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Research Article

Pharmacognostic, Phytochemical Screening of Different Solvent Extract of *Euphorbia humifusa*

Gomase Pravin V.*[1], Pawar Sunil P. [2], Khan G. J.[1], Shaikh Jishan[1]

*[1] Department of Pharmacognosy, Ali-Allana College of Pharmacy, Akkalkuwa. Dist- Nandurbar, India

[2] P.S.G.V.P.M.'s College of Pharmacy, Shahada. Dist. Nandurbar, India

ABSTRACT

Medicinal plants have bioactive compounds which are used to curing of various diseases. The aim of the study to Pharmacognostic, Preliminary phytochemical screening of different solvent extracts of *Euphorbia humifusa* were carried out. The traditional medicine involves the use of different plant extracts or the bioactive constituents, qualitative phytochemical analysis of these plants confirm the presence of various phytochemical like alkaloids, glycosides, flavonoids, tannins, Protein, Amino acid, Carbohydrate, phytosterols. The result suggest that the phytochemical properties for curing various ailments and possess potential antioxidant and reads to the isolation of new and novel compounds.

Keywords: Phytochemical Screening, Medicinal plants, *Euphorbia humifusa.*

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***Address for Correspondence:**

Gomase Pravin V., Department of Pharmacognosy, Ali-Allana College of Pharmacy, Akkalkuwa. Dist- Nandurbar, India

Corresponding author- pravingomase84@gmail.com

INTRODUCTION

Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts[1]. The importance of medicinal plant in drug development is known to us and humans have used them for different diseases from the beginning of human history[2].Medicinal and aromatic plants are potential source of raw materials used for manufacture of drugs and perfumery products more than a quarter of all the medicines used in the world today contain natural compounds derived from plants that often serve lead molecules whose activities can be enhanced by manipulation through combinations with chemicals and by synthetic chemistry that can be exploited in the field of new drugs research and development[3,4]. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals[5]. In addition, some medicinal plants are still obscured within the plants which need to be scientifically evaluated. The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment[6].

Phytoconstituents are the natural bioactive compounds found in plants. This phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions [7].

Euphorbia humifusa is annual growing to 0.2m. The species is monoecious i.e. individual flowers are either male or female, but both sexes are found in the same plant and is pollinated by insects. It is suitable for light (sandy) and medium (loamy) soils and prefers well drained soil .It cannot grow in the shade. It prefers dry or moist soil[8].

Taxonomical classification of *Euphorbia humifusa*[9, 10]

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Malpighiales

Family : Euphorbiaceae

Genus : *Euphorbia*

Species : *Euphorbia humifusa*

Fig. 1: ***Euphorbia humifusa*** **Wild Plant**

MATERIALS AND METHODS

Collection and identification of plant

Plant *Euphorbia humifusa* was collected from Akkalkuwa District Nandurbar, Maharashtra. The plant was authenticated by Dr. V. R. Jogdand, Dept. of Botany, RFNS, Senior Science College, Akkalkuwa, Dist- Nandurbar and submit the plant specimen for further reference.

Pharmacognostic Investigation of *Euphorbia humifusa*

Organoleptic / Macroscopic evaluation

In the present study the bark of *Euphorbia humifusa* was investigated for its macroscopic characteristics[11, 12]. Macroscopic characters, which were observed, are given follows.

Colour : Greenish red

Odour : Aromatic, Pleasant

Taste : Astringent, mucilaginous

Texture : Smooth

Standardization of *Euphorbia humifusa*Willd[12, 13]

The evaluation of a crude drug involves the determination of identity, purity and quality. Purity depends upon the absence off oreign matter whether organic or inorganic. The following standardization parameters were evaluated to obtain the qualitative information about the purity and quality of *Euphorbia humifusa*. The results are shown in Table 1.

Determination of foreign matter

Foreign matter in herbal drugs consists of either parts of the medicinal plant or it may be any organism, part or product of an organism. It may also include mineral admixtures not adhering to the medicinal plant materials e.g. soil, stones, dust etc. The specified quantity of plant material is spread on a thin layer of paper. By visual inspection or by using a magnifying lens (5X or 10X), the foreign matters are picked out and the percentage is recorded.

