

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 arborescence* 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, cynoglossin, 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 μ m 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 \times 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 \times 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 \times 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 \times 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.

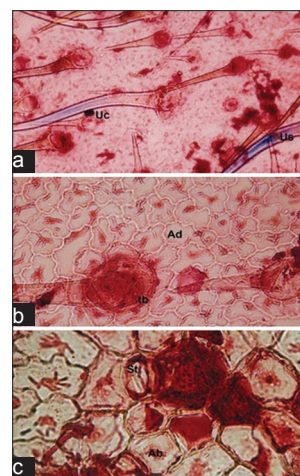


Figure 1: *Heliotropium peruvianum* (leaf). (a) Leaf adaxial surface showing unicellular conical hair, ×111, (b) leaf adaxial surface, ×263, (c) leaf abaxial surface, ×424 (Uc: Unicellular conical hair, Us: Uniseriate conical hair, Ad: Adaxial surface, Tb: Trichome base, St: Stomata, Ab: Abaxial surface)

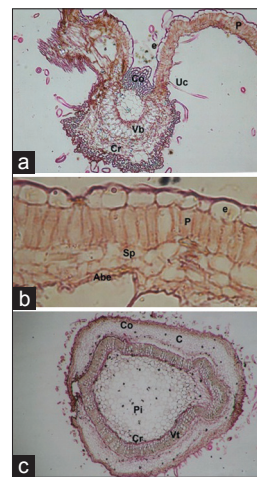


Figure 2: *Heliotropium peruvianum* (leaf and young stem). (a) TS of leaf at midvein, ×126, (b) TS of leaf lamina, ×521, (c) TS of stem, ×74 (P: Palisade, e: epidermis, Uc: Unicellular conical hair, Co: Collenchyma, Vb: Vascular bundle, Cr: Sphaeraphidal crystals, Sp: Spongy parenchyma, Abe: Abaxial epidermis, C: Cortex, Pi: Pith, Vt: Vascular tissue)

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

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

Table 2: Formulation of mother tincture (percolation technique^[9])

Alcohol	65% v/v
Drug strength	1/10
Preparation	
<i>Heliotropium peruvianum</i> in coarse powder	100 g
Strong alcohol	680 ml
Purified water	350 ml

To make one thousand millilitres of the mother tincture

Table 3: Standardisation of mother tincture

Parameters	Observations
Organoleptic profile	
Appearance	Clear, non-viscous, foaming on shaking
Colour	Yellowish brown
Odour	Aromatic and pleasant
Sediments	Absent
Weight per millilitre	Not more than 0.89 g
Total solids	Not less than 0.93% w/v
Alcohol content	61-64% v/v
pH	5.0-6.0
λ_{\max}	229, 254, 285, 326 nm

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_f 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_f 0.26, 0.73 and 0.86, respectively. At 254 nm, 14 spots appeared at R_f 0.07, 0.11, 0.14,

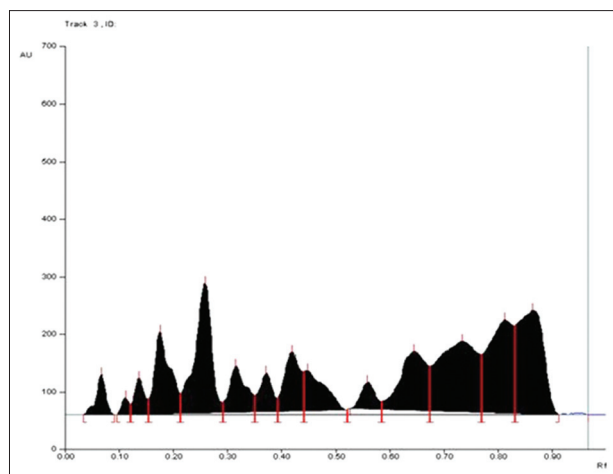


Figure 3: HPTLC densitogram (CHCl_3 :MeOH: NH_3 7.5:2.4:0.1 v/v) of *Heliotropium peruvianum* L. Q scanned at 366 nm

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 R_f 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

