

Pharmacognostic Evaluation and Analgesic Efficacy of Ethanolic Extract of *Euphorbia dracunculoides* L.

Umbreen Khattak¹, Rehman Ullah², Shafqat Ali Khan^{*1}, Barkatullah¹, Sami Ullah² and Saima²

ABSTRACT

Background: *Euphorbia dracunculoides* L is available in market in raw form. It is used by people for the treatment of warts, snake bite and epilepsy. Present study is about *Euphorbia dracunculoides* L. belonging to the family Euphorbiaceae, comprises pharmacognostic study, physiochemical analysis and their pharmacological efficacy. **Materials and Methods:** The macroscopic study was carried out through sensory organs like size, shape, texture etc. Physiochemical analysis was carried out through proper procedure from relevant literature, and analgesic activity was done through proper method by following literature. **Results:** Macroscopic study showed that the plant is an annual herb, stem is branched from the base, yellow green at the bottom and light green at the top, glabrous, smooth, contains white latex, its leaves are sessile, alternate, dark green, simple, stipulated, sub-acute or acute at the apex and entire margin. Root is yellowish in colour, conical in shape, smooth texture, downward in position. Powder drug study which was performed revealed various structures. Phytochemical screening includes both qualitative and quantitative analysis which was carried out indicated the presence of carbohydrates, proteins, saponins, sterols, alkaloids, phenolic compounds, glycosides, flavonoids and tannins. Proximate analysis showed proteins, crude fat, crude fibre, carbohydrates, moisture contents and ash. Elemental analysis revealed the presence of macro and micronutrients i-e Na, Zn, Mg and Fe, Cu, Ag and Au. Analgesic effect was dose dependent. Plant extract showed maximum inhibition of writhing 1.66 ± 0.32 (96.61%) at 300 $\mu\text{g/ml}$. **Conclusion:** The pharmacognostic study, physiochemical analysis and their pharmacological efficacy is helpful in the standardization of drug.

Key words: Pharmacognostic evaluation, Whole plant, *Euphorbia dracunculoides* L. macroscopic study, Powder drug study, Physiochemical analysis, Analgesic activity.

Umbreen Khattak,¹
Rehmanullah,² Shafqat Ali
Khan,^{*1} Barkatullah,¹ and
Sami Ullah²

¹Department of Botany, Islamia College
University, Peshawar, PAKISTAN.

²Department of Botany, University of Peshawar,
PAKISTAN.

Correspondence

Shafqat Ali Khan, Department of Botany,
Islamia College University, Peshawar,
PAKISTAN.

Tel.: +923439620689

E-mail: shafqatbotany3@gmail.com

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INTRODUCTION

Pharmacognosy signifies the importance of medicinal plants as cost-effective health factor of biodiversity and also their preservation and sustainable use (Rahman *et al.*, 2004). The knowledge of certain plants and their unique qualities has been passed on through the generations to ultimately reach us.¹ Natural products derived from plants have been traditionally important in prevention of illness and homeopathic treatment. They are also contained active ingredients for the production of health care products.² Medicinal plants contain some bioactive organic compounds i-e carbohydrates, steroids, tannins, flavonoids, alkaloids and terpenoides, which provide specific physiological action on the human body. Information of the chemical constituents of plants is advantageous because such information will be significant for the production of complex chemical substances.³⁻⁴ Medicinal plants possess enormous potential as beneficial drugs for human beings. Certain chronic and infectious diseases can be effectively treated using the substances present in these plants.⁵ However, lack of authentic documents and strict quality control has been detrimental to the

recognition and approval of traditional medicine in developed countries. To standardize and identify the plant material used in Traditional medicine we must invest in pharmacognostic and phytochemical studies. If the quality of the product is without blemish, it will be easier to ensure reproducible quality of herbal medicine which will ensure safety and effectiveness.⁶

Plants are considered basic nutritional source as they contain protein, carbohydrates, fats, oils, minerals, vitamins, and water, which are obligatory for growth and development in human and animals. These phytochemicals have been considered of crucial nutritional importance in the prevention of chronic disease such as cardiovascular disease, cancer, and diabetics.⁷⁻⁸ In human beings the macro and micro elements play a role in biochemical process. Plant secondary metabolites and minerals have a great role in metabolism. Fe, Ca, Mn, Mg, Zn and Cu are present in small amount in the hairs of Breast cancer patients.⁹ Analgesic drugs are pain relievers and generally act on the central and peripheral

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nervous system. Unlike anesthetics, which eliminate sensation, analgesics simply ease pain in the host. Traditional ethno-medicine used to relieve pain is a potential source for discovering novel anti-inflammatory, antipyretic and analgesic drugs. Natural products are a rich source of chemical substances with beneficial therapeutic capabilities.¹⁰

MATERIALS AND METHODS

Collection and preservation

Euphorbia dracunculoides was collected from the southern districts of Khyber Pakhtunkhwa, cleaned and jumbled thoroughly, dried in shade. A specimen was mounted in kept on a herbarium sheet, given a voucher number and kept in the department of Botany. Its macroscopic and microscopic characteristics were studied. An electric grinder was used to thrash the dried plants into powder and then stored in an air-tight bottle that protects it from hostile climate, insect attacks, molds and moisture. Physiochemical analysis is also carried out on a part of this powder and biological studies are performed on the rest.

