

Pharmacognostic Phytochemical and Physicochemical Evaluation of Rudraksha Leaves

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ABSTRACT

Objectives: The main aim of this investigation was to conduct a detailed Pharmacognostical and phytochemical characterization of *Elaeocarpus ganitrus* leaf material sourced from the East Sikkim region of India. **Materials and Methods:** A series of microscopical and physicochemical analyses were performed, encompassing transverse sectioning, powder microscopic examination, total ash quantification (acid insoluble and water-soluble determination), loss on drying determination, extractive value determination, pH determination, foaming index, swelling index, quantitative evaluation of phytochemicals and thin-layer chromatographic profiling of *Elaeocarpus ganitrus* leaf samples. **Results:** Microscopical examination revealed that the leaf transverse section comprises three distinct anatomical zones-the epidermis, mesophyll tissue, and vascular bundle. The epidermal cells exhibited an irregular, undulating arrangement accompanied by a notably thickened epidermal wall. Calcium oxalate crystals of prismatic, rosette, and cluster configurations were identified. Among the secondary metabolites detected, tannins and alkaloids were present in notably elevated quantities. **Conclusion:** This investigation delivers a detailed and well-documented pharmacognostical, physicochemical, and phytochemical profile of *Elaeocarpus ganitrus* leaves, which may serve as foundational data for the development of standardization benchmarks and inclusion in pharmacopoeial references.

Keywords: Microscopical, Pharmacognostic, Phytochemical, Rudraksha, Thin Layer Chromatography.

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INTRODUCTION

Elaeocarpus ganitrus Roxb., commonly known as Rudraksha, classified under the family of Elaeocarpaceae.¹ It is basically found in the region of Southeast Asia and in some part of the sub-himalayan belt. There are various studies done on the leaves of *Elaeocarpus ganitrus* previously, but the research attention has been increasing on this plant because of its high reservoir of secondary metabolites and its relevance to pharmacognostical quality assessment.²

Morphologically, the leaves are characterized by a simple, elliptic-lanceolate form, typically spanning 10-15cm in length. They display a bright green, glossy upper surface, contrasted by a comparatively duller, firm-textured lower surface.^{3,4} Powder microscopic analysis of *Elaeocarpus ganitrus* leaf materials

functions as a critical diagnostic instrument for pharmacognostic authentication and crude drug quality evaluation.^{5,6}

The leaves morphology is characterized by a simple, elliptic, lanceolate form, which is about 10-15cm in length and has a bright green glossy upper surface and firm-textured lower surface. Powder microscopy of *Elaeocarpus ganitrus* leaf plays an important role in authentication and crude drug quality evaluation. Phytochemical evaluation showed active secondary metabolites such as titeroenoids, flavonoids, tannins, alkaloids, phenolics, and glycosides constituents, it exhibits bioactive properties due to their anti-inflammatory and antioxidant activities.^{7,8}

Physiochemical analyses such as total ashvalue, both water and acid insoluble ash value, extractive value, and moisture content are important pharmacognostical evaluation performed. Although *Elaeocarpus ganitrus* leaves are known for their phytochemical significance, a detailed evaluation of their pharmacognostic and comprehensive chemical characterization of this plant is still limited in available literature. Therefore, a systematic and well-structured study is required to establish reliable identification standards and develop a detailed chemical profile for future research purposes.⁹



DOI: 10.5530/ijper.krupapharmacon.34

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BOTANICAL CLASSIFICATION

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Oxalidales
Family	Elaeocarpaceae
Genus	Elaeocarpus
Species	<i>Elaeocarpus ganitrus</i> Roxb. ^{10,11}

MATERIALS AND METHODS

Collection of *Elaeocarpus ganitrus*

The medicinal plant sample of *Elaeocarpus ganitrus* was directly procurement during the month of November from Sikkim. Both macroscopic and microscopic features are confirmed and identified through various evaluation parameters. Authentication of the plant sample was carried out by the Botanical Survey of India, Gangtok and a voucher number bearing accession 0165 was deposited.

