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# Pharmacognostical and phytochemical analysis Of *Bhringraj* (*Eclipta Alba* Hassk.)

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## ABSTRACT:

*Bhringraj* (*Eclipta alba* Hassk.) is an annual herbaceous plant found throughout world during rainy season. It is indicated in *krimi*, *kustha*, *switra*, *khalitya*, *palitya*, *kasa*, *swarbheda*, *darunaka* and as *rasayan* in *ayurveda* texts. The herb is known for its property like hepatoprotective, antiviral, antibacterial, hypotensive, anti-leprotic, analgesic, antioxidant, anti-haemorrhagic, anticancer, anti-hepatotoxic, promoter for blackening and growth of hair. Evaluation of pharmacognostical and phytochemical standards including powder microscopy, physio-chemical parameters, phytochemical screening and thin layer chromatography were done for identification, authentication and standardization of drug. The phytochemical analysis shows presence of carbohydrates, alkaloids, proteins, tannins, saponin and phenols.

**Keywords**: *Bhringraj* (*Eclipta alba* Hassk.), pharmacognostical, phytochemical, thin layer chromatography.

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## INTRODUCTION:

*Bhringraj* (*Eclipta alba* Hassk.) is an annual herbaceous plant known as false daisy belongs to Asteraceae family. It is mostly found in tropical and sub- tropical regions throughout world during rainy season. It is common weed of paddy fields. The genus *Eclipta* comes from Greek word which means to be deficient. The *alba* word derived from Latin word *albus* means white which refers to the colour of flowers[1]. The plant is commonly used in hair oil throughout India for healthy long and black hair. According to *ayurveda* text *Bhringraj* have *katu*, *tikta rasa*; *ushna virya*; *katu vipaka*; *ruksha*, *laghu guna* and it pacifies *vata* and *kapha dosha*[2]. Useful part of plant is *panchanga* (whole plant). Ample references of *Bhringraj* were found in various *samhita* which have explained its *keshya karma* by quoting its use in problems related to hair. It is mostly indicated in *krimi*, *kustha*, *switra*, *khalitya*, *palitya*, *kasa*, *swarbheda*, *darunaka* and as *rasayan*. Major chemical constituents are ecliptal, wedelolactone, nicotine, stigmasterol, hepatocosanol, hentriacontanol, steroidal alkaloids, ecliptalbine, 25 beta hydroxyverazine. *Bhringraj* is a hepatoprotective, antiviral, antibacterial, hypotensive, antileprotic, analgesic, antioxidant, antihaemorrhagic, anticancer, antihepatotoxic, promoter for blackening and growth of hair[3].

### TAXONOMIC CLASSIFICATION[4]-

Kingdom – Plantae

Subkingdom – Tracheobionta

Superdivision – Spermatophyta

Division – Magnoliophyta

Class – Magnoliopsida

Subclass – Asteridae

Order – Asterales

Family – Asteraceae

Genus – *Eclipta* L.

Species – *Eclipta prostrata* (L.) L.

Synonym- *Eclipta alba* Hassk.

**VERNACULAR NAMES[5]-**

English- Trailing eclipta

Hindi- *Bhamgra, Mochakand, Babri, Bhangra.*

Bengali- *Kesuti, Keshukti, Keshori, Keysuria, Keshwri, Kesaraya.*

Gujrati- *Bhangra, Kalughanthi, Dodhak, Kalobhangro.*

Kannada- *Garagada soppu.*

Malayalam- *Kannunni, Kayyonni.*

Marathi- *Bhringuraja, Maka*.

Tamil- *Kaikesi, Garuga, Kayanthakara*.

Telgu- *Galagara, Guntagalijeru*.

Arabi- *Kadim-el-bint*.

Oriya- *Kesara, Kesarda*.

Santhal- *Lal kesari.*

Sind- *Tik*.

Sing- *Kikirindi.*

**BOTANICAL DESCRIPTION[6]-**

*Eclipta alba* Hassk. is a small, branched annual herb with white flower heads and is native to the tropical and subtropical regions of the world.