Morphological observation

Macroscopic Study

The macroscopic observation of *Euphorbia dracunculoides* was carried out by organoleptic method, we analyzed the shape, size, odor, color, petiole, phylotaxis, lamina, surface, taste, Apex, Type, leaf margins, stipule and venation of the leaf, also analyzed colour, habit, taste, odor, size, surface, shape, branching, position, latex, texture, surface in stem, In root we analyzed size, shape, odor, taste, color, position, texture, surface and rootlets.¹¹

Microscopic Study

Powder microscopy

The organoleptic characteristics of *Euphorbia dracunculoides* in powder form were studied such as microscopical characteristic odor, color and taste. Following Wallis, (2005), who carried out powder drug study.

Procedure

Whole plant shade dried at 25°C for 7 days and grounded using electric grinder. Powders of dried parts were used for the observation of the microscopic characters. A pinch of fine powder was taken on a glass slide treated with chloral hydrate solution and iodine solution, then observed under a microscope using different objective lenses for observation of different anatomical structures, which were then photographed.

Elemental analysis

Elemental analysis of powder from the selected parts was carried out with atomic absorption spectrophotometry for the following trace and heavy metals.¹²

Preliminary qualitative phytochemical analysis

Phytochemical analysis was done by following¹³⁻¹⁷

Test for reducing sugars

- **Fehling's test:** A few drops of Fehling's reagent were added separately to ethanolic extracts of *Euphorbia dracunculoides* taken in test tubes. A red color appeared once the mixture was heated, thereby proving the presence of reducing sugars.
- **Benedict's test:** A few drops of Benedict's solution was added to ethanolic extract of *E. dracunculoides* taken in separate test tubes. The presence of reducing sugars was confirmed by the appearance of a brick red color.

Killere killiani test for steroidal Glycosides

- Glacial acetic acid was used to separately treat 4 ml of extract along with 5 % FeCl₃ and a few drops of concentrated H₂SO₄. A bluish green color appeared in the upper layer and a reddish brown appeared at the seam of the two liquids which confirmed the presence of steroidal glycosides.

Confirmative test for tannins

- **Ferric chloride (FeCl₃) test:** A few drops of FeCl₃ solutions were added separately to extract solution of the selected plant. The color transition from dark green to blue-black indicated the presence of Phenol heads, while the complete blue color meant hydrolysable tannin. The presence of condensed tannins in the test samples was confirmed by the green color.

Detection of Alkaloid

Hydrochloric acid was used to dissolve the ethanolic extracts separately, which were then filtered, and the filtrates were used for different tests for Alkaloid detection.

Dragondroff's test: A red precipitate was observed once the filtrate was treated with Dragondroff's reagent, which proved the presence of Alkaloids.

Hager's test: Yellow precipitation occurred once the Filtrates were treated with saturated picric acid solution, which is typical for Alkaloids.

Wagner's test: A reddish brown color appeared once the filtrates were treated with iodine in Potassium Iodide solution (Wagner's reagent), hence proving Alkaloid presence.

Saponins confirmation

Froth test: Distilled water was used to dilute the ethanolic extracts of the plant. A froth appears once they were vigorously shaken for more than five minutes, thus proving the presence of Saponins.

Detection of Triterpenes and Phytosterols

Salkowski's test: Chloroform was used to dissolve the extracts and the solution was then filtered. A few drops of concentrated H₂SO₄ were added to the filtrate, the solution was shaken and then kept still. Triterpenes were indicated by a yellow color while phytosterols were indicated by a red color in the lower layer.

Detection test proteins & aminoacids

Xanthoproteic test: A few drops of concentrated HNO₃ were added to 2 ml of extract solution. A yellow color appears once it is heated. The color soon turns orange.

Biuret test

- 2 ml of Biuret reagent was added to the test sample and a violet color is observed which represents the presence of protein.
- **Ninhydrin test:** To the plant extract solution 0.2% Ninhydrin solution was added resulted appearance of violet coloration which indicated the presence of protein.

Detection of Oil

Spot test

- A tiny amount of powdered drug was pressed between the folds of the filter paper which resulted in a translucent oily stain which represents the presence of fixed oil and fats.

Detection of flavonoids

Alkali test

- The test samples were treated with NaOH solution. A strong yellow color is observed. If dilute acid is added, it decolorizes which proves Flavonoid presence.

Lead acetate test

- If a yellow color is observed once lead acetate solution is added to the test samples, it means flavonoids are present.

Quantitative chemical analysis

Sterols, Saponins, Tannins and Alkaloids can be determined by quantitative analysis of the whole plant using standard method of ¹⁶⁻¹⁸

Alkaloids determination

Materials required

Ethanolic extracts, acetic acid, filter paper, beaker, balance, water bath

Procedure

Harborne method ¹⁶ was used to determine the quantity of Alkaloids in whole plant extract. 100 ml of 10 % acetic acid was added to 2 grams of ethanolic extract in a beaker, which was then covered and kept for four hours. The mixture was then concentrated to one-fourth of its original volume using a water bath. A precipitate formed once concentrated ammonium hydroxide was added drop by drop to the solution. Pre weighted (W1) Whitman filter paper was used to collect the precipitate. Dilute ammonium hydroxide was used to wash it. The filter paper containing the residue was dried and weighed (W2).

The amount of Alkaloid was calculated as such:

$$\text{Amount of alkaloid (mg/g)} = \frac{x}{\text{Weight of sample}}$$

$$\% \text{ Alkaloid} = \frac{x}{\text{Weight of sample}} \times 100$$

Where

$$X = \text{Weight of alkaloids} = W2 - W1$$

$$W1 = \text{Weight of filter paper}$$

$$W2 = \text{Weight of filter paper} + \text{ppt}$$

Saponin determination

Material required

A separatory funnel, balance, filter paper, water bath, 5 % sodium chloride solution, n-butanol, Ethanolic extract and Dimethyl ether.