Macroscopic Evaluation

Macroscopic inspection of medicinal plant materials is based upon shape, color, odor, size, shape, and texture appearance of the leaf surface. It is used to determine the identity and purity of the drug sample. It includes coloration, aromatic character, geometric form and taste were detected.¹²

Microscopic Evaluation

Leaves are placed to determine the microscopic evaluation by preparing the cross-sectional cutting method. Leaf sections are treated with potassium hydroxide reagent, followed by phloroglucinol and hydrochloric acid for staining for microscopic visualization. The quantitative micrometric analysis was performed on leaf samples pretreated with potassium hydroxide captured by lucida camera. Various parameters were evaluated such as palisade ratio, vein termination number, vein islet, stomatal number, stomatal index.¹³

Stomatal number and Stomatal index

The stomatal number of *Elaeocarpus ganitrus* was determined using standard microscopic methods. Fresh mature leaves were collected and the epidermal peel was taken from the lower surface of the leaves, as stomata are more abundant on this surface. The peel was cleaned, strained and mounted on a glass slide using glycerin. The prepared slide was then observed under a microscope, fields to obtain an average value. For the determination of the stomatal index, the following formula was used:

$$SI = S/E + S \times 100$$

Where, S=stomatal number and E= number of epidermal cells.

Vein Islet number

The vein islet number of *Elaeocarpus ganitrus* leaves was determined using standard microscopic method. Fresh leaves were collected, cleared and treated to make the vein network clearly visible. A small portion of the leaf was taken on a glass slide and glycerin was added on it, and observed under a compound microscope. The number of vein islets present in a defined area of the leaf was counted in different microscopic fields, and the average was calculated. It was expressed in the average number of vein islets per square millimeter of leaf area.

Vein termination number

For vein termination, fresh mature leaves of *Elaeocarpus ganitrus* were cleared to render the venation pattern clearly visible for detailed examination. A suitable portion of the leaf was mounted on a clean glass slide using glycerin and examined under the microscope. The vein ending of the leaf were subjected to a microscope in different fields. Then, the average count was calculated.¹⁴

Palisade ratio

The fresh, mature leaves of the plant sample were cleaned, and a small portion of epidermis was subjected to a previously washed, cleaned, dried glass slide by using glycerin and examined under the microscope. The number of palisade cells present below a single epidermal cell was counted, and the average value from various observations were recorded as the palisade ratio.¹⁵

Physicochemical parameters

The assessment of drugs was achieved through various physicochemical evaluation parameters that identify the drug's identity and purity. The various evaluation parameters and pH of the powdered leaf sample were evaluated according to the WHO guidelines.¹⁶

Determination of Ash Value

Total Ash: Accurately weighed 2 g of powdered drug sample and placed it in a previously washed, cleaned, pre-ignited crucible and subjected it to a muffle furnace for incineration at a temperature between 400-450°C until the residue changed from green to white colour, which indicates the complete removal of carbonaceous matter. Following cooling in a desiccator for 30 min, the remaining ash was weighed and the total ash percentage expressed as mg/g.

Acid-Insoluble Ash: The ash residue obtained above was refluxed with 25 mL of dilute hydrochloric acid for approximately 5 min. The acid-insoluble fraction was separated by filtration through ash-free filter paper, thoroughly rinsed with distilled water, dried, and re-ignited in the furnace to constant mass. Upon cooling, the

residue was taken and weighed for which the acid-insoluble ash percentage calculated as mg/g.

Water-Soluble Ash: The 25 mL of distilled water along with total ash are boiled for 5 min, and the remaining insoluble fraction was separated by filtration and washed with distilled water. The filter paper along with the insoluble matter was combusted in a furnace at 400-450°C. The mass of this insoluble fraction was then subtracted from the total ash value, and the obtained value-representing the water-soluble ash fraction-was expressed as a percentage (mg/g).^{12,16}

Determination of Extractive Values

Water-Soluble Extractive

Accurately take 4 g of dried powdered drug of *Elaeocarpus ganitrus* in a previously cleaned, dried, covered, sealed conical flask; on that, add 100 mL of distilled water and subject it to maceration over 24 hr, with vigorous shaking in an initial period of time and placed for 15-18 hr without any disturbance. The solution mixture was filtered and volume-adjusted to 100 mL. From that, 25 mL was taken and evaporated to dryness at room temperature in an evaporating dish. After evaporation, it is subjected to a desiccator and cooled at room temperature for 30 min. The residue is obtained, and the water-soluble extractive yield is calculated as mg/g.

Alcohol-Soluble Extractive

Weighed accurately 4 g of drug leaf sample and carefully placed it into a closed conical flask. On that, add 100 mL of alcohol, usually 90-95% ethanol, to a flask and stir continuously and set aside for maceration for 24 hr, stirring continuously in the 1st hr and then keeping it at room temperature without any disturbance for the remaining 15-18 hr.

