Vol. 5/2 (2005) 108 - 114

JOURNAL OF NATURAL REMEDIES

A Pharmacognostical study on *Convolvulus prostratus* Forssk.

Nayana S. Kapadia[a], Niyati S. Acharya[a], B. N. Suhagia[b], Mamta B. Shah[a]*

a. Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad
b. Department of Pharmaceutical Chemistry, L. M. College of Pharmacy, Ahmedabad

Abstract

Objective: Development of physico-chemical parameters and estimation of scopoletin, a chemical marker in herb of *Convolvulus prostratus* Forssk. (Family: Convolvulaceae). Materials and methods: Fresh flowering plants of *Convolvulus prostratus*, commonly known as Shankhpushpi, were studied for developing quality parameters and HPTLC method for quantification of scopoletin and fingerprinting of *C. prostratus* using a mobile phase toluene and ether (1:1, saturated with 10% glacial acetic acid) and instrument Camag Linomat IV with Camag TLC scanner. Results: Morphologically, the herb can be distinguished on the basis of hairy nature, sessile leaves, white-pale pink flowers and woody rootstalk. Leaves show striated cuticle, rubiaceous and cruciferous stomata, calcium oxalate prisms, and bicollateral vascular bundle. Stem shows unicellular covering trichomes, a discontinuous ring of pericyclic fibres and pitted cells in pith. Root is easily characterized by intra-xylary phloem. Spherical pollen grains and large number of unicellular trichomes are seen in powder of *C. prostratus*. HPTLC method was developed for determination of scopoletin content by scanning the plates at 366 nm. Conclusions: The histological characters and HPTLC method developed for quantification of scopoletin and fingerprinting of *C. prostratus* would serve as a useful tool in standardization of *C. prostratus*.

Key words: *Convolvulus prostratus*, scopoletin, HPTLC.

1. Introduction

Convolvulus prostratus Forssk. [1,2] (synonym: *Convolvulus pluricaulis* Choisy, *Convolvulus microphyllus* Sieb. ex. Spreng. and *Ipomoea microphylla* Roth.) belonging to family Convolvulaceae is commonly known as Shankhpushpi (Hind., Guj., Tam.), Shankhavali (Sans.), Shankhahuli (Mar.), Dankuni (Ben), and Dodak (Punj.) [3,4]. It is a small-diffused perennial herb with white-pale pink flower and woody rootstalk. The plant is found in tropical and temperate regions of the world. In India, it is distributed throughout the plains of Northern India, Bihar, Punjab and Kashmir to Deccan peninsula, and Saurashtra in Gujarat [5,6]. In Ayurvedic medicine, *C. prostratus* is described as medhya rasayana (nervine tonic) drug [7]

* Corresponding address
e-mail: mamta_b_shah@yahoo.com.

and a remedy for mental disorders [8] like insanity and epilepsy. It has utility as hypotensive [9], antiphlegmatic, anti-pholigistic, antianxiety and anthelmintic [6].

In Ayurveda, different plants are quoted under one common name Shankhpushpi visually *C. prostratus, Evolvulus alsinoides, Canscora decussata, Clitorea ternatea* and *Lavendula bipinnata* [7,8]. Amongst these plants *C. prostratus* is considered by majority of authors as Shankhpushpi [10,11]. Scopoletin, a non-condensed coumarin (6-methoxy-7-hydroxy coumarin) has been reported to be present in the plant [12].

No reports are available regarding systematic pharmacognostical study of this plant. In this regard, an attempt is made to study complete pharmacognosy of *C. prostratus.* Also, HPTLC method is developed for fingerprinting and quantification of scopoletin considering it as a chemical marker. The present study is of interest on account of differentiating plants, which go by that name or related names.

2. Materials and methods

2.1 Plant material

After taxonomic verification, fresh flowering herbs were collected from Gujarat University area in the month of October 2003. Voucher sample (LM 137) was deposited at Pharmacognosy department of L. M. College of Pharmacy, Ahmedabad. The sample after removal of soil and adhering material was dried at room temperature for 5-6 days, powdered to 60# and was used for the powder study and determination of scopoletin content. Free hand transverse sections of fresh leaf, stem and root were taken.