Procedure

Obadoni and Ochuko ¹⁸ method was used to determine the quantity of Saponin. 20 ml of distilled water was used to dissolve 2 grams of an ethanolic extract of *Euphorbia dracunculoides*, which was then moved to a 250 milliliter separating funnel. The mixture was shaken vigorously after a further addition of 20 ml diethyl ether. After draining out the layer of ether, 60 ml of n-butanol was mixed with the aqueous layer which resulted in the precipitation of saponin. Pre weighted (W1) Whitman filter paper was used to collect the Saponin, washed thoroughly with 10 ml of 5 % aqueous NaCl solution. An oven at 40 °C was used to dry the precipitate to constant weight (W2). The Saponin content and the initial weight of the sample were measured and calculated as given below:

$$\text{Amount of saponins (mg/g)} = \frac{x}{\text{Weight of sample}}$$

$$\% \text{ saponins} = \frac{x}{\text{Weight of sample}} \times 100$$

$$X = \text{Weight of saponin} = W2 - W1$$

$$W1 = \text{Weight of filter paper}$$

$$W2 = \text{Weight of filter paper} + \text{residue}$$

Tannins determination

Material required

Ethanolic extracts, Whitman filter paper, lead acetate solution, balance, hot air oven, sulphuric acid, water bath

Procedure

Van-Buren and Robinson, ¹⁷ was used to determine the % tannins in the plant extract. To get tannins first we took 2 gram extract of *Euphorbia dracunculoides* then dissolved it in distilled water (75ml), after dissolution the solution was then filtered with Whitman filter paper. Solution of lead acetate was added to it in order to get lead- tannate precipitate. 20 ml of water was added to the residue, dilute H₂SO₄ was also added to the solution which removed lead sulphate. The solution was filtered through pre weighted Whitman filter paper (W1) and tanins were collected. The filter paper was dried in oven (at 60 C). Filter paper was weighed again. % tannins were calculated by using the formula.

$$\text{Amount of Tannins (mg/g)} = \frac{x}{\text{Weight of sample}}$$

$$\% \text{ Tanins} = \frac{x}{\text{Weight of sample}} \times 100$$

$$\text{Where } X = \text{Weight of tannins} = W2 - W1$$

$$W1 = \text{Weight of filter paper}$$

$$W2 = \text{Weight of filter paper} + \text{residue}$$

Sterols determination

Materials

Whitman filter paper, Ethanolic extracts, petroleum ether, 10% potassium hydroxide solution, Balance, flasks, water bath, reparatory funnel.

Procedure

In this process, 75 ml of distilled water and 10%KOH were added to 2 grams of extract. The process changed the chlorophylls contents into chlorophyllins (water soluble salt). It was then treated thrice with petroleum ether (75ml) in a separating funnel. First, the flask was weighed (W1), ether portion was collected in the flask and then it was placed in a water bath. When it became concentrated and dried, the flask was placed in a desiccator for cooling. The flask was weighed again (W2) and % sterol was calculated using the following formula (Huang *et al.*, 2010)

$$\text{Amount of sterols (mg/g)} = \frac{x}{\text{Weight of sample}}$$

$$\% \text{ sterols} = \frac{x}{\text{Weight of sample}} \times 100$$

Where

$$X = \text{Weight of sterols} = W2 - W1$$

$$W1 = \text{Weight of flask}$$

$$W2 = \text{Weight of flask} + \text{residue}$$

Nutritional analysis

Determination of Moisture

Apparatus

Desiccator, Electric oven, Electric balance, petri dish with lid.

Procedure

2 gram of powder was taken in petri plate then weighted it (W1). The petri plate was covered with lid partially; the petri plate was then placed in oven (105C°) for 4 to 6 hours in order to remove moisture. Then place the petri dish in desiccator for cooling for 30 minutes. After that, weight the petri plate again.¹⁹

Calculation

$$(\%) \text{ Moisture} = \frac{W1 - W2}{\text{Weight of the sample}} \times 100$$

Determination of Ash

Equipment's required

Desiccator, burner, Muffle furnace, Blow pipe, Crucible, Electric balance,

Procedure

2 gram of sample powder was taken in a sterilized crucible (W1). Before placing in muffle furnace it was first burnt with burner with the help of blow pipe. Then place it in furnace (550°C) for many hours. The furnace was then turned off and the color of sample changed into grayish white. Then the crucible was transferred into desiccator in order to cool. Weight it again (W2). The % Ash was calculated by using the following formula.²⁰

Calculation

$$\% \text{Ash} = \frac{(W2 - W1) \times 100}{\text{Wt. of sample}}$$

W1 = Weight of empty crucible

W2 = Weight of crucible + Ash

Determination of Crude Protein by Micro Kjeldahl Method.

Reagents

4% boric acid solution, 40% NaOH, 0.1 N standard HCl solution, concentrated sulphuric acid, catalyst (7 gm K₂SO₄, 0.5g CuSO₄·5H₂O and 0.3 g HgO).

Indicator

- i. Methyl red, 0.25 percent in 95% Ethanol
- ii. Methylene blue, 0.2% in 95% ethanol. Mix(i) and (ii) in the ratio of 3:2

Apparatus

Volumetric flask, pipette, Kjeldahl flask, burette, digestion and distillation apparatus etc.

