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Research Article

Pharmacognostical Evaluation and Preliminary Phytochemical Screening of *Euphorbia neriifolia* Linn. Leaves

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ABSTRACT
The leaves of *Euphorbia neriifolia* Linn. showed high values of ash, calcium, potassium and fibres and microscopy reavealed presence of starch and calcium oxalate. The prelimnary phytochemical screening of leaves revealed presence of flavonoids,alkaloids, glycosides, saponins, carbohydrates, steroids & triterpenes etc. in different extracts.

Keywords: *Euphorbia neriifolia,* crude fibre, high ash value, calcium determination, calcium oxalate.

INTRODUCTION

Euphorbia neriifolia Linn. , also called as *Euphorbia ligularia* Roxb. , is a semisucculent perennial shrub or small tree commonly found in Central India (known as patton ki send or thuar) , western peninsular India and Orissa (known as thor or siju) in India, and in Ceylon, Baluchistan and Malay Islands.[1,2] The plant bears pairs of spines, stipular in origin, on tubercles of branchlets. The arrangement of these tubercles is such that 5 vertical or slightly spiral lines are formed, thus making the branch pentagonous in section, a feature that separates *E. neriifolia* from another very similar looking species, *E. nivulia*.[1,3]

Euphorbia neriifolia Linn. is traditionally used in abdominal troubles, loss of appetite, bronchitis, delirium, leucoderma, inflammation and several other conditions. Its leaves juice is used for earache treatment, whereas its latex is used in treatment of jaundice, leprosy, dyspepsia, skin erruptions, warts etc.[1,4,5] These medicinal uses can more or less be attributed to several phytoconstituents present in this plant like flavonoids, triterpenes (euphol, taraxerol, neriifolione, cycloartenol etc.), diterpenes (antiquorin, neriifolene) and their derivatives (ingenol triacetate, 12- deoxy euphorbol – 13,20 – diacetate etc.). [4,6,7,8] Various pharmacological studies have shown this plant to possess local anaesthetic[9], antidiabetic[10], wound healing[11], anti-inflammatory and analgesic[12], free radical scavenging and antioxidant[13], hepatoprotective[14] and varoius other pharmacological activities. The physical parameters (eg. ash values) of the leaves of this plant have not been reported yet, as well as very few reports are available on its microscopical features. The present study thus aimed at pharmacognostical evaluation and phytochemical screening of the leaves of *Euphorbia neriifolia* Linn.

MATERIALS AND METHODS

Collection & Authentication of Plant Material

The plant material i.e. *Euphorbia neriifolia* L. leaves were collected in the month of October 2011 from Saket Nagar, Bhopal (MP). It was identified and authenticated at the Department of Pharmacy, Barkatullah University, Bhopal(MP) by Dr. A.K. Pathak. Specimen of the plant parts were submitted as herbarium (Herbarium No. BUPH4059).

Processing of Plant Material / Sample Preparation

The *Euphorbia neriifolia* L. leaves were manually cleaned with the help of distilled water and the residual moisture evaporated at room temperature. The leaves were shade dried at room temperature, ground coarsely after drying and used for various analyses, except moisture content determination, where, fresh leaves were used. The residual moisture of dried crude drug was found with the help of IR moisture balance, which was later used to convert other parameters on 100% dry weight (DW) basis.

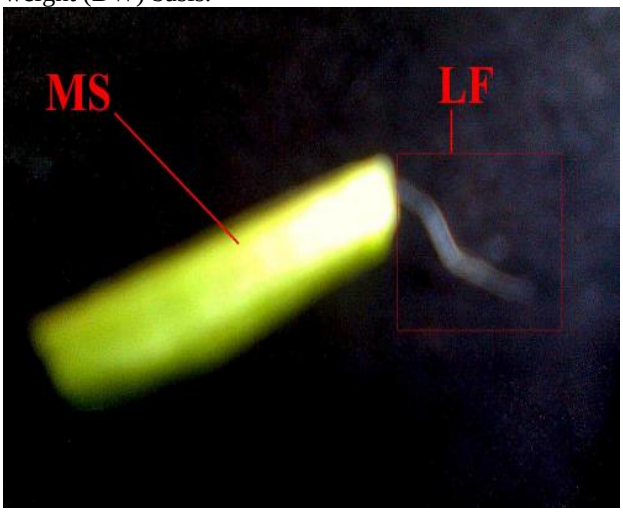

Figure 1: Part of midrib of leaf of *Euphorbia neriifolia* Linn.
MS : midrib section, LF : leaf fibre.

