ABSTRACT

Background: *Plectranthus fruticosus* (Benth.) Wight ex Hook. f, syn. *P. deccanicus* Brinq. is an under shrub belonging to the family Lamiaceae. Leaves and young stems of this plant are used as medicine in Homoeopathy.

Objective: To carry out pharmacognostic and physico-chemical studies to use authentic and correct species as standard raw materials to ensure purity, quality and its usefulness.

Materials and Methods: The leaves and young aerial parts of *Plectranthus fruticosus* were fixed in formaldehyde: acetic acid alcohol (F.A.A), processed for microtomy (paraffin method), sectioned and permanent slides prepared following Johansen. The microtome sections in T.S. and longitudinal section (L.S.) were obtained at 6-8 um thickness on Leica RM 2155 microtome. The powder microscopy characters were studied by boiling the powdered drug in distilled water, stained in saffranin and mounted with glycerine. Photomicrography was done with Olympus BX 53 research trinocular microscope.

Results: The leaves are large, ovate to cordate, thin, margins with rounded serrations, surface coarse with dense covering hair. Petiole is long and stem is quadrangular with nodes. Trichomes are predominantly uniseriate, macroform, conical besides few uniseriate filiform peltate and capitate hairs. Lamina is conspicuously thin. The hypodermal collenchyma is angularly thickened. Vascular bundles in midvein are arranged in an ‘arc’. Petiole is oblong to rounded, in transection (T.S.) and undulated. A ring of angular collenchyma is present. Cortical cells possess starch grains and crystals of calcium oxalate. A continuous vascular cylinder is present interrupted with 2-3 seriate medullary rays. The physicochemical properties and HPTLC fingerprints of this plant have been standardized.

Conclusion: The powder microscopic features and organoleptic characters along with the anatomical and physico chemical studies including HPTLC fingerprints are diagnostic to establish the pharmacopoeial standards for the drug.

Keywords: High performance thin layer chromatography, Pharmacognosy, Physico-chemical, *Plectranthus fruticosus*, Standardization

INTRODUCTION

*Plectranthus fruticosus* is an under shrub growing up to 4 m high.[4] It is cultivated in warmer parts of Africa and South America and also found in Western Ghats, Kodaikanal and Nilgiris in India. Leaves are large, ovate, cordate, rounded at base, margins with rounded serrations, villous on the nerves above, densely beneath, petioles long, stems spongy, flowers mauve, close in long racemes. Fruiting
calyx thick.\textsuperscript{[2]} Leaves and stems are used in the preparation of medicine. Its proving has been done by Australian Verein, Zeitf. (Est. 1862 Part 2) and also mentioned in Homoeopathic Pharmacopoeia of United States (HPUS). In Homoeopathy, it is used in cholera, cramps, dentition, fever, neuralgia, rheumatism, stiff neck and spasitic paralysis.\textsuperscript{[3,4]}

Chemically the leaves are reported to contain α-thuyene, sabinene, γ-terpinene, β-bourbonene, linalool, terpinen-4-ol, sabinyl acetate, α-humulene, aromadendrene, α-cubebene, β-bisabolene, γ-cadinene, α-elemene, trans-farnesol, trans-copaene, ent-15β,16β-epoxykauran-19-oic acid, ent-15β,16β-epoxykauran-19-ol and 10 (14)-aromadendrene-4β,15-diol.\textsuperscript{[5‑7]}

Earlier studies on \textit{P. fruticosus} pertaining to pharmacognosy and physico-chemical parameters in general and homoeopathic perspective in particular is not available. Hence, in the present paper, a detailed pharmacognostic and physico-chemical standardization studies on leaves and stem of the drug have been carried out as per protocol of Central Council for Research in Homoeopathy.

**MATERIALS AND METHODS**

**Plant Material**
The leaves and young aerial parts of \textit{Plectranthus fruticosus} were supplied by Survey of Medicinal Plants and Collection Unit of CCRH, Nilgiris, fixed in formaldehyde: Acetic acid: Alcohol (F.A.A.), processed for microtomy (paraffin method), sectioned, stained and permanent slides prepared following Johansen.\textsuperscript{[8]} The epidermal peels of leaf were obtained by gently scraping and peeling with razor blade. The microtome sections in T.S. and longitudinal section (L.S.) were obtained at 6-8 µm thickness on Leica RM 2155 microtome. The powder microscopy characters were studied by boiling the powdered drug in distilled water, stained in safranin and mounted with glycerine. Photomicrography was done with Olympus BX 53 research trinocular microscope.

