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Comparative pharmacognostical investigation of three different members of Sariva used in ayurveda

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Abstract

Hemidesmus indicus (L.) R. Br., is widely used in Ayurveda in Sri Lanka. The official part of it is the root. Due to the high demand, there is a known tendency for deliberate adulteration of it by *Ichnocarpus frutescens* (L.) R. Br. and *Cryptolepis buchananii* Roem. & Schult. All three plants are named as "Sariva" in Sanskrit. However, *H. indicus* is considered to be the true sariva. This study was carried out to establish morphological, anatomical and organoleptic and chromatographic characters which will enable *H. indicus* plant and its root to be distinguished from those of the other two plants. Fresh samples of all three plants were collected from natural habitats representing different ecological zones of the country, and were authenticated using herbarium specimens deposited at the National Herbarium. Absence of mechanical elements, absence of pith and presence of uniseriate medullary rays are the most distinguishable anatomical characters of *H. indicus* root. The root also has a characteristic odour and taste. The three plants give similar TLC patterns with differing relative intensities of spots. These results can be used to distinguish true sariva from the other two plants in the sariva group.

Keywords: Sariva, *Hemidesmus indicus*, *Ichnocarpus frutescens* (L.) R. Br. *Cryptolepis buchananii* Roem. & Schult. anatomical characters

Introduction

Hemidesmus indicus (L.) R. Br. belonging to family Periplocaceae is a plant well known for its cooling, blood purifying and appetizing properties and is used in Ayurveda for treating a variety of physiological disorders. The root of *H. indicus* is referred as true Sariva in Ayurveda Pharmacopeia [1]. Due to the increasing demand for roots of this species, there is a known tendency for deliberate adulteration by two other plants, *Cryptolepis buchananii* Roem. & Schult. (Periplocaceae) and *Ichnocarpus frutescens* (L.) R. Br. (Apocynaceae) [2, 3]. The plant is abundant throughout in deciduous scrub and forests of the dry and arid regions in Sri Lanka. The present investigation deals with the comparative pharmacognostical evaluation of three plant species of sariva. A comparative study of the morphology and anatomy of the root has been carried out with the aim of the pharmacognostic and taxonomic species identification. Correct identification of plants or plant parts are essential for the preparation of authentic ayurvedic drugs.

