Pharmacognostical studies on the rhizome and root of *Smilax zeylanica* Linn. –A potential alternate source for the Ayurvedic drug *Chopachinee*

V Madhavan¹, H T Hemalatha¹, M R Gurudeva² and S N Yoganarasimhan¹*

¹Department of Pharmacognosy, M S Ramaiah College of Pharmacy, Bangalore-560 054, Karnataka, India
²Department of Botany, V V Pura College of Science, Bangalore-560 004

Received 28 November 2008; Accepted 18 August 2009

*Chopachinee* is an important Ayurvedic drug used in several formulations and diseases. *Smilax* Linn. species are used as botanical source of *Chopachinee* while the accepted source is *Smilax china* Linn. *Smilax zeylanica* Linn., a potential alternate source for *Chopachinee*, occurring in South India is pharmacognostically investigated in this paper. It is used in the treatment of abscesses, skin disorders, sores, swellings, venereal diseases and as a substitute for Sarsaparilla. The present study comprises taxonomical, macroscopical, microscopical characters, physico-chemical and ultra-violet analysis besides chromatographic studies of the rhizome and root which not only help in the identification of the drug but also contribute towards establishing pharmacopoeial standards. HPTLC finger printing of diosgenin present in the drug is carried out to establish the biomarker compound.

**Keywords:** *Smilax zeylanica*, Smilacaceae, *Chopachinee*, Rhizome, Root, Pharmacognosy, Diosgenin, Ayurvedic Drug.

**IPC code:** Int. cl. A61K 36/00, A61K 36/90

**Introduction**

*Chopachinee* is an important Ayurvedic drug for which the accepted botanical source is *Smilax china* Linn. Its rhizome and roots are used in Ayurvedic formulations such as *Karpura sundari vati, Madhusnuhi rasayana* and *Nasarogahari vati* which are employed in the treatment of congenital diseases, epilepsy, hemiplegia, leprosy, parkinsonism, polyuria, psychosis, diseases of nervous system, urinary disorders and wasting diseases to name a few; it is also claimed to possess rejuvenator and blood purifier property.¹ The genus *Smilax* Linn. (Family-Smilacaceae) consists of more than 300 species, distributed all over the world, out of which 24 are found in India.² In South India, 4 species, viz. *Smilax aspera* Linn., *S. perfoliata* Lour., *S. wightii* A. DC. and *S. zeylanica* Linn. occur³. Different species such as *S. glabra* Roxb., *S. ovalifolia* Roxb. and *S. lanceifolia* Roxb. are used as substitutes of *Chopachinee*⁴. *S. zeylanica* is used in the treatment of abscesses, skin disorders, sores, swellings and venereal diseases⁵. Different species of the genus including *S. zeylanica* are also used as substitutes for Sarsaparilla in many parts of the world⁶⁷. The phytoconstituents reported in the leaves and roots of *S. zeylanica* are steroidal saponin glycosides like diosgenin, smilagenin and sarsapogenin⁸. Recently, the roots and rhizomes are reported to possess antiepileptic activity⁹.

* S. zeylanica Linn. is a dioecious climbing shrub; stem woody, sometimes armed with prickles; branchlets angular. Leaves 20 × 16 cm, variable in shape (ovate, elliptic or oblanceolate), acuminate at apex, rounded at base; petiole up to3 cm long; leaf sheath 8 mm long, narrow. Flowers greenish-white, in umbels: umbels 1 to 3. Perianth-lobes 6. Stamens 6, subequal. Pistillode absent. Ovary 3-locular; style short; stigmas 3; staminodes 3 (rarely 6), equalling stigmas. Berry globose (Plate I.1).