2.2 Chemicals

Standard scopoletin (Fluka Laboratory), Toluene, Solvent Ether, Glacial Acetic acid (AR grade)

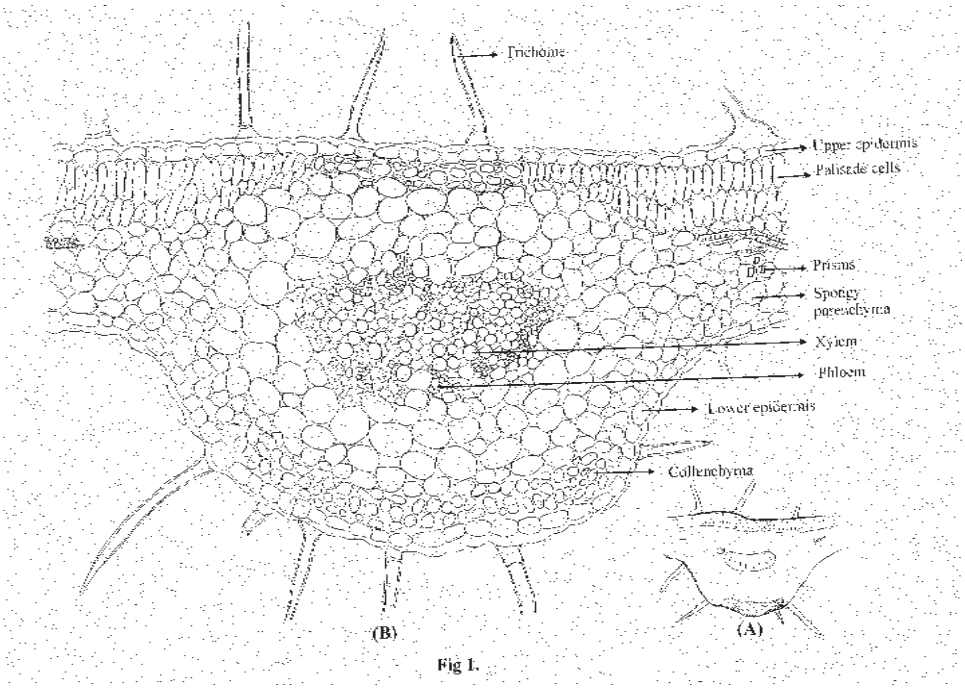


Fig. 1. *C.prostratus* leaf (A) Diagrammatic T.S. (x42) (B) Detailed T. S. (x350)

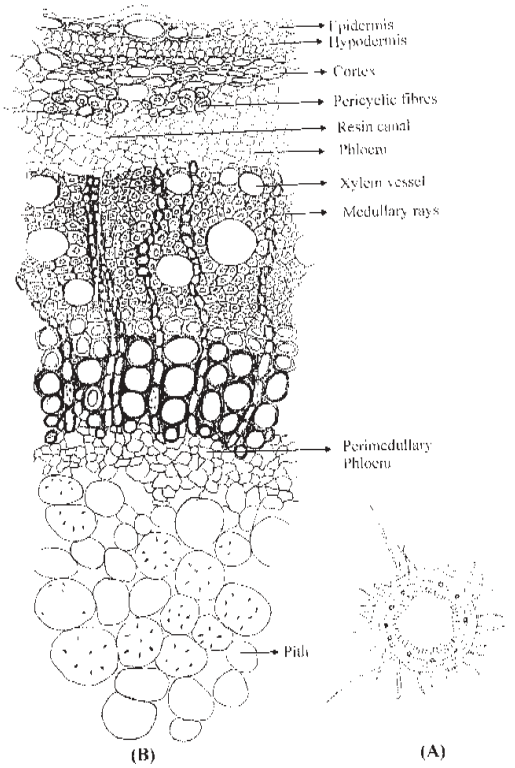


Fig. 2. *C. prostratus* Stem (A) Diagrammatic T. S. (x42) (B) Detailed T. S. (x350)