**Preparation of Extracts**
The air dried plant materials were coarsely powdered to 10/44 (sieve size) and subjected to determination of loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physico-chemical constants, UV aspects of mother tincture following official methods.\textsuperscript{[9,10]} Mother tincture was prepared as per Homoeopathic Pharmacopoeia of India (HPI) by Percolation method.

**High Performance Thin Layer Chromatography (HPTLC) Analysis**
25 mL mother tincture was evaporated on water bath to remove alcohol. The residue was extracted with 3 × 25 mL chloroform. Concentrated chloroform extract was used for the HPTLC study. The concentrated chloroform extract was spotted in the form of band of width 4 mm with a Camag microlitre syringe on pre coated silica gel aluminum plate 60F - 254, (5 × 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai). A constant application rate of 6 mL/sec. was employed. The slit dimension was kept at 4 × 0.45 mm and 20 mm/sec scanning speed was employed. The mobile phase consisted of chloroform: Methanol (9:1 v/v) and 10 mL of mobile phase was used for chromatography. Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase at room temperature for 20 minutes. The length of the chromatogram run was 8 cm and subsequent to the development, the TLC plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III) at 254 nm and 366 nm by reflectance scanning and operated by Wincats software (Camag) resident in the system.\textsuperscript{[11‑13]}

**OBSERVATIONS AND RESULTS**

**Pharmacognosy**

**Macroscopy**
The leaves of the plant are large, 10-12 cm long and 6-8 cm broad, ovate to cordate, thin, margins serrate, serrations rounded, surface rough with covering hair, all over, more beneath petiole up to 5 cm. long.

**Microscopy**
The epidermal cells over the surface of the leaf are polygonal isodiametric to anisodiametric, also polygonal linear on abaxial; sides thick, sinuate while deeply on abaxial. Epidermal cells 1480 per sq.mm on adaxial and 1600 per sq.mm on
abaxial. Costal cells 6 - 7 sided, linear, sides thin, straight to curved and surface striated. Stomata confined to abaxial [Figure 1b], diacytic, commonly dispersed all over, 460 per sq.mm. Stomatal Index 22.3. Trichomes are of 3 types. (i) Uniseriate macroform conical hair, commonly dispersed all over, more on veins and margins. (ii) Uniseriate filiform peltate hair, few, dispersed all over and (iii). Uniseriate filiform capitiate hair; few dispersed, all over [Figures 1a and b].

In vertical section (V.S.) slightly ribbed on adaxial and prominently on abaxial, secondary and tertiary veins also similarly ribbed. Midvein shield like, 454-626 µm (530) thick and lamina thin, undulated, 32-96 µm (56) thick, surface dispersed with conical and peltate trichomes [Figures 2a and b].

The epidermis is 1- layered, cells polygonal isodiametric, adaxially larger, mostly papillate 8-25 µm (16) in diameter, abaxially small 9 -24 µm (14) in diameter [Figure 2-b]. Cells over midvein small, interspersed with conical, peltate and capitiate hairs. At midvein, hypodermis consists of 8-10 layered collenchyma on adaxial while 3-4 layered on abaxial, cells angularly thickened. Parenchyma towards adaxial is 3-5 layered and 4-6 layered on abaxial. Centrally the ground parenchyma inside the vascular ring is 8-10 layered [Figures 2a and b].

The vascular stele is made of 6 - 7, oval to spherical bundles arranged in an ‘arc’. Vascular bundles are endarch, conjoint and collateral with a cambium between xylem and phloem. Xylem consists of vessels/tracheids in radial rows and few isolated with helical or scalariform thickenings. Phloem is made of phloem parenchyma, few bast fibers and sieve cells. Small amount of internal phloem is present. Pith is present at centre [Figure 3a and b].