The vernacular names assigned to *S. zeylanica* are:

Hindi – Chobchini, Jangliaushbah, Ramdatun; Kannada – Kaadu hambu thaavare;Sanskrit – Chopachinee, Vanamadhusnuhi;Telugu – Kummeritheega, Kondadantena, Kondagarbhathige, Konda, Sithapa, Gurivatheega, Kondathaamara, Kummarabaddu, Kushtaputamara; Tamil – Ayadi, Tirunamappalai, Periyakanni, Karuvalanchikudam,
Materials and Methods

Fresh rhizome and roots were collected from the surroundings of Kalakkad forests, Tirunelveli district, Tamil Nadu during December, 2006, preserved in 70% ethyl alcohol for histological studies. Botanical identification was done using local floras. Voucher herbarium specimen (Hemalatha 020) was prepared and is preserved along with crude drug sample at the herbarium and museum of M S Ramaiah College of Pharmacy, Bangaluru. Pharmacognostical evaluation including histochemical and macerate studies were carried out by taking free hand sections following Johansen and Wallis while powder studies were done as per Evans. Photomicrographs were obtained by observing the free hand sections of drug under compound binocular microscope (Olympus-CH20i model) with built in analogue camera (CMOF, 1.4 mega pixel). Computer Images were captured using AV-Digitaliser having Grand VCD 2000-Capture Guard. Measurements of cells and tissues were carried out using Micro Image Lite Image Analysis Software (Cybernetics, Maryland, USA). Physicochemical constants, organic analysis, ultraviolet analysis and chromatographic studies were carried out from shade-dried powder following prescribed methods. Vernacular names are provided following Gurudeva.

For HPTLC studies a solution of the alcohol extract in chloroform and a solution of the aqueous extract in methanol and water (1:1) was prepared (5 mg/ml). A solution of standard diosgenin in chloroform was also prepared (5 mg/ml). The studies were carried out on alcohol, aqueous extracts and standard diosgenin, using Camag HPTLC system equipped with Linomat V sample applicator, Camag TLC scanner 3 and CATS 4 software for interpretation of data. An aluminium plate (10 × 10 cm) precoated with silica gel 60F254 (E Merck) was used as adsorbent. The plates were developed using toluene: ethyl acetate (7:3) in a Camag twin trough chamber to a distance of 8 cm each. All the solvents used were of HPLC grade obtained from MERCK. Post derivatisation was carried out using Liebermann Burchard reagent. The Rf values were recorded by using a software. The developed plates were photodocumented at 254 nm, and 425 nm. HPTLC fingerprinting of diosgenin was further confirmed by overlaying spectrum of standard diosgenin with that of sample at 254 nm and 425 nm.

Results

Macroscopical characters of rhizome

Rhizomes 5 to 7 cm long and 5 cm thick, woody, brownish to blackish externally, yellowish internally, rough, give rise to several roots at different points, fracture fibrous, without characteristic odour and with slightly bitter taste (Plate I. 2, 3).

Microscopical characters

Transverse section of rhizome exhibits epidermis, a wide yellowish ground tissue, where vascular bundles are found scattered. Epidermis is single layered, made up of compact rectangular cells. Next to epidermis lies a large ground tissue which can be differentiated into two regions. Outer ground tissue is parenchymatous, many layered, consists of oval, thin walled cells, measure 24-40-45µ long; some cells are tanniferous, some others contain raphide bundles consisting of acicular crystals while in a few other cells granular cell contents are found; each raphide measures 6.6-6.7-6.9µ long; lysigenous cavities are found scattered in outer ground tissue (Plate I. 4 to 7). Inner ground tissue consists of an outer one layered sclerenchyma, which consists of large, thick walled cells having large lumen, measuring 17-27-45 15-26-47µ; this is followed by a few layers of smaller compactly arranged sclerenchyma cells, measuring 43-48-62 × 26-35-54µ while the remaining part of ground tissue is parenchymatous; some cells of this region contain dark content which are tanniferous and some others yellow cell content (Plate I. 8 to 11). Vascular bundles are found scattered; they are conjoint, collateral, encircled by a sclerenchymatous bundle sheath; a few lysigenous cavities are conspicuous (Plate I. 8 to 12).

Macerate exhibit parenchyma cells which are pitted, of different size and shape, measure 117-132-160 × 42-48-58µ (Plate I. 13 to 16); fibres of different size, with pits and large lumen, measure 164-203-264 × 8-21-33µ (Plate I. 17 to 19); trachieds of various size, with scalariform thickenings, measure 667-882-1104 × 20-25-34µ (Plate I. 20, 21) and vessels which
Plate I—1 – 54: Pharmacognostical studies on the rhizome and root of *Smilax zeylanica* Linn.

