



## Comparative Pharmacognostic Study of *Clerodendrum phlomidis* and *Premna integrifolia*

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### Abstract

**Objective:** A comparative study was designed to develop physico-chemical parameters for roots of *Clerodendrum phlomidis* and *Premna integrifolia* (Family: Verbenaceae), commonly known as Arni. **Materials and Methods:** Roots of *C. phlomidis* and *P. integrifolia* were studied for macro and microscopical characters. Clerodendrin-A, a chemical marker was isolated from the root of *C. phlomidis*. HPTLC method was developed to generate fingerprint profiles for the two roots and to quantify isolated clerodendrin-A (in *C. phlomidis* and *P. integrifolia* roots) using n-hexane: ethyl formate (7:3) as a mobile phase, precoated TLC plates (silica gel 60 F<sub>254</sub>) as a stationary phase and H<sub>2</sub>SO<sub>4</sub> as derivatizing agent. **Results and Conclusion:** Morphologically both roots resemble each other except for their color and size. Microscopically they can be differentiated by presence of rhytidoma in roots of *P. integrifolia*. Further, starch grains are found distributed in *C. phlomidis*, only in xylem parenchyma and xylem rays, where as in *P. integrifolia* all tissues except cork show starch. Clerodendrin-A isolated from *C. phlomidis* was also found to be present in *P. integrifolia*. Considering clerodendrin-A as a chemical marker, the present study was designed to develop HPTLC method for generation of distinct chemoprofile and quantification of clerodendrin-A in roots of *C. phlomidis* and *P. integrifolia*. HPTLC study of n-hexane fraction (diterpenoid rich) of the two roots performed using silica gel 60 F<sub>254</sub> as a stationary phase, n-hexane: ethyl formate (7:3) as a mobile phase and H<sub>2</sub>SO<sub>4</sub> as derivatizing agent revealed presence of a clerodendrin-A, a major diterpenoid at R<sub>f</sub> 0.26 (violet color). Clerodendrin-A concentration was found to be 0.073% w/w in *C. phlomidis* and 0.04% w/w in *P. integrifolia*.

**Keywords:** Arni, clerodendrin-A, *Clerodendrum phlomidis*, HPTLC, *Premna integrifolia*, Verbenaceae.

### 1. Introduction

*Clerodendrum phlomidis* (syn.: *Volkameria multiflora*) and *Premna integrifolia* (syn.: *Premna obtusifolia*), the two different plants belonging to family Verbenaceae are described in literature [1,2,3] under the common name of Arni or Agnimantha. Roots of both plants

are considered as Arni / Agnimantha for the preparation of important Ayurvedic formulations visually dashmool kwatha, aristha and churna, chayawanprashavle, valued for the treatment of variety of afflictions [4].

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*C. phlomidis* is a large bush or a small tree, growing in the drier parts throughout India. Roots are valued as tonic, diuretic, febrifuge, anti-diabetic, anti-inflammatory and antitussive [5].

*P. integrifolia* is a large shrub or a small tree distributed on the western sea coast from Bombay to Molucca, Srilanka and the Andaman. Root is used in the treatment of diabetes, chyluria, inflammations, swellings, bronchitis, dyspepsia, liver disorders, piles, constipation and fever [6].

Previous phytochemical studies include report of presence of  $\beta$ -sitosterol and  $\gamma$ -sitosterol, ceryl alcohol, clerodin, clerosterol, clerodendrin-A in root [7] and flavonoids, pectolinarigenin, hispidulin, apigenin and luteolin in flower [8] of *C. phlomidis*. In *P. integrifolia*, alkaloids premnine [9] ganikarine [10] and premnazole alkaloid [11] are reported from roots; while flavonoid luteolin [12], sterols and triterpene [13] are reported from the leaves. Pharmacognostical studies have been limited to leaves of *C. phlomidis* [14]. No reports regarding systematic identification of both the drugs are available. The present communication, deals with development of comparative quality parameters for both *C. phlomidis* and *P. integrifolia*.

## 2. Materials and Methods

### 2.1 Plant material

The roots of *C. phlomidis* were collected from Ayurvedic garden, Gandhinagar, Gujarat and *P. integrifolia* roots were collected from Pharmacognosy garden of Ayurtirth, division of Gujarat Ayurveda University, Timba, Gujarat. Herbarium specimens (LM 139 and 140) were deposited in the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad. After drying the roots were powdered to 60 # separately and stored in airtight containers.