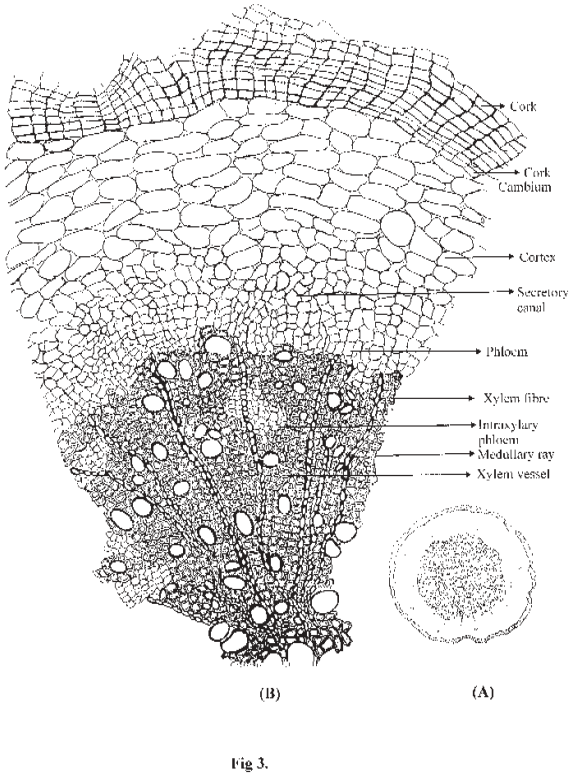


Fig 3.

Fig. 3. *C. prostratus* Root (A) Diagrammatic T. S. (x42) (B) Detailed T. S. (x350)

Table 1.
Leaf constants of *C. prostratus*

Parameters		Constants
Stomatal index	Upper surface	16
	Lower surface	20.5
Palisade ratio		6
Vein islet number		10
Vein termination number		16

2.3 Extraction of plant material

20 g of the powdered herb was refluxed with 150 ml ethanol (95%) for 2 h; extract was filtered and was evaporated to dryness. The residue was treated with 40 ml of 10% NaOH for 1 h at room temperature. After removing the non-saponifiable liquid fraction with $CHCl_3$, the aqueous alkaline layer was acidified with 10% HCl and the extracted with $CHCl_3$ (3 x 25 ml).

2.4 Chromatographic conditions

Instrument: Camag Linomat IV (semi automatic spotting device) equipped with Camag TLC scanner 3 and Camag CATS 4 integration software.

Stationary phase: precoated TLC plate of silica gel 60 F_{254} (Merck)

Mobile phase: toluene: ether (1:1) saturated with 10% glacial acetic acid.

Spotting parameter: calibration curve: 10-30 ml of standard solutions of scopoletin (40 µg/ml)

Table 2.
Dimensions of xylem vessels, trichomes and pollen grains

Sample	Xylem vessel (Diameter in µ)	Trichomes		Pollen grains (Diameter in µ)
		Length (µ)	Width (µ)	
C. prostratus	12-48.6	80-300	16-20	28-68