Materials and Methods

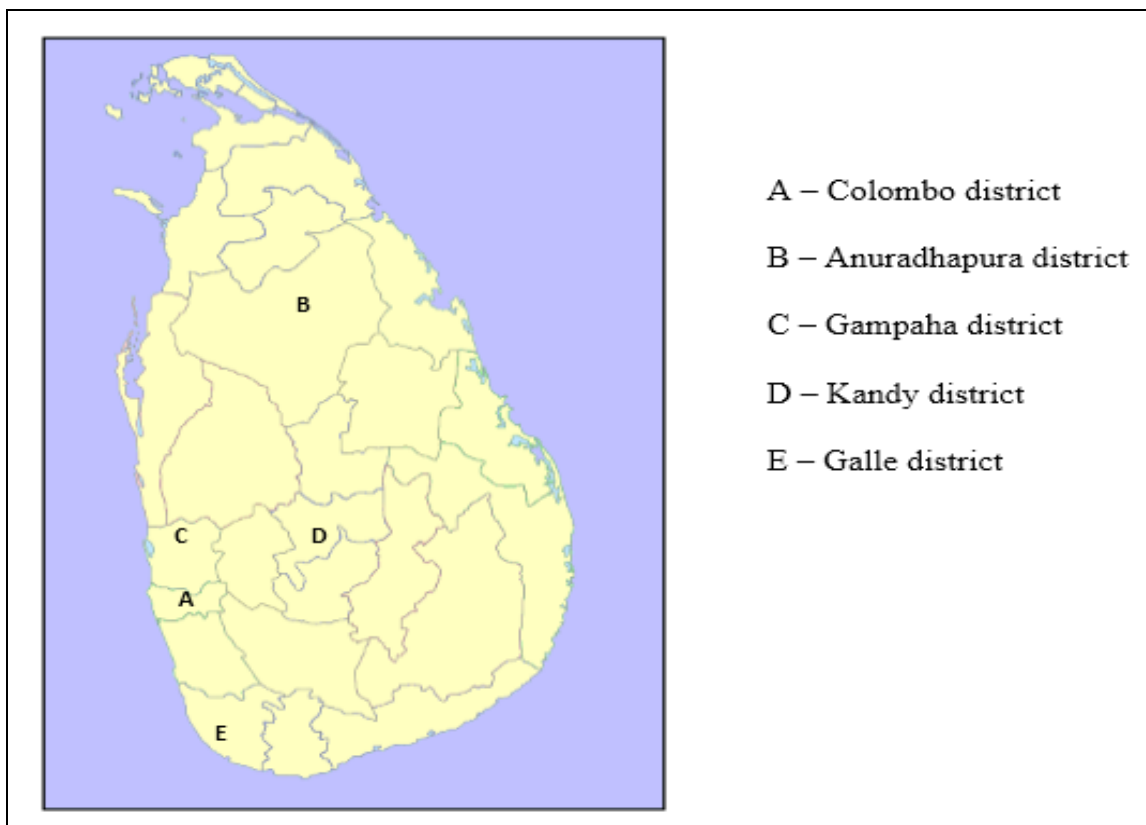
Collection of Plant material

Twenty five fresh samples per plant species collected from natural habitats in five provinces, representing different ecological zones of the country (Map 1).

Botanical identification

Botanical identification of the three plant species were done using available literature [3] and their identities were confirmed with the help of authenticated herbarium specimens deposited at the National herbarium, Peradeniya. Voucher herbarium specimens of all three plants were deposited (Acc. 1565a, 1565b, 1565c, 1565d, 1565e, 1566a, 1566b 1566c, 1566d, 1566e, 1567a, 1567b, 1567c, 1567d, 1567e) in the herbarium at the Bandaranaike Memorial Ayurveda Research Institute.

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Map 1: Map of Sri Lanka showing sampling locations of authentic sariva species

Studies on macroscopic and organoleptic characters

Leaf, stem, root, flower and fruit morphology of authentic *H. indicus* and its adulterants were studied and recorded. Organoleptic characters such as appearance, shape and size, colour, surface characteristics, texture, odour and taste of roots of *H. indicus* and its adulterants were recorded following the methods described in WHO guidelines [5].

Microscopical studies

Free hand sections were made from three different maturity positions of each root sample. Transverse Sections (TS), Tangential Longitudinal Sections (TLS) and Radial Longitudinal Sections (RLS) were stained using Safranin and Light green SF, by counter stain permanent slide method [6]. Micromorphological characters were drawn as seen through compound light microscope (model Kyowa, Biolux-12) and microphotographs were taken using Nikon 115 manual camera to illustrate the important features observed.

Powder microscopical analysis

Root samples collected from different localities were air dried at room temperature, powdered using a grinder (Kinematica - CH-6014, Switzerland) and passed through steel sieve mesh No 355 (Retsch®, AS 200, Germany). Powder microscopical studies were carried out following standard method [8, 9]. Samples were touched with a moistened tip of a needle and the powder that adhered to the needle was transferred on the glass slide containing 1 - 2 drops of water with saffranin, covered with a cover slip and warmed gently to remove air bubbles. Then the samples were studied, observing elements in ten microscopic fields. Microphotographs of different elements present were taken and the relevant data were recorded. The organoleptic characters of the powder were recorded following guidelines of World Health Organization [5].

Study of macerated root samples

Root samples collected from five different localities were subjected to maceration following Schultze's method [6]. Roots were cleaned with tap water and cut into 0.5 cm long pieces. These were immersed in concentrated nitric acid in a beaker. Few crystals of potassium chlorate was added and warmed up the solution till the material turned completely bleached. Subsequently, the macerated material was separated with a needle and stained with 1% saffranin solution for few minutes in a watch glass. Two drops of macerated sample was transferred on the glass slide and observed under light microscope. In each sample, different elements present in ten microscopic fields were observed. Length, width and thickening of tracheids, xylem vessels, fibers and stone cells were measured and recorded using stage micrometer and ocular micrometer. In addition, types of thickening present in the secondary wall of xylem elements were also recorded. Microphotographs were taken to illustrate important features.

Preparation of ethanol extracts.

One gram each of air dried and powdered roots of three plant species were extracted separately in 10 mL of absolute ethanol at room temperature (27 ± 5 °C) for 2 hours. The extracts were filtered kept in air until the solvent was completely evaporated. The residue was dissolved in 2 mL of absolute ethanol and was subjected to TLC analysis.

Chromatography

Chromatography was performed on pre-coated (Merck) analytical high performance Silica gel 60F 254 TLC plates (0.25 mm layer thickness). Plates were pre washed with methanol and activated by heating to 110°C for 30 minutes prior to use. TLC of the ethanol extracts of the three plants were developed using toluene: ethyl acetate (9.3: 0.7) as the solvent. The plates were sprayed with freshly prepared

anisaldehyde sulphuric acid reagent followed by heating at 110 °C for a few seconds for colour development.