### 2.2 Chemicals

*n*-Hexane, ethyl formate,  $H_2SO_4$ , methanol (AR grade), TLC Aluminum sheets pre-coated with silica gel 60 F<sub>254</sub> (E. Merck).

### 2.3 Pharmacognostic study

Thin sections and powdered materials were studied and cell contents and wood elements measurements were carried out according to the specifications of Evans [15]. Ash values and extractive values were determined as per the method given in Ayurvedic Pharmacopoeia [16]. The roots were subjected to preliminary phytochemical analysis [17].

### 2.4 Isolation and identification of clerodendrin-A

200g of root powder (*C. phlomidis*) was extracted with 500ml methanol exhaustively. Methanolic extract was concentrated till it retained a clear consistency and made aqueous by adding water (10% of total volume of the extract) and then extracted with *n*-hexane (3 x 100ml) to yield 0.175% of yellowish brown solid. 500mg of this solid was loaded on a glass column (60cm x 3cm) using silica gel (40g) as a stationary phase. Gradient elution was performed using *n*-hexane containing increasing amounts of ethyl acetate. The fractions, each of 10ml elutes were collected and monitored simultaneously on TLC plate using silica gel as a stationary phase and *n*-hexane: ethyl formate (7:3) as mobile phase.

The fractions eluted with *n*-hexane: ethyl acetate (80:20) showing only one spot on TLC [*n*-hexane: ethyl formate (7:3);  $R_f = 0.25$ ] were pooled and evaporated to dryness at  $25 \pm 2^\circ C$ , yielded fine white needles. Chromatographically pure compound was obtained by this method and was recrystallized from ethanol (95%) to yield 25mg of pure clerodendrin-A. The purity was confirmed by TLC and identity was

confirmed by comparing data of MP, IR and Mass spectral (using LCMS) analysis with the data given in literature [18].

*2.5 Estimation of clerodendrin-A in roots of C. phlomidis and P. integrifolia by HPTLC method:*

*Instrument:* CAMAG LINOMAT IV (semi automatic spotting device) equipped with Camag TLC Scanner 3 and Camag CATS 4 integration software

*Stationary Phase:* precoated TLC plates of silica gel 60 F<sub>254</sub> (Merck)

*Mobile phase:* *n*-hexane: ethyl formate (7:3)

*Spray reagent:* 5% aqueous H<sub>2</sub>SO<sub>4</sub>

*Calibration curve of clerodendrin-A:* Standard stock solution of clerodendrin-A was prepared by dissolving accurately weighed 5mg of clerodendrin-A in 5ml of *n*-hexane in a volumetric flask (1.0mg/ml). A fixed volume of standard solution (1, 2, 3, 4, 5, 6 µl) was spotted. Calibration curve of peak area vs. concentration of clerodendrin-A was plotted.

*Preparation of test solutions:* 5g of dried root powder of both plants were exhaustively extracted using methanol (2 x 25ml). The methanolic extract after concentrating to 15ml was made aqueous by adding 10% water. This 90% aqueous methanolic extract was then extracted with *n*-hexane (3 x 25ml). Hexane soluble fraction was concentrated to 25ml and 20µl of these sample solutions were used for estimation of clerodendrin-A.

After development, the plate was sprayed with 5% aqueous H<sub>2</sub>SO<sub>4</sub> followed by heating at 110°C, the bands were scanned at 396nm.

The method was validated in terms of linearity, precision, repeatability, specificity, limit of detection and limit of quantification.

### 3. Results and Discussion

Morphologically roots of *C. phlomidis* and *P. integrifolia* resemble each other but show different characters microscopically.