- Root - Well developed, a number of secondary branches arise from main root, upto about 7 mm in diameter, cylindrical, greyish.
- Stem - Herbaceous, branched, occasionally rooting at nodes, cylindrical or flat, rough due to oppressed white hairs, node distinct, greenish, occasionally brownish.
- Leaf - Opposite, sessile to subsessile, 2.2 - 8.5 cm long, 1.2 - 2.3 cm wide, usually oblong, lanceolate, sub-entire, sub-acute or acute, strigose with oppressed hairs on both surfaces.
- Flower - Solitary or 2, together on unequal axillary peduncles; involucral bracts about 8, ovate, obtuse or acute, herbaceous, strigose with oppressed hairs; ray flowers ligulate, ligule small, spreading, scarcely as long as bracts, not toothed, white; disc flowers 21 tubular, corolla often 4 toothed; pappus absent, except

occasionally very minute teeth on the top of achene; stamen 5, filaments epipetalous, free, anthers united into a tube with base obtuse; pistil bicarpellary; ovary inferior, unilocular with one basal ovule.

- Fruit - Achenial cypsella, one seeded, cuneate, with a narrow wing, covered with warty excrescences, brown.
- Seed - 0.2 - 0.25 cm long, 0.1 cm wide, dark brown, hairy and non endospermic.

## MATERIAL AND METHODS-

Microscopic, physio-chemical and phytochemical study including quantitative analysis of *Bhringraj* (*Eclipta alba* Hassk.) were done to determine the diagnostic features for the identification and standardization of powdered drug. All the standard references of procedures were followed from authentic books and sources during the study.

**Method of preparation of sample-**

The whole plant of *Bhringraj* (*Eclipta alba* Hassk.) was collected from village Ramgarh, Jaipur, Rajasthan after proper identification and a herbarium was prepared by drying plant specimen. The botanical authentication of plant was done by Department of Botany, University of Rajasthan, Jaipur with authentication no. RUBL 211724 as *Eclipta alba* Hassk. belongs to Asteraceae family. After this for study purpose whole plant of *bhringraj* was washed with running water and kept for drying under shade. The procured dried parts were powdered, labelled, packed and subjected for organoleptic and other analytic studies.

**PHARMACOGNOSTICAL STUDY-**

Pharmacognostical study was carried on the basis of morphological characters such as colour, odour, taste etc. and findings were recorded.

**PHYSICO-CHEMICAL PARAMETERS-**

**Determination of Moisture Content[7]:** Moisture content was determined by placing weighed sample of 5 g of drug in oven at 105º for 5 hours, and calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

**Determination of pH[8]:** The pH of test solution was measured by using digital pH

meter. First standardize the pH meter. Tablets of different pH were taken and each tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH. The instrument was switched on and left for some time until required different pH solutions appeared. Buffer solution was taken in the beaker and the electrode was dipped in it. Same procedure was repeated for the other buffer solution after washing the electrode thoroughly with distilled water. The sample was taken (10% aqueous solution) and electrode was dipped in it and the value of pH was noted.

**Determination of Extractive values[9]:**

**Determination of Alcohol Soluble Extractive:** 5 g coarsely powdered air dried drug was macerated with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish was kept in oven at 105º, to constant weight and weigh. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

**Determination of Water Soluble Extractive**: Procedure was same as that of alcohol soluble extractive value and it was preceded using distilled water instead of alcohol.

**Determination of Ash value[10]:**

**Total Ash: -** Weighed accurately 2 g of the air-dried drug in a silica crucible and incinerated at a temperature not exceeding 450ºC until free from carbon. Then, cooled and weighed. Percentage of ash value was calculated on the basis of air - dried drug.

**Acid Insoluble Ash: -** Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignite, cool in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

**Water Soluble Ash: -** Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch‘s Crucible or on an ash less filter paper. Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450˚ C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water

soluble ash. The percentage of water soluble ash was calculated with reference to the air - dried drug.

**PRELIMINARY PHYTOCHEMICAL SCREENING[11]:**

Phytochemical examinations were carried out for the extracts as per the standard methods.

**Tests for Carbohydrates:**

**Molisch's Test:** 2 ml of test solution was taken in a test tube and 2 ml of the Molisch's reagent was added and shaken carefully and then about 1ml of conc. $H_2SO_4$ is poured from side of the test tube and allowed to stand for one 1 minute. A purple colour ring at the junction of the two layers if formed indicated the presence of carbohydrate.

**Benedict's test:** It is used for reducing sugars and composed of mainly copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedict's solution was added and heated almost to boiling. Solution appears green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar, due to formation of cuprous oxide.

**Fehling solution test:** It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of sodium potassium tartarate.

Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath. Formation of reddish brown coloured precipitate due to formation of cuprous oxide indicates presence of reducing sugar.

**Tests for Alkaloids:**

**Dragendorff's reagent test:** 2 ml of test solution was taken in a test tube in which 2 ml of the Dragendorff's reagent (Mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of alkaloids.

**Wagner's Test:** Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formation of reddish-brown precipitate.

**Hager's Test:** A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow

precipitate was obtained which indicates the presence of alkaloids.