Results and Discussion

Macroscopical and microscopical studies

The detailed systematic pharmacognostical and phytochemical evaluation of plant and plant material provides means of standardization of an herb that can be used as drug

or as raw material. During the present study it was observed that these three plants, even though they share some similarities in their habit and habitat possess salient diagnostic characters that could be used to differentiate one another, when the whole plant is available (Table 1). It should be noted that the morphological characters were not change with climatic zones.



(a 1) *H. indicus*



(a 2) *H. indicus* – flowering stage



(c) *C. buchananii*



(b) *I. frutescens*

Fig 1: Habit of plants referred to as Sariva

Table 1: Morphological features of *Hemidesmus indicus* and its adulterants.

Character	<i>H. indicus</i>	<i>I. frutescens</i>	<i>C. buchananii</i>
Habit	Laticiferous twining herb with milky latex	Rusty villous climber with yellowish latex	Laticiferous twining shrub with milky latex
Leaves	Polymorphous elliptic oblong to linear lanceolate leaves greenish with white streak above.	Elliptic oblong leaves, greenish	Elliptic oblong or oblong lanceolate, greenish
Flower	Purplish flowers in axillary cyme.	Greenish white flowers in axillary or terminal panicle	Yellow green flowers in paniculate cyme.
Fruit	Follicle	Follicle	Cylindrical follicle
Seeds	ovate-oblong, black	Seeds many, black	Many seeds

The leaves are polymorphic in *H. indicus*. The leaves in the immature portion of the plant are linear and dark green with a white streak along the midrib where as those in the flowering regions, are shorter and broader without any variegate. The above features could be used to identify *H. indicus* and

the two adulterants at the time of collection in the field so that the risk of collecting incorrect plant material could be avoided.

The macroscopic and sensory character differences among roots are shown in Table 2.

Table 2: Macroscopic and organoleptic characters of *Hemidesmus indicus* roots and its adulterants

Character	<i>H. indicus</i>	<i>I. frutescens</i>	<i>C. buchananii</i>
Shape	Cylindrical, Woody, long. Very strong lateral roots and rootlets present.	Cylindrical, Woody, long. Lateral roots few or absent.	Cylindrical, Woody.
Color	Purplish brown and yellow center,	Rusty or pinkish brown in color.	Dark brown or blackish external
Surface	Fairy smooth, soft and non - exfoliating. It appears firmly, longitudinally wrinkled in dry roots. Easily peelable.	The surface skin is very thin, soft. Not easily peelable.	Longitudinal ridges and wrinkles present. Easily peelable.
Odour	Characteristic Aromatic.	Indistinct.	Indistinct.
Taste	Sweetish warm aromatic taste.	Acrid.	Slightly sweetish.

According to the observations odour and taste seem to be the most convenient characters distinguishing *H.indicus* from its adulterants. The above characters could be used to identify raw material at the time of purchase.

The characters available in the powder are much fewer than the potentially available characters in the whole specimen (organoleptic and anatomical). Starch grains are the most

prominent character in powder of all three Sariva species, but in *H. indicus*, both simple and compound starch grains are more abundant than in the adulterants.

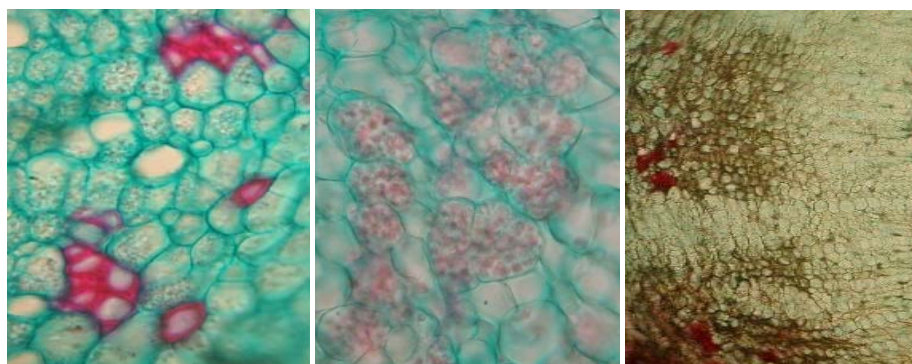
Various microscopical features to be used as quality standards in raw material identification are summarized in Table 3 and illustrated in plates 2, 3 and 4. This is the first record of such characters of these plants.