*3.1 Diagnostic characters of root of C. phlomidis*

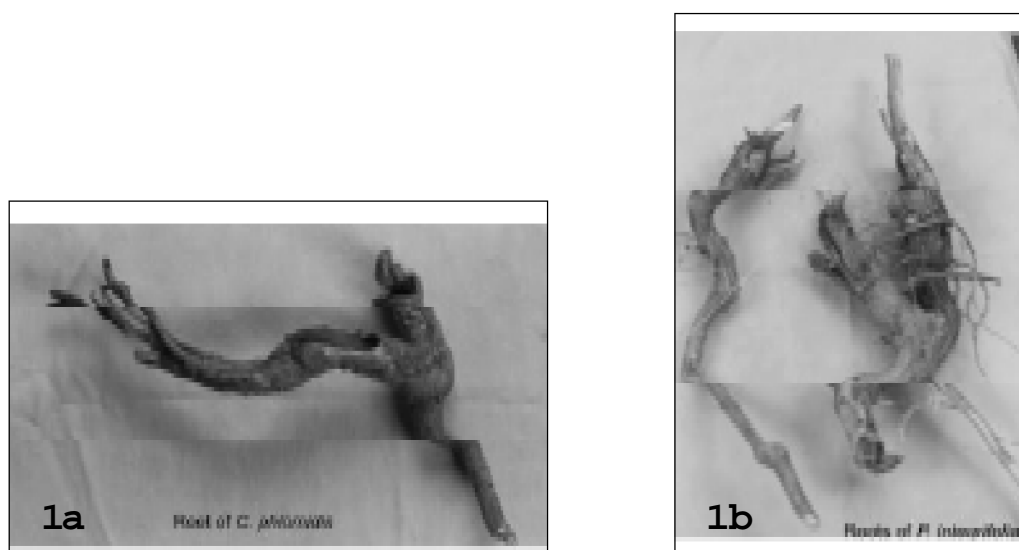
Roots are light brown in color, woody, branched and cylindrical in shape. Outer surface is exfoliated at some places and otherwise shows longitudinal striations and wrinkles. Roots possess bland taste and a slight aromatic odour.

The transverse section shows cork, consisting of 8-10 rows of tangentially elongated and radially arranged suberised cells. Cortex shows two to three discontinuous layers of stone cells (143-200µ) that are thick walled, lignified and lodged with prisms of calcium oxalate. A few cortical cells contain yellowish brown pigment. Phloem shows stone cells with prisms of calcium oxalate. The xylem vessels are of varying size, lignified, found isolated or in the group of 2-3. Medullary rays are 2-3 seriate and the cells are pitted and lignified. Starch is found in wood only.

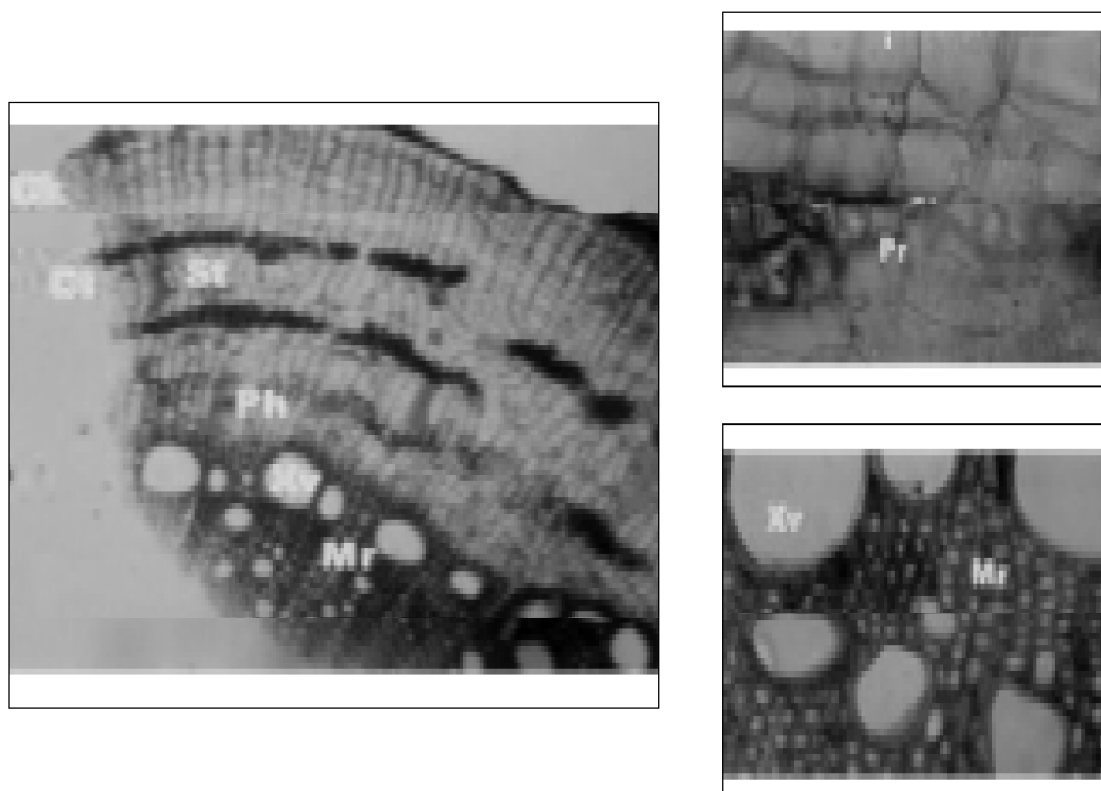
Powdered root is light brown, with slight aromatic odour and bland taste. Starch (8-25µ) is abundant, simple, spherical and cup shaped. Stone cells, rectangular to oblong and lodged with 3-8 prisms of calcium oxalate (12-25µ), which are found scattered also. The vessels (60.25-145.6µ) and fibres of the xylem are lignified and found in the groups of interlocking cells. The vessels are bordered pitted.