1. Fruiting twig; 2. Fresh rhizome and roots; 3. Dried rhizome and roots; 4. T.S. of rhizome showing different regions; 5. Outer parenchymatous ground tissue enlarged showing tanniferous cells; 6. Outer ground tissue showing raphide bundles and granular cell content; 7. Outer ground tissue showing lysigenous cavities; 8. Inner ground tissue showing outer sclerenchyma; 9. Inner ground tissue showing compactly arranged cells; 10. Inner ground tissue with dark tanniferous cell content; 11. Inner ground tissue with yellow cell content; 12. Ground tissue showing vascular bundles and lysigenous cavity
13 to 16. Parenchyma cells; 17 to 19. Fibres; 20, 21. Tracheids. 22 to 25; Vessels. 26 to 28. Young root. 26; T.S. of young root showing different regions. 27; T.S of young root showing epiblema, cortex and stellar regions; 28. Portion of stele enlarged
29 to 41. Mature root (without cortex) and macerate: 29. T.S. details without cortex; 30. Portion of stele enlarged; 31. Portion showing remnants of endodermis and pericycle; 32. Portion of stele showing radially arranged xylem and phloem; 33. Portion of stele showing vessels with cell content; 34. Portion showing phloem

Abbreviations: CC-cell content; COR-cortex; END-endodermis; EPB-epiblema; EPI-epidermis; GCC-granular cell content; ISGT-inner sclerenchymatous tissue; LYC-lysigenous cavity; OLSC-outer layer of sclerenchyma cells; OPGT-outer parenchymatous tissue; PGT-parenchymatous ground tissue; PC-pericycle; PH-phloem; PI-pith; PP-pitted parenchyma; RAP-raphide; RB-raphide bundle; SBS-sclerenchymatous bundle sheath; SCL-sclerenchyma; SG-starch grains; TFC-tanniferous cell; VB-vascular bundle; VE-vessel; VEC-vessel content; XY-xylem; YC-yellow content.
are long, narrow and with scalariform thickenings, measure 1199-2077-2753 × 38-60-78µ (Plate I. 22 to 25).

Macroscopical characters of root

Roots 10 to 20 cm long and 2 mm thick, adventitious, several, arising from different points of rhizome, run either vertically or horizontally, wiry, very hard, surface smooth, brownish to blackish externally, yellowish internally, without any characteristic odour and with slightly bitter taste, fracture fibrous. Fresh young roots are white (Plate I. 2, 3).

Microscopical characters

Transverse section is circular in outline. Young fresh root exhibits a single layered epidermis made up of compactly arranged rectangular cells measuring 12.5-19-26 × 12-18-26µ. This is followed by many layered cortex and stellar region with central pith. Cortex is broad, multilayered, consisting of parenchymatous cells measuring 21-54-75 × 26-49-71µ having intercellular spaces (Plate I. 26 to 28). In mature roots the epidermis and cortex peels off very early. Transverse section of mature root shows endodermis which is blackish, one layered, cells measure 23-31-35 × 16-20-23µ. Pericycle lies next to the endodermis which is sclerenchymatous with cells having large lumen, measuring 10-30-43 × 6-15-22µ (Plate I. 29, 31). Xylem and phloem are radically arranged, found embedded in the sclerenchyma; xylem is exarch, measure 7-13-8 × 7-12-16µ (Plate I. 32). Vessels measure 30-41-49 × 20-27-34µ; some vessels contain darkly stained cell contents (Plate I. 33, 34). Phloem cells measure 93-120-167 × 58-65-80µ, consists of phloem parenchyma, sieve tubes and companion cells (Plate I. 34). Pith is large, consists of parenchymatous cells with intercellular spaces, measuring 22-49-72 × 26-41-58µ (Plate I. 30). Raphide bundles of acicular crystals occur in some larger pith cells; each raphide measuring 43-57-82 × 3-3.2-3.7µ; some pith cells are pitted (Plate I. 35, 36). Starch grains are simple, found in some pith cells and phloem parenchyma, measure 3.5-7-9µ (diameter) (Plate I. 37 to 39).

