MACROSCOPICAL, ANATOMICAL AND PHYSICO- CHEMICAL STUDIES OF EUPHORBIA HIRTA LINN. GROWING WIDELY ON EASTERN UTTAR PRADESH REGION OF INDIA

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Abstract
Traditional knowledge and ethno-botanical use of plants have been widely acknowledged all over the world. Certain people of India, using whole plant of *Euphorbia hirta* L. as an effective remedy for various diseases. The documentation of traditional knowledge from eastern Uttar pardesh area reveals that it is highly widely used in *Ayurveda*. Pharmacological and physico- chemical details of *E.hirta* not available in authentic literature including API (Ayurvedic Pharmacopea of India) So the validation and standardisation of whole plant of *Euphorbia hirta* was carried out to establish its macro- and microscopical standards, physicochemical parameters, preliminary phytochemical investigation and TLC profiles to evaluate the characters of the plant. Macroscopic characters of *E. hirta* leaves shows composition of leaf is simple with dark green color about 2-6cm. long in size, T.S. of the leaf revealed the presence of stomata, upper and lower epidermis, vascular bundle. Powder characteristics revealed the presence of starch granules, covering trichomes. The various diagnostic characteristic of leaf powder was coarse, dark green, which revealed the presence of lignified xylem vessel,anomocytic type of sto-mata, Physicochemical parameters were also evaluated. Preliminary phytochemical analysis indicated a high percentage of quercetine and flavonoids and this may be one of the reasons behind the anti diabatic and hepatoprotective activity of this plant. The above parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material used *Ayurveda* for various diseases in eastern Uttar Pradesh.

Keywords: *Euphorbia hirta* Linn, Dudhi, Pharmacognostic standardisation, Physico-chemical parameters.

1. Introduction
As a result of the side effects associated with synthetic drugs, people started looking back at the ancient healing systems like Ayurveda, Siddha and Unani. Herbal drugs play an important role in health care programs especially in developing countries. However, obstacle behind the acceptance of alternative medicines in developed countries is the lack of documentation and stringent quality control. So the documentation and standardization of the raw materials used in herbal medicine is very essential for the world wide acceptance of this system of medicine. Correct identification and quality assurance of plant material is indispensable to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy. Pharmacognostic standardisation of plant material include its morphological (organoleptic), anatomical and biochemical characteristics. The Eastern Uttar Pradesh is using many plants by by Ayurvedic practicener for their health care and day to day ailments but many of them are not yet scientifically validated. This plant called *dudhi* as an effective remedy for various ailments. It is identified as *Euphorbia hirta* Linn. of the family Euphorbiaceae. , they prefer methanolic extract of whole plant of *E. hirta* Linn.as a remedy for various disease. As far as the available literature is concerned, this plant has not yet been scientifically validated. *E. hirta* Linn. asthma herb plant in English, is a slender-stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color. Leaves are opposite, elliptic - oblong to oblong-lanceolate, acute or subacute, dark green above; pale beneath, 1- 2.5 cm long, blotched with purple in the middle, and toothed at the edge. The fruits are yellow, three- celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds. Its distributed throughout the hotter parts of India and Australia, frequently found in waste places along the roadsides. Study, pharmacognostic standardisation of *E. hirta* Linn. was carried out to establish its macro- and microscopical
standards, physicochemical parameters, preliminary phytochemical investigation and TLC profiles to characterise the plant material

2. Materials and Methods

2.1 Collection and Authentication: The plant collected from garden of National Botanical Research Institute (NBRI), Lucknow and was authenticated at NBRI, the voucher specimen (NBRI-SOP-202, CIF-RB-2-153, 20-10-2011) were deposited at NBRI herbarium.

2.2 Pharmacognostic Standardization: Organoleptic characters such as shape, size, colour, odour, taste of stem were determined. Microscopic studies were carried out by preparing thin hand section of leaf and stem with chloral hydrate solution, stained with Phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine. Histochemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material.

2.3 Physico-chemical Evaluations: Moisture content, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried out as described in Indian Pharmacopoeia.