Determination of Physical Evaluation

Loss on drying

Loss on drying is the amount of both water and volatile mater which evaporates during drying. For determination loss on drying accurately weighed flat and thin porcelain dish was dried and 2g of sample was transferred, the weight was taken and sample was distributed evenly. Then loaded porcelain dish was kept in oven at 100^{0}C. The sample was dried to constant weight. After drying it was collected to room temperature in desiccators. Weighed and calculated loss on drying in terms of percent w/w.

Determination of ash value

Ash is the residue remains after incineration. Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes inorganic variables like calcium oxalate, silica and carbonate content of the crude drug affects total ash value. Such variables are then removed by treating with acid and then acid insoluble ash value is determined.

1. Determination of total ash

Accurately weighed 2gm of the air-dried crude drug was taken in a tarred silica dish and incinerated at a temperature not exceeding 450 ^{0}C until free from carbon, cooled in a desiccators and weight was taken. The process was repeated till constant weight was obtained. The percentage of ash was calculated with reference to air-dried drug.

2. Determination of water soluble ash

The ash, obtained as per the method described above boiled for 5 minutes with 25 ml of water, filtered, and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450^{0}C and weight was taken. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

3. Determination of acid insoluble ash

The ash obtained as per method described above and boiled with 25 ml of 2 M hydrochloric acid for 5 minutes, filtered, and collected the insoluble matter on an ash less filter paper, washed with hot water, ignited, and cooled in a desiccators and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

Table 1: Data showing physical evaluation of *Euphorbia humifusa*

Sr. No.	Physical Evaluation	Results
1	Foreign matter	0.3 ± 0.20 %
2	Loss on drying	0.91± 0.32 %
3	Total Ash Content	0.71± 2.0 %
4	Acid Insoluble Ash	0.24 ± 0.70 %

Preparation of plant extracts

The entire fresh plant sample was collected and washed under the running tap water to remove soil particles and other dust particles. The entire plant was cut into small pieces dried under the shed for 15 days at room temperature. The entire shaded and dried plant shifted for grinding to get crude powder.

200g of crude powdered were extracted successively with Petroleum ether, chloroform and methanol using Soxhlet apparatus at 55-85 °C for 8-10 h in order to extract the polar and non-polar compounds [13]. For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room temperature and stored at 4 °C for further use for Preliminary Phytochemical Screening.

Phytochemical screening of Extracts of *Euphorbia humifusa*

All the reagents and solution used in phytochemical analysis were prepared by following standard procedure and perform phytochemical investigation[13, 14]. Phytochemical examinations were carried out for different extracts of plant as per the standard methods.

Test for carbohydrates

- **Molisch test**

2 ml of test solution was treated with few drops of 15% ethanolicα- napthol solution in a test tube and 2 ml of concentrated sulphuric acid was added carefully along the side of tubes. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

- **Fehling's test**

5 ml of test solution was mixed with 5 ml Fehling's solution (equal mixture of Fehling's solution A and B) and boiled on water bath. Development of brick red precipitate indicates the presence of reducing sugars.

- **Benedict's test**

To 2 ml of Benedict's reagent, 1 ml of test solution was added, warmed, and allowed to stand. Formation of red precipitate indicates presence of reducing sugars.

Test for proteins

- **Biuret test**

The equal volume of biuret reagent was mixed with test solution. The formation of purple violet color indicates the presence of proteins.

- **Million's test**

The equal volume of Million's reagent was mixed with test solution and boiled. Formation of white precipitate indicates the presence of proteins.

- **Xanthoprotein test**

The equal volume of sulphuric acid was mixed with test solution and boiled. Formation of white precipitate indicates the presence of proteins.

- **Test for sulphur containing protein**

5ml of test solution was mixed with 2ml of 40% sodium hydroxide and few drops of 10% lead acetate solution and boiled. Formation of deep black or brownish color indicates presence of proteins.

- **Precipitation test**

The equal volume of test solution was treated with absolute alcohol, 5% copper sulphate and 5% lead acetate solution separately. Formation of white colloidal precipitate indicates presence of proteins.

Test for amino acids

- **Ninhydrin test**

The 2 ml of test solution was treated with 1 ml of ninhydrin reagent and boiled for 5 min in water bath. Formation of purple color indicates the presence of amino acids.

- **Tyrosine test**

The 2ml of test solution was treated with 1ml of Million's reagent and boiled. Formation of dark red color indicates the presence of amino acids.

- **Tryptophan test**

The 2ml of test solution was treated with 1ml of glyoxalic acid concentrated sulphuric acid. The formation of reddish violet ring at the junction of two layers indicates the presence of aminoacids.