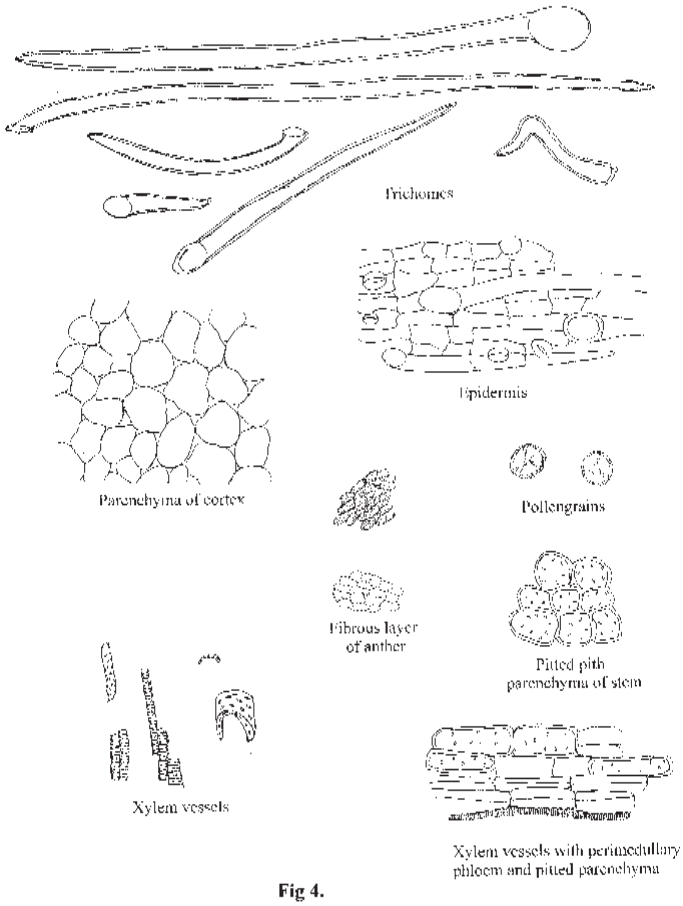


Fig. 4. Powder Study of *C.prostratus* Herb (x350)

Test sample: 25µl
Temperature: 25 ± 2ºC
Migration distance: 11 cm
Detection: 366 nm

2.5 Fingerprinting and estimation of scopoletin

Graded concentration of standard scopoletin solution (40 µg/ml), 10, 12, 15, 20, 25 and 30µl volume were spotted on methanol washed silica gel 60 F_{254} TLC plates (Merck) with Camag Linomat IV automatic spotter. 25µl of test sample solutions were used for spotting.

The plate was developed in mobile phase, toluene: ether (1:1) saturated with 10% glacial acetic acid.

After development the plate was dried and scanned at 366 nm. Data of peak height and peak area of each spot of scopoletin was recorded. Standard curve of peak area *vs*. concentration of scopoletin was plotted.

3. Results and discussion

The lamina of leaf shows striated cuticle, number of simple and unicellular, thick walled trichomes. Palisade tissue consists of two layers of compactly arranged cells containing chlorophyll. Plenty of calcium oxalate crystals are found throughout the mesophyll. Midrib shows a well-developed bicollateral vascular bundle consisting of radial rows of xylem vessels and parenchymatous phloem as patch on either side of xylem (Fig. 1 & Table 1).

Transverse section of stem is characterized by presence of long slightly curved unicellular covering trichomes, collenchymatous cortex, secretory canal and pericyclic fibres in the cortical region and the phloem. Xylem consists

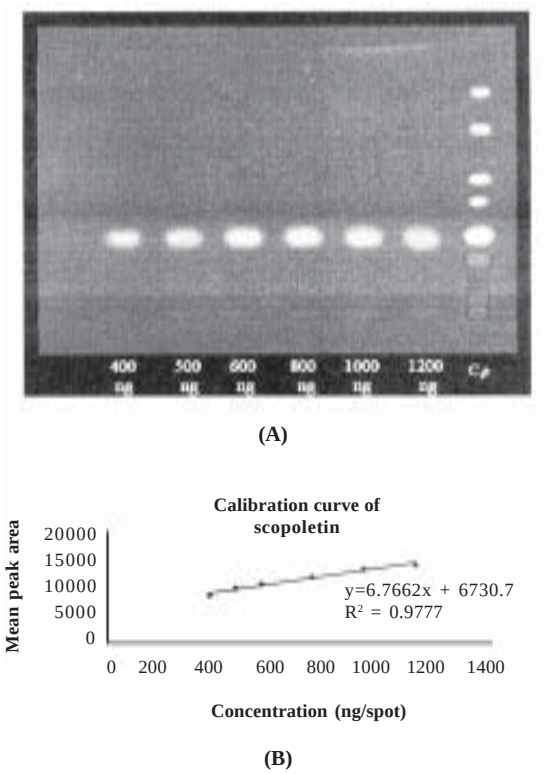


Fig. 5.
A. Chromatogram of Standard Scopoletin and *C.prostratus*
B. Calibration Curve of Scopoletin