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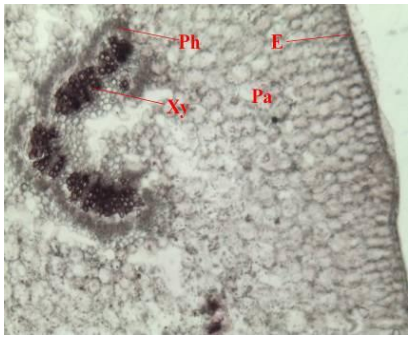

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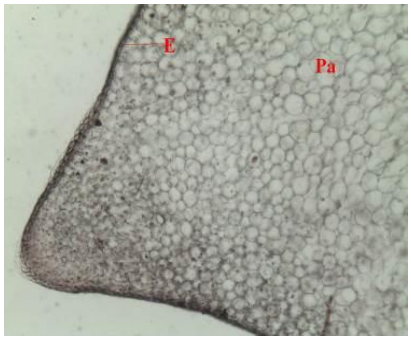

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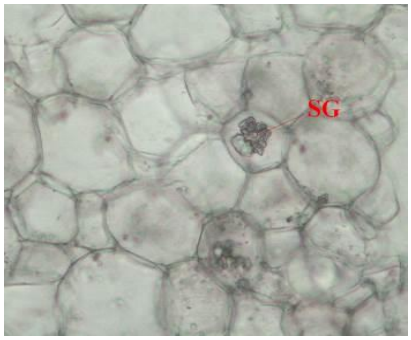

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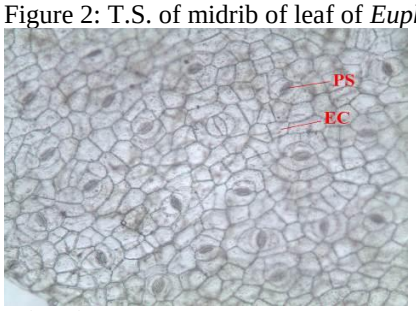
Figure 2: T.S. of midrib of leaf of *Euphorbia neriifolia* Linn



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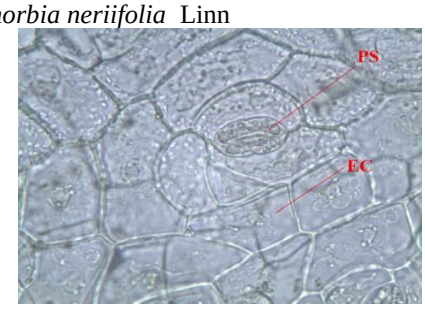

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Figure 3: Lower epidermis of leaf of *Euphorbia neriifolia* Linn PS : paracytic stomata, EC : epidermal cells.

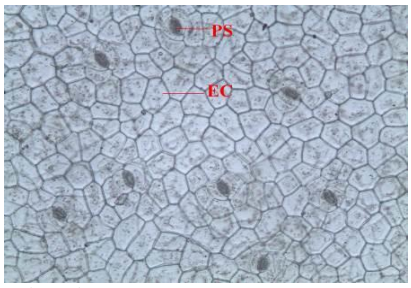

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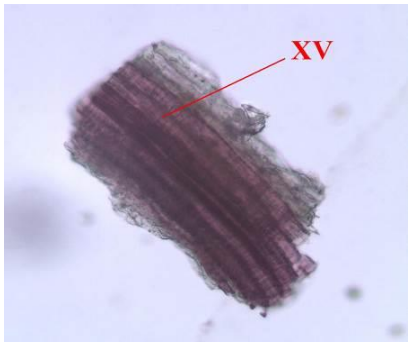
Figure 4: Upper epidermis of leaf of *Euphorbia neriifolia* Linn
PS : paracytic stomata, EC : epidermal cells.