Procedure

0.3g of the powder sample was taken in KJELDAHL flask. 7 ml of conc. H₂SO₄ and 1.5 g of catalyst were added to the flask. It was mixed thoroughly by spinning the flask. After this, it was digested in digestion flask on the heater till the solution may became clear. The process continued for 30 minutes. When the digestion completed, the heater was turned off in order to cool the solution. The digest was then diluted with 50ml distilled water in a volumetric flask. 5 ml of the digest was then taken by

using pipette. The solution was then transferred into distillation tube. Then, 5 ml of 40 % Sodium hydroxide solution was added through the funnel. Distillate was distilling for five minutes. 20ml of 4% boric acid solution was taken in a flask and some drops of indicator were added into the flask and then, collected the distillate. During distillation, the color changed from pink to blue.

After that, the distillate was titrated against 0.1 N HCl taken in a burette till the pinkish color restored. The reading of the volume of HCl was noted and % protein was calculated.²¹

Calculation

$$\% \text{ protein} = \frac{(S - B) \times N \text{ of acid} \times 0.014 \times \text{dilution} \times 100}{\text{Wt. of sample} \times \text{aliquot of digest taken for distillation}}$$

Where

S = Sample titration reading

B = Blank titration reading

0.14 = Millequivalent of nitrogen

Determination of fat or ether extract:

Apparatus: Water bath, petroleum ether (B.P 40-60C°), Soxhlet extraction apparatus, Extraction Thimble (Whatman).

Procedure

For the extraction of crude fat, the Soxhlet apparatus was used. Here in this process, first 2 gm moisture free sample powder was taken in a clean thimble. The thimble was than plugged with cotton then placed it in extractive tube. Weighted the 250-ml round bottom flask then added petroleum ether solvent up to 1/3rd of the flask after that fixed it in the extractive tube. Turned on burner and tap water to flow. Run the apparatus for 3 to 4 hours. After that, thimble was removed from the tube; the flask was then placed on the water bath to heat up and to remove the moisture. The flask was then placed in oven at 105C° in order to dry for 1hr. It was allowed to cool and weighted again (W2).

Fats percentage was then calculated by the following equation ²²

$$\% \text{ fat (Ether extract)} = \frac{x}{\text{Weight of sample}} \times 100$$

Where X = Weight of the fats = W2 - W1

W1 = Weight of the empty flask

W2 = Weight of the empty flask + sample after evaporation of solvent

Crude fibre determination:

Reagents: Asbestos, Ethyl Alcohol, Petroleum ether, Sodium hydroxide (2%), HCl (2%)

Apparatus

Muffle furnace, crude fibre extraction apparatus, suction pump, oven, electric balance, Gooch crucible, funnel, beaker, filter paper or cotton cloth

Procedure

2 gram of sample powder was taken in a beaker and added 200 ml of 2% NaOH kept on water bath for 30 minutes till it boils then filtered it with a cloth. The residue was then transferred into the beaker and add 200 ml of 2% HCl keep on water bath and boil for 30 minutes. Filter it with a cloth again and the remaining residue was then washed with 200ml hot water in order to remove the acid. The residue was then transferred into the crucible and placed in oven at 105C° for 4 hours. After that it was

transferred to dessicator for 30 minutes to cool and weighed (W1). Then the crucible placed in a muffle furnace for 4 hours at 550 C° burned till white and weighed again (W2). Crude fibre was then calculated.²³

$$\% \text{ crude fibre} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

Where

W2 – W1 = Crude fiber

W1 = Weight of sample after placing in oven

W2 = Weight of sample after placing in muffle furnace

Carbohydrates determination:

Carbohydrates can be determined by adding the weights of all the other contents then subtracted from 100.²⁴

% carbohydrates= (crude fibre+ crude fat+ crude protein+ moisture contents+ Ash) – 100

Analgesic activity by writhing induced method

Requirements

Acetic acid, plant extract and silver nanoparticles, diclofenac sodium, Albino mice (about 22g b.w), normal saline solution and 3 and 5 cc syringes

Procedure

The animals were not fed anything for at least 24 hours before the start of the experiment. There was a total of six groups. Group I was treated with diclofenac sodium (10mg/kg b.w, p.o) used as positive control. Group II was treated with normal saline (10mg/kg b.w, i.p) used as negative control. Group III was treated with 100mg/kg b.w, i.p of plant extract. Group IV was treated with 200mg/kg b.w, i.p of plant extract. Group V was treated with 300mg/kg b.w, i.p route of plant extract. Group VI was treated with only acetic acid. After 30 minutes of drug administration such as diclofenac sodium, normal saline solution, plant extract solution to the mice then injected 1 % acetic acid into the peritoneal cavity in order to cause pain. Recorded the writhing (extension of hind limbs, contraction of abdomen and turning of trunk) in the mice after 5, 10 and 15 minutes in various groups. % inhibition was recorded by using the following formula.²⁵⁻²⁶

Pharmacognostic study of *Euphorbia dracunculoides* L. was carried out to standardized the quality and quantity of the drug.

RESULTS

Pharmacognostic studies of *Euphorbia dracunculoides* L.

Whole plant extract of *Euphorbia dracunculoides* L. were evaluated for pharmacognostic study i-e Macroscopic, powder drug study, phyto-chemical analysis, nutritional and elemental analysis.

Macroscopy

The macroscopic character of the plant *Euphorbia dracunculoides* L. were observed for odor, shape, taste, colour and texture (Table 1, 2, 3). It helped in the correct identification of the plant. It is an annual herb. Its stem is light green at the top and yellow green at the bottom and 15-30 cm tall, it is much branched from the base. Its leaves are alternate, sessile, simple, dark green in color, its roots are yellowish in color, conical shape, smooth with rootlets.

