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Phytochemical and anatomical screening of *Eclipta prostrata* L. An important medicinal herb from Chandigarh

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Abstract
Eclipta prostrata L. is commonly known as False Daisy or Bhringaraj. It is a creeping and moisture loving herb commonly found on roadsides and waste lands. It is also popularly known as "King of hairs" used in indigenous system of medicine as a hepatoprotective drug. It is small branched annual herbaceous plant with a long history of traditional medicines uses in many countries especially in tropical and subtropical regions. The herb has known for its curative properties and has been utilized as analgesic, antibacterial, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, immunomodulatory properties and it is considered as a good rejuvenator too. A wide range of chemical compounds including alkaloids, flavonoids, polyacetylenes, triterpenes and their glycosides have been isolated from this species. Extracts and metabolites from this plant have been known to possess pharmacological properties. In the present investigation anatomical, phytochemical and HPLC studies of *Eclipta alba* were carried out. In order to ensure the use of only genuine and uniform material of such herbal drugs, work on plant identifying features assumes vital significance. Preliminary phytochemical analysis showed that alkaloids, flavonoids, glycosides, steroids, terpenoids were present in the plant material. The results of the anatomical studies and chromatogram of HPLC, would serve as standard reference for identification of the medicinal herb.

Keywords: Phytochemical screening, *Eclipta prostrata,* hepatoprotective, anatomical studies, HPLC

1. Introduction
Eclipta prostrata L. is an annual herbaceous plant, commonly known as King of hairs. It is an erect, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteraceae (Kirtikar and Basu, 1999) [1]. It is also known as Bhringaraj. This plant has been traditionally used as a liver tonic in Ayurveda and is commonly used as deobstruent to promote bile flow and to protect the liver. It is used in hair oil preparations as it promotes hair growth and maintains hair black. The herb is also known for its medicinal value as an analgesic, antiseptic, antiviral, antibacterial, antioxidant, antihaemorrhagic and anti-hyperglycemic. The juice of the plant with honey is given to infants for catarrh. The chloroform extracts also exhibit significant antidiabetic activities. The plant also shows immunomodulatory action and is therefore, used as a potential memory modulator [2-3]. It is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation (Saxena, 1993) [4]. It is used as a tonic and diuretic in hepatic and spleen enlargement (Chandra *et al.*1987) [5]. It is also used in catarrhal jaundice and for skin diseases (Kapoor, 2001) [6]. Important active constituent present in this plant is Wedelolactone. In present studies an attempt has been made to provide referential information for the correct identification and standardisation of this plant material for qualitative evaluation.

2. Material and methods
2.1 Collection and processing of plants: Plants were collected from different localities of Chandigarh during the flowering period in order to enable easy identification. The roots, stem and leaves were separated from the plant and preserved for future study. Roots were washed with a solution of 5% mercuric chloride for 5 minutes and then washed with distilled water, dried and stored in the dry packets. Stems were trimmed smaller pieces and stored in polybags. The leaves were washed, dried in shade, powdered and then stored in air sealed brown bottles. The various species were subjected to the following study:

2.2 Anatomical study: The plant material collected for anatomical study was fixed in F.A.A. (*i.e*. Formalin acetic acid-alcohol, 1:1:18) after trimming to correct dimensions. Hand sections of fresh stem and root (mature) were cut using a sharp blade. Thin transverse sections were stained in safranin and then fast green, passed through alcohol grades for dehydration, and then mounted in D.P.X. Observations were taken from these sections using light microscope. Selected sections were also photomicrographed for making plates. Detailed anatomical features of root, stem and leaves of various species were studied and special identifying features of each part of each species were identified.

2.3 Phytochemical study

2.3.1 Qualitative analysis: The powdered plant material was mascerated with 80% methanol. The solvent was then evaporated at a constant temperature of 72 ^{0}C until a very concentrated extract was obtained. The identification tests for the various phytochemicals present in the species were carried out to test their presence. The various phytochemicals that have been studied here includes: Alkaloids, Anthraquinone derivatives, Flavonoids, Glycosides, Terpenoids and Steroids. These phytochemicals were selected because of their importance in pharmaceutical industry. The phytochemicals along with the method of their detection is given below:

i) Alkaloids: Dragendroff's test: 0.1ml of dil. HCl and 0.1 ml of Dragendroff's reagent were added in 2 ml solution of extract in a test tube. Development of orang brown coloured precipitate suggested the presence of alkaloid. (Manu and Kuttan, 2009) [7].

ii) Anthraquinone derivatives: Modified Borntrager's test: 5ml of extract solution was hydrolysed with dilute sulphuric acid and extracted with benzene. 1ml of dilute ammonia was added to it. Rose pink coloration suggests the positive response for anthraquinones. (Njoku and Obi, 2009) [8].

iii) Flavonoids: Shinoda test: About 0.5 g of extract portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A red to purple colouration indicates the presence of flavonoids. (Siddiqui and Ali, 1997) [9].

iv) Glycosides: to the solution of extract in glacial acetic acid, few drops of ferric chloride and conc. H_2SO_4 acid were added and observed reddish brown colour at junction of two layers and bluish green colour in upper layer shows the presence of glycosides. (Siddiqui and Ali, 1997) [9].

v) Steroids: Sulphuric acid test: the fraction of extract was treated with ethanol and H_2SO_4 and violet green colour observed for the presence of steroid. (Siddiqui and Ali, 1997) [9].

vi) Terpenoids: Liebermann-Burchard test: 1ml extracts was treated with 0.5 ml of chloroform, 0.5 ml of acetic anhydride. Then conc. H_2SO_4 was added slowly and formation of dark green colour shows the presence of terpenoids. (Siddiqui and Ali, 1997) [9].

