Pharmacognostical studies on the leaves of *Cocculus hirsutus* (Linn.)
Diels – *Chilahinta*, an Ayurvedic drug

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*Cocculus hirsutus* (Linn.) Diels known as *Chilahinta* in Ayurveda and *Kattu kodi* in Siddha is an important medicinal plant belonging to the family Menispermaceae. The leaves are used to treat several diseases like polyuria, fevers, piles and is said to possess aphrodisiac property. The present study provides taxonomical, pharmacognostical and physico-chemical details helpful in laying down standardization and pharmacopoeial parameters. Some diagnostic characters are presence of unicellular ribbon shaped trichome both on lamina and petiole, presence of sunken stomata, excretory sacs in mesophyll. Physico-chemical studies revealed total moisture content (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%). Ultraviolet analysis exhibited considerable variation and preliminary organic analysis revealed presence of alkaloids, flavonoids, fixed oils, fats, mucilage, glycosides and phytosterols. HPTLC profile of alcoholic extract of leaves gave 16 phytoconstituents.

**Keywords:** *Cocculus hirsutus*, Menispermaceae, Leaves, Pharmacognosy, Physico-chemical analysis, HPTLC, Alcohol extract.

**IPC code:** Int. cl.⁸—A61K 36/59, A61K 127/00

**Introduction**

*Cocculus* Linn. (Menispermaceae) consists of 20 species of mostly scandent or rarely suberect herbs or shrubs, distributed in tropics and subtropics⁴. *Cocculus hirsutus* (Linn.) Diels is a scandent shrub, known as *Chilahinta* in Ayurveda and *Kattu kodi* in Siddha system of medicine; the roots and leaves are used to treat polyuria, eczema, dysuria, abdominal disorders, rheumatoid arthritis, fevers, piles, syphilis, disorders of blood and as an aphrodisiac⁵,⁶. Several isoquinoline alkaloids are present in leaves and roots; the important ones are cohirsine, cohirsinine, cohirsitinine, hirsutine and jamtine⁷-¹¹. Some histological studies on the root, rhizome and leaf has been carried out¹²,¹³ but detailed pharmacognostical studies including macerate details, powder study exhibiting the various elements and fluorescence analysis besides HPTLC studies on the leaf is not available¹⁴-¹⁶. Hence, the present investigation which helps not only in the identification of the drug but also provides requisite material for establishing the biomarker/bioactive compound.

**Materials and Methods**

Fresh leaves were collected from Kudur village, Bangalore Rural district, Karnataka, during March, 2006, preserved in 70% ethyl alcohol for histological studies. Botanical identification was carried out using local floras¹⁷,¹⁸, identified by senior Plant Taxonomist and authenticated at the herbarium of the Regional Research Institute (Ay), Bangalore (RRCBI). Voucher herbarium specimen (Sajid Ullah 001) was prepared and is preserved along with crude drug sample at the herbarium of M S Ramaiah College of Pharmacy, Bangalore.¹⁹ Pharmacognostical evaluation including histochemical, macerate and powder studies were carried out by taking free hand sections following Johansen²⁰, Wallis²¹ and Evans²². Safranin (4%) was used to stain transverse sections. Reagents like potassium iodide, ferric chloride, Sudan III,
concentrated HCl, ruthenium red and phloroglucinol with dilute HCl were used for histochemical tests. Concentrated nitric acid (50%) with pinch of potassium chlorate crystals was used as the macerating fluid. Photomicrographs were obtained by observing 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). Physico-chemical constants, organic analysis, ultra-violet analysis and chromatographic studies were carried out from shade-dried powder following prescribed methods. HPTLC studies were carried out on alcohol extract 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 (5x10 cm) precoated with silica gel 60F254 (E Merck) was used as adsorbent.

Results

Botanical description


Hirsute climbers or stragglers. Leaves 2.5-5x2-2.5 cm, simple, ovate, obtuse, mucronate, truncate at base, softly villous. Flowers small, greenish-yellow. Male flowers in 5 to 6 cm long axillary cymes, rarely in racemes. Fruits globose, green, shining (Plate 1 Fig. A). Distributed throughout Tropical Africa and India in most districts.

Macro- and microscopical characters of leaves

Leaves dorsiventral, variable, simple, ovate to ovate-oblong or slightly lanceolate with truncate to cordate base, apex sometimes mucronate, margins entire or slightly wavy, lamina hairy, greyish-white tomentose beneath. Veination reticulate with 5 to 6 pairs of alternating lateral veins; first two pairs of lateral veins arise basally giving a multicostate appearance; secondary and tertiary veinlets anastomose to form reticulation with free end included in meshes. Petiole greyish-tomentose, with a distinct swelling at proximal and distal ends. Leaves greenish, odourless and mucilaginous when fresh, brittle and powdery on drying, without characteristic taste, prolonged contact produces itching sensation (Plate 1 Fig. B).