- **Cystein test**

The 3ml of test solution was boiled with few drops of 40% sodium hydroxide and 10% lead acetate solution. Formation of black precipitates indicates the presence of amino acids.

Test for fats and oil

- **Sudan red III test**

  Thick section of sample was treated with sudan red III and observed under microscope. Oil globules gets red indicates presence of oil.

- **Saponification test**

  The ethanolic solution of sample was treated with few drops of copper sulphate and sodium hydroxide solution. Formation of blue color indicates presence of fats.

Test for steroids

- **Salkowski test**

  The equal volume of test solution was treated with concentrated Sulphuric acid and chloroform. A reddish brown color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

- **Liebermann-Burchard reaction**

  The equal volume of chloroform extract of sample was treated with acetic anhydride and few drops of conc. Sulphuric acid were added from the side of the test tube. First red, then blue and finally green color indicates the presence of steroids.

- **Liebermann's reaction**

  The equal volume of test solution was boiled with acetic anhydride and cooled. Few drops of conc. Sulphuric acid were added from the side of the test tube. Formation of blue color indicates the presence of steroids.

Test for cardiac glycosides

- **Keller-Killiani test**

  The equal volume of test solution was treated with glacial acetic acid and few drops of 5 % Ferric chloride and conc. Sulphuric acid. Formation of reddish brown color at junction of two liquid layers and upper layer appeared bluish green indicates presence of cardiac glycosides.

- **Baljet's test**

  Thick section of sample was treated with sodium picrate solution and observed under microscope. Yellow to orange color indicates presence of cardiac glycosides.

- **Legal's test**

  The 2 ml of test solution was treated 1 ml pyridine and 1 ml sodium nitroprusside solution. Formation of pink red color indicates presence of cardiac glycosides.

- **Raymond's test**

  The equal volume of test solution was treated hot methanolic sodium hydroxide. Formation of blue color indicates presence of cardiac glycosides.

Test for anthraquinone glycosides.

- **Borntrager's test**

  The test solution was boiled with dil. H_2SO_4 and filtered. The equal volume of benzene was mixed, dilute ammonia solution was added in separated organic layer. Ammonical layer turns pink or red indicates presence of anthraquinone glycosides.

- **Modified Borntrager's test**

  The test solution was boiled with ferric chloride solution and dilutes hydrochloric acid then filtered. The equal volume cold filtrate was mixed benzene and dilute ammonia solution was added in separated organic layer. Ammonal layer shows pinkish red color indicates presence of anthraquinone glycosides.

Test for saponin glycoside

- **Foam test**

  The test solution was vigorously mixed with water. Formation of foam for more than 15seconds indicates presence of saponin glycosides.

Test for cyanogenetic glycoside

- **Guignard test**

  The equal volume of test solution was treated dilute sulphuric acid in test tube and sodium picrate treated filter paper was suspended in test tube. Filter paper gets brick red color indicates presence of cyanogenetic glycosides.

- **Mercurous nitrate test**

  The equal volume of test solution was treated with 3% aqueous mercurus nitrate solution. Formation of metallic mercury indicates presence of cyanogenetic glycosides.

Test for coumarin glycoside

- **Alkali test**

  The equal volume of test solution was treated with aqueous sodium hydroxide solution. Formation of blue green color indicates presence of coumarin glycosides.

- **Fluorescence test**

  The test solution was covered in test tube with alkali moist filter paper then test tube subjected to heating and filter paper was observed under UV light. Yellowish green fluorescence indicates presence of coumarin glycosides.

Test for flavonoids

- **Shinoda test**

  The test solution was treated with magnesium powder and few drops of concentrated hydrochloric acid. Formation of orange, pink or red color indicates presence of flavonoids.

- **Sulfuric acid test**

  The test solution was treated with sulfuric acid. Formation of orange, pink or red color indicates presence of flavonoids.

- **Lead acetate test**

  The test solution was treated with lead acetate solution. Formation of yellow precipitates indicates presence of flavonoids.

- **Alkali test**

  The test solution was treated with Sodium Hydroxide solution. Formation of yellow color which disappears after addition of acid indicates presence of flavonoids.

Test for alkaloids

- **Dragendorff's Test**

  The equal volume of test solution was treated with Dragendorff's reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

- **Mayer's test**

  The equal volume of test solution was treated with Mayer's reagent in test tube. Formation of yellowish buff precipitate indicates the presence of alkaloids.

