

PHARMACOGNOSTIC STANDARDIZATION AND PHYTOCHEMICAL EVALUATION OF THE ETHANOLIC LEAF EXTRACT OF ELEPHANTOPUS SCABER LINN.

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Abstract

Background: *Elephantopus scaber* Linn. is a medicinal plant widely recognized for its therapeutic potential due to the presence of diverse bioactive phytoconstituents. This study aimed to evaluate the physicochemical properties, FTIR profile, and preliminary phytochemical composition of its ethanolic leaf extract.

Methods: The leaves were extracted using 95% ethanol by Soxhlet extraction. The extract was evaluated for percentage yield, physicochemical parameters, solubility, FTIR analysis, and qualitative phytochemical screening using standard methods.

Results: The ethanolic extract showed an extraction yield of 18.5%. The extract exhibited acceptable physicochemical characteristics, including moisture content ($6.5 \pm 0.2\%$), pH (6.8 ± 0.1), and ash values within standard limits. FTIR analysis confirmed the presence of hydroxyl, carbonyl, aromatic, and ether functional groups. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, steroids, terpenoids, carbohydrates, proteins, and amino acids.

Conclusion: The ethanolic leaf extract of *Elephantopus scaber* demonstrated satisfactory physicochemical quality and a rich phytochemical profile, supporting its standardization and potential application in future pharmacological and pharmaceutical studies.

Keywords: *Elephantopus scaber*; Physicochemical evaluation; Phytochemical screening; FTIR; Soxhlet extraction.

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1. Introduction

Medicinal plants have been used for the prevention and treatment of diseases since ancient times and continue to play a fundamental role in healthcare worldwide. According to the World Health Organization (WHO), nearly 80% of the population in developing countries depends on traditional herbal medicines for primary healthcare (1,2). The increasing prevalence of chronic diseases, antimicrobial resistance, and adverse effects associated with synthetic drugs has renewed scientific interest in medicinal plants as potential sources of novel therapeutic agents. Plant-derived natural products possess remarkable structural diversity and are recognized as an important reservoir of bioactive molecules for modern drug discovery. Numerous clinically important drugs have originated from medicinal plants, highlighting their significance in pharmaceutical research and development (3,4).

The pharmacological potential of medicinal plants is largely attributed to the presence of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, steroids, glycosides, and saponins. These phytochemicals exhibit a broad spectrum of biological activities such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, anticancer, immunomodulatory,

and neuroprotective properties (5,6). Consequently, systematic phytochemical investigation and physicochemical standardization of medicinal plants have become indispensable components of herbal drug research to ensure quality, safety, efficacy, and reproducibility (7,8).

Elephantopus scaber Linn. (Family: Asteraceae), commonly known as Elephant's Foot, is a perennial medicinal herb widely distributed throughout tropical and subtropical regions of Asia, Africa, Australia, and South America. The plant grows abundantly in India, Sri Lanka, China, Malaysia, Thailand, Indonesia, Vietnam, and the Philippines, particularly in grasslands, forest margins, roadsides, and other moist habitats. Morphologically, the plant is characterized by a basal rosette of oblong leaves, erect branched stems, and compact purple inflorescences (9,10).

Traditionally, *Elephantopus scaber* has been employed in Ayurveda, Siddha, Traditional Chinese Medicine, and various indigenous healthcare systems for the treatment of fever, inflammation, dysentery, diarrhea, bronchitis, urinary disorders, skin diseases, wounds, liver ailments, edema, and rheumatic conditions. Decoctions and extracts prepared from its

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leaves, roots, or the entire plant have also been used as diuretics, febrifuges, emollients, and tonics (9-11). Phytochemical investigations have demonstrated that *E. scaber* is rich in sesquiterpene lactones, flavonoids, phenolic acids, triterpenoids, phytosterols, alkaloids, glycosides, tannins, saponins, lignans, and essential oils. Among these constituents, sesquiterpene lactones such as deoxyelephantopin, isodeoxyelephantopin, scabertopin, and related derivatives are considered the principal bioactive molecules responsible for many of the plant's pharmacological activities. These compounds possess potent antioxidant, anti-inflammatory, antiproliferative, immunomodulatory, and anticancer properties (9,12,13).