Transverse section of petiole is circular in outline with trichomes emerging all over the surface, consists of epidermis, cortex and stellar region with prominent pith. Epidermis single layered, made up of thick walled rectangular cells. Hairs or trichomes emerge from some epidermal cells. Hypodermis is made up of collenchymatous cells, measure 5-7-10µ. Cortex consists of hexagonal cells, demarcated into inner region of larger cells and outer region of smaller cells, cells contain abundant chloroplast and sparsely distributed starch grains. Endodermis is single layered. Pericyclic is multilayered, heterogeneous, sclerenchymatous over the vascular bundle, parenchymatous in between vascular bundle; conjunctive tissue is found in between vascular bundle (Plate 1 Fig. C-D). Stele consists of vascular bundle varying from 6 to 8, arranged in a ring; vascular bundle conjoint; collateral, endarch (Plate 1 Fig. E). Xylem cells measure 5-7-8µ, consists of vessels and fibres; phloem cells measure 5-7-8µ, consists of sieve tube and companion cells.

Transverse section of leaf consists of lamina and midrib regions (Plate 1 Fig. F). Lamina exhibits upper and lower epidermis; lower epidermal cells smaller, measure 5-6-7µ, upper epidermal cells measuring 8-9-10µ; epidermal cells rectangular, filled with chloroplast. Stomata anomocytic, found on lower epidermis, many, sunken, each surrounded by 4 to 6 epidermal cells. Mesophyll comprises of palisade and spongy parenchyma, cells filled with chloroplast and starch grains. Palisade cells columnar, 1-layered, except near midrib where it is 2 to 3-layered. Spongy parenchyma 2 to 3-layered, cells elongated, thin walled and enclose air spaces in between and excretory sacs (Plate 1 Fig. G). Midrib exhibits crescent shaped vascular bundle enclosed by sclerenchymatous bundle sheath. Next to bundle sheath lies parenchymatous ground tissue; some peripheral cells are collenchymatous; vascular bundle

Methods, results, and discussions...
Plate 1: Figs. A-U. Macro- and microscopical characters of the leaves of *Cocculus hirsutus*.


(Abbreviations: CJT-conjunctive tissue; COL-collenchyma; COR-cortex; END- endodermis; EP-epidermis; EPC-epidermal cell; EXS-excretory sac; GT-ground tissue; HYP-hypodermis; LAM-lamina; LOE-lower epidermis; MID-midrib; PH-phloem; PPA-pitted parenchyma; PPER-parenchymatous pericycle; SBS-sclerenchymatous bundle sheath; SCLP-sclerenchymatous pericycle; SPP-spongy parenchyma; ST-stomata; TRI-trichome; TRIB-trichome base; UPE-upper epidermis; XY-xylem; XYP-xylem parenchyma; VE-vessel).
consists of xylem and phloem (Plate 1 Figs. F, H, I). Macerate of leaf exhibit epidermal cells measuring 6-8-9µ, with trichome base and sunken anomocytic stomata (Plate 1 Figs. J, K, L); unicellular trichome which are long and ribbon shaped, measuring 104-177-270µ (Plate 1 Fig. O); thick walled collenchyma cells measuring 123-138-160µ (Plate 1 Fig. P); fibres with pointed ends measuring 93-138-204µ (Plate 1 Fig. Q, R); vessels which are narrow and with reticulate thickening measuring 73-91-113µ (Plate 1 Fig. S, T); fragment of lamina showing veination pattern (Plate 1 Fig. U). Stomatal index was found to be 13.3, average number of stomata was 14, vein islet number 6.2 and vein termination number 12.5.

Powder study
Leaf powder is mucilaginous, greenish, odourless, bitter, fibrous; when treated with chloral hydrate solution, stained in 1% safranin for 5-10 minutes, mounted in 50% glycerine shows fragments of unicellular, long, ribbon shaped trichomes, fibres with pointed ends, narrow cylindrical vessels, bits of veins with trichome base and groups of epidermal cell.

Histochemical tests
Sections were treated with different reagents and results provided in Table 1.

Physico-chemical studies
The % 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 % of successive extractive values and consistency of extracts were: petroleum ether (60-80ºC) (2.80) (sticky mass), benzene (1.75) (sticky mass), chloroform (2.16) (sticky mass), acetone (3.10) (sticky mass), ethanol (’0.36) (semi solid) and water (4.54) (semi solid).

Preliminary organic analysis
A known quantity of dried powder was extracted in a Soxhlet with petroleum ether (60-80ºC), benzene, chloroform, acetone and ethanol (95%) and finally macerated with chloroform-water (2%) for 24h successively and tested for different constituents; it revealed presence of alkaloids, flavonoids, fixed oils, fats, mucilage, glycosides and phytosterols (Table 2).