Loss on drying

The 2g of drug sample was taken into a pre-weighed petri plate and subjected to a heated air oven for drying approximately at 100°C. After drying, it was cooled in a desiccator, and the drug sample was reweighed. The cycle was continued until a uniform weight was obtained and the moisture level present in the sample was calculated.^{13,16}

Swelling index

Weigh accurately 2 g of powdered drug sample of *Elaeocarpus ganitrus* and introduce it into 50 mL of measuring cylinder; on that, add 50 mL of distilled water. The cylinder was subjected for 24 hr to permit full hydration, after which the final volume occupied by swollen material was measured and recorded.¹⁶

Foaming index

1 g of leaf powdered was transferred to a 500 mL conical flask filled with 100 mL of hot water. The mixture was heated for 30

min, allowed to cooled, and then passed through filter paper into a 100 mL volumetric flask with volume adjustment to 100 mL. Ten numbered test tubes were prepared with serial dilutions from 1 mL to 10 mL of the decoction, each adjustment to 10 mL total volume using distilled water. All tubes were agitated in a lengthwise manner twice every 15 sec and kept undisturbed to stand for 15 min, after which height of the foam were recorded.^{14,16}

Preparation of extract

Leaves are dried for 15 days in the lab and powdered. About 100 g of powdered leaf was taken separately and extracted by Soxhlet method using distinct solvents i.e. petroleum ether, chloroform and ethanol. After extraction the solution mixture is filtered and concentrated. The extract was evaporated in the laboratory at room temperature for 3-5 days to produce a semi-solid extract.¹⁶

Phytochemical screening

Standard analytical reagents are used to detect the presence or absence of phytoconstituents including alkaloids, saponins, carbohydrates, sterols, terpenoids, and coumarin.^{14,16}

Quantitative evaluation of phytochemicals

Sample Preparation: The leaf-powdered drug was subjected to successive extraction using different solvents in a specific order from nonpolar to polar. After extraction, extract was cooled and filtered through a separatory funnel and various quantitative phytochemicals are evaluated, which are as follows:¹⁷

Determination of Total Phenolic Content

Standard Preparation

The stock solution was prepared by using 0.1 g of gallic acid dissolved in 100 mL of solvents to obtain a 100 µg/mL concentration, which was used as a standard for evaluation of total phenolic content. Standard solutions of 20 µg/mL to 100 µg/mL are formulated from that stock solution and could be used for plotting the calibration curve of gallic acid.

Procedure

A standard solution, along with 0.1 mL of the ethanolic leaf extract, were prepared separately into 10 mL volumetric flasks. To each flask, 0.05 mL of Folin-Ciocalteu reagent was introduced into each flask for 5 min. Thereafter, 0.2 mL of a 20% sodium carbonate solution was prepared and added into it, and the volume was adjusted to 10 mL with distilled water. The prepared reaction mixture was incubated at room temperature for 20-30 min, which facilitates the light blue colour development. Absorbance was then recorded at 650 nm using a UV-visible spectroscopy. A standard graph of gallic acid calibration were constructed and calculated.^{17,18}

Determination of total alkaloids

Approximately 1g of *Elaeocarpus ganitrus* leaf powder was weighed into a conical flask, combined with 10 mL ethanol, thoroughly mixed, and filtered. Dilute hydrochloric acid (1 mL) was incorporated into the filtrate, followed by titration against 0.1N sodium hydroxide. Bromocresol green indicator was employed; a color transition to yellow marked the titration endpoint.¹⁹

$$\text{Total alkaloids} = (V_1 - V_2) \times N \times 10 \div W$$

Were,

V_1 = Volume of NaOH consumed for blank,

V_2 = Volume of sodium hydroxide utilized for sample analysis,

N = Normality of sodium hydroxide and,

W = Weight of sample taken.

Determination of total tannins

Stock solution and tannic acid standards were prepared identically to the total phenolic's method. Total tannin content was assessed by the casein precipitation, about 2 g of casein were mixed to 12 mL of extract, and continuously stirred at ambient temperature for 3 hr prior to filtration through Whatman filter paper. Residual phenolics in 0.1 mL of the filtrate were quantified using the Folin-Ciocalteu technique. The tannin content was derived by subtracting the post-precipitation phenolic value from the total phenolic content.²⁰

Determination of total flavonoids

Standard preparation

A stock solution was formulated by adding 10 mg of quercetin in alcohol, followed by dilution to obtain a 100 mg per litre concentration. From this standard stock; 20, 40, 60, 80 and 100 mg per litre was prepared.

