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# A pharmcognostic study on *Sphaeranthus indicus*

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**Abstract**

The study is aimed at development of Physico-chemical parameters and estimation of 7-hydroxy eudesmanolide, a major sesquiterpene lactone of *Sphaeranthus indicus*, (Family: Astraceae), commonly known as Gorakhmundi. *S. indicus* is a branched, hairy and strongly scented herb with spathulate, sessile leaves and pinkish purple flowers. Leaf shows both simple (uni-multicellular) and glandular (Club and clavate) type of trichomes. Stem shows a ring of deltoid vascular bundles and well-developed pith with few pitted cells. Root shows metaderm, and radially arranged fibers and secretory canal placed alternatively in cortical region. Powdered drug shows large number of various types of trichomes, pollen grains in pollen sacs and cruciferous stomata. HPTLC method was developed to quantify 7- hydroxy eudesmanolide in *S. indicus* using a mobile phase n-hexane: diethyl ether (3:7) and scanning the plate at 213 nm. The amount of 7-hydroxy eudesmanolide was found to be 0.0658% w/w. The present study is an effort to develop a pharmacognostic report for *S. indicus*.

**Key words:** Astraceae, Eudesmanolide K, Gorakhmundi, HPTLC, *Sphaeranthus indicus*

## 1. Introduction

*Sphaeranthus indicus* Linn. (Synonym: *S. hirtus Willd*.) belonging to family Astraceae, is commonly known as Munditika, Sravani (Sans.), Gorakhmundi (Guj., Hindi), Mundi (Hindi, Mar., Kan.), and Indian Globe thistle (Eng.). The plant is found in damp and shady places in plains all over India, Sri Lanka, Malaya, Australia and Africa [1, 2]. The entire plant is valued as a general tonic, alterative, bitter, aphrodisiac, anthelmintic and emollient [2, 3, 4]. Flowers are useful as blood purifiers in skin diseases [4]. Root bark is a valuable remedy for piles [4]. The plant is reported to contain deep cherry coloured volatile oil having cadiene, oscimene, citral, p-methoxycinnamaldehyde, geraniol, eugenol, and geranyl acetate as major constituents [5]. Number of eudesmanolides [6, 7, 8], an alkaloid sphaeranthine [9] and an isoflavone 5,4'-dimethoxy-3'- prenylbiochanin 7-O-β-D-galactoside [10] are reported to be present in *S. indicus*.

The present study is aimed to carry out pharmacognostic studies for *S. indicus*. 7-Hydroxy eudesmanolide, a major sesquiterpenoid, was isolated and estimated by TLC method using HPTLC.

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## 2. Materials and Methods

### *2.1 Plant material*

Fresh, fully-grown flowering plants of *Sphaeranthus indicus* were collected from Gujarat University campus in the month of November 2005. The plants collected were authenticated by taxonomist of Botany Department, Gujarat University, Ahmedabad, Gujarat. A voucher sample (LM 258) was deposited at the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat. The plant material was cleaned, dried under shade and powdered to 60 #.

### *2.2 Pharmacognostic study*

Microscopic studies were carried out for leaf, stem, root and powdered drug. Ash values and extractive values were determined by the method of Ayurvedic Pharmacopoeia [11].

### *2.3 Chemicals*

Petroleum ether (40-60 °C), *n*-hexane, diethyl ether, anisaldehyde and $H_2SO_4$. All solvents and reagents were of AR grade.

### *2.4 Estimation of 7- hydroxy eudesmanolide by HPTLC method*

*Preparation of test sample and working standard:*

5 g of powdered plant material after removing volatile oil through steam distillation was extracted with 50 % ethanol (3 X 20 ml) exhaustively. The hydro-alcoholic extract (11.23 % w/w) after striping off alcohol was fractionated using ethyl acetate (4 X 20 ml) to get ethyl acetate soluble fraction (1.21 %w/w) (Ext B). 1 mg/ml solution of ethyl acetate soluble fraction was used for HPTLC study.