**Test for Amino acids:**

**Ninhydrin test:** The Ninhydrin test was used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecules and nitrogen of free amino acid.

**Tests for Proteins:**

**Biuret test:** A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.

**Xanthoproteic test:** A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.

**Millon's test:** A small quantity of test sample was taken and 2 to 3 ml of millons reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

**Test for saponin:**

**Foam test:** A small quantity of the test sample (about 1 ml) was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicates the presence of saponins.

**Test for glycosides:**

**Borntrager's test:** 1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the ethanolic extract and was observed for the formation of reddish pink colour.

**Test for Phenolic Compound:**

The extract was taken in water and warmed; to this 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

**Test for Steroids:**

**Salkowski reaction:** Few mg of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. The development of red colour indicates the presence of steroids.

**Test for Tannins:**

**Ferric chloride solution:** A 5 percent solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate.

Appearance of dark green or deep blue colour indicates the presence of tannins.

**Lead acetate:** A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicates the presence of tannins.

**Potassium Dichromate:** A solution of potassium dichromate was added to the filtrate. Appearance of dark colour indicates the presence of tannins.

**THIN LAYER CHROMATOGRAPHY (TLC)[12]:**

Thin layer chromatography is a tool for separation and identification of chemical constituent. Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid.

**Chromatography plates:** T.L.C. plate coated with 0.25 mm layer of silica gel 60 $F_{254}$ with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

**Activation of pre-coated Silica gel 60 $F_{254}$:** Plate was dried in hot oven at $105^0$ C for one and half hour.

**Preparation of mobile solution:** Toluene: Acetone: Formic acid (11: 6: 1).

**Preparation of test solution:** 4 gm powdered test drug was extracted with 100 ml of ethanol (90 percent) in Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

**Sample application:** Sample was applied with the help of capillary 1 (one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1 (one) cm below the top of the T.L.C. plate

**Visualization:** p-anisaldehyde sulphuric acid spray.

**Rf Value:** Measured and recorded the distance of each spot from the point of its application and calculated Rf value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

**OBSERVATIONS AND RESULTS:**

The different pharmacognostic parameters were studied and evaluated in order to standardize the drug. The results of pharmacognostic parameters i.e. microscopic

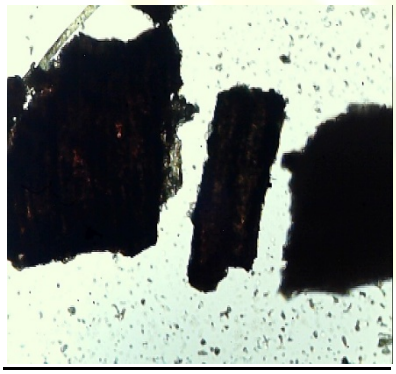
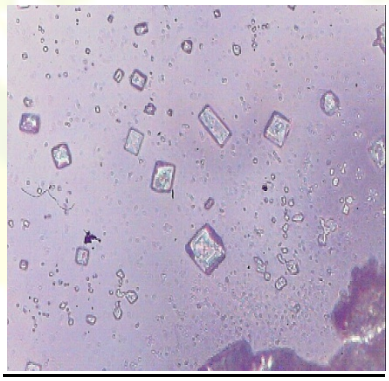
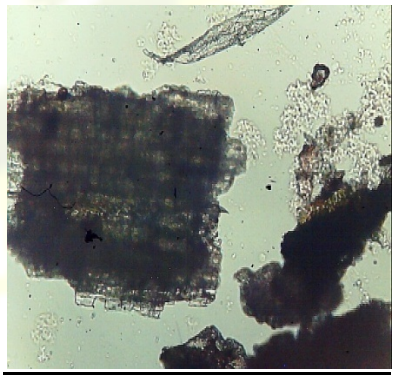
study, physicochemical parameters, phytochemical analysis have been recorded.

**Table 1: Macroscopic study of powder of *Bhringraj* (*Eclipta alba* Hassk.) whole plant**

| S. No. | Observed | *Bhringraj* (*Eclipta alba* Hassk.) |
|---|---|---|
| 1. | Colour | Brownish green |
| 2. | Odour | Characteristic |
| 3. | Taste | Slightly bitter |

**Powder microscopy of *Bhringraj*:** In powder microscopy, structure like tracheids, starch grains and fragments were seen.

