

Pharmacognostic Evaluation of *Epilobium hirsutum* Linn.

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ABSTRACT

Background: *Epilobium hirsutum* Linn. (Onagraceae) is used widely as traditional medicine for its various medicinal values. Despite the popular utilization of this plant, still no conclusive study has been reported so far regarding the pharmacognostical standardization. **Aim:** Thus, the present study was aimed to scientifically establish a standard monograph of *E. hirsutum* on the basis of pharmacognostical aspects. **Material and methods:** Macroscopic, qualitative and quantitative microscopic characters of *E. hirsutum* were analyzed. **Results:** The diagnostic characters of the *E. hirsutum* were evaluated based on the macroscopical and microscopical characters. **Conclusion:** The obtained qualitative and quantitative standards will provide referential information for correct identification, purity, standardization and preparation of monograph of *E. hirsutum*.

Key words: *Epilobium hirsutum* Linn, Onagraceae, Great willowherb, Hairy willowherb, Great hairy willowherb.

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INTRODUCTION

Epilobium hirsutum Linn. is a flowering plant belonging to family Onagraceae, commonly known as great willowherb and great hairy willowherb or hairy willowherb.¹ *E. hirsutum* is a large, annual, densely covered with white hair. The leaves are 1-3 in number, 2-12 cm long, 0.5-3.5 cm wide, lanceolate, stem-leaves usually opposite, sessile, stem clasping and small teeth shaped. The flowers are 10-16 mm long, pink purple, axillary in long and short leafy racemes at the end of stem and branches, calyx-tube liner, 4 angled, lobes 4 short pointed, soon falling off. Petals 4, erect or spreading, notched, stamens 8, alternate ones longer, stigmas 4, distinct, erect at first, recovered afterwards. The capsule is 4-9 cm long. There are many seeds which are, small, tipped, with a tuft of long white hairs.²

The native range of *E. hirsutum* includes Mediterranean region, Europe, Asia, Africa and India particularly in Jammu and Kashmir, it is distributed in the western Himalaya up to an altitude of 7,000 ft.³⁻⁴ Common habitats include marshland, ditches and the banks of rivers and streams. Earlier claim shows that the *E. hirsutum* has antinociceptive,⁵ anti-inflammatory,⁶ antioxidant,⁷ it alters the protein synthesis by alteration in enzyme and mRNA,⁸ *in-vitro* iron chelation activity.⁹ The plant is also used as an antimicrobial, antitumor and in the treatment of enlarged prostate, prostatitis, cystitis, burning feeling when urinating, and burning feeling after prostate operation.¹⁰ Despite the popular utilization of this plant, still no conclusive study has been reported so far regarding the pharmacognostical standardization. Hence, the present work was focused to investigate the pharmacognostical properties of *E. hirsutum*.

MATERIAL AND METHODS

Plant collection and authentication

E. hirsutum was collected in the flowering stage from the fields of Chatterhama, Hazartbal, Srinagar, Jammu and Kashmir, India during August 2013. It was authenticated by Mr. Akhtar H. Malik, Curator, Centre for biodiversity and taxonomy, Department of Botany, University of Kashmir, Jammu and Kashmir, India (1914-KASH).

Chemicals and instruments

All reagents and chemicals used for pharmacognostic screening were analytical grade. Compound microscope was used for the study. The photography was done by using microscope camera using Toupview software. Camera lucida was used for determination of quantitative microscopical characters.

Morphology and Microscopy

The morphological studies were carried out by using simple determination technique like shape, size, colour, odour, taste, surface and fractures. Microscopic studies were carried out by preparing thin hand section of leaf, stem and root. The sections were cleared with alcohol and stained. Histochemical reactions were applied with concentrated hydrochloric acid and phloroglucinol for identification of lignified elements, iodine solution for starch grains, Sudan red-III for cuticle layer and oil globules, Ruthenium red for mucilage and acetic acid for calcium oxalate crystals. The leaf constants like stomatal number, stomatal index, vein termination number, vein islet number and palisade ratio were measured using camera lucida.¹¹

Powder microscopy

The powder microscopy of shade dried *E. hirsutum* was carried out by using various reagents for the identification of powder characteristics.¹²⁻¹³