*3.2 Diagnostic characters of root of P. integrifolia*

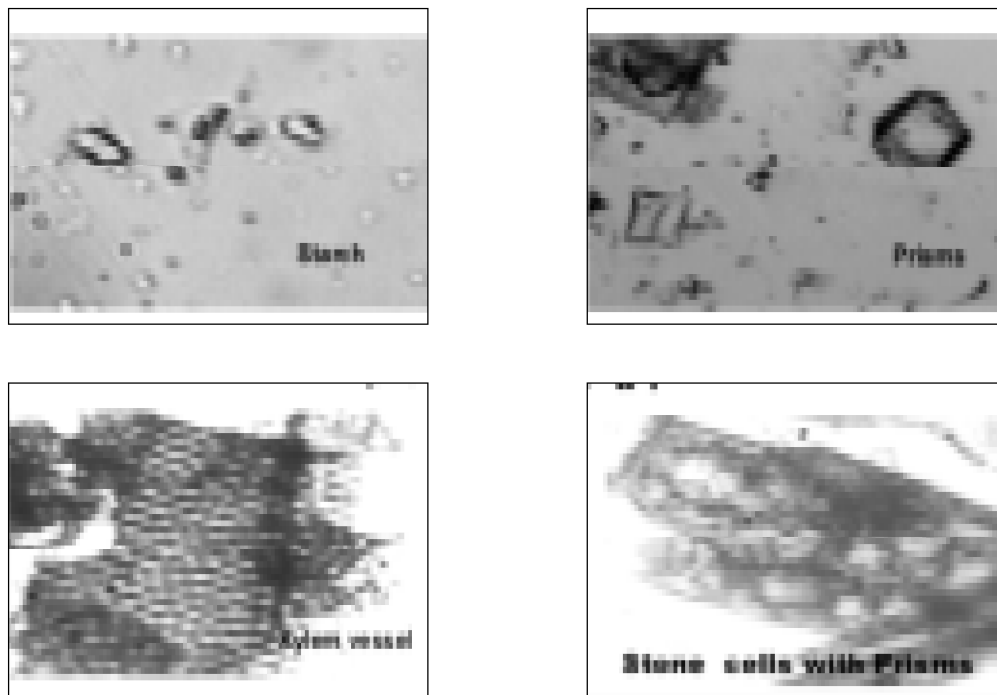
Roots are yellowish brown in color, woody, branched and somewhat tortuous to cylindrical in shape. Surface gets exfoliated easily and shows prominent longitudinal striations and wrinkles. Roots possess bland taste and slightly aromatic odour.