Procedure

From the stock, 1 mL of each standard and plant test sample was poured into another 10 mL volumetric flask containing 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. Before the addition of 0.3 mL of 10% aluminum chloride, the mixture is set aside for incubation for 5-6 min. For the neutralization of the reaction mixture, 2 mL of 1 M sodium hydroxide was added and left for incubation for 5 min at room temperature, and the volume was made up with water. For a blank preparation, replaced the aluminum chloride reagent with distilled water. The absorbance of both standard and test sample was recorded at 420 nm.²¹

Thin layer chromatography

The TLC were performed to evaluate the phytochemical constituents of the sample. Silica gel GF254 was taken as the stationary phase. The mobile phase for the petroleum ether

extract consisted of toluene:ethyl acetate (9.3:0.7); for the chloroform extract, hexane:acetic acid (9:1); and for the ethanol extract, chloroform:methanol:ammonia solution (6.5, 3.0, 0.5).

Development of the chromatogram: The TLC plate was spotted using a capillary tube and placed in a mobile phase chamber. The plate was left until the solvent front reached approximately three-fourths of the plate length.

RESULTS

Authentication

The leaves of *Elaeocarpus ganitrus* was obtained from Sikkim, India, during the month of November, and botanical identification and authentication were done by BSI, Gangtok with voucher specimen number 0165.

Macroscopic Characteristics

The leaves are simple with an elliptic outline, which is approximately 10 to 12 cm long in length and has a serrated leaf margin with an apical tip with a green upper surface (Figure 1).

Microscopic Characteristics

Cross-sectional examination of *Elaeocarpus ganitrus* leaves revealed an organized tissue architecture comprising a surface cuticle, palisade and spongy mesophyll layers, bicollateral vascular bundles, and epidermal cells bearing anisocytic-type stomata. Powder microscopic analysis demonstrated the characteristic presence of lignified fibres, parenchymatous cell fragment, stone cell, stomatal fragment, xylem vessel fragment, epidermal cell fragment epidermal cell fragment, calcium oxalate crystals, trichome, trichome base and epidermal cell fragment (Table 1 and Figures 2-6).

Physicochemical Parameters

Results of physicochemical profiling are summarized in Table 2. Total ash content was recorded at 4.5%, water-soluble ash at 2.0%, and acid-insoluble ash at 0.5%, the last value being comparatively low. The alcohol-soluble hot extractive value (17%) exceeded the water-soluble cold extractive value (11%). Moisture loss on drying was recorded at 6%. Both swelling and foaming indices were below 1%, and the leaf extract exhibited an acidic pH.

Table 1: Microscopical characteristics of leaf constants.

Sl. No.	Leaf constants	Per square millimeter
1.	Stomatal number	12-16
2.	Stomatal index	18-22
3.	Vein islet number	15-18
4.	Vein termination number	10-12
5.	Palisade ratio	5.25-6.5

Phytochemical Screening

Qualitative chemical analysis of petroleum ether, chloroform, and ethanolic leaf extracts confirmed the detection of alkaloids, sterols, tannins, and flavonoids across the tested solvent fractions (Table 3 and Figures 6, 7).

Quantitative Evaluation of Phytochemicals

Spectrophotometric analysis, employing gallic acid as the calibration standard, was carried out to quantify the total phenolic and total alkaloid content present in the crude leaf extract. The calibration curves enabled accurate estimation of these phytoconstituents and indicated the presence of appreciable amounts of both compound classes in the leaf material (Figure 5). The chemical test of the leaves was performed to assess the contents of total flavonoids, total phenolic, total tannins and total alkaloids based on the occurrence of these bioactive constituents. The total phenolic, total tannin and total flavonoid contents in the leaf sample were calculated using the respective standard calibration equations. For total phenolics, the equation obtained from the gallic acid calibration curve was ($y=0.005x - 0.0457$, $R^2=0.9976$) and the final concentration is $81.1 \pm 0.19 \mu\text{g/mL}$ equivalent to gallic acid. The total tannin content was determined using the tannic acid standard equation, ($y=0.0048x-0.023$, $R^2=0.9838$) and the concentration of plant extract is $48.9 \pm 0.16 \mu\text{g/mL}$ equivalent to tannic acid, while the total flavonoid content

was estimated from the quercetin calibration curve equation, ($y=0.0078x - 0.03$, $R^2=0.9853$) and the concentration of plant extract is $38.2 \pm 0.12 \mu\text{g/mL}$ equivalent to quercetin. The percentage of total alkaloids detected in the leaf test sample was found to be $4.0 \pm 0.18\%$ (Figures 7-13).