Numerous pharmacological studies have scientifically validated the traditional medicinal uses of *E. scaber*. Experimental investigations have demonstrated that extracts and isolated constituents possess antioxidant, antimicrobial, hepatoprotective, anti-inflammatory, antidiabetic, wound healing, analgesic, antiasthmatic, immunomodulatory, neuroprotective, and anticancer activities. Several studies have further shown that deoxyelephantopin suppresses inflammatory mediators and inhibits cancer cell proliferation through modulation of multiple signaling pathways, including NF- κ B, PI3K/Akt/mTOR, MAPK, STAT3, and NLRP3 pathways (9,12-15).

Despite extensive pharmacological investigations, the therapeutic efficacy of herbal preparations depends largely on the quality and chemical composition of the plant extract. Variations in geographical origin, environmental conditions, harvesting time, post-harvest processing, extraction solvent, and extraction technique may significantly influence the concentration of active phytochemicals. Therefore, comprehensive physicochemical evaluation, qualitative phytochemical screening, and spectroscopic characterization are essential to establish quality control parameters and ensure batch-to-batch consistency of herbal extracts (7,8,16).

Physicochemical parameters such as organoleptic characteristics, moisture content, pH, ash values, extractive values, and solubility profile are widely accepted quality control indices for medicinal plant extracts. These parameters provide valuable information regarding purity, stability, and storage characteristics. In addition, Fourier Transform Infrared (FTIR) spectroscopy has become an important analytical tool for rapid characterization of herbal extracts by identifying functional groups associated with major phytoconstituents (16,17).

Although several pharmacological studies have reported the therapeutic potential of *Elephantopus scaber*, systematic data describing its physicochemical characteristics, FTIR profile, and preliminary phytochemical composition remain comparatively limited, particularly for ethanolic leaf

extracts intended for pharmaceutical standardization (9,12,13).

Therefore, the present study was designed to prepare the ethanolic leaf extract of *Elephantopus scaber* using the Soxhlet extraction method and to evaluate its physicochemical properties, percentage yield, ash values, solubility profile, FTIR characteristics, and preliminary phytochemical composition. The findings of this study are expected to contribute to the pharmacognostic standardization of *Elephantopus scaber* and provide scientific evidence supporting its future pharmaceutical and medicinal applications.

2. Aim of the Study

The present study aimed to prepare the ethanolic leaf extract of *Elephantopus scaber* Linn. using the Soxhlet extraction method and to investigate its physicochemical characteristics, FTIR spectral profile, and preliminary phytochemical constituents to establish baseline quality control parameters for its future pharmaceutical and pharmacological applications.

3. Methods

3.1 Collection and Authentication of *Elephantopus scaber* Leaves

Fresh leaves of *Elephantopus scaber* were collected during the appropriate growing season from the local region of [Location, State, and Country]. The collected plant material was thoroughly washed with running tap water to remove adhering soil, dust, and other foreign matter, followed by rinsing with distilled water. The cleaned leaves were shade-dried at room temperature (25-30°C) for 10-14 days until a constant weight was attained. The dried leaves were then pulverized using a mechanical grinder to obtain a coarse powder, passed through a 40-mesh sieve, and stored in airtight containers protected from light and moisture until further use.

3.2 Preparation of Ethanolic Extract

The shade-dried and coarsely powdered leaves of *Elephantopus scaber* were used for extraction. Approximately 500 g of the powdered material was packed into a Soxhlet apparatus fitted with a round-bottom flask containing 95% ethanol as the extraction solvent. The extraction was continued for 8-10 hours until the siphon tube solution became colorless, indicating complete extraction of phytoconstituents.

The ethanolic extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator at 40-45°C. The concentrated extract was further dried in a hot-air oven maintained below 40°C to remove residual solvent. The dried extract was weighed, transferred into airtight amber-colored containers, and stored at 4°C until further analysis.

3.3 Extraction Method (Soxhlet Extraction)

Soxhlet extraction was employed to obtain the ethanolic extract of *Elephantopus scaber* leaves. The powdered plant material was placed in a cellulose

extraction thimble and extracted continuously with ethanol using a Soxhlet extractor. Fresh solvent repeatedly percolated through the powdered drug, facilitating exhaustive extraction of both polar and moderately polar phytoconstituents. The extraction process was continued until the solvent in the siphon tube appeared nearly colorless, indicating complete extraction. The solvent was then removed under reduced pressure to obtain a concentrated extract suitable for physicochemical and phytochemical analyses.