Microscopic study

Powder drug study

Powder drug study of whole plant powder of *Euphorbia dracunculoides* L. was carried out (Figure 1). It shows different structures in powder form

Table 1: Macroscopic studies of *Euphorbia dracunculoides* stem

S.No	Characteristic	Observation
1	Color	Yellow green at bottom, light green at top
2	Habit	Annual
3	Taste	Bitter
4	Odor	Odorless
5	Size	15 to 30 cm
6	Branching	From the base it is much branched
7	Position	Vertical
8	Latex	Present, white
9	Surface	Glabrous
10	Texture	Smooth
11	Shape	Cylindrical

Table 2: Macroscopic studies of *Euphorbia dracunculoides* Root

Root	Characteristic	Observation
1	Size	13 cm
2	Shape	Conical
3	Odor	Cephaceous
4	Taste	Almost tasteless
5	Color	Yellowish
6	Position	Downward
7	Texture surface	Smooth
8	Rootlets	Present

Table 3: Macroscopic studies of *Euphorbia dracunculoides* Leaf

S. No	Characteristic	Observation
1	Size	1.5- 8cm × 1.5-5mm
2	Petiole	Absent, sessile
3	Phyllotaxis	Alternate
4	Taste	Slightly bitter
5	Odor	Odorless
6	Color	Dark green
7	Lamina	Simple
8	Stipule	Stipulated
9	Texture	Soft coriaceous
10	Apex	Acute, subacute or obtuse
11	Margin	Entire
12	Venation	Reticulate
13	Surface	Glabrous

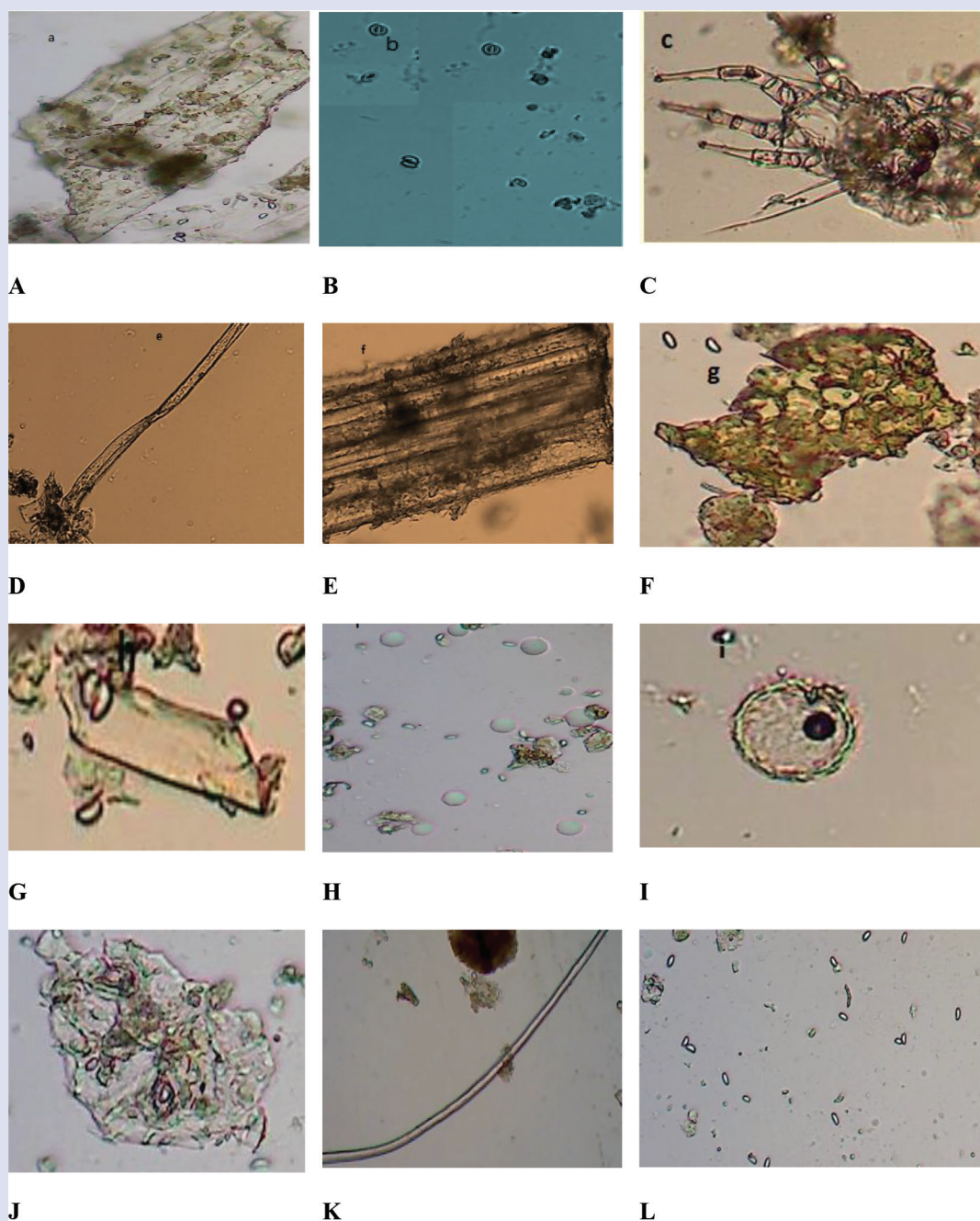


Figure 1: Powder drug study of *Euphorbia dracunculoides* L.

