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Pharmacognostical Evaluation of *Sphaeranthus indicus* (Linn.)

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Abstract – This study presents a detailed pharmacognostical study of the underground portion of the crude drug *Sphaeranthus indicus* Linn. (Asteraceae), an important plant in the Indian system of medicine. The root and stolon were studied using procedures of light, conofocal microscopy, WHO recommended-physicochemical determinations, and authentic phytochemical procedures. The physicochemical, morphological, and histological parameters presented in this paper may be proposed as parameters to identify and establish the authenticity of *S. indicus* root and stolon.
Keywords – *S. indicus*, pharmacognosy, adulterants

Introduction

In recent years, there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance (Reddy *et al.*, Venkatesh *et al.*, 1994). The use of plant drugs is subject to their correct identification. In general potent drugs are always either adulterated or substituted depending on morphological characters or biological activity. Despite the modern techniques, of investigations, identification of plant drugs by pharmacognostical studies is more reliable.

S. indicus has been used traditionally as an antibacterial (Mahajan *et al.*, 1999), antifungal (Kasera *et al.*, 1983), antmicrobial (Dubey *et al.*, 2002) and repellent (Tiwari *et al.*, 1993). The demand for this drug has led to an increase in the adulteration of *S. indicus* with inferior species like *S. africans* and other species resembling to the genuine drug. However there is no pharmacognostical report on the anatomical and physico-chemical standards required for the quality control of the underground plant parts of the crude drug.

Materials and methods

Plant material – The underground portions of *S. indicus* were collected during July 2005 from Manipal. The plant was identified by Dr. Gopalakrishna Bhat, Botanist, Poorna Prajna College, Udupi, Karnataka, India.

Chemicals and instruments – The different materials used for the study include basic microscopical equipments *viz;* compound microscope, glass slides, cover slips, watch glass and other common glass wares. Microphotographs were taken using a Leicia DMLS microscope attached with Leitz MPS 32 camera. Common solvents *viz;* ethanol (95%), hexane, petroleum ether, solvent ether, n-butanol, chloroform and reagents *viz;* phloroglucinol, glycerin, hydrochloric acid, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai (India).

Macroscopic and microscopic analysis – The macroscopy and morphology of the plant were studied according to the method of (Brain and Turner 1975a). For the microscopical studies, cross-sections were prepared and stained as per the procedure of (Johansen1940). The micro-powder analysis was done according to the method of (Brain and Turner 1975b) and (Kokate 1986a).

Physicochemical analysis – Physicochemical values such as the percentage of ash values and extractive values were performed according to the official methods prescribed in Indian Pharmacopoeia, 1996 and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992).

Preliminary phytochemical screening – Preliminary phytochemical screening was carried out by using methods described by (Kokate 1986b and Harborne 1984).

Experimental

Macroscopic characteristics (Fig. 1) – A large shrub upto 9 m in height with small leaves 1 ~ 5 cm in length and 1 ~ 2.5 cm in breadth. Flowers are globose purple in colour. Stem is greenish in colour. Roots are brown in colour and internally light brown in colour. They are

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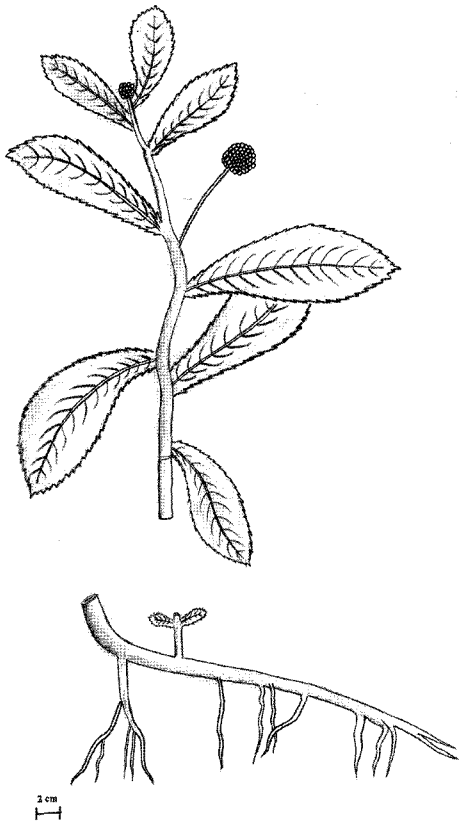

Fig. 1. Macroscopy of the aerial aspect and root of *S. indicus*.