A major sesquiterpene lactone, 7-hydroxy eudesmanolide isolated from the plant was characterized by comparing its M.P., UV, IR, NMR and Mass spectral data with the data given in literature [12]. It was used as a standard to develop HPTLC method for its quantification in *S. indicus*.

*Chromatographic conditions*

*Instrument:* Camag Linomat V (semiautomatic spotting device) equipped with Camag TLC Scanner 3, Camag WINCATS integration software and Camag Reprostar-3

*Materials:* precoated silica gel 60G $F_{254}$ plate (E. Merck)

*Mobile phase*: *n*-hexane: diethyl ether (3:7)

*Spotting parameters:* calibration curve: 3-8 µl of working standard solution of 7-hydroxy eudesmanolide (100 µg/ml)

For test sample: 10 µl

Temperature: 25 ± 2 °C

Band width: 4 mm

Space between two bands: 5 mm

Migration distance: 7.5 cm

Detection: 213 nm

*Estimation of 7-hydroxy eudesmanolide:*

Graded concentration of standard 7-hydroxy eudesmanolide (0.1 mg/ml), 3, 4, 5, 6, 7, 8 µl and10 µl of test sample were spotted on silica gel 60 $F_{254}$ TLC plate (E. Merck) using Camag Linomat V automatic spotter. After developing in mobile phase, n-hexane: diethyl ether (3:7), the plate was scanned at 213 nm. Data of peak area of each spot of 7-hydroxy eudesmanolide was recorded. Standard curve of peak area vs. concentration of 7-hydroxy eudesmanolide was plotted.

The method was validated in terms of linearity, precision, repeatability, specificity, limit of detection (LOD), and limit of quantification (LOQ). LOD and LOQ were determined at