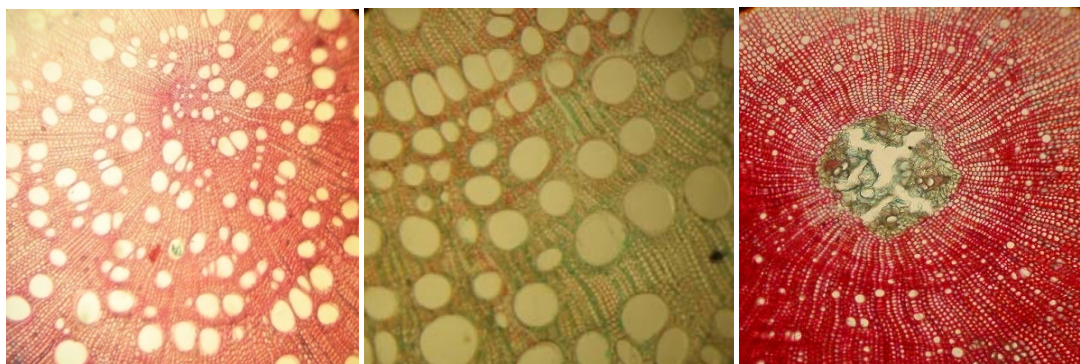
Table 3: Anatomical features of roots of *Hemidesmus indicus* and its adulterants

Character	<i>H. indicus</i>	<i>I. frutescens</i>	<i>C. buchananii</i>
Cork	Outer part of the cork is thick, hard crustaceous. It is deep purplish and composed of about 20 – 30 rows of thick walled brownish cells. These cells are radially flattened and rectangular in appearance. Easily peelable from the rest of the roots.	The surface skin is very thin and soft. Composed of limited number of thin walled cells. Not easily peelable and not specified as cell layers.	Deep brownish in colour 8 – 15 layers of cells. These are radially flattened and rectangular in appearance. The outer bark is easily peelable.
Cortex	Composed of several rows of thin walled cells and richly loaded with starch grains. Branched laticiferous ducts present in the cortex.	Cortical cells are scanty loaded with starch grains, and secretory cells. Branched laticiferous ducts	Starch grains scanty. laticiferous ducts and secretory cells are present in the cortex.
Mechanical elements(fibers) in the cortex	Completely absent	Present	Fiber cells present.
Phloem	It is not clear as a zone. Thin walled elements with narrow medullary rays present.	Phloem is clearly differentiated as an annular zone.	Phloem is differentiated as a clear zone.
Wood Vessel arrangement Medullary rays Vessels and parenchyma Pith	Wood part comparatively small. Diffuse porous Majority of are uniseriate and rich in starch in the form of grains. Rarely biseriate rays are scattered in the xylem tissue. Apotracheal. Absent in older roots.	Wood part comparatively large semi ring porous Both uniseriate and multiseriate medullary rays rich in starch in the form of grains present. Apotracheal. A pith like structure present in older roots.	Wood part comparatively large ring porous Both uniseriate and multiseriate medullary rays rich in starch in the form of grains present. Paratracheal. A pith like structure present in older roots.

Multilayered thick walled cork, cortical cells filled with starch grains, absence of mechanical elements (plate 2), comparatively small wood part, absence of a clear zone of phloem, diffuse porous vessel arrangement (plate 3),

uniseriate medullary rays (plate 4) and absence of pith (plate 5) are the most distinguishable anatomical characters of *H. indicus*.

*I. frutescens**H. indicus**C. buchananii***Plate 2:** T. S. of root cortex showing mechanical elements (x400)

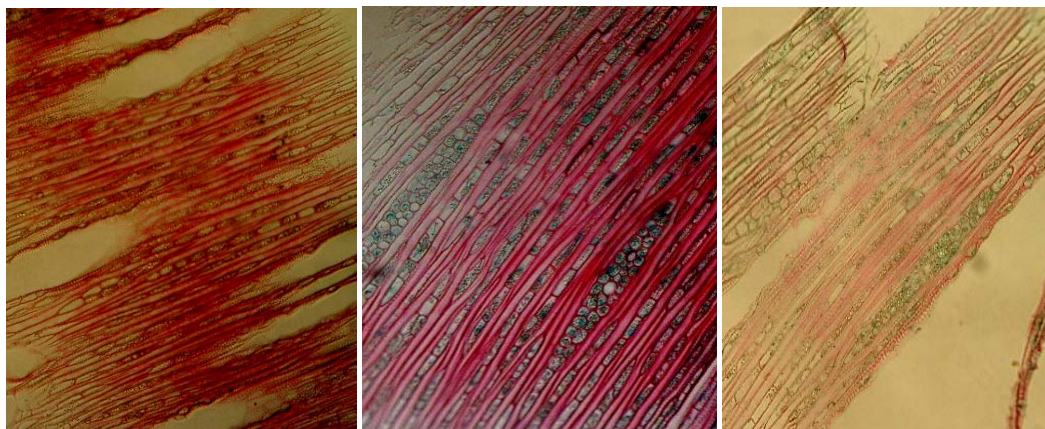


H. indicus (diffuse porous)

I. frutescens (semi ring porous)

C. buchananii (ringporous)

Plate 3: T. S. of root wood showing the arrangement of xylem vessels (x100)