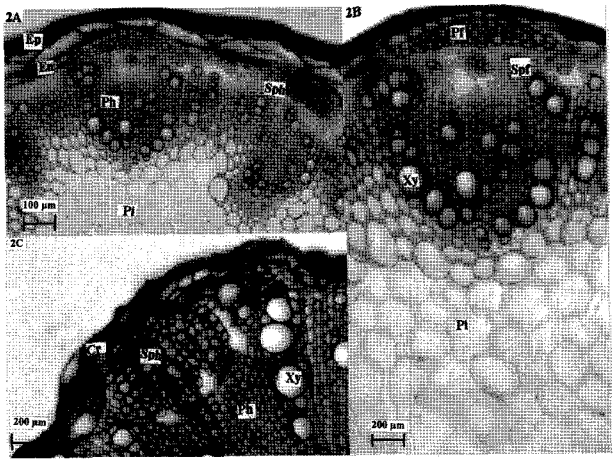

Fig. 2. Microscopy of the stolon and root of *S. indicus*.

A. Photomicrograph showing transverse section of the stolon of *S. indicus*.

B. A portion of vascular bundle of the stolon (enlarged) **Ep**-epidermis, **En**-endodermis, **Pf**-pericyclic fibres, **Ph**-phloem, **Xy**-xylem, **Pi**-pith, **Sph**-secondary phloem, **Spf**-secondary phloem fibres.

C. Microscopy of the root of *S. indicus* **Cr**-cork, **Xy**-xylem, **Ph**-phloem, **Sph**-secondary phloem.

tuberous with 10 ~ 15 cm in length and 0.1 ~ 0.4 in diameter. Longitudinal striations are seen with transverse scars at regular interval. It has no taste and the odour is characteristic.

Microscopic characteristics (Fig. 2)

i) Root – The root shows secondary characters and has a circular outline.

i) Epiblema: It is the outermost layer covering the root and consists of a single layer of barrel shaped cells.

ii) Endodermis: This is single layered made up of barrel shaped cell with casparian thickening.

iii) Cambium: In primary condition there is no cambium as such. The commencement of secondary growth starts with the formation of arch cambium in between the xylem and phloem patches of vascular bundle. These arches are joined and appear in form of wavy rings, which start cutting the xylem and phloem outwardly and inwardly and are called secondary xylem and secondary phloem, respectively.

iv) Secondary phloem: It consists of sieve tubes, companion cells and phloem parenchyma. The whole phloem, since fragile gets removed every season. Its function is conduction of food material.

v) Secondary xylem: As growth of secondary xylem begins the pith gets eliminated and the central portion becomes occupied by secondary xylem. It consists of tracheids which gives mechanical strength to roots, while vessels help in conduction of water.

vi) Cork: In the root of this drug, its presence is a remote chance. Even if this layer of cork is formed it is peeled off due to mechanical piercing through soil.

ii) Stolon – The stolon is a creeping stem, which grows at ground level. It strikes root below and stems above at each node. Through the internodal region of stolon if we take a cross section the following anatomical features are observed.

i) Epidermis: The young stem is covered by a single layer of cells having a cuticular lining on the outer tangential wall. Next to the epidermis is the cortex consisting of air cavities and a number of canals, which may contain mucilage.

ii) Endodermis: It is a single layer made up of rectangular or barrel shaped cells with casparian thickening.

iii) Pericycle: It is multicellular and is made up of alternate bands of sclerenchyma and parenchyma. The sclerenchyma patches appear as a cap to each vascular bundle and hence known as bundle cap.

iv) Secondary xylem: It consists of tracheids, vessels, fibres and xylem parenchyma.