Thin Layer Chromatography

TLC profiling across three solvent extract types yielded chromatographically distinct separation patterns indicative of multiple phytoconstituents. For petroleum ether extract, the solvents system is toluene: ethyl acetate (9.3:0.7) resolved 6 distinct spots of different Retention factor values of 0.17, 0.37, 0.42, 0.50, 0.65, and 0.77. The chloroform extract, developed in hexane: acetic acid (9:1), yielded 4 spots at R_f values of 0.13, 0.25, 0.37, and 0.48. The ethanolic extract in chloroform: methanol: ammonia (6.5:3.0:0.5) produced 3 spots at R_f values of 0.25, 0.35, and 0.46. These findings collectively confirm the chemical diversity of the extracts and provide solvent system guidance for future compound isolation.

Table 2: Values are expressed as means $\pm$ SEM of triplicate determination.

Sl. No.	Physicochemical Characteristics	Observation%
1.	Total Ash Value	4.5 ± 0.17
2.	Total Acid insoluble ash	0.5 ± 0.06
3.	Total Water-soluble ash	2.0 ± 0.07
4.	Loss on drying	6.0 ± 0.06
5.	Water soluble hot extractive value	25.0 ± 0.12
6.	Alcohol soluble hot extractive value	17.0 ± 0.12
7.	Water soluble cold extractive value	11.0 ± 0.06
8.	Alcohol soluble cold extractive value	39.0 ± 0.17
9.	Swelling index	Less than 1
10.	Foaming index	Less than 100
11.	Ph	Acidic



Figure 1: Leaves of *Elaeocarpus ganitrus*.



Figure 2: Leaves powder.

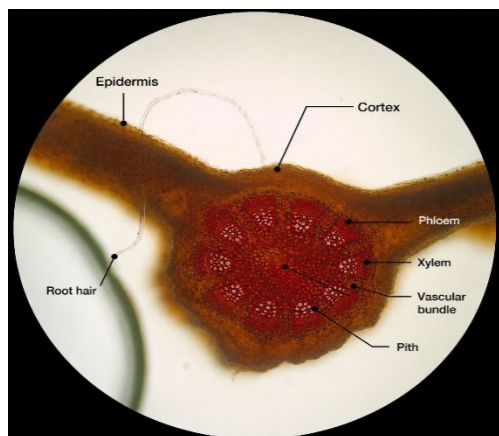
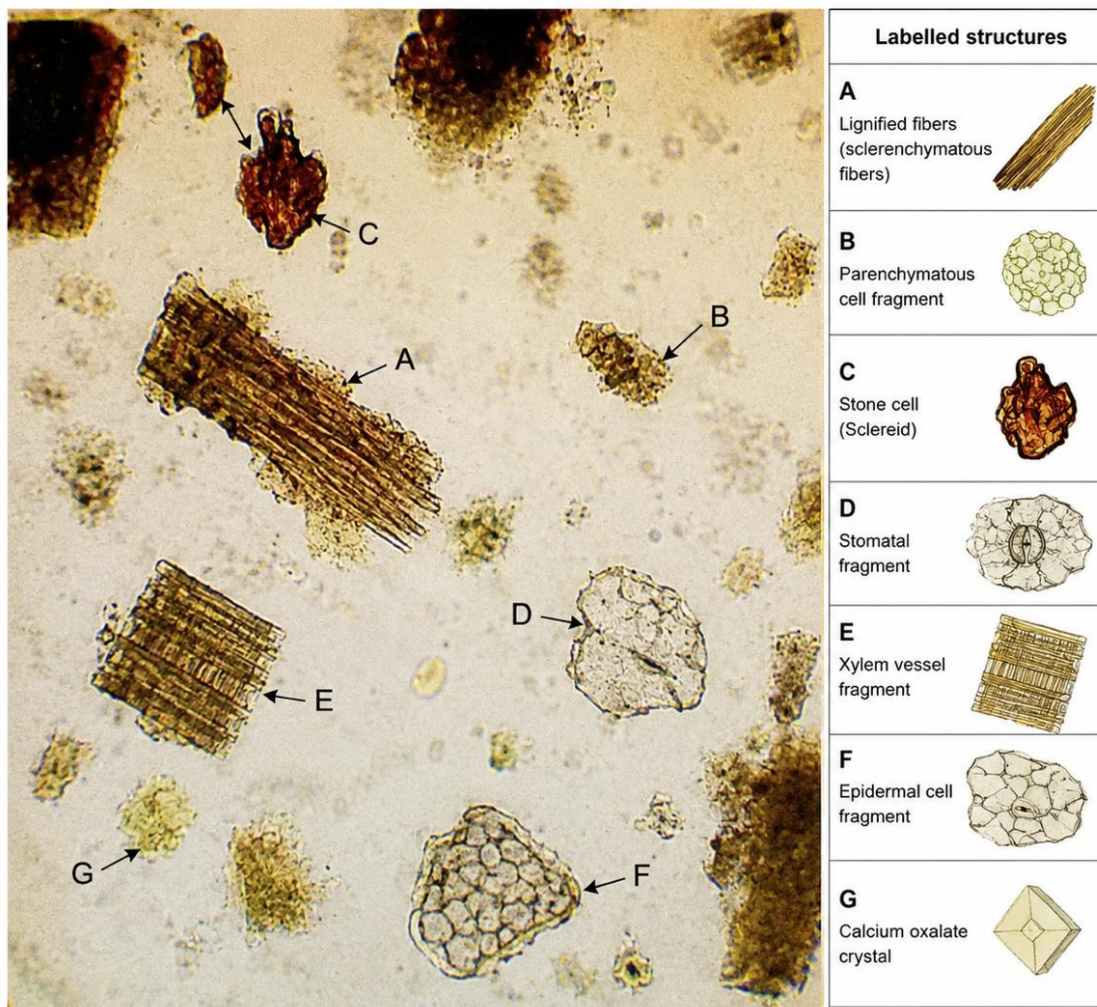