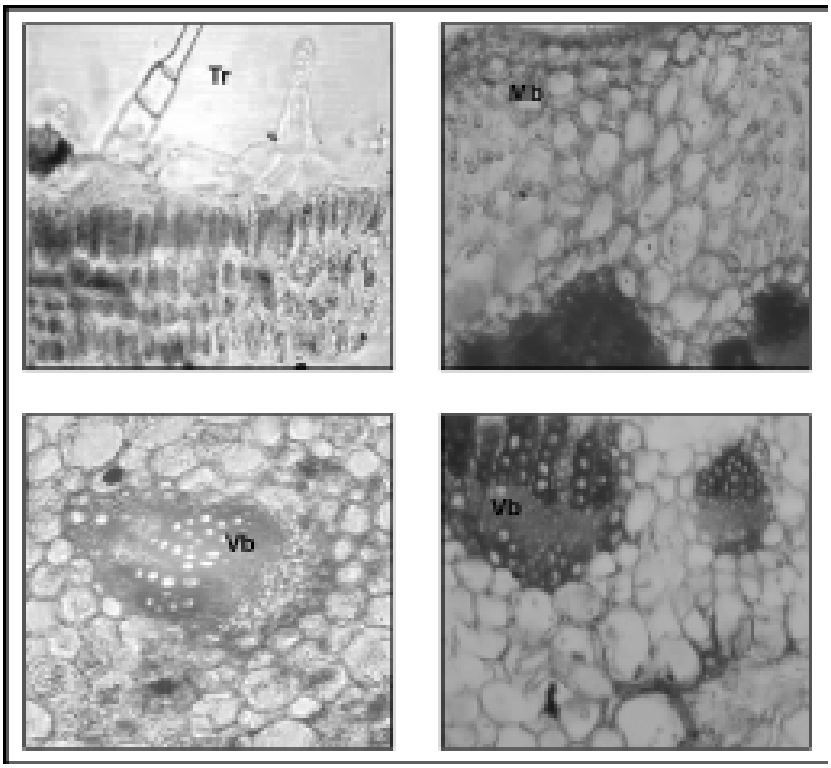


**Fig. 1:**
**T.S.** of leaf of *Sphaeranthus indicus*.
**Mb.:** midrib, **Tr.:** trichome, **Vb.:** vascular bundle

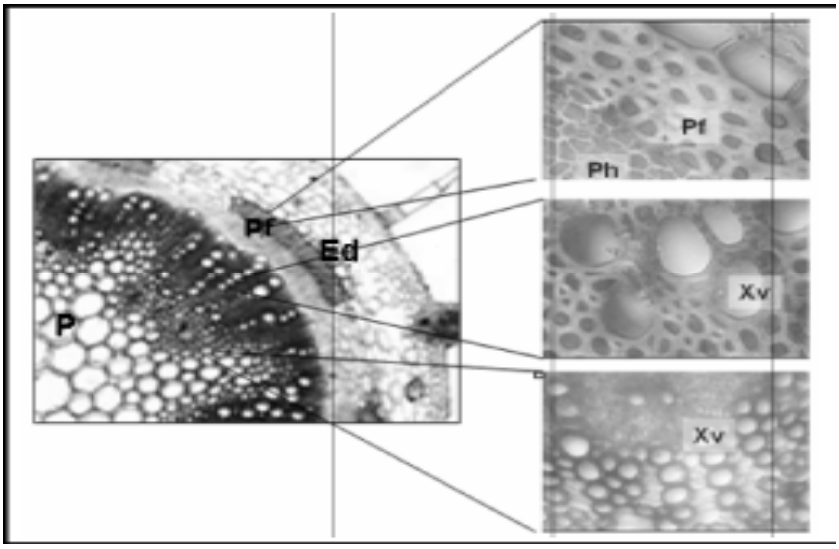


**Fig. 2:**
**T. S.** of stem of *Sphaeranthus indicus*.
**P.:** pith, **Pf.:** pericyclic fibres, **Tr.:** trichome, **Vb.:** vascular bundle.

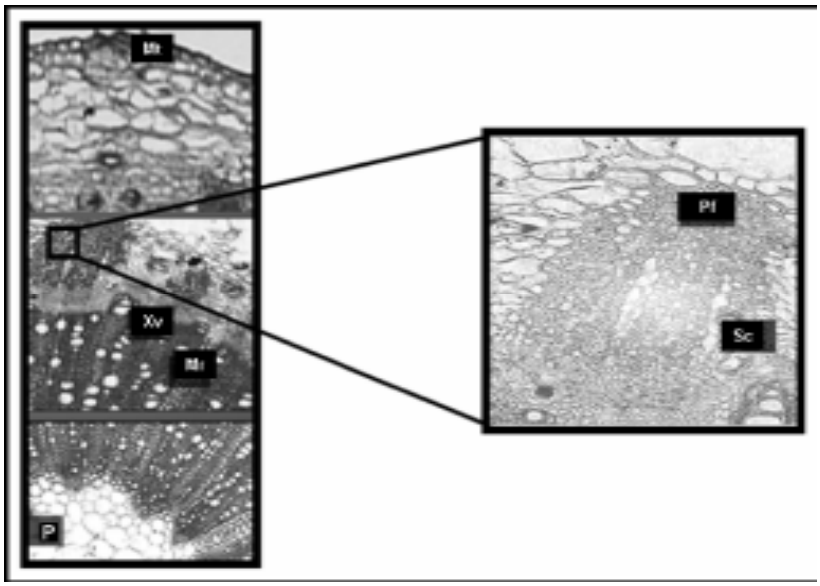


**Fig. 3:**
**T. S.** of root of *Sphaeranthus indicus.*
**Mr.:** medullary rays, **Mt.:** metaderm, **Ph.:** phloem, **Sc.:** secretory canal