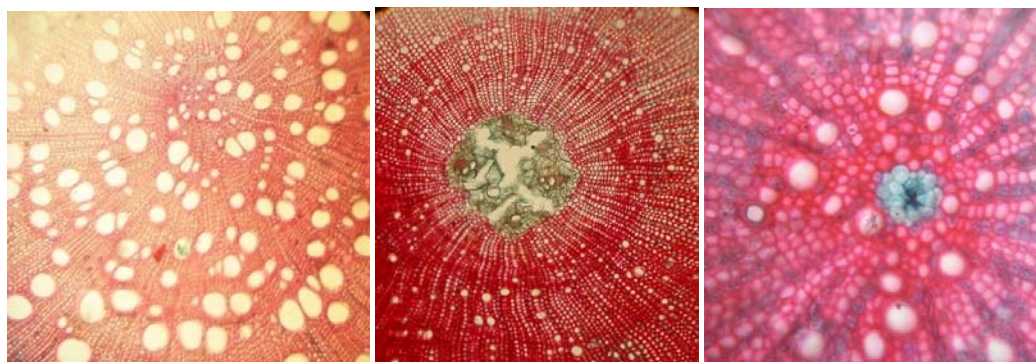


H. indicus (uniseriate)

I. frutescens (both uni and multiseriate)

C. buchananii (both uni and multiseriate)

Plate 4: T.L.S. of wood showing medullary ray cells (x100)



H. indicus

C. buchananii

I. frutescens

Plate 5: T.L.S. of wood showing pith (x100)

When the macerated root materials are concerned, *H. indicus* could be differentiated from its adulterants by the presence of typical, drum-shaped xylem vessels (not drum shaped in adulterants) and the absence of stone cells (stone cells were abundantly observed in adulterants).

TLC analysis

The chromatogram of the ethanolic extracts of the roots of *H. indicus*, *C. buchananii* and *I. frutescens* after spraying with anisaldehyde sulphuric acid reagent, is given in plate 6.

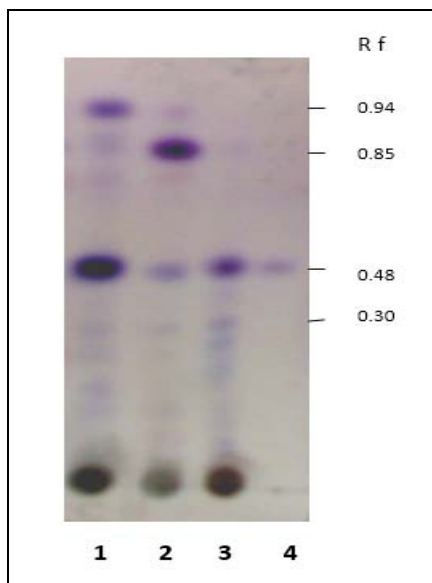


Plate 6: Chromatogram of the ethanol extracts of *H. indicus* and its adulterants

Track 1	<i>C. buchananii</i>
Track 2	<i>H. indicus</i>
Track 3	<i>I. frutescens</i>
Track 4	Lupeol

While there are similarities in the pattern of spots observed for the three plants, they can be distinguished from each other by considering the differences in the relative intensities of the spots. Thus, *C. buchananii* exhibits two prominent spots at rf 0.48 and 0.94, while the most prominent spot for *H. indicus* is at rf 0.85. *I. frutescens* has its most prominent spot at rf 0.48, which corresponds to lupeol which has been reported from all three plants previously.

Conclusion

Three plants of sariva can be easily distinguished from one another morphologically. Their roots are not easy to distinguish from each other.

However, The root of *H. indicus* which is considered to be true Sariva, can be distinguished from its adulterants *C. buchananii* and *I. frutescens*, by its characteristic odour and taste, and multilayered thick walled cork, cortical cells filled with starch grains, comparatively small wood part, diffuse porous vessel arrangement, uniseriate medullary rays, absence of a clear zone of phloem, pith and mechanical elements. The differences in the relative intensities of spots observed in the tlc of the ethanol extracts of the three plants can be also used to distinguish them from each other.

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