A. Epidermal cells. B. Stomata. C. Multicellular Trichomes. D. E. Phloem Fibers, F. Cortical cells, G. Xylem vessel H. Oil droplets, I. Pollen grain, J. Epidermal cell with stomata, K. Single trichome, L. Starch grains

which helps in the authentication and standardization of crude plant extract drug. Following were some of the structures observed under microscope.

- Fragments of epidermal cells
- Stomata
- Xylem vessels
- Phloem fibres
- Single and multicellular trichomes
- Cortical cells
- Starch grains
- Oil droplets
- Epidermal cell with stomata
- Pollen grain

Phytochemical Analysis.

Both qualitative and quantitative phytochemical analysis of the ethanolic extract of whole plant *Euphorbia dracunculoides* L. were carried out. Qualitative analysis of *Euphorbia dracunculoides* L. revealed the presence of carbohydrates, proteins, flavonoids, phenolic compounds, tannins, alkaloids, glycosides, sterols etc (Table 4).

Quantitative analysis of some of the secondary metabolites such as saponins, flavonoids, tannins and alkaloids were also carried out in the ethanolic whole plant extract of *Euphorbia dracunculoides* L. The quantitative result revealed the presence of sterols (20.5%), tanins (18.5%), saponins (17%) and alkaloids (10%). (Table. 5)

Nutritional analysis

In the present study, the nutritional analysis of *Euphorbia dracunculoides* L. were carried out and the result presented in the table. 6. Result of proximate analysis of *Euphorbia dracunculoides* L. showed the highest quantity of fibres (28.13 ± 0.7094) followed by proteins (26 ± 2.6457), carbohydrates (17 ± 0.5), ash (9.01 ± 0.015), fat (7.1 ± 0.3605), and moisture contents (14%).

Elemental analysis

In the present study, the elemental analysis of *Euphorbia dracunculoides* L. conducted which showed the presence of both macro and micro nutrients given in the table. 7.

The concentration of macronutrients i-e Magnesium (2.156 ± 0.0071), Sodium (1.032 ± 0.0034 ppm) and Zn (0.428 ± 0.0160) while the concentration of micronutrients i-e Iron (3.043 ± 0.0168), Cu (0.088 ± 0.0036), Ag (0.008 ± 0.0036) and Au (0.347 ± 0.4262) were found in *E. dracunculoides* L.

Table 4: Qualitative analysis of *Euphorbia dracunculoides* L.

S.No	Constituents	Test name	Whole plant extract
1.	Carbohydrates	Benedict's Test	+
		Fehling's Test	+
2.	Proteins and amino acids	Ninhydrin Test	+
		Biuret test	+
		Xanthoproteic Test	-
3.	Flavonoids	Alkali reagent test	+
		Lead acetate test	+
4.	Saponins	Froth Test	+
5.	Alkaloids	Wagner's Test	+
		Hagner Test	+
		Dragon draff's Test	+
6.	Sterols and triterpenoids	Salkowski test	+
7.	Tanins and Phenolic compounds	Ferric chloride test	+
8.	Glycoside	Killaerkilani Test	+
9.	Oil	Spot test	-

Table 5: Quantitative analysis of *Euphorbia dracunculoides* L

Tanins	Sterol	Saponins	Alkaloids
18.5%	20.5%	17%	10%

Analgesic activity by writhing induced method

Crude ethanolic extract of *Euphorbia dracunculoides* at various doses through i.p. route (100, 200, 300mg/kg b.w) showed decrease in the mean number of writhing in different test groups at different time as shown in the table 8.

After five minutes, In saline treated group mean writhing was 31.66 ± 1.66 . The percent writhing inhibitory effect produced by different test doses of crude extract of *E. dracunculoides* L. was 64.21% (100mg/kg), 74.73% (200mg/kg) and 95.79% (300mg/kg) compared to saline control. After 10 minutes, in saline treated group the mean writhing was 49 ± 1.73 , the writhing inhibitory effect of crude extract was 51.02% (100mg/kg), 70.08% (200mg/kg), 96.61% (300mg/kg) while after 15 minutes, in saline treated group the mean writhing was 68 ± 1.15 , the writhing inhibitory effect of crude extract was 53.92% (100mg/kg), 66.17% (200mg/kg) and 95.58% (300mg/kg). The effect produced by crude extract was dose dependent. The maximum writhing inhibition was shown by diclophenac sodium at 10mg/kg b.w (99.32%) and crude extract at 300mg/kg (96.61%). This result showed that the crude plant possess strong analgesic effect.

DISCUSSION

Macroscopy

That reduces the wrong recommendations of medicinal plants and traditional medicines to high extent, stepwise pharmacognostic investigations, including morphology, anatomy, quantitative microscopy determination such as stomata number, index, vein islet r, veinlet termination number, palisade ratio and qualitative and quantitative phytochemical screening give standardization of the crude drugs. Correct identification, authentication and quality assurance of the preliminary resources as an important requirement to make sure the reproducible quality of phytomedicine which will show the safety and effectiveness of herbal products.