2.4 Preliminary Phytochemical Screening: The methanolic, petroleum ether, chloroform and aqueous extract of E. hirta Linn. was subjected to tests for the presence or absence of the major class of compounds by standard methods.

2.5 TLC profile: Powdered E. hirta Linn., 10 g was extracted by refluxing with aqueous and methanol (50 ml × 3) sequentially for a period of 30 minutes each and the combined extract of each were filtered and concentrated to 10 ml. Apply 10 ml each extract as bands at a height of 10 mm from the base of a 5 × 10 cm coated silica gel plate 60 F254 using CAMAG Automatic sampler (ATS4) and developed up to 80 mm from the base of the plate in an Automatic Developing Chamber (CAMAG ADC2) using the mobile phase toluene :ethyl acetate: acetic acid (3:7:1) v/v for aqueous, Toluene :Ethyl acetate: Formic acid (3:1:1) v/v for methanol extract. Dry the plate in air and profile pictures were taken after derivatization with anisaldehyde sulphuric acid reagent (ANS).

3. RESULTS

3.1 Morphological characters: is a slender-stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color. Leaves are opposite, elliptic - oblong to oblong-lanceolate, acute or subacute, dark green above; pale beneath, 1- 2.5 cm long, blotched with purple in the middle, and toothed at the edge. The fruits are yellow, three- celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds.

3.2 Anatomical characters: the detail and systemic pharmacognostical evaluation would give valuable information for the future studies. Macroscopic characters of E. hirta leaves shows composition of leaf is simple with dark green color having no odour about 2-6cm. long in size, shape is ovate, texture is hairy, apex is acute and midrib is distincts on both the side. T.S. of the figure-1) leaf revealed the presence of stomata,upper and lower epidermis, trichomes vascular bundle and collenchyma. Powder characteristics revealed the presence of starch granules, scariform vessels, covering trichomes, lignified fibres, pericyclic fibres, epidermal cells with trichomes. The various diagnostic characteristic of leaf powder was coarse, dark green, odourless, with bitter test which revealed the presence of lignified xylem vessel, anomocytic type of stomata, epidermal cells with stomata, pericyclic fibers, scalariform vessels and trichomes. The quantitative determination of some pharmacognostic parameters is useful for setting standard for crude drugs the vein islet, vein termination and other parameters determined in the quantitative microscopy are relatively constant for the plants and can be used to differentiated closely
related species. The physical constant evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth. The total ash is particularly important in evaluation of purity of drugs.

3.3 Powder microscopy: Powder characteristics revealed the presence of starch granules, scleriform vessels, covering trichomes, lignified fibres, pericyclic fibres, epidermal cells with trichomes.

3.4 Physicochemical parameters: The value of loss on drying at 110°C showed the presence of moisture content in the sample, which is 8.5%. The total ash, acid insoluble ash and water soluble ash were found to be 5.5%, 2.20 and 4% respectively. The ash contents showed the amount of inorganic matter present in the sample and the acid insoluble ash almost within 2.2%, which expresses low siliceous matter present in the sample. Extractive values are tabulated in Table -1

| Table-1 Extractive values of E. hirta L. |
|----------------------------------------|
| **Cold Extractive value (% w/w)** |
| Plant | Petroleum Ether extract | Chloroform extract | Methanolic extract | Water extract |
|-------|------------------------|--------------------|--------------------|---------------|
| Euphorbia hirta L. | 1.2% | 2.5% | 9.80% | 13.41% |
| **Hot extractive value (% w/w)** |
| Plant | Petroleum Ether extract | Chloroform extract | Methanolic extract | Water extract |
|-------|------------------------|--------------------|--------------------|---------------|
| Euphorbia hirta L. | 2.0% | 2.45% | 12.2% | 16.6% |
| **Successive Extractive value (% w/w)** |
| Plant | Petroleum Ether extract | Chloroform extract | Methanolic extract | Water extract |
|-------|------------------------|--------------------|--------------------|---------------|
| Euphorbia hirta L. | 1.9% | 2.30% | 11.2% | 16.46% |
3.5 Preliminary phytochemical investigation: The result of phytochemical analysis tabulated in Table-2