RESULTS

Morphological characteristics

The morphological studies revealed (Figure 1) that the leaves was simple, sessile, green in colour, oblong-lanceolate in shape, 2-12 cm long, 0.5-3.5 cm wide, aromatic odour with characteristic and aromatic taste. Leaves had sharply serrulate margin, acute apex, symmetrical base, reticulate pinnate venation, thin texture and pubescent surface. The stem was green in colour, cylindrical in shape, about 0.5-1 m long, aromatic odour



Figure 1: Plant of *E. hirsutum*.

with characteristic and aromatic taste. The surface was pubescent with short fracture. The root was brownish in colour, cylindrical in shape, about 8 cm long and 0.5-1 cm in diameters, the root was odourless and tasteless. Longitudinal ridges and rootlets with short fracture were present on surface of root. The flowers were large and have 4 notched petals. The size of flower was 10-16 mm long and purple pink in color. The sepals were green; stigma was white with 4 lobes. The fruits were capsular and slender 5-8 cm long, brownish in color and containing numerous seeds embedded in fine silky fluff. The seeds were oblong and flattened in shape, 1 mm long and had nearly white hairs.

Qualitative microscopical characteristics

Transverse section of leaf

The transverse section of leaf shows that upper and lower epidermis of lamina consists of single layered, rectangular cells, distinct cuticle, it also contains uniseriate, unicellular covering trichomes (Figure 2 A-B), whereas Figure 2 C shows anomocytic type of stomata, lower epidermis contains more stomata. Mesophyll consists of palisade single layered, compact cells, with several layered spongy parenchyma, oil globules and vacuoles were present throughout mesophyll. Midrib consists of upper and lower epidermis same as that of lamina, collenchyma present below upper epidermis and above lower epidermis, vascular bundles were arc shaped hydrocentric and were present more towards dorsal side, rest of midrib was filled with normal parenchymatous cells (Figure 2 D). It also contains brownish matter, needle shaped calcium oxalate crystals and starch grains (Figure 2 E-F).

Transverse section of stem

Transverse section of stem shows that epidermis consists of single layered, quadrangular cells and thick cuticle. It also contains uniseriate, unicellular covering trichomes. Hypodermis was present below epidermis consists of few layers of collenchyma and sclerenchyma cells. Cortex consists of several layers of thin walled cellulose parenchyma cells. Mature stem contains brownish matter in cortex. Unlignified group of fibers were present outside the phloem. Vascular bundle was closed collateral type; phloem contains sieve tubes and companion cells. Xylems were well developed, consists of xylem parenchyma and vessels. Pith was large, thin walled, polygonal parenchyma cells (Figure 3 A-B). Cortex

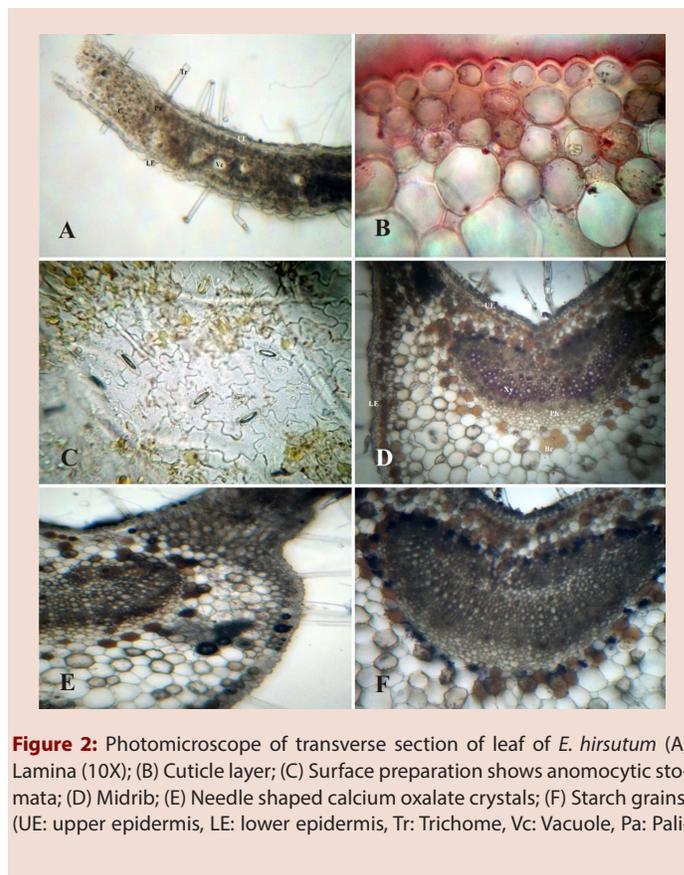


Figure 2: Photomicroscope of transverse section of leaf of *E. hirsutum* (A) Lamina (10X); (B) Cuticle layer; (C) Surface preparation shows anomocytic stomata; (D) Midrib; (E) Needle shaped calcium oxalate crystals; (F) Starch grains. (UE: upper epidermis, LE: lower epidermis, Tr: Trichome, Vc: Vacuole, Pa: Pali-