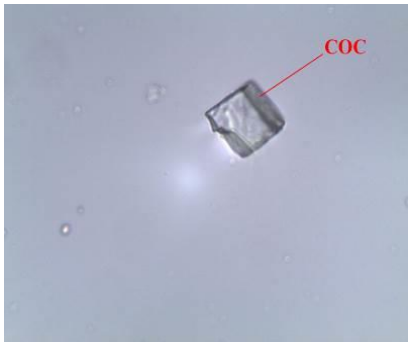


Figure 5: Powder microscopy of leaf of *Euphorbia neriifolia* Linn

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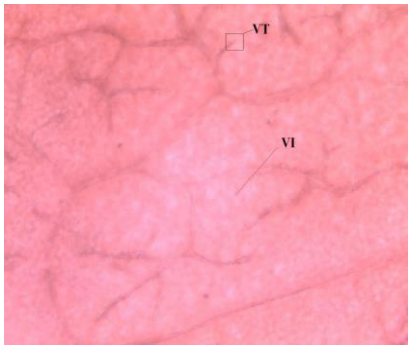

Figure 6: Lamina of leaf of *Euphorbia neriifolia* Linn.
VT : vein termination, VI : vein islet

Physical Parameters[15]

Loss on Drying

About 10 g. of accurately weighed crude drug was dried at 105ºc for 5 hours. The drying and weighing was continued at one hour interval until difference between two successive weighings corresponded to not more than 0.25%. Loss on drying was calculated as :

$$\text{Loss on drying} = \frac{W_1 - W_2 \times 100}{W_1}$$

Where, W_1 = Weight of crude drug before drying, W_2 = Weight of crude drug after drying, Crude drug = leaves of *Euphorbia neriifolia* L.

Ash Values

Total Ash

About 2 g. accurately weighed, ground drug was taken in a tared silica crucible and was incinerated at a temperature not exceeding 450ºc until free from carbon. It was then cooled and weighed. The percentage of ash with reference to the air-dried drug was calculated as :

$$\text{Total ash} = \frac{\text{Weight of ash obtained} \times 100}{\text{Amount of drug taken}}$$

Acid Insoluble Ash

The ash obtained in (2.1.) was boiled for 5 minutes with 25 ml of *dilute hydrochloric acid.* The insoluble matter was collected on an ashless filter paper, washed with hot

Table 1: Physical Parameters of Leaves of *Euphorbia neriifolia* L.

S.No.	Parameter	Result
1.	Loss on Drying	93.81 ± 0.690 %
2.	Total Ash	19.39 ± 0.528 %
3.	Acid Insoluble Ash	03.10 ± 0.294 %
4.	Water Soluble Ash	02.66 ± 0.235 %
5.	Water Soluble Extractive	31.20 ± 0.400 %
6.	Alcohol Soluble Extractive	07.31 ± 0.365 %
7.	Crude Fibre Content	15.36 ± 0.500 %

The data are mean value ± standard deviation (SD) of three replicates.

Table 2 : Calcium, Sodium, Potassium Content in Leaves of *Euphorbia neriifolia* L.

Calcium	Sodium	Potassium
41.25	00.18	21.74

Table 3 : Qualitative Analysis of Crude Fibre

S.No.	Test	Observation
1.	5 % potassium hydroxide	Insoluble
2.	Concentrated HCl	Insoluble
3.	5 % sodium citrate	Insoluble
4.	80 % sulphuric acid	Insoluble
5.	60 % sulphuric acid	Insoluble
6.	Molisch reagent	Dark blue-violet colouration
7.	Chlor-zinc-iodine	Dark blue-black colouration
8.	Phloroglucinol + HCl	Red colouration

Table 4: Qualitative Chemical Examination of Various Extracts of Leaves of *Euphorbia neriifolia* L. (obtained by Successive Solvent Extraction)