Table-2 Preliminary phytochemical investigation

| S.No. | Constituents                  | Petroleum Ether extract | Chloroform extract | Methanolic extract | Aqueous extract |
|-------|-------------------------------|-------------------------|-------------------|-------------------|-----------------|
| 1     | Alkaloids                     | +                       | +                 | -                 | +               |
| 2     | Carbohydrates                 | -                       | -                 | -                 | -               |
| 3     | Glycosides                    | -                       | -                 | +                 | +               |
| 4     | Phenolic compounds and Tannins| -                       | -                 | +                 | +               |
| 5     | Flavonoids                    | -                       | +                 | +                 | +               |
| 6     | Terpenoids                    | -                       | -                 | -                 | -               |
| 7     | Saponins                      | -                       | -                 | +                 | -               |
| 8     | Sterols                       | +                       | +                 | +                 | +               |
| 9     | Proteins                      | -                       | -                 | -                 | -               |
| 10    | Resins                        | -                       | -                 | +                 | -               |
| 11    | Oil / Fats                    | -                       | -                 | -                 | -               |

3.6 TLC profile: Well resolved TLC profiles were recorded: For the future reference and identity of the plant material, Aqueous extract TLC profile showed four spots under UV 254 nm, after derivatization in visible light. Methanolic extract the profile showed three spots under UV 254 nm, All the above parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material. The result obtained from TLC are depicted in Table-3

Table-3 TLC analysis of Aqueous and methanolic extract of E. hirta L.

| S. No. | Extracts       | Solvent system                        | Number of spots | Rf value |
|--------|----------------|--------------------------------------|-----------------|----------|
| 1.     | Aqueous extract| Toluene : Ethyl acetate: Acetic acid (3:7:1) | 4               | 0.52     |
|        |                |                                      |                 | 0.73     |
|        |                |                                      |                 | 0.84     |
|        |                |                                      |                 | 0.86     |
| 2.     | Methanolic extract| Toluene : Ethyl acetate: Formic acid (3:1:1) | 3               | 0.28     |
|        |                |                                      |                 | 0.45     |
|        |                |                                      |                 | 0.74     |

3.7 Fluorescence analysis: The observation are presented in Table-4

Table-4. Fluorescence analysis of Euphorbia hirta L.

| Solvent used | Day Light | U V light | 254nm | 366nm |
|--------------|-----------|-----------|-------|-------|
| Powder as such | Dark green | Slight green | Blackish green | |
| 1N HCl      | Light green | Green colour | Dark green | |
| 50% HCl     | Green     | Brownish green | Light green | |
| 50% HNO3    | Slightly green | Brownish | Light green | |
| 50% H2SO4   | Light yellow | Green colour | Moderate green | |
| 1N NaOH     | Light green | Green colour | Dark green | |
| Alcoholic NaOH | Light green | Green colour | Dark green | |
| Methanol    | Light green | Dark green | Dark green | |
| Benzene     | Slightly yellow | Slight buff | Green | |
| FeCl3       | Brownish yellow | White | Green | |
| 1% KOH      | Brownish black | Light buff | Dark green | |
| Lead acetate | White | Florescent white | White | |
| Distilled water | Clear | Green | Green | |
3.8 Powdered drug reaction with different reagent: The results are tabulated in Table 5.

Table 5: Powdered drug reaction with different reagent

| Treatment          | Observation                          |
|--------------------|--------------------------------------|
| Conc. HCL          | Dark green                           |
| Conc. HNO₃         | Light brown with whitish foam        |
| Conc. H₂SO₄        | Greenish yellow                      |
| Glacial Acetic acid| Light yellow                         |
| Iodine solution    | Dark brown                           |
| NaOH in Methanol   | Light green                          |

4. Discussion and Conclusion

WHO has emphasised the need to ensure quality control of the raw materials used for Ayurvedic medicines by using modern techniques and by applying suitable parameters and standards. In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation and TLC profiles in aqueous and methanol extracts were studied, which are being reported for the first time in this plant and could be helpful in authentication and preparation of a suitable monograph for the proper identification of *E. hirta* Linn. for the future.

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