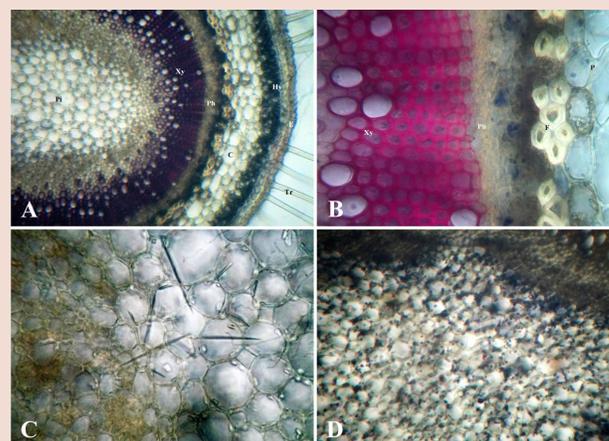


Figure 3: Photomicroscopy of transverse section of root of *E. hirsutum* (A) Root (10X); (B) Needle shaped calcium oxalate crystal. (Co: Cork, Br: Brownish matter, C: Cortex, Ph: Phloem, Xy: Xylem, Pi: Pith).

and pith also contains needle shaped calcium oxalate crystals and starch grains (Figure 3 C-D).

Transverse section of root

The transverse section of root revealed that cork consists of several layers of thin walled, polygonal cells with brown content. Cortex consists of several layers of thin walled, polygonal parenchymatous cells, brownish matter and needle shaped calcium oxalate crystals. The phloem shows phloem parenchyma, sieve tubes and unlignified phloem fibers. Xylem

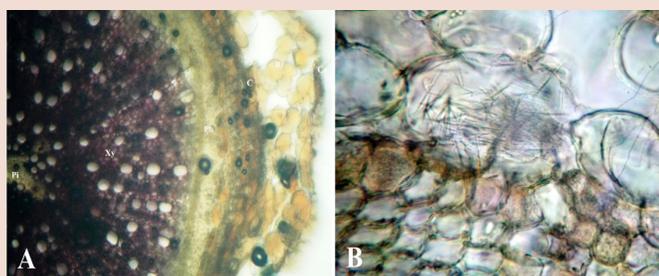


Figure 4: Photomicroscopy of transverse section of root of *E. hirsutum* (A) Root (10X); (B) Needle shaped calcium oxalate crystal. (Co: Cork, Br: Brownish matter, C: Cortex, Ph: Phloem, Xy: Xylem, Pi: Pith).

Table 1: Quantitative microscopy of leaf of *E. hirsutum*

| Leaf constant | Mean value |
|-------------------------|--------------|
| Stomatal number | |
| Upper surface | 46.25 ± 2.50 |
| Lower surface | 57.50 ± 2.89 |
| Stomatal index | |
| Upper surface | 14.54 ± 1.56 |
| Lower surface | 18.04 ± 2.15 |
| Vein islet number | 72.50 ± 2.89 |
| Vein termination number | 28.75 ± 2.50 |
| Palisade ratio | 7.25 ± 0.50 |

consists of xylem parenchyma, vessels and medullary rays. Pith was very small and consists of parenchyma cells (Figure 4 A-B).

Powder characteristics

The shade dried powder of *E. hirsutum* shows greenish brown colour having characteristic odour and sweet, bitter, sharp and sour taste. Epidermis consists of thin walled, irregular parenchymatous cells with wavy outline. Trichomes were uniseriate, unicellular. Parenchyma shows thin walled polyhedral cells with intercellular space. Cork shows polygonal isodiametric cells with brownish matter. Mesophyll consists of spongy-parenchyma with vascular strands. Xylem vessels were lignified spiral shaped. Fibres were lignified, thick walled and cylindrical in shape. Starch was simple, oval and rounded shaped. It also contains brownish matter whereas the stomata were of anomocytic type (Figure 5 A-J).

Quantitative microscopical characteristics

These values were especially useful for identifying the different species of genus and also helpful in determination and authenticity of the plant. The results were shown in Table 1.