S. No.	Phytoconstituent category	Test	PE	CE	EE	WE
1.	Alkaloids	Dragendorff test	-	+	-	-
2.	Glycosides	General test	-	-	+	-
3.	Anthraquinone glycosides	Modified Borntrager's test	-	-	-	-
4.	Cardiac glycosides	Legal's test	-	-	-	-
5.	Coumarin glycosides		-	-	+	-
6.	Flavonoids	Shinoda test	-	+	+	-
7.	Dihydro flavonoids	Zinc chloride reduction test	-	+	+	-
8.	Steroids and triterpenoids	Salkowaski test	+	+	+	-
9.	Proteins	Xanthoproteic test	-	-	-	-
10.	Proteins	Biuret test	-	-	-	-
11.	Amino acids	Ninhydrin test	-	-	-	-
12.	Tannins	5 % ferric chloride	-	-	-	-
13.	Tannins	Nitric acid	-	-	-	-
14.	Carbohydrates	Molisch's test	-	-	-	+
15.	Carbohydrates	Fehling's test	-	+	+	+
16.	Carbohydrates	Benedict's test	-	-	-	+
17.	Starch	Iodine solution	-	-	-	+
18.	Saponins	Froth test	-	-	-	+

water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated as :

$$\text{Acid insoluble ash} = \frac{\text{Weight of ash obtained} \times 100}{\text{Amount of drug taken}}$$

Water Soluble Ash

The ash obtained in (2.1.) was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temprature not exceeding 450ºc. The weight of the insoluble matter was substracted 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 the air-dried drug as :

$$\% \text{ Ash} = \frac{\text{Wt. of ash taken} - \text{Wt. of ash obtained}}{\text{Amount of drug taken}} \times 100$$

Extractive Values

Water Soluble Extractive

5 g. of the air dried drug was coarsely powdered and macerated with 100 mL. of chloroform water in a closed flask with frequent shaking for six hours and then allowed to stand for eighteen hours. It was then filtered and 25 mL. of the filtrate was evaporated and then dried at 105ºc, to constant weight and weighed. The percentage of water-soluble extractive was calculated as :

$$\% \text{ extractive} = \frac{\text{Wt.of extract obtained (100 mL.)}}{\text{Amount of drug extracted}} \times 100$$

Alcohol Soluble Extractive

The procedure and formula used to determine the alcohol soluble extractive was same as used for water-soluble extractive, except for the use of alcohol instead of chloroform water.

Determination of Calcium, Sodium, Potassium Content [16]

The dried and powdered leaves were incinerated and the resultant ash was digested with suitable amount of dilute hydrochloric acid. It was then filtered with Whatmann no. 42 filter paper and this sample was compared with standard solutions of $CaCO_3$, KCl and NaCl using flame photometer. The calcium, sodium & potassium content were calculated with the help of calibration curves of standard solutions.

Morphological Evaluation [17]

Various morphological parameters like colour, size, surface appearance and texture, shape, margin, apex, base, venation etc. were studied studied in the leaf of *Euphorbia neriifolia* L.

Microscopical Evaluation [15]

The transverse section of the midrib (safranin stained) and the lower and upper epidermis of the leaf was studied under the microscope. Powder microscopy was done after staining the powdered leaves with safranin, iodine solution etc. The Stomatal index, Vein islet and Vein termination number were determined by studying the upper and lower epidermis and safranin stained lamina fragments, respectively, under the microscope. The vein termination number was calculated as the number of vein termination per mm. sq. area. The vein islet number was calculated as the number of vein-islets per square millimeter and the stomatal index was calculated as :

$$\text{Stomatal index} = \frac{S \times 100}{E + S}$$

Where

S = the number of stomata in a given area of leaf ; and

E = the number of epidermal cells (including trichomes) in the same area of leaf.