The stem is quadrangular with thick nodes; pale yellowish to grey, vertically striated. In T.S. outer most epidermis consists of narrow elongated cells, 22 – 65 µm (44) long and 6 – 32 µm (16) wide, often dispersed with conical and few peltate hairs. The hypodermal cortex is distinguished into 3 zones, outer, middle, and inner. The outer cortex is 4 – 6 layered often with patches of sclerenchyma. The medium cortex consists of 8 – 10 layered collenchyma with characteristic angular thickenings [Figures 4c]. And the inner cortex

**Figure 1:** (a) Leaf abaxial surface with macroform conical and peltate glandular hair x 139  (b) Leaf abaxial surface showing conical hair, stomata and glandular hair x 173  (c) Leaf adaxial surface with sinuate sides x 730 ch = conical hair, ph = peltate hair, st = stomata, ade = adaxial epidermal cells

**Figure 2:** (a) V.S. of leaf at midvein x 144  (b) V.S. of leaf lamina x 176  c = collenchyma, p = parenchyma, m = mesophyll, x = xylem, phl =phloem, ade = adaxial epidermis, abe = abaxial epidermis, pl = palisade tissue, sp = Spongy tissue, vb = vascular bundle
is 5 - 7 celled thick made of polygonal to spherical cells, 54 - 130 µm (86) in diameter. The cortical parenchyma cells possess slightly dense contents with starch grains and crystals of calcium oxalate in few. The secondary phloem is continuous enclosing the xylem cylinder. Phloem consists of phloem parenchyma, bast fibers and sieve cells. Secondary xylem is extensive, consisting of vessels which are mostly isolated and few in radial rows of 2 - 3. Vessels in L.S. are bordered pitted and in alternate rows. Fibers occur in radial rows. Medullary rays are 2 - 3 seriate and interrupt the xylem. Ray cells are large, polygonal to tangentially elongated and often contain starch grains. Pith is abundant [Figure 4a-c].

**Powder Microscopy**
- Pieces of broken uniseriate conical hairs with pustulate surface, several
- Pieces of abaxial epidermis with diacytic stomata, few
- Pieces of adaxial epidermis with sinuate walls, few
- Isolated vessels with bordered pits, few
- Few pieces of cortex with collenchyma cells
- Isolated fibers, broken
- Isolated starch grains numerous
- Pieces of leaf with peltate hairs over it.

**Organoleptic characters**
- Colour – Dark green/moss green
- Touch – Smooth
- Odour – Not characteristic, tingling on standing
- Taste – Slightly pungent.

**Physico-chemical studies**
The study of loss on drying reveals the presence of water and also some volatile organic matter.

The determined data under the physico-chemical study for the raw drug is summarized in Table 1 and that of mother tincture preparation and its standardization in Tables 2 and 3 respectively.

**HPTLC finger printing**
The profile of chromatographic separation scanned at 254 nm, reveals ten spots [Figure 4] out of which 9, 7 and 5 spots possess maximum composition with $R_f$ at 0.88, 0.74 and 0.61 respectively. While, chromatogram scanned at 366nm, revealed 10 spots with 4, 5 and 6 spots showing maximum composition at $R_f$ 0.56, 0.61 and 0.66 respectively. It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the mother tincture. These are considered as valuable standards for the pharmacopoeia. At 254nm, ten spots appear at $R_f$ 0.37 (yellowish brown), 0.43 (yellowish brown), 0.49 (brown), 0.56 (brown), 0.61 (yellowish brown), 0.66 (brown), 0.74 (light blue), 0.81 (yellowish brown), 0.88 (yellowish brown), 0.94 (yellowish brown) with various concentrations while at 366 nm, ten spots at $R_f$ 0.37, 0.43, 0.49, 0.56, 0.61, 0.66, 0.74, 0.81, 0.88 , 0.94 (all pink to red). On exposure to iodine, ten spots appear at $R_f$ 0.37, 0.43, 0.49, 0.56, 0.61, 0.66, 0.74, 0.81, 0.88, 0.94 (all yellow colour). These are vital finger print parameters to ensure the reliability and reproducibility of the drug [Figure 5].