Figure 3: Transverse section of leaf.

DISCUSSION

The current investigation was to create phytochemical and pharmacognostical criteria for *Elaeocarpus ganitrus* leaves to enable their identification, purity, and quality control. Their finding shows different macroscopic and microscopic traits with defined physicochemical parameters and the presence of phytoconstituents. So, quality assessment is necessary to establish the legitimacy and repeatability of plant-based formulations. Important diagnostic characteristics of the leaves were validated by macroscopic and microscopic analyses. The straightforward, glossy, elliptic to oblong shape with serrated edges is consistent with previous observations. Anisocytic stomata, palisade and spongy

Powder microscopy of Rudraksha leaves (*Elaeocarpus ganitrus*)

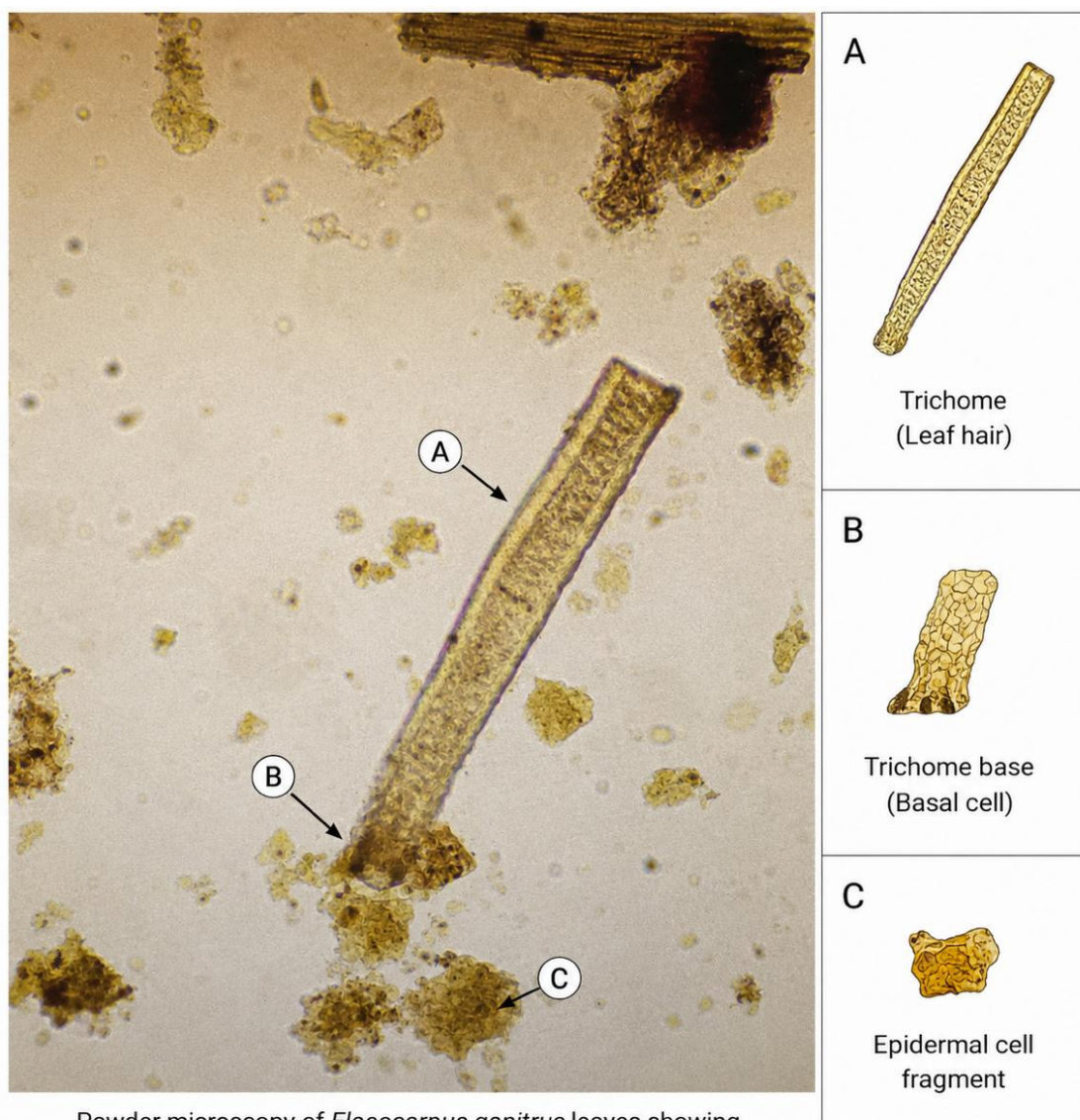


