ORIGINAL ARTICLE

Pharmacognostic evaluation of *Heliotropium peruvianum* L.: A homoeopathic drug

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ABSTRACT

**Background:** *Heliotropium peruvianum* L., popularly known as “Heliotrope” in English, is a trailing plant belonging to the family Boraginaceae. The leaves and young aerial parts are used as medicine in headache, tension in inner canthus, pressure in the pit of stomach and sternum, uterine displacement and dysmenorrhoea.

**Objective:** Pharmacognostic and Physico-chemical studies were carried out to facilitate use of correct species and standard raw material to ensure uniformity.

**Materials and Methods:** Pharmacognostic studies of leaf and young stem of *Heliotropium peruvianum* were carried out. Physico-chemical parameters of the raw drug, viz., extractive values, ash values, formulation, besides weight per milliliter, total solids, alcohol content, along with high performance thin layer chromatography (HPTLC) and ultraviolet visible (UV–vis) studies were worked out for mother tincture.

**Results:** The leaves are elliptically long, dark green above and silvery white beneath. The sides of epidermal cells of leaf in surface view are straight to curved and wavy on the adaxial side and wavy to sinuate on the abaxial side. Papillate cells occur on the adaxial side. Stomata are few, anomocytic and restricted to the abaxial side. Unicellular conical and uniseriate conical hairs with thick walls occur on the leaf. In transverse section (TS), the leaf at midvein is shield like, slightly ridged on the adaxial surface and prominently ribbed towards the abaxial surface. Epidermal cells on the adaxial side are larger and interrupted by large encircling epidermal cells around trichomes. Parenchyma is four to five layered and often contains sphaeraphides. The phloem also possesses sphaeraphidal idioblasts. The young stem is oval in TS. Vascular tissue form of a continuous ring.

**Conclusion:** The powder microscopic features and organoleptic characters along with anatomical and Physico-chemical studies, besides HPTLC fingerprinting are diagnostic to establish the standards for the drug.

**Keywords:** Drug standardisation, Heliotropium peruvianum L., High performance thin layer chromatography, Homoeopathic pharmacognosy, Physico-chemical

INTRODUCTION

*Heliotropium peruvianum* L. (Syn. *Heliotropium arborescense* L.), commonly known as “Heliotrope” or Cherry Pie in English, belongs to the family Boraginaceae. It is a native of Peru and is also grown as an ornamental plant in Indian gardens.[¹] The aerial parts are used in the preparation of homoeopathic
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Medicine. The young aerial parts are used in sore throat, uterine displacement with active bearing-down sensation and dysmenorrhoea.[2] It is also known to be used for pain in forehead, feeling of tension in the left inner canthus, pressure in the pit of stomach, pressure and oppression of the external parts of the chest and pain in outer portion of the leg above the ankle.[3] The homoeopathic authority is given in Archiv. f. Hom, 19, pt. 1 p. 188. It also finds mention in Homoeopathic Pharmacopoeia of United States.[4] Chemically, it is reported to contain heliotropin, vanillin, cymoglossin, heliotrine, benzaldehyde, benzyl acetate, p-anisaldehyde, lithospermic acid, lithospermic acid B and caffeic acid.[5,6] Pyrrolizidine alkaloids, viz., heliotrine, present in the plant are reported to possess hepatotoxic effect in humans and is also responsible for causing “Gulran disease,” an epidemic in Afghanistan.[7]

Earlier studies on *Heliotropium peruvianum* pertaining to pharmacognostic and Physico-chemical parameters, in general, and with a homoeopathic perspective, in particular, are not available. Hence, the authors have conducted detailed pharmacognostic and Physico-chemical standardisation studies on the leaves and young stem of the drug as per the protocols suggested by Central Council for Research in Homoeopathy (CCRH).