DISCUSSION

The pharmacognostic evaluation is the first step towards the confirmation of identity and to assess the purity and quality of plant. Before the use of plant it is necessary that standards should be available to control and check the identity of the plant along with its quality. As per WHO the macroscopic and microscopic description of medicinal plant is the initial step towards establishing its identity, it should be carried out before any tests are undertaken.¹⁴

CONCLUSION

The present work deals with the pharmacognostic study of *E. hirsutum*. The morphological and microscopical study of plant and powder were

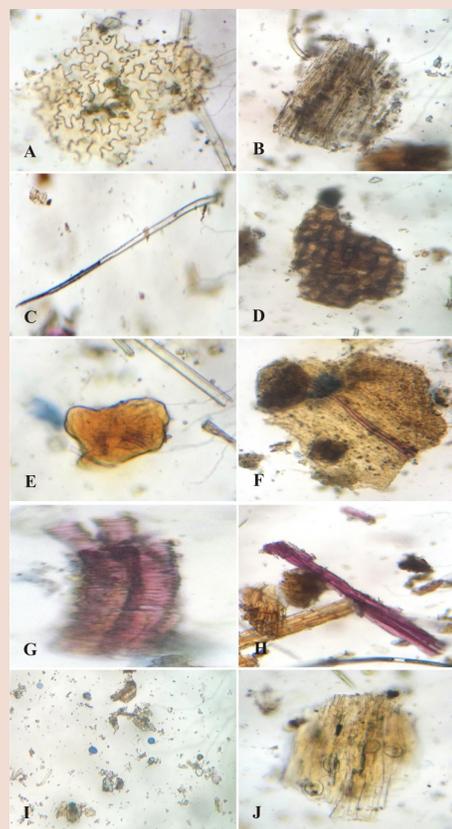


Figure 5: Photomicroscopy of powder of *E. hirsutum*.

studied for their specific characters. The present study will provide useful information for correct identification of plant. In addition the results of the present study could be useful for preparation of a monograph of the *E. hirsutum*.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ABBREVIATION USED

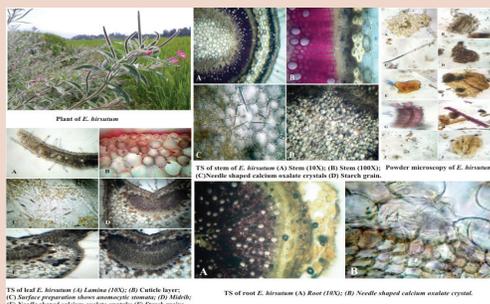
mm: millimeter; **cm:** centimeter; **m:** meter; **ft:** feet; **E:** Epidermis; **UE:** upper epidermis; **LE:** lower epidermis; **Hy:** Hypodermis; **Tr:** Trichome; **C:** Cortex; **F:** fibers; **Xy:** Xylem; **Ph:** Phloem; **Pi:** Pith; **Vc:** Vacuole; **Pa:** Palisade; **Br:** Brownish matter.

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PICTORIAL ABSTRACT



TS of leaf *E. hirsutum* (A) Lamina (10X); (B) Cuticle layer; (C) Stomata (10X); (D) Mesophyll; (E) Stomata (10X); (F) Stomata (10X); (G) Stomata (10X); (H) Stomata (10X); (I) Stomata (10X); (J) Stomata (10X); (K) Stomata (10X); (L) Stomata (10X).

TS of root *E. hirsutum* (A) Root (10X); (B) Needle shaped calcium oxalate crystal.

SUMMARY

- The morphological characters of *E. hirsutum* were established.
- The transverse section of leaf shows lamina with single layered epidermis, trichomes, anomocytic stomata, vacuole, palisade, cortex, mesophyll, midrib with single layered epidermis, vascular bundles, brownish matter, needle shaped calcium oxalate crystals and starch grains.
- Stem contains single layered epidermis, hypodermis, cortex with several layers, pith, trichomes, fibers, phloem, xylem, needle shaped calcium oxalate crystals and starch grains.
- Root shows the presence of cork, cortex, pith, phloem, xylem, brownish matter and needle shaped calcium oxalate crystals.
- The powder characteristics of *E. hirsutum* reveals the presence of Epidermis, Parenchyma, trichomes, cork, brownish matter, mesophyll, xylem, Starch grain, fibers, stomata.
- Various quantitative microscopy parameters of *E. hirsutum* were also established.

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