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ISOLATION AND ESTIMATION OF CLERODENDRIN A IN *CLERODENDRUM PHLOMIDIS* AND *PREMNA INTEGRIFOLIA* ROOT

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ABSTRACT

Clerodendrin A, a chemical marker was isolated from the root of *Clerodendrum phlomidis* (V*erbanaceae*). We here report first time, the presence of clerodendrin A in another V*erbanaceae* drug, *Premna integrifolia*. Further, HPTLC method was developed to quantify isolated clerodendrin A in *C. phlomidis* and *P. integrifolia* roots using n-hexane: ethyl formate (7:3) as mobile phase, pre-coated TLC plates (silica gel 60 F_{254}) as stationary phase and H_2SO_4 as derivatizing agent. The method was validated in terms of linearity, precision, repeatability and accuracy. The limit of detection and limit of quantification was determined on the basis of signal to noise ratio. Content of clerodendrin A was found to be 0.073 and 0.04% w/w in *C. phlomidis* and *P. integrifolia* respectively. The developed HPTLC method was easy, simple, and precise.

Key words: *Clerodendrin A; Clerodendrum phlomidis; HPTLC; Premna integrifolia; Verbanaceae.*

INTRODUCTION

Clerodendrum phlomidis and *Premna integrifolia*, the two different plants belonging to family *verbanaceae* are described in literature [1-3] under the common name of Arni. Roots of both plants are considered as Arni/ Agnimantha for the preparation of important Ayurvedic formulations like Dashmool kwatha, aristha and churna, Chayawanprashavleh, valued for the treatment of variety of afflictions [4].

C. phlomidis is a large bush or a small tree, growing in the dry region throughout India. Roots are valued as tonic, diuretic, febrifuge, anti-diabetic, anti-inflammatory and anti-tussive [5]. *P. integrifolia* is a large shrub or a small tree distributed in the western ghat. Roots are used in the treatment of diabetes, chyluria, inflammation, swelling, bronchitis, dyspepsia, liver disorders, piles, constipation and fever [5, 6].

Previous phytochemical studies include report of presence of â- sitosterol and ã-sitosterol, ceryl alcohol, clerodin, clerosterol, clerodendrin A in root[7,8] and flavonoids, pectolinarigenin, hispidulin, apigenin and luteolin in flower[9,10] of *C. phlomidis.* In *P. integrifolia*, alkaloids premnine[11], ganikarine[12] and premnazole[13] are reported from roots; while flavonoids luteolin[14,15], sterols and terpenoids[15-18] are reported from the leaves.

The present communication, deals with new method of isolation and estimation of clerodendrin A. Clerodendrin A was isolated from *C. phlomidis* and was observed here to be present in *P. integrifolia* also. HPTLC method was developed to quantify the same in both the roots.

EXPERIMENTAL

Plant material

The fresh, well-developed plants of *C. phlomidis* were collected from the Ayurvedic garden, Gandhinagar in the month of October- 2005 and plants of *P. integrifolia* were collected in the month of September- 2005 from the Pharmacognosy garden of Timba Ayurvedic Pharmacy College, Timba. The authenticity of plants was confirmed by a taxonomist of Gujarat Ayurveda University, Jamnagar. Voucher specimens of both the samples (LM 108 & LM 109) were deposited in L. M. College of Pharmacy, Gujarat. Roots of both plants after drying in the sun were reduced to (60 #) powder separately.

Apparatus

Analytical balance: AB204-S, Metler Toledo, FT-IR – Perkin Elmer, GX-FTIR, LC-MS (Q-TOFF) - LC (WATERS), MS (MICROMASS), a Hamilton 100 ml HPTLC syringe, a Camag Linomat IV (semiautomatic spotting device), a Camag twin-trough chamber, a Camag TLC Scanner 3, a Camag CATS 4 integration software.

Chemicals

n-Hexane, ethyl acetate, ethyl formate of AR grade, TLC Aluminum sheets pre-coated with silica gel 60 F_{254} (E. Merck).

Isolation and characterization of clerodendrin A

200 g of root powder (*C. phlomidis)* was extracted with 500 ml methanol exhaustively. Methanolic extract was concentrated till it retained a clear consistency and was kept in refrigerator (4-8 °C) for a week, which led to deposition of D-mannitol confirmed by co-TLC using standard mannitol. After removing mannitol the extract was made aqueous by adding water (10 % of total volume of extract) and then extracted with n-hexane (3 X 100 ml) to yield 0.175% of yellowish brown solid.