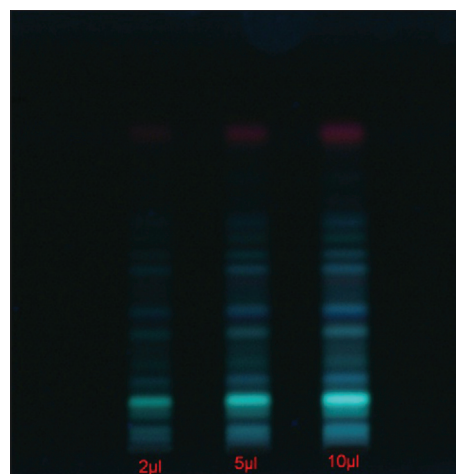


Figure 4: HPTLC fingerprinting of *Heliotropium peruvianum* L. Q under 366 nm

values given for absorption at 254 nm and 366 nm of the mother tincture will be specific for the selected solvent system and R_f 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.

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हेलिओट्रोपियम पेरुवियानम

पृष्ठभूमि: हेलिओट्रोपियम पेरुवियानम एल. बोराजिनासीए परिवार से संबंधित एक अनुगामी पौधा है, जो अंग्रेजी में हेलिओट्रोप के नाम से लोकप्रिय है। इसकी पत्तियों और युवा वायुविषयक भाग को सिरदर्द, भीतरी कैंथस भाग में तनाव, पेट और उरोस्थि में दबाव, गर्भाशय विस्थापन और कष्टार्तव में उपयोग किया जाता है।

उद्देश्य: सही प्रजातियों के प्रयोग की सुविधा और मानक अनिर्मित सामग्री की एकरूपता सुनिश्चित करने के लिए भेषजज्ञान और भौतिक अध्ययन किया गया।

सामग्री एवं विधि: हेलिओट्रोपियम पेरुवियानम की पत्तियों और नई डंठल पर भेषजज्ञान अध्ययन किया गया। मदर टिंचर के लिए कच्ची सामग्री के भौतिक मापदंडों अर्थात् निष्कर्षण मूल्य, निरूपण, प्रति मिलीलीटर के अलावा वजन, कुल ठोस, अल्कोहल की मात्रा, उच्च प्रदर्शन पतली परत में क्रोमेटोग्राफी (एचपीटीएलसी) और पराबैंगनी विज्वल (यूवी-विज़) पर अध्ययन किया गया।

परिणाम: पत्तियाँ दीर्घवृत्त रूप से लंबी, ऊपर से गहरे हरे रंग की और नीचे से सफेद चमकीले रंग की होती हैं। सतह से पत्ती की एपिडर्मल कोशिकाओं की तरफ सीधी घुमावदार और मध्यतल की ओर लहरदार अभ्यक्ष होती हैं। मध्यतल की ओर किनारे पर अंकुरक कोशिका पाई जाती हैं। रंध्र कुछ अनियमित और अपाक्ष की ओर सीमित होती हैं। पत्तियों पर मोटी परत के साथ कोशिकीय शंक्वाकार और कमबद्ध शंक्वाकार बाल होते हैं। अनुप्रस्थ अनुभाग (टीएस) में, मध्यशिरा पर पत्ती की सतह ऊपर की ओर ढाल की तरह विशिष्टता से धारीदार होती हैं। मध्यतल की ओर एपिडर्मल कोशिका बड़ी होती हैं और ट्रोइकोम्स के आसपास बड़े घेरे के साथ एपिडर्मल कोशिकाओं से बाधित होती हैं। पैरेन्काइमा 4.5 स्तर की होती हैं और अक्सर स्फर्इराफाइड्स मौजूद होता है। फ्लोएम के पास भी स्फर्इराफाइडल आइडियोब्लास्ट पाया जाता है। नई डंठल में रूपान्तरित अनुभाग अंडाकार होती हैं। संवहनी ऊतक एक सतत वेलनीय है।

निष्कर्ष: दवा के लिए मानकों को स्थापित करने हेतु एचपीटीएलसी फिंगरप्रिंटिंग के अलावा पाऊंडर सूक्ष्म विशेषताओं और ऑर्गेनोलेप्टिक संकेतों पर शारीरिक और भौतिक अध्ययन के लिए निदान किया।

खोजशब्द: औषध मानकीकरण, हेलिओट्रोपियम पेरुवियानम एल. एचपीटीएलसी, होम्योपैथी भेषजज्ञान, भौतिक रसायन।