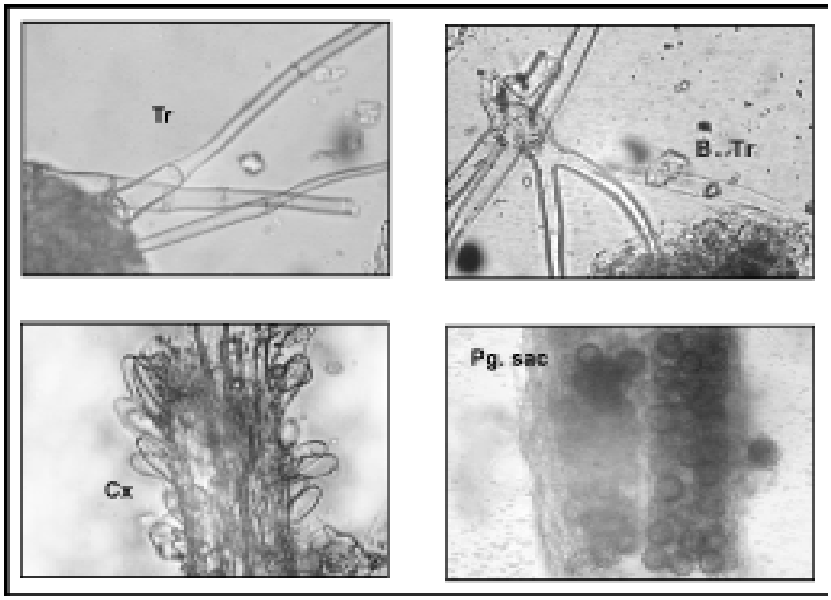


**Fig. 4:**
Powder study of *Sphaeranthus indicus.*
**B. Tr.:** branched trichome, **Cx.:** Calyx with Balloon shaped trichomes,
**Pg.:** Pollen grains, **Pg.Sac.:** pollen sac, **St.:** stomata, **Xv.:** xylem vessel

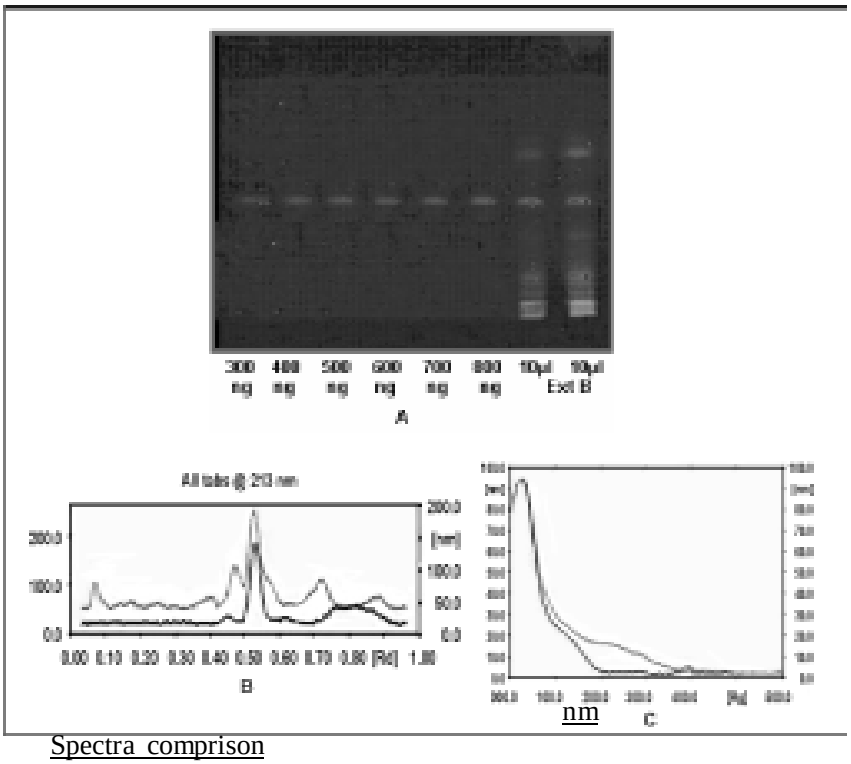


**Fig. 5:**
HPTLC Study
**A.** Co-Chromatography of 7-hydroxy eudesmanolide, Ethyl acetate fraction of *Sphaeranthus indicus* under 215 nm.
**B.** TLC densitometric chromatogram scanned at 213nm.
**C.** Overlay spectra of standard 7-hydroxy eudesmanolide and of the same in *Sphaeranthus indicus*.