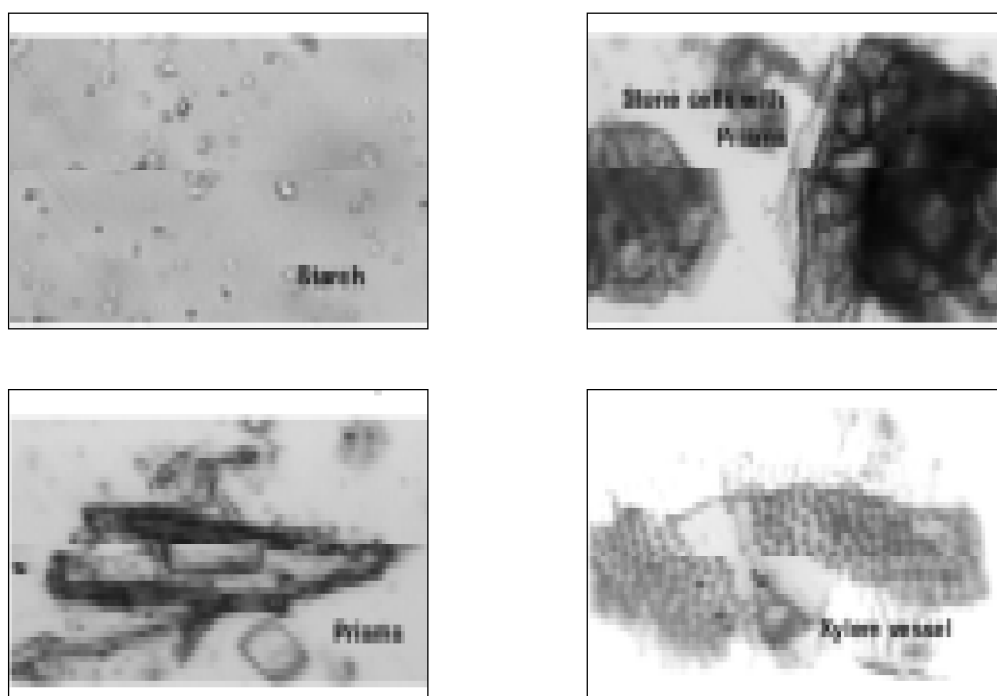
**Fig. 1a.** Root of *C. phlomidis*.  
**1b.** Root of *P. integrifolia*.



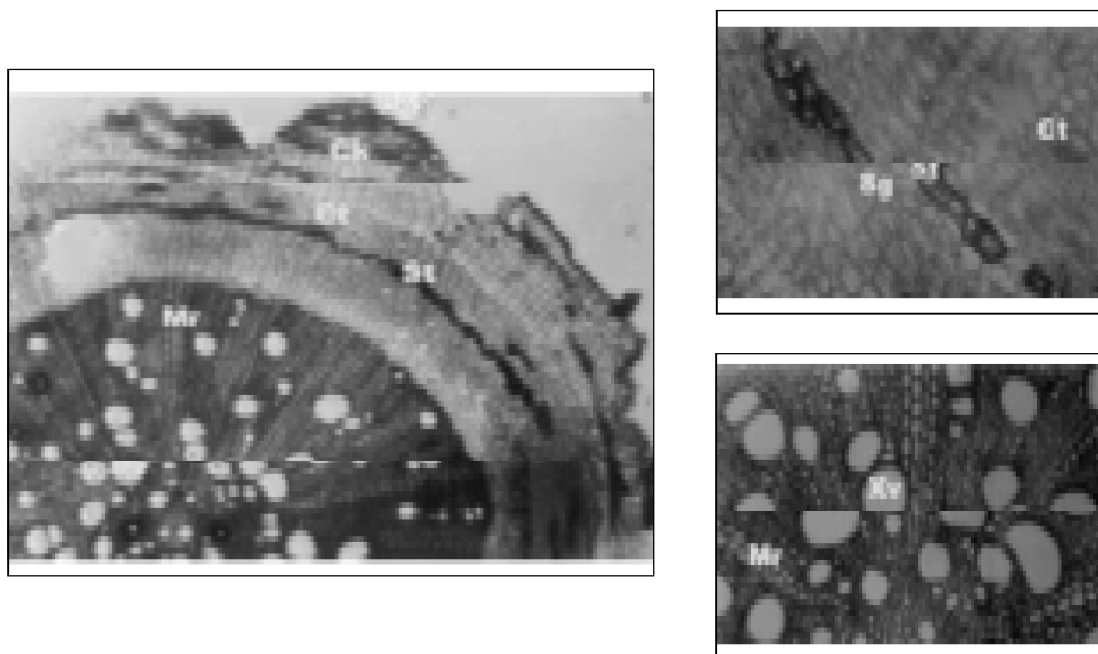
**Fig. 2.** T. S. of *C. phlomidis* root.