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500 mg of this solid was loaded on a glass column (60 cm X 3 cm) using silica gel (60-120 mesh) as a stationary phase. Gradient elution was performed using hexane containing increasing amounts of ethyl acetate. The fractions, each of 10 ml elutes were collected and monitored simultaneously on TLC plate using n-hexane: ethyl formate (7:3) as mobile phase. The fractions eluted with hexane: ethyl acetate (80:20) showing only one spot on TLC [R_f: 0.25] were pooled and evaporated to dryness at 25 ± 2 °C, yielded fine white needles. The compound was re-crystallized in aqueous ethanol to yield 25 mg of pure clerodendrin A. The purity was confirmed by TLC and identity was confirmed by comparing data of MP, IR and Mass spectra (using LCMS) analysis with the data given in literature[8].

Estimation of clerodendrin A

Preparation of standard solution

Standard stock solution of clerodendrin A was prepared by dissolving accurately weighed 5 mg of clerodendrin A in 5ml of n-hexane in a volumetric flask (1.0 mg/ml).

Preparation of sample solutions

5 g of dried root powders of both plants were exhaustively extracted using methanol (2 X 25 ml). The methanolic extracts after concentrating to 15 ml were made aqueous by adding 10% water. This 90% aqueous methanolic extract were then extracted with n-hexane (3 X 25 ml). Hexane soluble fractions were separated and concentrated to 25 ml and used for present study.

Calibration curve of clerodendrin-A

A fixed volume of standard solution (1, 2, 3, 4, 5, 6 ìl) and sample solutions (n-hexane fraction of ***C.***

phlomidis and ***P. integrifolia*** -20 ml) were spotted and the plate was developed in mobile phase, n-hexane: ethyl formate (7:3). After development, the plate was sprayed with 5 % aqueous H_2SO_4 followed by heating at 110 °C, the bands were scanned at 396 nm. Calibration curve of peak area vs. concentration of clerodendrin A was plotted. After applying suitable dilution factor and comparing peak height and peak area of standard and sample solutions, the amount of clerodendrin A was calculated.

Validation of HPTLC method

ICH guidelines were followed for the validation of the HPLC method. The method was validated in terms of linearity, precision, repeatability and accuracy. The range of the concentration of the clerodendrin A was determined for the linearity, expressed in terms of correlation co-efficient (r^2) of the linear regression analysis. The intra-day precision was determined by analyzing clerodendrin A for three times on the same day. The inter-day precision was determined by analyzing clerodendrin A daily for 5 days. Results were expressed as co-efficient of variance (% CV). Instrumental precision was checked by repeated scanning of the same spot of clerodendrin A seven times and expressed as % CV. The repeatability of the method was affirmed by analyzing 1 µg/spot of clerodendrin A after application on TLC plate (n=7) and was expressed as % CV. Accuracy of the method was determined by performing recovery studies at four levels (50, 100, 150, 200 % addition) and the percent recovery was calculated. The limit of detection (LOD) and the limit of quantification (LOQ) was determined by applying different dilution of standard clerodendrin A along with hexane as blank and determined on the basis of signal to noise ratio.

RESULTS AND DISCUSSION

Characterization of isolated clerodendrin A

The isolated clerodendrin A was identified by comparing the data of melting point (m.p.) (160-162 °C), and IR and LCMS spectral analysis with the data given in literature [8].

Spectral data

IR: 3500 -3600 cm^{-1}(-OH stretching), 1850 -1870 cm^{-1} (C=O stretching), 1710 cm^{-1}(C=O stretching), 1620 cm^{-1} (CH=CH stretching), 1390 cm^{-1}(C-O stretching), 1140 cm^{-1}(OH bending).

LCMS: The isolated compound showed an intense peak at 111.

HPTLC

HPTLC study indicated presence of clerodendrin A in both *C. phlomidis* and *P. integrifolia* roots (Fig.1).

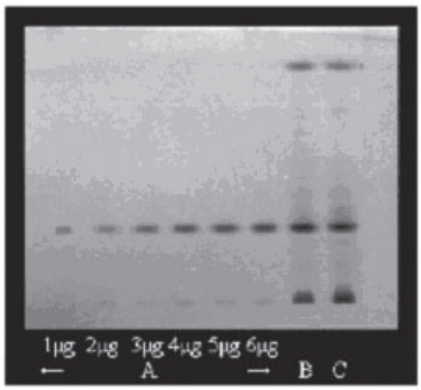

Fig 1 : Chromatogram of Clerodendrin A, n-hexane fraction of C.phlomidis and P. integrifolia (A- Clerodendrin A, B- C.phlomidis and C- P. integrifolia)

Clerodendrin A resolved at R_f 0.25, showing a single peak having absorption maxima at 396 nm. The chromatogram further revealed almost similar chemo-profile for n-hexane fractions of roots of *C. phlomidis* and *P. integrifolia* (Fig.2). The method of chromatography using n-hexane: ethyl formate (7:3) as a mobile phase and 5% aqueous H_2SO_4 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*. The calibration curve of clerodendrin A was linear with

r^2=0.99 in the concentration range (Fig.3). 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). The proposed method was validated in terms of linearity, precision, repeatability and accuracy. The limit of detection and limit of quantification were found to be 0.5 µg/spot and 1 µg/spot respectively (Table 1).