Powder microscopy of *Elaeocarpus ganitrus* leaves showing lignified fibers, parenchymatous cells, sclereids, stomatal and xylem fragments.

Figure 4: Powder microscopy of *Elaeocarpus ganitrus* leaves showing lignified fibers, parenchymatous cell fragments, stone cells, stomatal fragments, and xylem vessel fragments.

Table 3: Preliminary phytochemical Test.

Phytoconstituents	Pet. Ether Extract	Chloroform Extract	Ethanol Extract
Alkaloids	-	+	+
Carbohydrates	-	-	+
Glycosides	-	-	-
Sterols	+	+	+
Phenolic	-	-	+
Flavonoids	-	-	+
Saponins	-	-	-
Tannins	-	-	+



Powder microscopy of *Elaeocarpus ganitrus* leaves showing trichome (leaf hair), trichome base (basal cell) and epidermal cell fragment.

Figure 5: Powder microscopy of leaves showing trichome, trichome base and Epidermal Cell.



Figure 6: Soxhlet extraction.

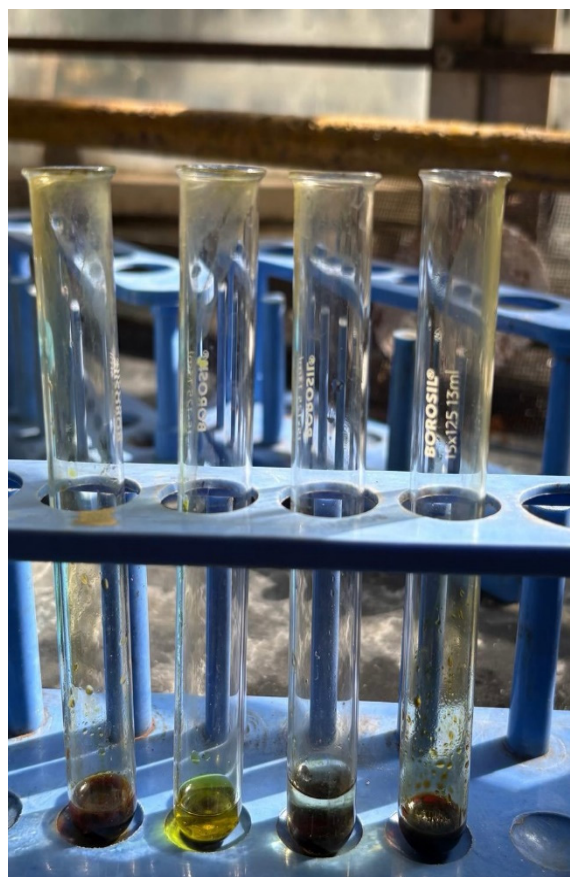


Figure 7: Preliminary phytochemical test.

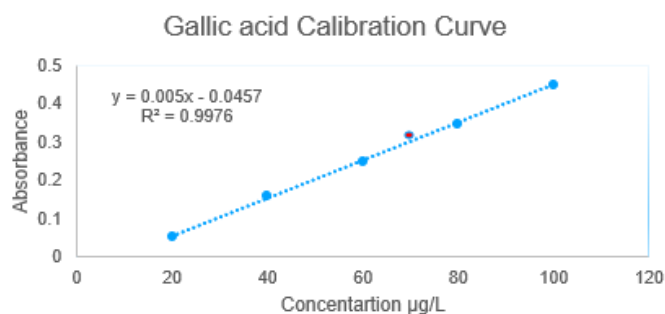
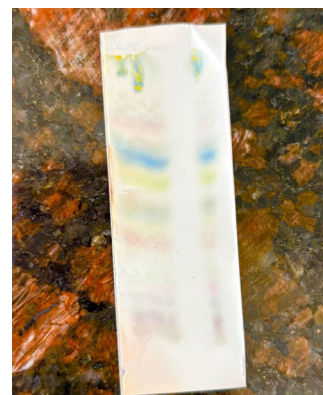