**Table 2: Powder microscopy of whole plant of *Bhringraj* (*Eclipta alba* Hassk.)**

| ***Bhringraj*** | | |
|---|---|---|
|  |  |  |
| **Tracheid** | **Starch Grain** | **Fragment** |

**Physico-chemical parameters:**

**Table 3: Physico-chemical analysis of *Bhringraj* (*Eclipta alba* Hassk.)**

| S. no. | Physiochemical Standards | Results |
|---|---|---|
| 1. | Moisture content | 9.23% |
| 2. | pH | 6.4 |
| 3. | Water soluble extractive | 15.50% |
| 4. | Alcohol soluble extractive | 3.56% |
| 5. | Total ash | 17.43% |
| 6. | Acid insoluble ash | 8.94% |
| 7. | Water soluble ash | 9.39% |

**Phytochemical analysis:**

The preliminary phytochemical investigations of aqueous and alcoholic extract of whole plant of *Bhringraj* (*Eclipta alba* Hassk.) were carried out and result was tabulated as below:

**Table 4: Phytochemical analysis of extracts of *Bhringraj* (*Eclipta alba* Hassk.)**

| **Name of test** | ***Bhringraj*** | |
|---|---|---|
| | **Aqueous extract** | **Alcoholic extract** |
| **Carbohydrate test** | | |
| Molisch test | -ve | -ve |
| Benedict test | +ve | +ve |
| Fehling test | -ve | +ve |
| **Alkaloids** | | |
| Dragendorff test | +ve | +ve |

| | | |
|---|---|---|
| Wagner's test | -ve | +ve |
| Hager's test | -ve | -ve |
| **Amino acids** | | |
| Ninhydrin test | -ve | -ve |
| **Proteins** | | |
| Biuret test | -ve | -ve |
| Xanthoproteic test | +ve | +ve |
| Millon's test | +ve | +ve |
| **Saponin** | | |
| Foam test | -ve | +ve |
| **Glycosides** | | |
| Borntrager's test | -ve | -ve |
| **Phenolic compound** | | |
| Phenolic test | -ve | +ve |
| **Steroids** | | |
| Salkowski test | -ve | -ve |
| **Tannins** | | |
| $FeCl_3$test | +ve | +ve |
| Lead acetate test | +ve | +ve |
| Pot. Dichromate test | -ve | -ve |

**Thin Layer Chromatography:**

Test solution of *Bhringraj* (*Eclipta alba* Hassk.) showed 16 spots (with $R_f$ value 0.07, 0.15, 0.23, 0.28, 0.39, 0.45, 0.48, 0.54, 0.58, 0.61, 0.69, 0.75, 0.81, 0.85, 0.91, 1.0).

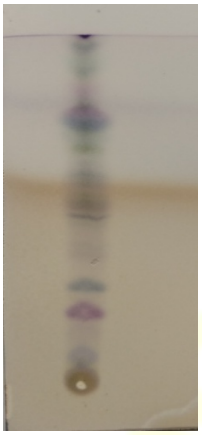


**Fig-1: TLC plate of *bhringraj* (*Eclipta alba* Hassk.)**

## DISCUSSION

*Bhringraj* (*Eclipta alba* Hassk.) has brownish green colour, characteristic odour and slightly bitter in taste. Powder microscopy revealed presence of tracheids, starch grains and fragments after observation under microscope. Loss on drying is water holding property of test substance revealed sample has 9.23% moisture content. pH value was found 6.4 shows acidic nature. Extractive value is directly relative to strength or potency of drug which estimates in different solvents. Water soluble extractive value was found 15.50% and alcoholic extractive value was found 3.56%. Ash value is the indicator of the presence of inorganic and earthy matter in the plant material. High ash value is suggestive of thermo-non labile/heat stable nature. The total ash value in sample was found 17.43%%. The acid insoluble content which indicates the presence of siliceous matter and heavy metals in sample was 8.94%.Water soluble ash estimates the inorganic water soluble salt in sample was 9.39%. The results of phytochemical analysis in aqueous and alcoholic extracts showed presence of carbohydrate, alkaloids, proteins, tannins, saponin and phenols. Thin layer chromatography

establishes the phytochemical fingerprint profiling of drug for identity.

## CONCLUSION

Different physicochemical parameters such as loss on drying, water soluble extract, alcohol insoluble extractive value, total ash, acid insoluble ash, water soluble ash and $R_f$ value were observed. The phytochemical analysis confirmed presence of carbohydrate, alkaloids, proteins, tannins, saponin and phenols. These values can be useful to detect adulteration. All studied standardization parameters like pharmacognostic study, physicochemical parameters and phytochemical screening provides the knowledge in the identification and authentication of whole plant of *Bhringraj* (*Eclipta alba* Hassk.).

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