**MATERIALS AND METHODS**

**Pharmacognosy**

The leaves and young aerial parts of *Heliotropium peruvianum* were supplied by the Survey of Medicinal Plants and Collection Unit of CCRH at Nilgiris, fixed in formaldehyde: Acetic acid: Alcohol (FAA), processed for microtomy (paraffin method), sectioned, stained and permanent slides were prepared following the procedure of Johansen.[8] The epidermal peels of leaf were obtained by gently scraping and peeling with razor blade. The microtome sections in transverse section (TS) and longitudinal section (LS) were obtained at 6-8 μm thickness on Leica RM 2155 microtome. The powder microscopy characters were studied by boiling the powder drug in distilled water, staining in saffranin and mounting with glycerine. Photomicrography was done with Olympus B × 53 research trinocular microscope.

**Physico Chemical Properties**

The air-dried young aerial parts of the drug were coarsely powdered to 10/44 (sieve size) and subjected to determination of moisture content (loss on drying at 105°C), total ash content, water-soluble ash content, acid-insoluble ash content, extractability in different solvents, Physico-chemical constants, High-Performance Thin Layer Chromatography (HPTLC) and ultraviolet (UV) aspects of mother tincture, following official methods.[9-12] Mother tincture was prepared as per Homoeopathic Pharmacopoeia of India (HPI).[9] In this method, 100 g of coarse powder of the drug was suspended in 680 ml of 95% alcohol and 350 ml purified water for 24 hour at room temperature. It was filtered and made up to 1000 ml using the same solvent ratio. Percolation method[9] was used for the preparation of mother tincture.

**HPTLC Analysis**

Mother tincture was used for the HPTLC study. The mother tincture was diluted in methanol and was spotted in the form of a band with 4-mm width with a Camag microlitre syringe on pre-coated silica gel aluminium 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 Technologies, Mumbai, India). A constant application rate of 6 mL/sec was followed. The slit dimension was kept at 4 × 0.45 mm, and a scanning speed of 20 mm/sec was employed. The mobile phase consisted of chloroform: methanol: Ammonia (7.5:2.4:0.1 v/v/v). Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag) at room temperature. The length of the chromatogram run was 9 cm. Subsequent to the development, the thin layer chromatography (TLC) plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Derivatisation was done using anisaldehyde–sulphuric acid reagent. The plate was scanned using Camag TLC scanner III in visible light by reflectance scanning operated by CATS 4 software (v 4.05, Camag) resident in the system.[10-12]

**OBSERVATIONS AND RESULTS**

**Pharmacognosy**

**Macroscopy**

Leaves are elliptically long, up to 5 cm, apex acute, margin entire, dark green above and silvery white
beneath, rough, undulated, with dense stiff hair; young stem is slender, dark green, densely hairy.

**Microscopy**

**Leaf surface**

Epidermal cells six to seven sided, polygonal, isodiametric and anisodiametric, sides thin, straight to curved and wavy on adaxial side and wavy to sinuate on abaxial side [Figure 1b and c]; a few cells on the adaxial surface are papillate. Contents scanty with rod-like structures in a few cells on the adaxial side [Figure 1b], while they are dense on the abaxial side. Cell number is 3133/mm² (on the adaxial side) and 2616/mm² (on the abaxial side).

Trichomes are of two types: (1) unicellular conical, distributed common, all over, more on veins, surrounded by conspicuously thick-walled encircling cells and (2) uniseriate conical, two to five celled, rare and distributed all over [Figure 1a and b].

Stomata are on the abaxial surface only, anomocytic of both small and large size, subsidiaries indistinct, rare, stomatal frequency (SF) 83/mm². Stomatal Index (SI) 3.086, size 16-32 μm (26) in diameter [Figure 1c].

**Transection**

Leaf at midvein is shield-like, slightly ribbed adaxially and prominently ribbed on the abaxial side, 238-410 μm (330) thick. Lamina is dorsiventral, undulated, 43-130 μm (82) thick with covering hairs over the surface [Figure 2a and b].

Epidermis is single layered, cells are large on the adaxial side and are interspersed by thick-walled encircling cells surrounding the trichomes; they are tabular to barrel-shaped, oval to spherical; cells are conspicuously small on the abaxial side and are interspersed with stomata and conical hair [Figure 2a and b].