Figure 8: Gallic acid calibration curve.



mesophyll, trichomes, and calcium oxalate crystals are examples of microscopic identification markers. In pharmacognosy, these anatomical characteristics are frequently employed to differentiate authentic plant material from adulterants.^{22,23}

Identification is further strengthened by quantitative microscopy, which provides continuous features such vein morphology and stomatal index. These values are helpful tools for crude drug standardization and comparison with published literature because they are thought to be stable and less impacted by environmental fluctuations.

Total ash (4.5%), loss on drying (6%), among other physicochemical parameters found in this investigation, show good purity and little contamination. Controlled moisture content guarantees stability, whereas low acid-insoluble ash indicates insignificant siliceous materials. Prior standardization investigations have revealed similar results.²⁴

Higher solubility in alcohol than in water was shown by extractive values, indicating the prevalence of rather polar molecules. Based on the present finding the alcoholic extracts include phenolics, Alkaloids, sterols, phenolics, flavonoids, and tannins all of which

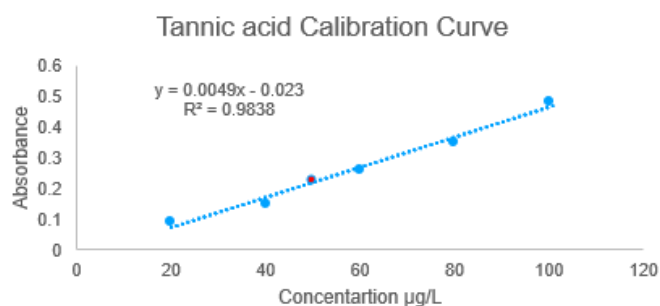


Figure 9: Tannic acid calibration curve.

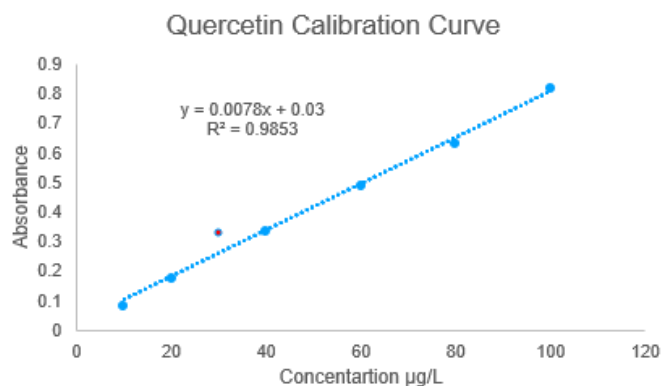


Figure 10: Quercetin calibration curve.



have medicinal potential were detected using phytochemical screening. Because phenolics scavenge free radicals, quantitative assessment of phenolics supports antioxidant potential.²⁵

CONCLUSION

The present study shows a comprehensive Pharmacognostic and phytochemical profile of *Elaeocarpus ganitrus* leaves. It shows an important parameter that are used for identification and assessment of plants microscopic, macroscopic, and physiochemical parameter such as ash value, extractive value, and leaf constant will help in establishing the standard quality for ensuring the identity and authenticity of the crude drug. The TLC and quantative evaluation are useful for the separation, phytochemical characterization, quality control and standardization of herbal drug. Furthermore, this data can be used as a monograph and will serve as a reference for future research on pharmacological research on *Elaeocarpus ganitrus*.

ACKNOWLEDGMENT

I sincerely express my gratitude to principal, teaching and non-teaching staff of Krupanidhi College of Pharmacy, for suggesting the research topic, encouragement for continuous guidance and support during my project work. I also thanks to my parents for their unwavering motivation and support, entire the period of this research work.

ABBREVIATIONS

BSI: Botanical Survey of India; **pH:** Potential of Hydrogen; **R_f:** Retention factor; **SEM:** Standard Error of the Mean; **TLC:** Thin Layer Chromatography; **UV:** Ultraviolet; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Chettri B, Sharma S, Bihani M, Dang R. Pharmacognostic Phytochemical and Physicochemical Evaluation of Rudraksha Leaves. *Indian J of Pharmaceutical Education and Research*. Krupapharmacon 2026;168-77.