- **Wagner's test**

  The equal volume of test solution was treated with Wagner's reagent in test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

- **Hager's test**

  The equal volume of test solution was treated with Hager's reagent in test tube. Formation of yellowish precipitate indicates the presence of alkaloids.

- **Tannic acid test**

  The equal volume of test solution was treated with tannic acid solution in test tube. Formation of buff color precipitate indicates the presence of alkaloids.

- **Murexide test**

  The test solution was treated with concentrated nitric acid and evaporated to dryness and treated with ammonium hydroxide after cooling. Formation of purple color indicates the presence of alkaloids.

Test for tannins and phenolic compounds

- **Ferric Chloride test**

  The 5 ml of test solution was treated 1 ml of 5 % ferric chloride solution. Greenish black coloration indicates the presence of tannins and phenolic compounds.

- **Lead acetate test**

  The 5 ml of test solution was treated 1 ml of 10 % aqueous lead acetate solution. Formation of yellow colored precipitate indicates the presence of tannins and phenolic compounds.

- **Gelatin test**

  The 5 ml of test solution was treated 1 ml of gelatin solution. Formation of white colored precipitate indicates the presence of tannins and phenolic compounds.

- **Bromin test**

  The 5 ml of test solution was treated 1 ml of bromin solution. Decoloration of bromine water indicates the presence of tannins and phenolic compounds.

- **Acetic acid test**

  The 5 ml of test solution was treated 1 ml of acetic acid solution. Formation of red colored solution indicates the presence of tannins and phenolic compounds.

- **Potassium dichromate solution test**

  The 5 ml of test solution was treated 1 ml of dilute potassium dichromate solution. Formation of red colored precipitate indicates the presence of tannins and phenolic compounds.

- **Dilute Iodine test**

  The 5 ml of test solution was treated 1 ml of dilute iodine solution. Formation of red colored precipitate indicates the presence of tannins and phenolic compounds.

- **Dilute nitric acid test**

  The 5 ml of test solution was treated 1 ml of dilute nitric acid solution. Formation of reddish to yellow color indicates the presence of tannins and phenolic compounds.

- **Potassium permanganate solution test**

  The 5 ml of test solution was treated 1 ml of dilute potassium permanganate solution. Discoloration of solution indicates the presence of tannins and phenolic compounds.

RESULTS

Physical Evaluation

The Physicochemical parameters were investigated and reported in Table 1. The above studies enable the identification of the plant material for future investigation and form an important aspect of drug studies.

Phytochemical Analysis

The phytochemical test was done on various plant extracts like Petroleum ether; chloroform and methanol were done and identify the active constituents. The results were presented in following the Tables. 2. From this analysis, Methanol extract found to have more active constituents compare to other extracts. In Methanol extract presence of Carbohydrate, Proteins, Amino acids, Glycosides, Flavonoids, Tannins and Saponins are determined.

Table 2. Phytochemical analysis of Extracts of *Euphorbia humifusa*

Sr. No.	Parameter	Pet. ether Extract	Chloroform Extract	Methanol Extract
1	Carbohydrate	-	-	+
2	Protein	-	-	+
3	Amino acid	-	-	+
4	Steroids	+	+	-
5	Cardiac glycosides	-	-	+
6	Anthraquinone glycosides	-	-	-
7	Saponin glycosides	-	-	+
8	Cyanogenetic glycosides	-	-	-
9	Coumarin glycosides	-	-	-
10	Alkaloids	-	+	+
11	Flavonoids	-	+	+
12	Tannins	-	+	+
+ : Present			- : Absent	

DISCUSSION AND CONCLUSION

The phytochemical analysis showed that the *Euphorbia humifusa* plant extract contains a mixture of phytochemical as Carbohydrate, Proteins and Amino acids, Glycosides, Flavonoids, Tannins and Saponins. These secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses. From the three extract to indicate the more positive result of methanolic plant extract from the other solvent of plant extract. The medicinal plants have been used to treatment of so many disease and their medicinal roles of these plants have such a secondary product and identified the bioactive compounds. This paper reveals that above medicinal plant *Euphorbia humifusa* gives a basis of its use in medicine and develop to further drugs in pharmaceutical area and also contains different biologically active constituents, and secondary product are valuable of further analysis.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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