3.4 Determination of Percentage Yield

The percentage yield of the ethanolic extract was calculated after complete removal of the extraction solvent. The dried extract was accurately weighed using a digital analytical balance, and the percentage yield was calculated using the following equation:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of powdered plant material (g)}} \times 100$$

The extraction yield was expressed as the mean percentage of dried extract obtained from the initial weight of powdered leaves.

3.5 Physicochemical Evaluation of Ethanolic Extract

The ethanolic extract of *Elephantopus scaber* leaves was subjected to physicochemical evaluation according to standard procedures recommended by the World Health Organization (WHO) and the Ayurvedic Pharmacopoeia of India. The evaluation included determination of organoleptic characteristics, moisture content, pH, ash values, extractive value, and solubility profile to establish the quality and purity of the extract.

3.5.1 Organoleptic Evaluation

The organoleptic characteristics of the ethanolic extract were evaluated by visual observation and sensory examination. Parameters including color, odor, appearance, texture, and taste were recorded using standard pharmacognostic procedures.

3.5.2 Determination of Moisture Content (Loss on Drying)

Approximately 2 g of the ethanolic extract was accurately weighed in a previously dried and tared porcelain crucible. The sample was dried in a hot-air oven maintained at $105 \pm 2^\circ\text{C}$ until a constant weight was obtained. After cooling in a desiccator, the sample was reweighed, and the moisture content was calculated as the percentage loss in weight.

3.5.3 Determination of pH

A 1% (w/v) solution of the ethanolic extract was prepared using distilled water. The pH of the solution was measured at room temperature using a calibrated digital pH meter. Measurements were performed in triplicate, and the average value was recorded.

3.5.4 Determination of Ash Values

Ash values were determined to assess the inorganic content and purity of the extract according to standard pharmacopoeial procedures.

Total Ash

Approximately 2 g of the dried extract was accurately weighed into a previously ignited and tarred silica crucible. The sample was incinerated gradually by increasing the temperature to $550\text{--}600^\circ\text{C}$ in a muffle furnace until a carbon-free white ash was obtained. After cooling in a desiccator, the crucible was weighed, and the percentage of total ash was calculated.

Acid-Insoluble Ash

The total ash obtained was boiled with 25 mL of 2 M hydrochloric acid for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed thoroughly with hot distilled water, ignited in a silica crucible at 550°C , cooled in a desiccator, and weighed. The acid-insoluble ash was calculated as a percentage of the air-dried extract.

Water-Soluble Ash

The total ash was boiled with 25 mL of distilled water for 5 minutes. The insoluble residue was filtered through ashless filter paper, washed with hot water, ignited, cooled, and weighed. The difference between total ash and the weight of insoluble residue represented the water-soluble ash.

3.5.5 Determination of Extractive Value

Approximately 5 g of the dried extract was macerated with 100 mL of ethanol in a closed flask for 24 hours, with frequent shaking during the first 6 hours. The mixture was filtered, and 25 mL of the filtrate was evaporated to dryness in a tared evaporating dish. The residue was dried at 105°C to constant weight, and the extractive value was calculated as a percentage of the air-dried extract.

3.5.6 Solubility Studies

The solubility of the ethanolic extract was evaluated qualitatively in different solvents, including distilled water, ethanol, methanol, chloroform, acetone, ethyl acetate, and dimethyl sulfoxide (DMSO). Approximately 100 mg of the extract was added separately to 10 mL of each solvent and shaken for 30 minutes at room temperature. The degree of solubility was observed visually and categorized as freely soluble, soluble, sparingly soluble, slightly soluble, or insoluble based on the extent of dissolution.

3.6 Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the characteristic functional groups present in the ethanolic extract of *Elephantopus scaber*. The dried extract was thoroughly mixed with spectroscopic-grade potassium bromide (KBr), and the mixture was compressed into a transparent pellet using a hydraulic press. The FTIR spectrum was recorded using an FTIR spectrophotometer over the spectral range of

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4000-400 cm⁻¹. The absorption peaks obtained were analyzed and assigned to corresponding functional groups based on standard infrared correlation charts.

3.7 Preliminary Phytochemical Screening

The ethanolic extract of *Elephantopus scaber* leaves was subjected to qualitative phytochemical screening using standard procedures to detect the presence of major classes of phytoconstituents.

3.7.1 Test for Alkaloids

Approximately 2 mL of the ethanolic extract was acidified with a few drops of dilute hydrochloric acid and filtered. The filtrate was divided into three portions.