Ground tissue of midvein consists of collenchyma and parenchyma tissues. Collenchyma is present as a group of cells in the adaxial ridge and is three to four layered on the abaxial side, with cells angular thickened. Parenchyma is present as a group of cells on the adaxial side and is four to five layered on the abaxial side; cells are polygonal to spherical, 10-30 μm (25) in diameter, with thin walls, and often with sphaeraphides, 16-28 μm (22) in diameter [Figure 2a].

Palisade is one layered, occupying two-third area of the mesophyll. Cells are 14-22 μm (20) long and 5-14 μm (10) wide and are interspersed with secretory canals. Spongy tissue is two to four layered in tiers or loosely arranged with air spaces at some regions [Figure 2b].

Vascular tissue of midvein consists of an arc or crescent-shaped vascular bundle, laterally 80-150 μm (120) long and vertically 65-130 μm (90) wide, endarch, conjoint and collateral. Xylem is in radial rows or laterally aligned. Xylem parenchyma is scanty. Phloem is interspersed with sphaeraphides [Figure 2a]. In LS, the vessels/tracheids possess mostly helical and a few scalariform thickenings.
Young stem

In TS oval, up to 2-4 mm thick, surface is undulated and covered by conical trichomes. The epidermis is one layered, often with papillate cells and interrupted by large encircling cells surrounding trichomes. The collenchyma is five to seven layered, cells are polygonal to spherical and angular thickened. Cortical parenchyma is 8-12 layered, in a few cells containing sphaeraphides [Figure 2c].

The vascular tissue is in the form of a continuous ring [Figure 2c]. The xylem cells in radial rows are interrupted with rays. A vascular cambium is present. In LS, the vessel elements are bordered pitted, helical and scalariformly thickened. The phloem is outside the xylem with phloem parenchyma, bast fibres and sieve elements. The phloem is also interspersed with sphaeraphides. The pith at the centre is abundant and has sphaeraphides in a few cells [Figure 2c].

Powder Microscopy

1. Numerous unicellular and uniseriate trichomes, whole or broken
2. Pieces of cortical tissue with attached tracheary elements
3. Pieces of brownish stem with epidermis and hypodermal tissues
4. Pieces of leaf lamina with underlying mesophyll
5. Few Sphaeraphides
6. Pieces of epidermis of the adaxial side with cells having curved to wavy walls and conical hair
7. Few cortical cells with sphaeraphides

Organoleptic characters

Colour - Brownish green
Touch - Smooth
Odour - No characteristic
Taste - No characteristic

Physico-chemical studies

The data obtained in the Physico-chemical study of the raw drug are summarised in Table 1, and the mother tincture preparation and its standardisation are presented in Tables 2 and 3, respectively.

Qualitative Phytochemical Tests

Preliminary phytochemical study indicates the presence of alkaloids, saponins, triterpenoids and tannins, besides traces of fixed oils. Moisture content reveals the presence of water in the plant, besides some volatile organic matter. Results of Physico-chemical studies are summarised in Tables 1-3.

### Table 1: Standardisation of raw drug

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantitative values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (loss on drying at 105°C)</td>
<td>Not more than 9.50</td>
</tr>
<tr>
<td>Total ash</td>
<td>Not more than 9.70</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>Not more than 1.10</td>
</tr>
<tr>
<td>Water-soluble ash</td>
<td>Not more than 6.90</td>
</tr>
<tr>
<td>Alcohol-soluble extractive</td>
<td>Not less than 4.90</td>
</tr>
<tr>
<td>Water-soluble extractive</td>
<td>Not less than 11.3</td>
</tr>
<tr>
<td>Extractive values in Hexane</td>
<td>Not less than 0.35</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Not less than 3.20</td>
</tr>
<tr>
<td>Methanol</td>
<td>Not less than 6.70</td>
</tr>
</tbody>
</table>

