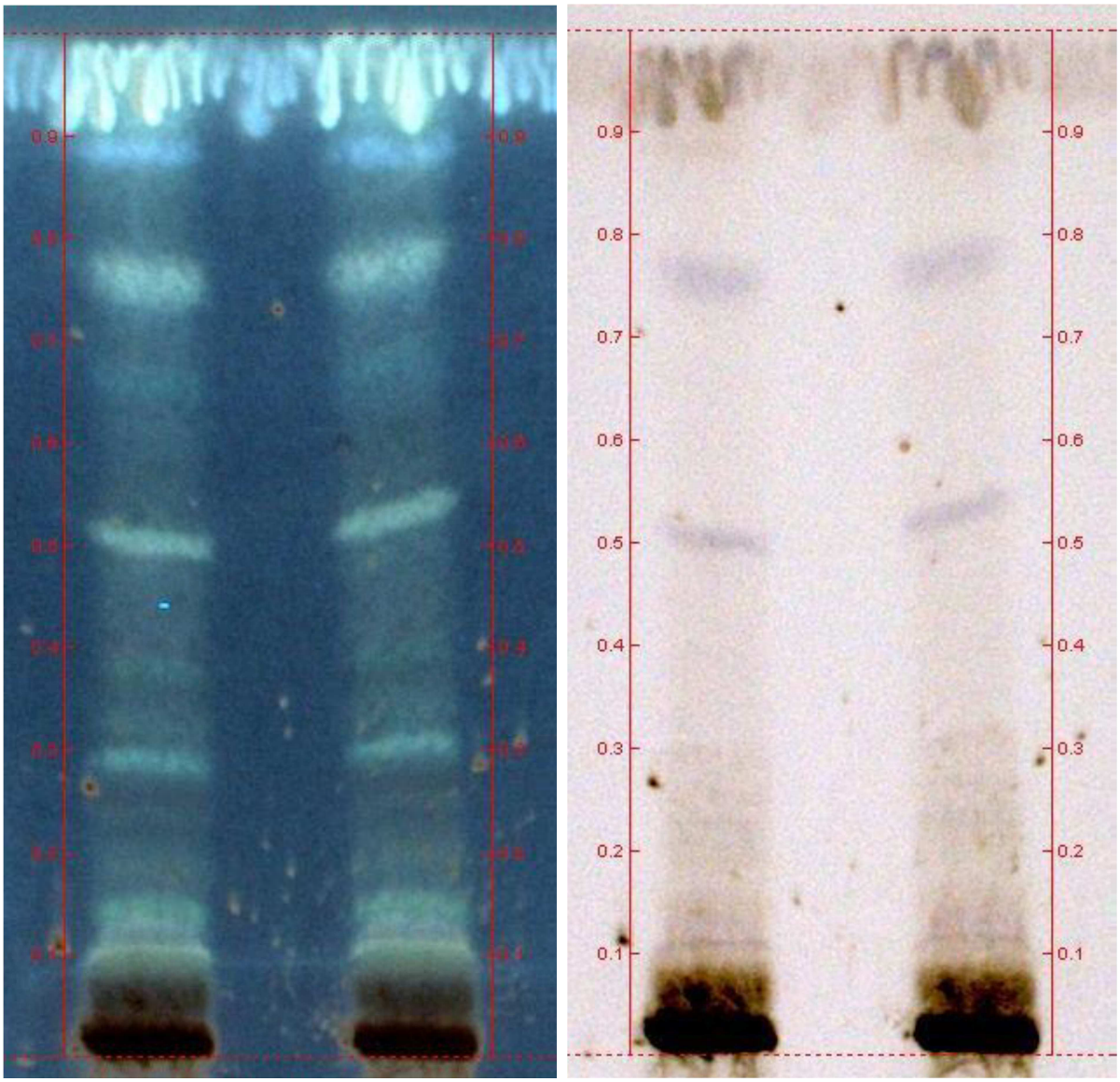
Plate 2. HPTLC Fingerprints of *Clerodendrum phlomidis* (root)



254nm before derivatization



366 nm before derivatization



366nm after derivatization **UV light after derivatization**

CONCLUSION

The macroscopic, microscopic and powder microscopic dignostic features have been established to identify *Chlerodendrum phlomidis* (root). The pharmacognostic and physico-chemical parameters can be used for checking the adultration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug here by substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate important phyto-constituents. These finding could be helpful in identification and authentication.

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