Table 3.
Validation parameters for estimation of scopoletin

Parameters	Results
1. Linearity	0.9777
2. Precision (% C.V.)	
Repeatability of Measurement	0.618
Repeatability of Application	1.76
Interday	2.05-3.99%
Intraday	2.35-3.80
3. Range	400-1200 ng/spot
4. Limit of Detection	200 ng/spot
5. Limit of Quantification	400 ng/spot
6. Accuracy	99.37-99.96%
7. Specificity	Specific

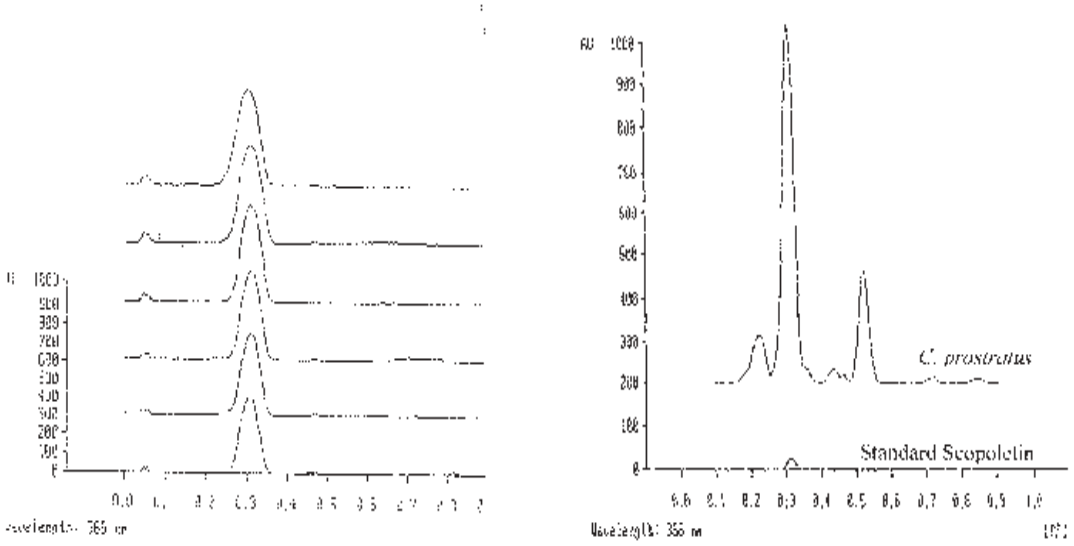
of two regions. Outer zone consists of scattered xylem vessels with bigger diameter, while inner zone consists of radially arranged xylem vessels with smaller diameter. Medullary rays are uniseriate and pitted. Pith is surrounded by perimedullary phloem and it characteristically shows few cells with lignification and pits (Fig. 2).

The root shows 6-7 layers of suberised cork with cork cambium, collenchymatous parenchyma in cortex and secretory canal in phloem. Wood region shows presence of intraxylary phloem (Fig. 3).

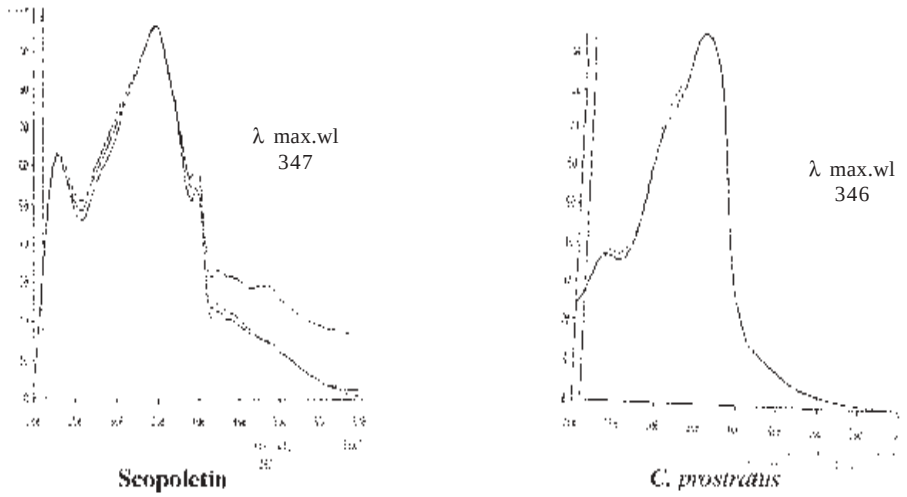
Powder of *C. prostratus* can be identified with long, unicellular pointed trichomes, striated cuticle and spherical pollen grains with three distinct wings like appendages (Fig. 4 & Table 2).