### Table 2: Formulation of mother tincture (percolation technique)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>65% v/v</td>
</tr>
<tr>
<td>Drug strength</td>
<td>1/10</td>
</tr>
<tr>
<td>Preparation</td>
<td>Heliotropium peruvianum in coarse powder</td>
</tr>
<tr>
<td>Strong alcohol</td>
<td>680 ml</td>
</tr>
<tr>
<td>Purified water</td>
<td>350 ml</td>
</tr>
</tbody>
</table>

To make one thousand millilitres of the mother tincture

### 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, foaming on shaking</td>
</tr>
<tr>
<td>Colour</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Odour</td>
<td>Aromatic and pleasant</td>
</tr>
<tr>
<td>Sediments</td>
<td>Absent</td>
</tr>
<tr>
<td>Weight per millilitre</td>
<td>Not more than 0.89 g</td>
</tr>
<tr>
<td>Total solids</td>
<td>Not less than 0.93% w/v</td>
</tr>
<tr>
<td>Alcohol content</td>
<td>61-64% v/v</td>
</tr>
<tr>
<td>pH</td>
<td>5.0-6.0</td>
</tr>
<tr>
<td>$\lambda_{max}$</td>
<td>229, 254, 285, 326 nm</td>
</tr>
</tbody>
</table>

### HPTLC Fingerprinting

The profile of chromatographic separation scanned at 254 nm reveals 14 spots. Among them, 5, 13 and 14 spots possess maximum composition at $R_1$ 0.26, 0.73 and 0.85, respectively. It is clear 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 in pharmacopoeia. While the chromatogram scanned at 366 nm [Figures 3 and 4] reveals 14 spots with 5, 12 and 14 showing maximum composition at $R_1$ 0.26, 0.73 and 0.86, respectively. At 254 nm, 14 spots appeared at $R_1$ 0.07, 0.11, 0.14,
0.17, 0.26, 0.32, 0.35, 0.42, 0.48, 0.53, 0.60, 0.63, 0.73 and 0.85 with variable concentrations, while at 366 nm, 14 spots appeared at Rf 0.07, 0.11, 0.14, 0.17, 0.26, 0.32, 0.37, 0.42, 0.45, 0.56, 0.64, 0.73, 0.81 and 0.86. This vital fingerprint parameter with ensure the reliability and reproducibility of the heliotropium peruvianum.

**DISCUSSION**

The diagnostic pharmacognostic characteristics of *Heliotropium peruvianum* include the following. In TS, midvein is shield like and prominently ribbed on abaxial side. Lamina is dorsiventral, palisade is one layered and interspersed with secretory canals. Hypodermal collenchyma in midvein possesses angularly thickened cells and the parenchyma is scanty often containing sphaeraphides. A single arc or crescent-shaped vascular bundle is present. Phloem is interspersed with sphaeraphides in a few cells. Young stem is oval in TS and covered by conical hairs. The epidermis is one layered and often papillate. The hypodermal collenchyma is angularly thickened. Few cortical cells contain sphaeraphides. The vascular tissue is a continuous ring interrupted by rays. The vessels in LS show bordered pits, helical and scalariform thickenings. The phloem also possesses sphaeraphidal cells. Pith is abundant with sphaeraphides in a few cells.

**Physico-chemical Analysis**

Physico-chemical analysis of *Heliotropium peruvianum* is presented in Tables 1-3. HPTLC fingerprinting is a precise and accurate tool to authenticate and characterise the drug, which in turn ensures the therapeutic efficacy. The chromatographic scanned values given for absorption at 254 nm and 366 nm of the mother tincture will be specific for the selected solvent system and Rf values. Physical parameters, extractive values and the chemical assays carried out are important aspects for standardisation. UV–visible study exhibits three prominent peaks which serve as characteristic standards.

**CONCLUSION**

The determined Physico-chemical data, macroscopic and microscopic characters, and the methodology employed in the study help to identify heliotropium peruvianum, the plant authenticity as well as quality, purity and efficacy during the manufacturing of the drug.

**REFERENCES**


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