Macerate exhibit parenchyma cells of different size, some pitted, measuring 134-181-228 × 16-46-78µ (Plate I. 40, 41); sclerenchyma cells of different size and shape, measuring 196-226-257x 37-41-46µ (Plate I. 42 to 44); fibres with narrow ends and pitted thickenings, measuring 689-1023-1357 × 17-24-31µ (Plate I. 45, 46); Vessels of different size, with scalariform or pitted thickenings, measuring 266-270-276 × 42-44-47µ (Plate I. 47, 48).

Powder analysis

Powder is brownish, slightly bitter, odourless, fibrous; when treated with chloral hydrate solution, stained in 1% safranin for 5 to 10 minutes, mounted in 50% glycerine shows the following elements: fragments of sclerenchyma cells, vessels and parenchyma cells (Plate I. 49-51); fragments of fibres which are long and with pointed ends (Plate I. 52) and fragments of vessels with scalariform thickenings (Plate I. 53, 54).

Histochemical tests

The sections of rhizome and root were treated with different reagents and results provided in Table 1.

Physico-chemical studies

The percentage of moisture content was 6.67, total ash 5.07, acid insoluble ash 0.57, water soluble ash 0.65, alcohol soluble extractive 32.63 and water soluble extractive 26.85; the colour, consistency and percentage of successive extractive values of extracts were petroleum ether (60-80°C) (tata mimosa, sticky mass, 1.6), benzene (olive green, sticky mass, 0.9), chloroform (midbuff, sticky mass, 1.4), acetone (leaf brown, semi solid, 1.72), ethanol (70%) (brown, semi solid, 2.28) and chloroform water (golden brown, sticky mass, 0.96).

Preliminary organic analysis

A known quantity of dried powder was extracted in a Soxhlet with petroleum ether (60-80°C), benzene, chloroform, acetone and ethanol (95%) and finally macerated with chloroform-water (2%) for 24 h successively and tested for different constituents; it revealed the presence of alkaloids in chloroform, ethanol and water extracts, carbohydrates in ethanol and water extracts, phytosterols in petrol ether, benzene, acetone extracts, fixed oil and fats in petrol ether and acetone extracts, phenols and tannins in ethanol and water extracts, saponins in ethanol extract, flavonoids, proteins and amino acids in ethanol and water extracts, and gums and mucilage in water extracts.

Chromatographic studies

HPTLC fingerprint profile of alcohol extract revealed 5 phytoconstituents at Rt 0.24, 0.29, 0.56, 0.63, and 0.87 at 254 nm (Fig. 1) while aqueous extract gave two at Rt 0.24 and 0.56 (Fig. 2) whereas
standard diosgenin gave R$_f$ at 0.55 (Fig. 3). Under visible light alcohol extract gave 4 constituents at Rf 0.06, 0.55, 0.62 and 0.88 (Fig. 4), aqueous extract also gave 4 constituents at Rf 0.06, 0.44, 0.49 and 0.54 (Fig. 5) whereas standard diosgenin revealed Rf at 0.54 (Fig. 6). The Rf at 0.56 in alcohol and aqueous extracts corresponded to that of R$_f$ 0.55 diosgenin standard when scanned at 254 nm. When scanned at 425 nm, alcohol and aqueous extracts revealed spot at R$_f$ 0.55 and 0.54, respectively, corresponding to that of diosgenin standard (R$_f$ 0.54). This was further confirmed by overlay spectrum of standard diosgenin, alcohol and aqueous extracts of S. zeylanica scanned at 425 nm (Fig. 7).

**Ultra-violet analysis**

Powdered drug under ultra-violet and ordinary light when treated with different reagents emitted various colour radiations which help in identifying the drug in powder form (Table 2).

**Diagnostic characters**

S. zeylanica plant is identified by its climbing habit and reticulate venation in leaves which is rare in monocotyledons. Rhizome is identified by the presence of outer parenchymatous and inner sclerenchymatous ground tissue, vascular bundles scattered, conjoint and collateral, presence of tanniferous cells, raphide bundles of acicular crystals and conspicuous lysigenous cavities. Roots are identified by their adventitious nature, presence of sclerenchymatous pericycle with cells having large lumen, raphide bundles in pith cells, darkly stained cell content in vessels, vessels with scalariform thickenings.