**Table 1.** Validation parameters for estimation of 7-hydroxy eudesmanolide

| Parameters | Results |
|---|---|
| Linearity | 0.9968 |
| Precision (% C.V.) | |
| • Repeatability of Measurement | 0.20 % |
| • Repeatability of Application | 0.41 % |
| • Interday | 2.48-2.95 % |
| • Intraday | 1.98-2.53 % |
| Range | 300-800 ng/spot |
| Limit of Detection | 200 ng/spot |
| Limit of Quantification | 300 ng/spot |
| Accuracy | 97.56-101.89 % |
| Specificity | Specific |

signal-to-noise ratio of 3:1 and 10:1 respectively. The accuracy of an analysis was determined by calculating systemic error involved. Accuracy of the above method was ascertained by adding known concentration of 7-hydroxy eudesmanolide to the pre-quantified sample solution and estimating the quantity of the same using proposed method.

## 3. Results and discussion

*Transverse section of leaf*

Leaf is dorsiventral and shows abundant trichomes of varying types on both the epidermii. Simple trichomes are 3-4 celled, thick-walled and measure130.8 – 145.2µ in length and 29.0 – 43.5µ in width. Trichomes are straight/knee shaped, with swollen base, and with collapsed cell at the middle or at the apex. Branched trichomes are with 3-5 arms. Club and clavate shaped glandular trichomes measure 43.5 – 72.5µ in length and 30.2 – 43.5µ in width. Midrib shows 3-4 collateral vascular bundles, associated with group of sclerenchymatous cells on either side.

*Transverse section of stem*

Stem shows under developed cork with 2-3 layers of parenchymatous cells covered with papillose cuticle having trichomes and can be distinguished by the presence of discontinuous ring of lignified pericyclic fibres and a well-developed ring of bicollateral vascular bundle surrounding the pith. Medullary rays are pitted, lignified and about uni-tetraseriate.

*Transverse section of root*

Root shows on its outer side metaderm, a typical brown colored tissue. It consists of suberised cells, arranged irregularly and forms a protective layer. Radial groups of pericyclic fibres and few stone cells are seen alternating with radially arranged secretory canals in the secondary cortex. Phloem is parenchymatous and radially arranged. Medullary rays are pitted, lignified and about 2-5 seriate.

Powder is light greenish brown with aromatic odor and bitter or slightly pungent taste. *S. indicus* can be identified with trichomes of above mentioned types. Large number of anisocytic stomata, pollen grains (22.5 – 27.0 µ in diameters), scattered as well as in pollen sac; balloon shaped trichomes on the calyx are important identifying characters of powdered drug. Xylem vessels are lignified, and bordered pitted and usually co-occur with other wood elements.

Limits of total ash, acid insoluble ash and acid soluble ash were found to be 18.9, 11.4, 6.2 % w/w respectively. Alcohol soluble extractive matter was 17.4 % w/w, while water soluble extractives were 21.7% w/w.

A major sesquiterpene lactone, 7-hydroxy eudesmanolide was quantified by TLC densitometric method using HPTLC, using *n*-hexane: diethyl ether (3:7) as a mobile phase and precoated TLC plates of silica gel 60 G $F_{254}$. The compound resolved as a bluish green spot at Rf 0.5 after derivatisation with anisaldehyde sulphuric acid reagent and heating at 110°C for 10 min. Content of 7-hydroxy eudesmanolide was found to be 0.0658 % w/w in plant material.

The aim of this investigation was to develop identification parameters for the plant and TLC densitometric method for estimation of 7-hydroxy eudesmanolide content, which has been achieved. The TLC densitometric method was validated for specificity, linearity, accuracy, precision, repeatability and reproducibility (Table 1).

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