**Fig. 3a.** Powder study of *C. phlomidis* root.



**Fig. 3b.** Powder study of *P. integrifolia* root.



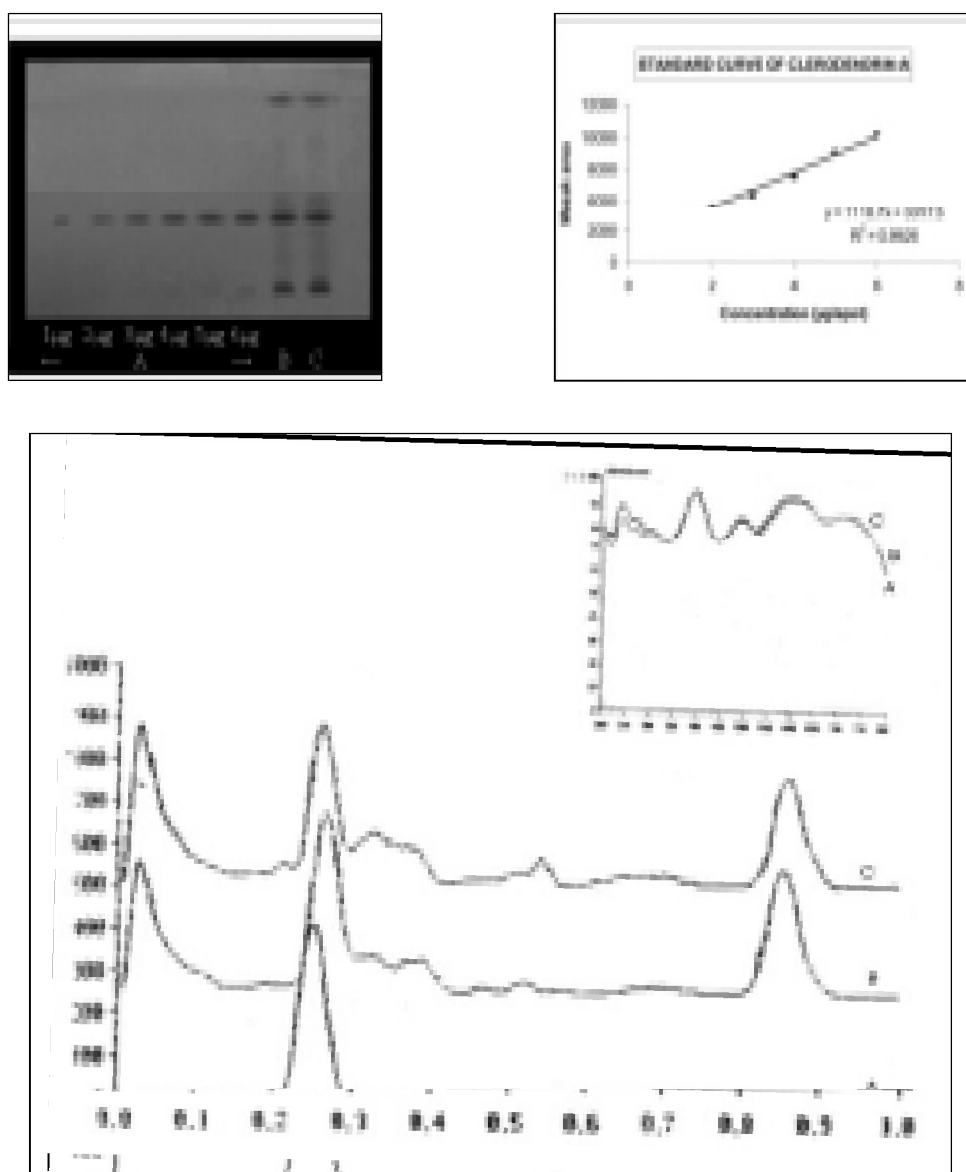
**Fig. 4.** T. S. of *P. integrifolia* root.

**Table 1.** Ash and extractive values of *C. phlomidis* and *P. integrifolia* roots.

Sample	Ash values % w/w			Extractive values % w/w	
	Total Ash	Acid insoluble	Water soluble	Alcohol soluble	Water soluble
<i>C. phlomidis</i>	6.25	0.16	2.3	13.8	14.4
<i>P. integrifolia</i>	8.9	8.2	0.77	2.04	13.8

**Table 2.** Phytochemical screening of *C. phlomidis* and *P. integrifolia*.

Phytoconstituent	<i>C. phlomidis</i>	<i>P. integrifolia</i>
Alkaloids	-ve	+ve
Flavonoids	+ve	+ve
Saponins	+ve	-ve
Sterols, terpenoids	+ve	+ve
Carbohydrates	+ve	+ve



**Fig. 5a.** Co-Chromatography of clerodendrin-A, *n*-hexane fraction of *C. phlomidis* and *P. integrifolia*.  
 b. Calibration curve of clerodendrin-A.  
 c. HPTLC chromatogram of scanned at 396nm.  
 d. HPTLC spectra of clerodendrin-A in *C. phlomidis* and *P. integrifolia* root