2.3.2 Quantitative analysis: The quantity of active compound present in each plant sample was measured by High Performance Liquid Chromarography (HPLC) by the methods discussed by Dhalwal *et al.,* (2010) [10] and Anjum *et al.,* (2011) [11].

2.3.3 Chromatographic conditions and procedure: High Performance Liquid Chromatography (HPLC, Shimadzu, LC 2010A, Japan), Auto sampler, UV-Detector was used for the analysis of active compound. The data was acquired on the LC solution administrator data system (Japan). Phenomenex C_{18} column (250 mm × 4.6 mm, 5 µm) (California, USA) and a isocratic mixture of methanol and water containing 0.1% v/v formic acid in the ratio of 30:70. The mobile phase was filtered through 0.45µm millipore filter and degassed by sonication for 30 min. The flow rate was adjusted to 1.0 ml/min. Injection volume was adjusted to 20µl and detection was made at 300 nm (can vary in different plant samples). The total run time for all the samples found to be 30 min. When the same drug solution was injected 5-6 times, the retention time of the peaks was found to be same.

2.3.4 Extraction procedure: The air-dried samples of the plant material were powdered and passed through 20 mesh sieve. The sieved material (100g) was extracted with 400ml methanol (99%), 50% alcohol and water separately at the temperature of 80-850 C for 1-2 hrs on a water bath. The material was filtered and further refluxed three times with methanol, 50% alcohol and water separately. Following this all the extracts were pooled together, concentrated under vacuum using rota-vac (Heidolph, Schwalbach, Germany). Finally the material was air-dried after removal of the above solvents.

2.3.5 Preparation of standard solution: Standard solution of pure phytocompound was prepared by dissolving 10mg in 100 ml (100 ppm) of methanol in a volumetric flask (stock solution). For the calibration curves the standard solutions were prepared by dilution of stock solution with mobile phase to reach a concentration range 4-20 ug/ml. and calibration curves were prepared by plotting peak area vs concentrations. For the estimation of markers compound peak areas were plotted at a particular wavelength.

2.3.6 Preparation of sample solution: Approx. 20 mg grinded powder of three extracted samples were taken and dissolved with50 ml methanol separately. The samples were sonicated for 20 min. After sonication the volume was made up to 50 ml with HPLC grade methanol and filtered through 0.45µm membrane filter.

3. Results and Disscussion

3.1 Stem and leaf anatomy

i) Stem

Transverse section of stem shows an outermost layer of epidermis, cortex, vascular bundle covered with per cyclic fibers and pith (Plate I, Fig.1). The epidermis is single-layered, formed of quadrangular cells. The cortex shows presence of layers of parenchyma (Plate I, Fig. 2). Below the cortex, lignified pericyclic fibers are present which cover the vascular bundle. Below this, a well developed vascular bundle is present (Plate I, Fig. 3). Innermost layer of pith consists of large, thin-walled, rounded cells (Plate VI, Fig. 2). Large air cavities are present below the epidermal region (Plate V, Fig. 4).

ii) Leaf

Transverse section of the leaf shows the presence of upper and lower epidermis, mesophyll and midrib region. Both epidermal layers made up of single layer of rectangular cells. The upper as well as lower epidermis unicellular trichomes

and few glandular trichomes (Plate I, Fig. 6, 7). The mesophyll region is composed of single layered palisade and spongy parenchyma tissue. The midrib portion consists of collenchymas and vascular bundle. Palisade cells single-layered, compact, radially elongated and present beneath the upper epidermis but not in a continuous layer. The spongy parenchyma is made up of loosely arranged parenchymateous cells and shows the presence of intercellular spaces. The midrib region possesses 2-3 layers of collenchyma cells, just between the two layers of epidermis. A well developed vascular bundle is found embedded in the midrib region (Plate I, Fig. 5, 7).