Table 1. Preliminary phytochemical screening of the powder of *S. indicus*

test	hexane	benzene	chloroform	acetone	ethanol	water
alkaloids	+	+	+	–	+	+
carbohydrates	–	–	–	–	+	+
phenolics	–	–	–	+	+	+
gums and mucilages	–	–	–	–	–	+
volatile oil	+	–	–	–	+	+
flavonoids	–	–	–	+	+	+

+ denotes the presence of the respective class of compounds.

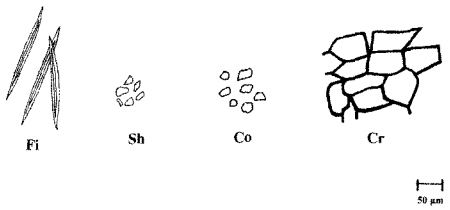

Fig. 3. Powder microscopy of *S. indicus*. **Cr**-cork, **Co**-calcium oxalate, **Fi**-fibres, **Sh**-starch grains.

v) Secondary phloem: It consists of sieve tubes, companion cells, phloem parenchyma, and phloem fibres.

vi) Bark formation: The bark formation take place in dicot plants. Cells of cork cambium consist of meristematic cells which cut off continuously as new cells both inside and outside. The inner cells differentiate into secondary cortex or phelloderm where as outer cells form cork or phellem. The phellem, phelloderm, and phellogen together constitute the bark. The plant bark is not cumulative but instead gets peeled off every year. Hence the bark tissues are not prominently seen.

vii) Pith: When secondary growth reaches its climax the pith gets eliminated by crushing which is done by hard xylem cells.

Powder microscopy (Fig. 3) – The following microscopic characters are observed in the powder.

i) Fibres are few in numbers and in a group of 2 ~ 3. They are thick walled, lignified and measures 450 ~ 525 µm in length and 30 ~ 33 µm in width.

ii) Fragments of cork are present.

iii) Starch grains are frequently seen, simple and measures 25 ~ 28 µm.

iv) Calcium oxalate crystals are few in number and are prismatic.

Preliminary phytochemical screening – Preliminary phytochemical screening of the root and stolon (Table 1) showed the presence of alkaloids, flavonoids, volatile oil and gums.

Physicochemical constants – Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 2) of the powdered drug revealed high concentration of sulphated ash (8.0%), whereas acid insoluble ash was found to be very low since the drug was collected fresh.

The extractive values are important for the quality control of crude drugs. The water soluble extractive (Table 3) was high in *S. indicus*. Moisture content of the drug was found to be 10%. Swelling index was found to be 12, mucilage content 12%, No bitterness was detected. The drug contained 2% v/w of volatile oil (Table 4).

Successive solvent extractive constants – Of the total extractive (Table 5) the major portion was found to be ethanol extract (6.64%) followed by acetone, aqueous, benzene, hexane and chloroform extracts.

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Table 2. Ash values of the powder of *S. indicus*

parameters	values % (w/w)
total ash	5.5
acid insoluble ash	0.5
water soluble ash	2
sulphated ash	8

Table 3. Extractive values of the powder of *S. indicus*

parameters	values % (w/w)
hot extraction	14.6
cold maceration	
a) water soluble extractive	10
b) ethanol soluble extractive	14
c) non volatile ether soluble extractive	4

Table 4. Physicochemical constants of the powder of *S. indicus*

parameters	values
bitterness value	nil.
swelling index	12
moisture content	10% (w/w)
volatile oil content	2% (w/w)

Table 5. Successive solvent extractive constants of the powder of *S. indicus*

solvents	consistency and color	average value of extractives % (w/w)
hexane	non-sticky solid/yellowish brown	2
benzene	sticky semisolid/dark green	3
chloroform	sticky semisolid/dark brown	1.92
acetone	sticky semisolid/brown	2.62
ethanol	sticky semisolid/reddish brown	6.64
water	solid/brown	2.36

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