**Table 3.** Clerodendrin-A content in roots of *C. phlomidis* and *P. integrifolia*.

Sample	Mean Peak Area (n = 4)	Average amount of clerodendrin-A (µg/spot)	Average % of clerodendrin-A ± S.D.	% C.V.
<i>C. phlomidis</i>	6418.6	2.772	0.073 ± 0.004	5.7
<i>P. integrifolia</i>	4991.5	1.496	0.040 ± 0.003	5.3

**Table 4.** Validation parameters for clerodendrin-A.

Sl. No.	Parameters	Results
	Precision (% C.V.)	0.071
1	* Repeatability of Measurement	0.114
	* Repeatability of Application	0.061 - 0.072
	* Interday	0.060 - 0.075
	* Intraday	
2	Linearity	0.9926
3	Range	1-6 µg/spot
4	Limit of Detection	0.5 µg/spot
5	Limit of Quantification	1 µg/spot
6	Accuracy	97.02 - 99.92 %
7	Specificity	Specific

The transverse section of *P. integrifolia* root shows rhytidoma made up of 15-20 layers of interrupted cork and 2-3 layers of cortex containing small stone cells packed with calcium oxalate prisms. Stone cells are pitted and show thickening on three sides. Inner cork is made up of about 8-10 layers of thin walled tangentially elongated suberised cells. The cortex is made up of collenchymatous parenchyma and shows a single discontinuous layer of elongated lignified, thick walled stone cells (80-125µ) lodged with 3-5 prisms of calcium oxalate (16-30µ). Phloem is comparatively wide and parenchymatous. The elements of wood occur in thin radial wedges. Xylem vessels are small (50-138.1µ) and numerous. Medullary rays are 1-4 seriate, lignified and pitted. Starch (8-30µ) is found in cortex, phloem and xylem.

Powder of root of *P. integrifolia* is brown in color having slight aromatic odor and bland taste. Starch is simple, spherical and cup shaped with distinct hilum. Stone cells are small, rectangular to oblong in shape and lodged with prisms, which are found scattered also. Physical parameters were developed by estimating ash

and extractive values are mentioned in Table 1. Qualitative chemical examination of root powders of both the drugs indicated the presence of alkaloids only in *P. integrifolia*, presence of saponins in *C. phlomidis*. Flavonoids, sterols and terpenoids were found in both of them (Table 2).

### 3.3 Identity of isolated clerodendrin-A

The identity of isolated clerodendrin-A was established by comparing the data of melting point (160-162°C), and IR and LCMS spectral analysis with the data given in literature [18].

**Spectral data:** IR: 3500 -3600 cm<sup>-1</sup>(-OH stretching), 1850 -1870 cm<sup>-1</sup> (C=O stretching), 1710 cm<sup>-1</sup> (C=O stretching), 1620 cm<sup>-1</sup> (CH=CH stretching), 1390 cm<sup>-1</sup> (C-O stretching), 1140 cm<sup>-1</sup> (OH bending).

**LCMS:** The isolated compound showed a molecular ion base peak at the 620 [18].

### 3.4 HPTLC Study

HPTLC study indicated presence of clerodendrin-A in both *C. phlomidis* and *P. integrifolia* roots. Clerodendrin-A resolved at R<sub>f</sub>=0.25, showing a single peak having



absorption maxima at 396nm. The chromatogram further revealed almost similar chemoprofile for *n*-hexane fractions of roots of *C. phlomidis* and *P. integrifolia*. The method of chromatography using *n*-hexane: ethylformate (7:3) as a mobile phase and 5% aqueous H<sub>2</sub>SO<sub>4</sub> as detecting agent gave good resolution of clerodendrin-A without any interference of the other compounds present in root samples of *C. phlomidis* and *P. integrifolia*. Clerodendrin-A was found to be more in *C. phlomidis* roots (0.073% w/w) as compared to that of roots of *P. integrifolia* (0.04% w/w) (Table 3). This is the first report

of histology and clerodendrin-A content of root of *P. integrifolia*. The proposed method was validated in terms of linearity, precision, repeatability, accuracy, specificity and limit of detection (Table 4). The HPTLC method was easy, simple, precise and helps to serve as a handy tool for authentication of roots of *C. phlomidis* and *P. integrifolia*.

#### 4. Acknowledgement

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