This is the first report of clerodendrin A content of root of *P. integrifolia*. The HPTLC method was easy, simple, and precise and helps to serve as a handy tool for authentication of roots of *C. phlomidis* and *P. integrifolia*.

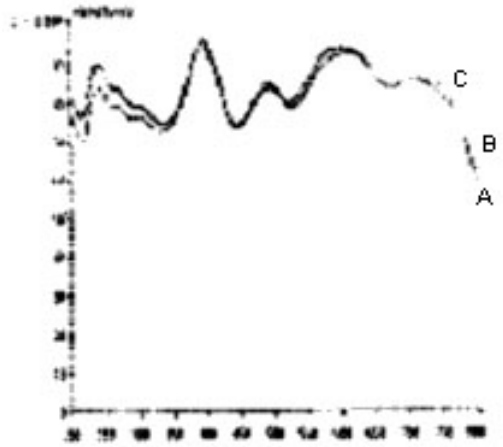

Fig 2 : HPTLC Chromatogram scanned at 396nm. (A- Clerodendrin A, B- C.phlomidis and C- P. integrifolia)

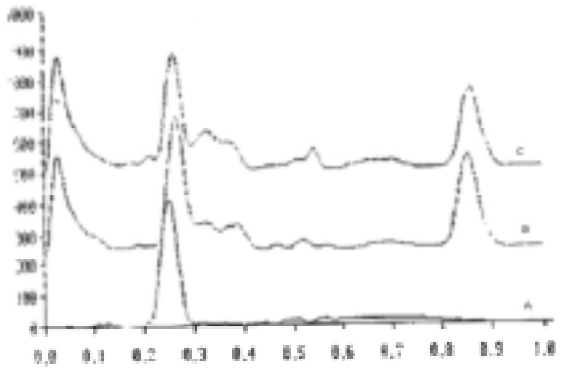

Fig 3 : HPTLC spectra of Clerodendrin A in C.phlomidis and P. integrifolia root. (A- Clerodendrin A, B- C.phlomidis and C- P. integrifolia)

Table 1: *Validation parameters for HPTLC method*

No.	Parameters	Results
1	Precision (% C.V.)	
	• Intraday	0.061-0.072
	• Inter day	0.060-0.075
	• Repeatability of Measurement	0.071
	• Repeatability of Application	0.114
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 - 99 %

References

1. Sharma MP. In Supplement to Cultivation and Utilization of Medicinal Plants. Handa SS, Kaul MK, eds. Regional Research Laboratory, Jammu- Tawi, India 1996, p 703.
2. Bishnupriya D, Billore KV, Gupta PO. In Recent Progress In Medicinal Plants. Singh VK, Govil JN, Singh G, eds. Vol. 1. SCI Tech Publishing, LLC, U. S. A. 2003, p 297.
3. Vaidya BG. Nighantu Adarsh (Uttarardh). Shri Swami Atamanand Sarsavati Ayurvedic, Government Pharmacy Ltd., Surat 1965, p 815.
4. Anonymous. The Ayurvedic Pharmacopoeia of India. Part 1, Vol. III, Edn 1st, Government of India, Ministry of Health and Family Welfare, Dept. of ISM and Homoeopathy, New Delhi 2001, p 3.
5. Nadkarni AK, Nadkarni KM. Indian Materia Medica. eds. 3rd. Popular Prakashan Private Ltd., Bombay 1982, p 353.
6. Anonymous. The Wealth of India, Vol. 8. Publications and Information Directorate, CSIR, New Delhi, 1969, p 239.
7. Joshi KC, et al. Planta Med. 1979; 37(1): 64.
8. Kato N, et al. J Chem Soc Chem Commu. 1971; 24: 1632.
9. Seth KK, et al. Pharmazie. 1982; 37(1): 74.
10. Anam EM. Indian J Chem. 1999; 38 B(11): 1307.
11. Basu NK and Dandiya PC. J Am Pharm Ass Sc. edu. 1947; 36: 389.
12. Basu NK and Joneja AN. Indian J Pharm. 1949; 11**:** 191.
13. Barik BR, et al. Fitoterapia. 1992; 63(4): 295.
14. Dasgupta B, et al. Planta Med. 1984; 50(3): 281.
15. Purushothaman KK and Vasanth S. Indian Drugs. 1986; 23(8): 482.
16. Debelmas AM, et al. Plant Med Phytotherapy. 1973; 7: 104.
17. Rao BC, et al. Indian J Chem. 1984; 24B(4): 403.
18. Rao PVS, et al. Indian J Chem. 1987 ; 26B (2): 191.