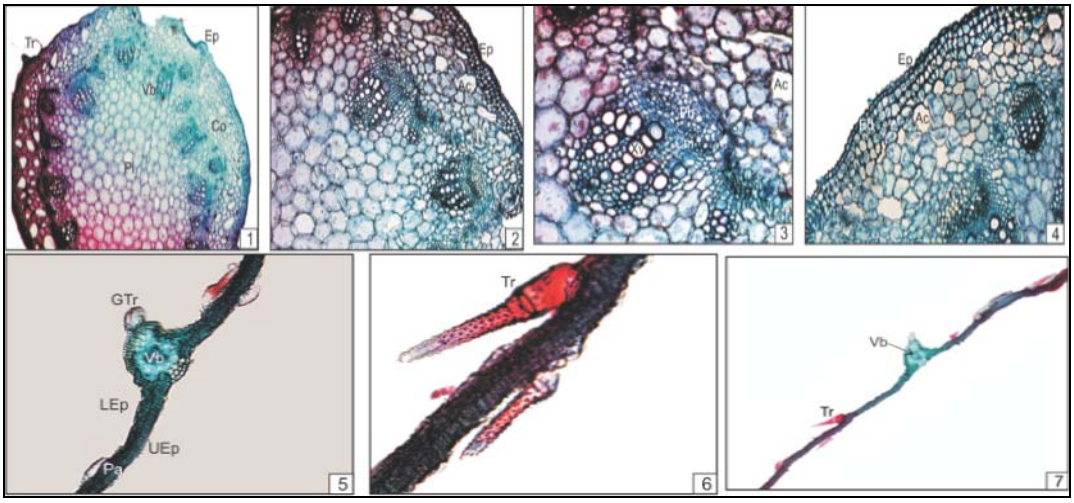

Plate-1(Figs. 1-7)
1. T.S. of stem showing epidermis, cortex, vascular bundles, trichomes and pith (x10).
2. T.S. of stem showing enlarged view of developed periderm and pith (x20).
3. Stem showing, large xylem vessels, air cavities (x40).
4. Enlarged view of epidermal portion of stem with large air cavities (x20).
5. T.S. of mid rib of leaf showing vascular bundle in centre and the parenchymatous cells between upper epidermis and palisade cells (x20).
6. T.S. of lamina of leaf showing trichomes, palisade and spongy parenchyma cells with upper and lower epidermis (x40).
7. T.S. of leaf showing trichomes and midrib region (x10).

Abbreviations: (Ep) Epidermis; (Co) Cortex; (P) Pith; (UEp) upper Epidermis; (LEp) lower Epidermis (Tr) trichome; (Ac) Air cavities; (GTr) glandular trichome; (Vb) Vascular bundle.

3.2 Phytochemical analysis

i) Qualitative analysis: Leaves and stem extracts showed the presence of alkaloids, terpenoids, flavonoids, glycosides and steroids. Anthraquinone derivatives not detected. The active compound in this species is Wedelolactone (Chokotia *et al.,* 2013) [12].

ii) Quantitative analysis by HPLC: Leaves were used for quantitative analysis. For assaying the active compound, standard Wedelolactone (WDL 95%) was purchased from Natural Remedies Pvt. Ltd, Bangalore. With UV detector at 254 nm using gradient mixture of orthophosphoric acid and acetonitrile as mobile phase a well resolved symmetric peak appeared on chromatogram at 12.76 mins in methanol (Fig.8b). The retention time of reference standard was observed at 13.08mins (Fig.8a).

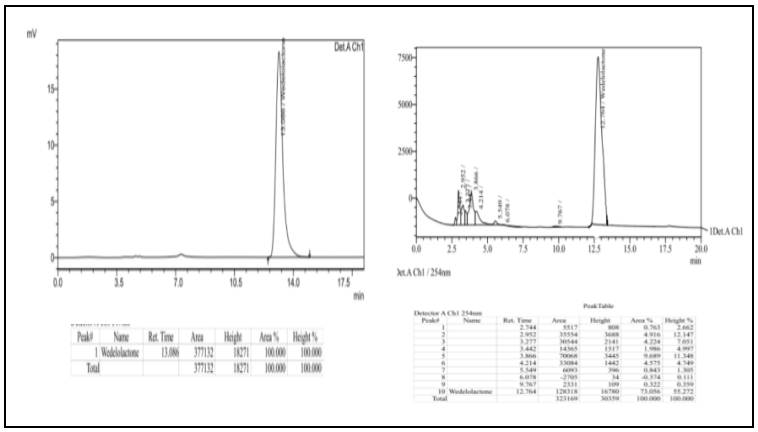

Fig 8: HPLC Chromatogram (a) reference standard Wedelolactone (b) *Eclipta prostrata.*

The percentage of prominent active compound found in the leaves was 0.323% (ug/ml) which is comparatively higher than the previous estimations (0.08% and 0.09%) by Murali *et al.,* (2002) [13]. The unique identifying features of morphology found were: stem light-green to purple; leaves slender with hair; flower heads (capitulum) arise from axils of leaves, and bear white short ray petal and whitish disk florets with yellow anthers, which are useful in the correct identification of the herb.

4. Conclusion

The plant under study is also used in various commercial formulations like Abana, Geriforte, Pilex, Protec and Purim, also used in protein conditioner. Approaches like screening, phytochemical profiling of these plants helped to get elite species. Since the drug is primarily obtained from stem and leaves, these have been studied in detail to avoid and prevent adulteration of commercial drug by users. Certain diagnostic features of morphology and anatomy have been found to be useful in the correct identification of the herb. The correct botanical identification of the herbal drugs of commerce shall help to check piracy of these drugs and hence make available true botanicals to the consumer.

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