**DISCUSSION**

*P. fruticosus* belongs to Lamiaceae is an under shrub growing upto 4 m high. The leaves and young stems are useful in Homoeopathy. The leaves are large,
petiolate, broad, ovate to cordate. Stem is quadrangular with thick nodes and vertical striations over the surface. Stomata are confined to abaxial side and diacytic. The trichomes are of three types viz., i) uniseriate macroform conical hair ii) uniseriate filiform peltate and iii) uniseriate filiform capitate hair. The ratio of lamina to midvein thickness is 1:10. The epidermis is 1-layered and frequently papillate on adaxial. The hypodermis in midvein is predominantly collenchymatous, angularly thickened, while cortical parenchyma is scanty. The vascular stele consists of are 6-7 V. bundles in an 'arc'. The petiole is oblong or rounded in T.S. and undulated, distributed with conical and peltate hairs on the surface. Collenchyma is conspicuous with angular thickenings. The vascular strand is described as dissected arc\textsuperscript{[14]} which is presently confirmed and occurs in the form of a ring. Internal phloem is present towards the pith. The stem is quadrangular; a closed ring of collenchyma in the cortex has been reported\textsuperscript{[14]} and is presently confirmed and occurs as middle cortex with characteristically angular thickenings. Further, occurrence of cork arising in the sub-epidermal layers as reported\textsuperscript{[14]} is not presently found. Cortical cells are found to possess starch grains and crystals of calcium oxalate. Secondary xylem is extensive; 2 - 3 seriate medullary rays interrupt the xylem. Pith is present at the centre. The aim of standardization is to improve the quality and enhance the therapeutic efficacy and safety of remedies. The reliability of the method employed for analysis of a given drug, should have higher level of precision and sensitivity, to ensure the quality control of the products. The physico-chemical properties [Tables 1-3] help to identify the impurity and estimate the amount of active compounds present in the drugs. In HPTLC finger printing, the developed chromatogram will be specific with the selected solvent system and R\textsubscript{f} value. UV- spectroscopic study exhibits, four prominent peaks which serve as characteristic standards.

**CONCLUSION**

The macro and microscopical, organoleptic along with HPTLC fingerprinting are diagnostic and establish the standards. HPTLC finger prints of Plectranthus can provide standard finger prints and will be used as a reference tool for identification, authentication, quality control and standardization of this important plant.

**Table 1: Standardization of raw drug**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantitative values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105° C</td>
<td>Not more than 6.77% w/w</td>
</tr>
<tr>
<td>Total ash</td>
<td>Not more than 12.4% w/w</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>Not more than 1.96% w/w</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>Not more than 6.86% w/w</td>
</tr>
<tr>
<td>Alcohol soluble extractive</td>
<td>Not less than 12.25% w/w</td>
</tr>
<tr>
<td>Water soluble extractive</td>
<td>Not less than 23.03% w/w</td>
</tr>
<tr>
<td>Extractive values in</td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td>Not less than 1.07% w/w</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Not less than 5.07% w/w</td>
</tr>
<tr>
<td>Methanol</td>
<td>Not less than 16.52% w/w</td>
</tr>
</tbody>
</table>

**Table 2: Formulation of mother tincture**

**(percolation technique used)**

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>65% v/v (based on HPUS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug strength</td>
<td>1/10</td>
</tr>
<tr>
<td>Preparation</td>
<td></td>
</tr>
<tr>
<td>Plectranthus fruticosus in coarse powder</td>
<td>100 g</td>
</tr>
<tr>
<td>Strong alcohol</td>
<td>677 ml</td>
</tr>
<tr>
<td>Purified water</td>
<td>350 ml</td>
</tr>
<tr>
<td>To make one thousand millilitres of the mother tincture</td>
<td></td>
</tr>
</tbody>
</table>

HPUS: Homoeopathic pharmacopoeia of united states

**Table 3: Standardisation of mother tincture**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organoleptic profile</td>
<td>Clear, non-viscous and foaming on shaking</td>
</tr>
<tr>
<td>Appearance</td>
<td>Dark greenish brown</td>
</tr>
<tr>
<td>Colour</td>
<td>Pleasant and aromatic</td>
</tr>
<tr>
<td>Odour</td>
<td>light traces of sedimentation on standing</td>
</tr>
<tr>
<td>Sediments</td>
<td>Not more than 0.93 g</td>
</tr>
<tr>
<td>Weight per mL</td>
<td>Not less than 1.97% w/v</td>
</tr>
<tr>
<td>Total solids</td>
<td>61-64% w/v</td>
</tr>
<tr>
<td>Alcohol content</td>
<td>5.52</td>
</tr>
<tr>
<td>pH</td>
<td>209, 270, 326, 408 nm</td>
</tr>
</tbody>
</table>

Rao, et al.: Pharmacognosy of Plectranthus fruticosus

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