Similar study was also conducted by other researchers. Mughal *et al.*²⁷ studied the phytochemical analysis and pharmacognostic study of, *Euphorbia hirta*, *Euphorbia prostrata*, *Jatropha integerrima*, *Ricinus communis* and *Euphorbia splendens* (Family: Euphorbiaceae). Sharma and Pracheta,²⁸ carried out the macroscopic and powder drug study of the leaves of the *Euphorbia neriifolia*. They observed that the fresh leaves are simple, with leathery texture, dark green in colour, sub-acute apex, acute base, cuneate shaped, entire margins, glabrous surface, reticulate venation. Kumar *et al.*²⁹ evaluated pharmacognostically *Euphorbia Hirta* plant. The leaves of *E. hirta* are opposite, oblong or oblong-lanceolate, elliptical, its upper surface is darker, with a faintly toothed margin. Its flowers are numerous, small and about 1 cm in diameter. Kanthale and Panchal,³⁰ evaluated the pharmacognostic study of *Adhatoda vasica* Nees.

Microscopic study

Powder drug study

Powder microscopy help in the identification of the herbal drugs and detection of adulteration in crude drugs

Similar study was also carried by other researchers; Sharma and Pracheta,²⁸ carried out the macroscopic and powder drug study of the leaves of the *Euphorbia neriifolia*. Powder drug study revealed the presence of anomocytic stomata, spongy parenchyma, unicellate multicellular trichomes, xylm parenchyma, starch grains, xylm fibres, epidermal cells. Patil and Jadhav,³¹ carried out the macroscopic and microscopic study of the plant *Antidesma acidum* Retz. Its powder drug study of leaves showed unicellular trichomes, epidermal cells, phloem fibres, hexagonal sclerenchyma cells, xylem and stomata. Kumar *et al.*²⁹ investigated trichomes, vascular bundles, xylem cells, spongy parenchyma

Table 6: Proximate analysis of *Euphorbia dracunculoides* L.

Ash %	Carbohydrates %	Fibers%	Proteins %	Fats%	Moisture%
9.01±0.015	17±0.5	28.13±0.7094	26±2.6457	7.1±0.3605	14

Table 7: Elemental Analysis of *Euphorbia dracunculoides* L.

Macro-elements (ppm)				Micro-elements (ppm)		
Na	Zn	Mg	Fe	Cu	Ag	Au
Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1.032±0.0034	0.428±0.0160	2.156±0.0071	3.043±0.0168	0.088±0.0036	0.008±0.0036	0.347±0.4262

Table 8: Analgesic activity by writhing induced method of *Euphorbia dracunculoides*

Dose (mg/kg b.w)	Mean number of Writhing ± SEM (% of saline)		
	5 Min	10 min	15min
Normal saline			
10 ml/kg	31.66±1.66	49±1.73	68±1.15
Diclophenac sodium			
10	1.33±1.32 (95.79%)	0.33±0.32 (99.32%)	0.33±0.32 (99.32%)
Plant extract <i>Euphorbia dracunculoides</i>			
100	11.33±0.66 (64.21%)	24.66±3.47 (51.02%)	31.33±4.67 (53.92%)
200	8±2.51 (74.73%)	14.66±1.20 (70.08%)	23±1.52 (66.17%)
300	1.33±0.32 (95.79%)	1.66±0.32 (96.61%)	3±0.57 (95.58%)

and phloem cells in the leaves of *Holoptelea integrifolia*. Sundar and Pillai, ³² evaluated the pharmacognostical standards by studying macroscopic and microscopic characters of *Solanum virginianum* L.

Phytochemical Analysis

Phytochemicals are actually organic compounds which possess medicinal properties (Mir *et al.*³³ Medicinal plants contain some bioactive organic compounds i-e carbohydrates, steroids, tannins, flavonoids, alkaloids and terpenoids, which provide specific physiological action on the human body. Okeniyi *et al.*³⁴ confirmed the presence of reducing sugars, cardiac glycosides, flavonoids, saponins, triterpenes, tannins, steroids, glycosides, carbohydrates and phlobatanins in the stem and leaf extract of *Euphorbia heterophylla*. Qaisar *et al.*³⁵ studied secondary metabolites of the aerial parts of *Euphorbia helioscopia*, *E. hirta*, *E. milli* and *E. prostrata*. They showed that these plants contain flavonoids, tannins and terpenoids. Similar study has been carried by other researchers; Pranoothi *et al.*³⁶ carried out the Quantitative analysis of *Leucas indica* which revealed the presence of total flavonoids and phenols contents in the plant extract. Kumbhar and Godghate, ³⁷ conducted the qualitative and quantitative analysis of *Acacia nilotica* bark, *Bombax malabaricum* flower, *Butea monosperma* (flower, bark, pod, leaves) and *Eucalyptus globulus* bark. Subha *et al.*³⁸ carried out the qualitative and quantitative analysis of *Tanacetum parthenium* L. Among quantitative analysis phenols were found maximum in methanolic extract (52.01 mg/g) followed by terpenoids (53.65 mg/g), flavonoids (19.64mg/g) and tannin (16.53 mg/g). Jaradat *et al.* ³⁹ studied

the quantitative analysis of *Ephedra alata*. Their result showed the total phenolic and flavonoid contents present in the extract of *Ephedra alata*.

Nutritional analysis

Food is the basic need of all living organisms. The basic purpose behind eating is getting calories, maintaining, growing, reproducing and multiplying of cells. A food must have proteins, carbohydrates, lipids, mineral elements, vitamins and water. Proteins, fats and carbohydrates are the most important nutrients of life. Similar studies were conducted by other researchers; Offor *et al.*⁴⁰ analyzed crude fibre (17.3%), Ash (3.8%), moisture (14.8%), carbohydrates (50.6%), fat (4.5%) and protein (24.5%) in the dried leaves of *moringa*. Igwenyi *et al.*⁴¹ conducted the Proximate analysis of *Euphorbia hyssopifolia* which showed the quantity of crude fat (3.20%), protein (0.88%), ash content (3.80%), carbohydrate content (6.98%), crude fiber (2.15%) and high moisture content (83.00%). Ghani *et al.* ⁴² analyzed 4 medicinal plants i-e *Achryanthus aspera*, *Peganum hermala*, *Mentha longifolia* and *Solanum nigrum* for nutritional study. Offor and Uchenwoke,⁴³ examined the proximate analysis of *Gongronema latifolium* leaves.