**Table 1—Histochemical tests of sections of S. zeylanica**

<table>
<thead>
<tr>
<th>Test for</th>
<th>Reagent</th>
<th>Reaction</th>
<th>Root</th>
<th>Rhizome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>Phloroglucinol + dil.HCl</td>
<td>Majenta colour</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mucilage</td>
<td>Ruthenium red solution.</td>
<td>No red colour</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>Iodine solution</td>
<td>Blue color</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>Millon’s reagent</td>
<td>No brick red colour</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oil</td>
<td>Sudan red III solution</td>
<td>Red colour</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s reagent</td>
<td>No brownish colour</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Dil. ferric chloride solution</td>
<td>Bluish black</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Crystals</td>
<td>Con. HCl</td>
<td>Effer-vescence</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present; - = Absent

**Table 2—Ultra-violet analysis of the root and rhizome powder of S. zeylanica**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment of powder</th>
<th>Visible light</th>
<th>UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>short wave (254 nm)</td>
<td>long wave (365 nm)</td>
</tr>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Leaf brown</td>
<td>Leaf brown</td>
</tr>
<tr>
<td>2</td>
<td>Powder + methanol</td>
<td>Golden brown</td>
<td>Mid buff</td>
</tr>
<tr>
<td>3</td>
<td>Powder + acetone</td>
<td>Leaf brown</td>
<td>Mid buff</td>
</tr>
<tr>
<td>4</td>
<td>Powder + ethanol (95%)</td>
<td>No change</td>
<td>Mid buff</td>
</tr>
<tr>
<td>5</td>
<td>Powder + dil. ammonia</td>
<td>Leaf brown</td>
<td>Mid buff</td>
</tr>
<tr>
<td>6</td>
<td>Powder +1N methanolic NaOH</td>
<td>Leaf brown</td>
<td>Brilliant green</td>
</tr>
<tr>
<td>7</td>
<td>Powder +1N ethanolic NaOH</td>
<td>Leaf brown</td>
<td>Brilliant green</td>
</tr>
<tr>
<td>8</td>
<td>Powder +1N HCl</td>
<td>Leaf brown</td>
<td>Tata mimosa</td>
</tr>
<tr>
<td>9</td>
<td>Powder + 50% H$_2$SO$_4$</td>
<td>Golden brown</td>
<td>Jade green</td>
</tr>
<tr>
<td>10</td>
<td>Powder + 50% HNO$_3$</td>
<td>Leaf brown</td>
<td>Mid buff</td>
</tr>
<tr>
<td>11</td>
<td>Powder + 5% KOH</td>
<td>Golden brown</td>
<td>Tata mimosa</td>
</tr>
</tbody>
</table>

NOTE: The colour mentioned in the table is based on the Asian paints premium gloss enamel card, Asian Paints Limited, Mumbai.
Conclusion
The preliminary phytochemical analysis of the rhizome and root of *S. zeylanica* revealed presence of glycosides, saponins, phytosterols and tannins. HPTLC fingerprinting detected the presence of diosgenin, a biomarker compound. The work contributes towards utilizing *S. zeylanica* as a potential alternate source for the Ayurvedic drug Chopachinee.

Acknowledgement
The authors are thankful to the Gokula Education Foundation, Bangaluru and to the Principal, V V Pura College of Science, Bangaluru for evincing interest in this work. They are also thankful to Mr. V Chelladurai of Tirunelveli for help in collection of authentic plant material.

References
2 Santapau H and A N Henry, Dictionary of the Flowering Plants of India, Publication and Information Directorate, New Delhi, Reprint, 1976, 18.
5 Chopra R N, S L Nayar, and IC Chopra, Glossary of Indian Medicinal Plants, National Institute of Science Communication and Information Resources, CSIR, New Delhi, Reprint, 2002, 228.
12 Jain S K. and R R Rao, Field and Herbarium Methods, Today & Tomorrow Publishers, New Delhi, 1976, 22-61.