Table 1—Treatment of sections of C. hirsutus with different reagents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reagent</th>
<th>Test for</th>
<th>Reaction</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section</td>
<td>Iodine solution</td>
<td>Starch</td>
<td>No blue colour</td>
<td>-</td>
</tr>
<tr>
<td>Section</td>
<td>Ferric chloride</td>
<td>Tannin</td>
<td>No black colour</td>
<td>-</td>
</tr>
<tr>
<td>Section</td>
<td>Sudan III solution</td>
<td>Oil</td>
<td>Pinkish to reddish colour on the lower portion of trichomes</td>
<td>+</td>
</tr>
<tr>
<td>Section</td>
<td>Conc. HCl</td>
<td>Crystals</td>
<td>Effervescence observed</td>
<td>+</td>
</tr>
<tr>
<td>Section</td>
<td>Ruthenium red</td>
<td>Mucilage</td>
<td>Pink colour</td>
<td>+</td>
</tr>
<tr>
<td>Section</td>
<td>Pinch of phloroglucinol + dilute HCl</td>
<td>Lignin</td>
<td>Magenta color</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present; - = absent

Table 2—Preliminary phytochemical analysis of the leaves of C. hirsutus

<table>
<thead>
<tr>
<th>Test for</th>
<th>Pet. ether extract</th>
<th>Benzene extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Ethanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates and Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oil and Fats</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compound and Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins and Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Volatile Oils</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

+ = present; - = absent
Chromatographic studies

HPTLC fingerprint profile of alcohol extract of leaves revealed 16 phytoconstituents at Rf 0.04, 0.07, 0.11, 0.15, 0.21, 0.34, 0.38, 0.41, 0.49, 0.52, 0.56, 0.66, 0.77, 0.83, 0.93, 0.96 (Fig. 1). Out of these, peaks at Rf 0.21, 0.34, 0.49, 0.66, 0.77 and 0.83 were pronounced whereas peaks at Rf 0.04, 0.38, 0.41, 0.56, 0.93 and 0.96 were comparatively less pronounced and peaks at Rf 0.07, 0.11, 0.15 and 0.52 were least pronounced. All bands quenched fluorescence at 254 nm; under visible light bands at Rf 0.34, 0.49, 0.66 and 0.83 showed yellow colour (Fig. 2) and bands at Rf 0.04, 0.07, 0.11, 0.15, 0.21, 0.38, 0.41, 0.52, 0.56, 0.77, 0.93 and 0.96 showed green colour at 254 nm (Fig. 3) and all bands showed reddish orange colour fluorescence at 366 nm (Fig. 4).

Ultra-violet analysis

Powdered drug under ultra-violet and ordinary light when treated with different reagents emitted various colour radiations (Table 3) which help in identifying the drug in powder form.

Diagnostic characters

*C. hirsutus* plant and leaf drug is identified by:

- Straggling habit;
- Dorsiventral leaf with greyish tomentose lamina which is mucilaginous when fresh;
- Presence of unicellular ribbon shaped trichome both on lamina and petiole, with oil content in the lower half of trichome;
- Presence of sunken anomocytic

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visible light</th>
<th>UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder as such</td>
<td>Pista</td>
<td>Opaline green</td>
</tr>
<tr>
<td>In Methanol</td>
<td>Cascade green</td>
<td>Water green</td>
</tr>
<tr>
<td>In 1N Methanolic NaOH</td>
<td>Cascade green</td>
<td>Water green</td>
</tr>
<tr>
<td>In Ethanol (95%)</td>
<td>Cascade green</td>
<td>Mint green</td>
</tr>
<tr>
<td>In 1N Ethanolic NaOH</td>
<td>Cascade green</td>
<td>Mint green</td>
</tr>
<tr>
<td>In 1N HCl</td>
<td>Opaline green</td>
<td>Water green</td>
</tr>
<tr>
<td>50% H₂SO₄</td>
<td>Water green</td>
<td>Water green</td>
</tr>
<tr>
<td>50% HNO₃</td>
<td>Leaf green</td>
<td>Water green</td>
</tr>
<tr>
<td>5% KOH</td>
<td>Cascade green</td>
<td>Water green</td>
</tr>
</tbody>
</table>

The colour mentioned in the Table are based on the “Asian paints” premium gloss enamel, Asian paints limited, Mumbai.
stomata; presence of excretory sacs in mesophyll; presence of sclerenchymatous bundle sheath enclosing vascular bundle.

Conclusion
Pharmacognostical evaluation on the leaves of *C. hirsutus* (Linn.) Diels provide diagnostic characters useful for the identification of the drug. HPTLC profile of alcohol extract helps to establish marker compound and to isolate and identify the biomarker/bioactive constituent.

Acknowledgment
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