Crude Fibre Determination [17]

2 g. of powdered drug was put into a 100 mL. porcelain dish , and 50 mL. of 10% nitric acid was added and boiled with constant stirring. It was then filtered and residue was strained out through a piece of fine meshed cotton cloth and washed the residue on the cloth with 100 mL. boiling water. The material was removed from the cloth and put into porcelain dish. 50 mL. of 2.5% caustic solution was added and boiled with continuous stirring. The liquid was strained through cloth and washed with 100 mL. of boiling water. The crude fibre obtained was dried, further tested qualitatively, and was then incinerated. The crude fibre content was calculated as

$$\text{Content} = \frac{\text{Loss on incineration} \times 100}{\text{Original amount of crude drug}}$$

Prelimnary Phytochemical Screening [18,19]

Preparation of Plant Extracts

The air dried, powdered leaves of *Euphorbia neriifolia* L. were extracted by successive solvent extraction (SSE) method. First solvent to be used was petroleum ether (b.p. 60-80 ºc), next solvent was chloroform, then ethanol and lastly the powdered crude drug was extracted with chloroform water.

Qualitaive Analysis of the Extracts obtained by SSE

The extracts obtained by SSE were subjected to various qualitative tests to detect the presence of phytoconstituents like alkaloids, glycosides, carbohydrates, saponins, tannins & phenolic compounds, proteins & free amino acids, steroids & triterpenoids etc.

RESULTS AND DISCUSSION

The physical parameters determined of *E. neriifolia* leaves are presented in the following table. Such high values of total ash, and yet smaller values of acid insoluble and water soluble ashes indicate high amount of calcium salts (like calcium oxalate) in the crude drug, hence, amount of total calcium and some other elements were determined in the leaves of *Euphorbia neriifolia* L.

When morphologically evaluated, the leaves of *Euphorbia neriifolia* L. were found to be green, 20.5 cm. long and 5.4 cm wide (near apex), obovate shaped with glabrous surface, entire margin, cuneate base, acute apex (with rounded tip) and reticulate venation. Upon irregular sectioning of leaf midrib, numerous fibres were found, thus establishing, that these leaves are highly fibrous in nature.

Microscopical evaluation of these leaves revealed the presence of calcium oxalate crystals and starch grains. The calcium oxalate crystals appear to be rhomboidal in shape. The stomatal index of lower epidermis and upper epidermis was found to be 13.6 & 10.9 respectively. The vein termination number was found to be 15.2 & vein-islet number was found to be 2.1. The stomata was paracytic type. Transverse section of midrib of leaf showed single layer of barrel shaped cells forming epidermis. The vascular bundles, 4-5 in number, were collateral type, and were arranged as arrow or inverted v-shape in the centre, surrounded by parenchyma. The xylem fibre were found to be annular type.

Due to the highly fibrous nature and high ash value of leaf, crude fibre were extracted. Various qualitative tests of crude fibre before incineration showed that the crude fibre may contain suberin or cutin, some amount of carbohydrates, cellulose and lignin. The crude fibre content was found to be 15.36 %.

The prelimnary phytochemical screening of leaves revealed presence of flavonoids,alkaloids, glycosides, saponins, carbohydrates, steroids & triterpenes etc. in different extracts.

CONCLUSION

The leaves of *Euphorbia neriifolia* L. were found to be highly fibrous. Based on various qualitative analysis of the crude fibre extracted, it can be said that it may contain suberin or cutin, some amount of carbohydrates, cellulose and lignin. The crude fibre content was found to be 15.36 %. Unlike its stem, leaves of *Euphorbia neriifolia* L. showed much presence of calcium oxalate crystals and starch. Also, the determination of physical parameters showed high values of total ash, and yet smaller values of acid insoluble and water soluble ashes. Hence, amount of total calcium and some other elements were determined in the leaves of *Euphorbia neriifolia* L. The leaves of *Euphorbia neriifolia* L. were found to be rich in calcium and potassium (calcium = 41.25 mg/g of crude drug, potassium = 21.74 mg/g of crude drug). The prelimnary phytochemical screening of leaves revealed presence of flavonoids,alkaloids, glycosides, saponins, carbohydrates, steroids & triterpenes etc. in different extracts. Flavonoids are known for their various pharmacological actions like anti-inflammatory, anti-carcinogenic etc. Based on various analysis and their results mentioned above, we conclude that the leaves of *Euphorbia neriifolia* L. have immense potential as source of calcium, potassium and flavonoids in pharmaceuticals.

ACKNOWLEDGEMENT

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