Characteristic TLC fingerprint was developed along with standard scopoletin using toluene: ether (1:1) saturated with 10% glacial acetic acid as mobile phase and precoated TLC plate of silica gel 60 F_{254} (Fig. 5 & 6). Co-chromatography with standard scopoletin revealed presence of the same in *C. prostratus*, the spot of which being resolving at R_f 0.31 (both having UV maxima 347 nm) (Fig. 6). Further, besides scopoletin in *C. prostratus* three other fluorescent spots emitting same fluorescence (bluish, at 366 nm) of coumarins resolving at R_f 0.47, 0.55 and 0.63 were found to be major compounds (Fig. 5&6). The scopoletin content was found to be $3.05X10^{-3}$ % w/w.

The aim of this investigation was to develop identification parameters and HPTLC method for estimation of scopoletin content, which has been achieved and confirmed by validation



(A)



(B)

Fig. 6

A. HPTLC Chromatograms of Standard Scopoletin and *C. prostratus*

B. UV Spectra of Scopoletin and *C. prostrates.*

parameter like specificity, linearity, accuracy, precision, repeatability and reproducibility (Table 3).

A simple sample preparation method was generated, which was economic for the purpose of quality control. The HPTLC method was found to be sensitive and suitable for routine analysis of *C. prostratus.* In previously reported method, microphyllic acid was analysed employing GC-MS [13]. The present method was found to be relatively easy and accurate.

References

1. Srivastava RC. (1989) *Drug-Plant Resources of Central India (An inventory),* Today and Tomorrows Printers & Publishers: New Delhi; 36.

2. Rizk AM, Williamson EM, Evans FJ. (1985) *Int. J. Crude Drug Res.* 23(1): 1-4.

3. Parrota JA. (2001) *Healing Plants of Peninsular India,* CABI Publishing: New York; 241.

4. Vaidya BG. (1965) *Nighuntu Adarsh (uttarardh),* 2nd edition, Shri Swami Atmanand Sarswati Ayurvedic Sahakari Pharmacy Ltd.: Surat; 743.

5. Watt G. (1972) *Dictionary of Economic Products of India,* Vol. 2, Periodical Experts: New Delhi; 519.

6. Anonymous. (1950) *The Wealth of India* (Raw materials), Vol. 2, CSIR: New Delhi; 315.

7. Handa SS, Kaul MK. (1996) *Supplement and Utilization of Medicinal Plants,* CSIR: New Delhi; 508-524.

8. Venkata Rao E. (2003) In: Singh S, Govil JN, Singh VK. (Eds.) *Recent Progress in Medicinal Plants,* Vol. 2, (Phytochemistry and Pharmacology). Studium Press LLC: USA; 195-199.

9. Satyawati GV, Gupta AK, Tondon N. (1987) *Medicinal Plants of India,* Vol. 1, Indian Council of Medicinal Research, New Delhi; 276-277.

10. Sinha P, Kumar A, Wahi SP. (1986) *Bull. Med. Ethnobot. Res.* 7(1-2): 62-73.

11. Singh HB, Viswanathan MV. (2000) *J. Med. and Aro. Plant Sciences.* 22(1): 78.

12. Despande SM, Srivastava DN. (1969) *J. Ind. Chem. Soc.* 46(6): 759-760.

13. Wagner H, Hildehert, Schwarting G. (1977) *Phytochemistry* 16(6): 715-717.