Elemental analysis

Medicinal plants contain Macro and Micro elements that control biochemical processes in the human body. The active ingredients of these plants play an essential role in metabolism. Similar work was also performed by other researchers Saxena *et al.*⁴⁴ determined the elements in the roots, leaves and stem of *Uraria picta*. Henry *et al.*⁴⁵ demonstrated the elemental analysis of *Cayratia gracilis* (Guill. & Perr.). Barua *et al.*⁴⁶ studied the elemental analysis of 5 plants i-e *Bryophyllum pinnatum*, *Corchorous fascicularis*, *Ocimum sanctum*, *Acorous calamus* and *Plantago erosa*. Parvez *et al.*⁴⁷ analyzed the macro minerals i-e Ca, N, P, S, Mg and K and micro minerals i-e Zn, Fe, Cl, Mn, Cu, Mo, Se and Na in the *Euphorbia granulate*.

Analgesic activity by writhing induced method

Analgesia is the sensation of "pain". Usually a deeply unlikable sensation brought about by internal or external stimulation. An analgesic is used to relieve pain without effecting consciousness or eliminating complete sensation. They act on the CNS or the peripheral nervous system. Analgesic activity is the capability of a substance to ease pain. Acetic acid permeates the capillaries which releases endogenous constituents to give rise inflammatory pain by stimulating nerve endings. Similar study was shown by other researchers; Chandrashekar *et al.*⁴⁸ investigated the analgesic activity of the *Phyllanthus lawii*. They suggested that the methanolic extract of *P. lawii* reduces the writhing in mice by injecting acetic acid showing peripheral analgesic activity. It releases the pain so it showed excellent analgesic activity. Kadam and Bodhankar, ⁴⁹ observed the significant analgesic activity at the dose 400mg/kg b.w of the ethanolic

extract of *Entada phaseoloides* by performing acetic acid induced pain method. Ramamurthy and Sathiyadevi,⁵⁰ observed the analgesic effect by acetic acid induced writhing method in mice of the plant *Indigofera trita*. They showed that at the dose 200mg/kg and 400mg/kg b.w the plant extract are more potent to reduce writhing

CONCLUSION AND RECOMMENDATION

It is concluded from the present research work that *Euphorbia dracunculoides* is an erect glabrous annual or sometimes perennating herb. The powder microscopy revealed presence of various type of tissues that is a tool for the determination of adulterants in crude powder of *E. dracunculoides* available in herbal market. Qualitative analysis revealed the presence of carbohydrates, proteins, flavonoids, phenolic compounds, tannins, alkaloids, glycosides, sterols and saponins etc. The quantitative result of *Euphorbia dracunculoides* L. revealed the presence of highest amount of sterols (20.5%). The result of elemental analysis showed highest concentration of macronutrients i-e Magnesium while the highest concentration of micronutrients i-e Iron (3.043±0.0168). Analgesic activity of *Euphorbia dracunculoides* L. by writhing induced method showed excellent analgesic effect at 300 µg/ml. Hence it is recommended that further advance and high level work should be done on *E. dracunculoides* to use it as a natural, economic and safe drug instead of synthetic haphazard medicines.

CONFLICT OF INTEREST

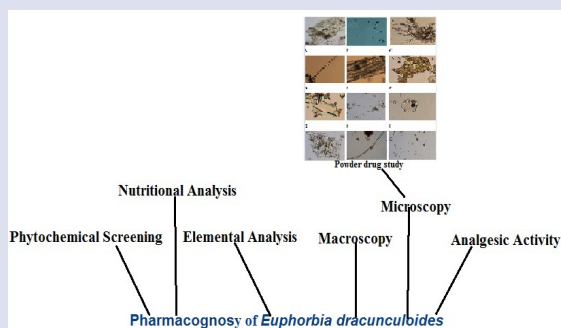
All the authors declare no conflict of interest.

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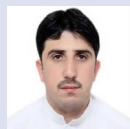
GRAPHICAL ABSTRACT



HIGHLIGHTS OF PAPER

- Pharmacognostic Evaluation and Analgesic Efficacy of Ethanolic Extract of *Euphorbia dracunculoides* L.
- Macroscopic study.
- Powder drug study.
- Phytochemical screening includes both qualitative and quantitative analysis.
- Proximate analysis showed proteins, crude fat, crude fibre, carbohydrates, moisture contents and ash.
- Analgesic activity using Acetic acid induced writhing.

AUTHOR PROFILE



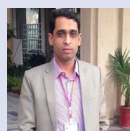
Shafqat Ali Khan: Ph.D. Scholar Department of Botany, Islamia College Peshawar, Pakistan.



Rehman Ullah: Assistant Professor Department of Botany, University of Peshawar, Pakistan.



Barkatullah: Assistant Professor Department of Botany, Islamia College Peshawar



Sami Ullah: Assistant Professor Department of Botany, University of Peshawar, Pakistan.

Umbreen Khattak: Ph.D. Scholar Department of Botany, Islamia College Peshawar, Pakistan.

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