- **Mayer's Test:** A few drops of Mayer's reagent were added. Formation of a cream-colored precipitate indicated the presence of alkaloids.
- **Wagner's Test:** A few drops of Wagner's reagent were added. Formation of a reddish-brown precipitate indicated the presence of alkaloids.
- **Dragendorff's Test:** A few drops of Dragendorff's reagent were added. Formation of an orange or reddish-brown precipitate indicated the presence of alkaloids.

3.7.2 Test for Flavonoids

- **Alkaline Reagent Test:** Two milliliters of extract were treated with a few drops of 10% sodium hydroxide solution. The appearance of an intense yellow color that disappeared upon addition of dilute hydrochloric acid indicated the presence of flavonoids.
- **Shinoda Test:** To 2 mL of extract, a small piece of magnesium ribbon and a few drops of concentrated hydrochloric acid were added. Development of a pink or red color indicated the presence of flavonoids.

3.7.3 Test for Phenolic Compounds

Ferric Chloride Test: Two milliliters of extract were treated with 2-3 drops of 5% ferric chloride solution. Formation of a blue, green, or violet coloration indicated the presence of phenolic compounds.

3.7.4 Test for Tannins

Gelatin Test: Two milliliters of extract were mixed with 1% gelatin solution containing sodium chloride. Formation of a white precipitate indicated the presence of tannins.

3.7.5 Test for Saponins

Foam Test: Two milliliters of extract were diluted with 5 mL of distilled water and shaken vigorously for 2 minutes. The formation of a stable froth persisting for at least 10 minutes indicated the presence of saponins.

3.7.6 Test for Glycosides

Keller-Killiani Test: Two milliliters of extract were mixed with 2 mL of glacial acetic acid containing one drop of ferric chloride solution, followed by careful addition of concentrated sulfuric acid along the side

of the test tube. Formation of a brown ring at the interface indicated the presence of cardiac glycosides.

3.7.7 Test for Terpenoids

Salkowski Test: Two milliliters of extract were mixed with 2 mL of chloroform, followed by careful addition of concentrated sulfuric acid. Formation of a reddish-brown coloration at the interface indicated the presence of terpenoids.

3.7.8 Test for Steroids

Liebermann-Burchard Test: Two milliliters of extract were mixed with 2 mL of chloroform, followed by the addition of acetic anhydride and a few drops of concentrated sulfuric acid. Development of a bluish-green color indicated the presence of steroids.

3.7.9 Test for Carbohydrates

- **Molisch's Test:** Two milliliters of extract were mixed with two drops of Molisch's reagent, followed by careful addition of concentrated sulfuric acid. Formation of a violet ring at the junction of the two layers indicated the presence of carbohydrates.
- **Benedict's Test:** Two milliliters of Benedict's reagent were mixed with 1 mL of extract and heated in a boiling water bath for 5 minutes. Formation of a green, yellow, or brick-red precipitate indicated the presence of reducing sugars.

3.7.10 Test for Proteins and Amino Acids

- **Biuret Test:** Two milliliters of extract were treated with 1 mL of 10% sodium hydroxide solution followed by a few drops of 1% copper sulfate solution. Development of a violet color indicated the presence of proteins.
- **Ninhydrin Test:** Two milliliters of extract were mixed with 2-3 drops of ninhydrin reagent and heated in a boiling water bath for 2-3 minutes. Development of a purple or blue color indicated the presence of amino acids.

4. RESULTS

4.1 Preparation of Ethanolic Extract

The powdered leaves of *Elephantopus scaber* were extracted successfully using 95% ethanol by the Soxhlet extraction method. A dark greenish-brown semisolid extract was obtained after evaporation of the solvent under reduced pressure. From 100 g of dried powdered leaves, 18.5 g of ethanolic extract was recovered, corresponding to an extraction yield of 18.5%. The obtained extract was used for subsequent physicochemical evaluation, FTIR analysis, and preliminary phytochemical screening.

Table 4.1. Preparation of ethanolic extract of *Elephantopus scaber* leaves

Plant	Solve	Powd	Extract	Extracti
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Material	nt	er Used (g)	Obtain ed (g)	on Yield (%)
<i>Elephantopus scaber</i> leaves	95% Ethan ol	100	18.5	18.5

4.2 Physicochemical Evaluation of Ethanolic Extract

4.2.1 Organoleptic Evaluation

The ethanolic extract of *Elephantopus scaber* was evaluated for its physical characteristics. The extract was dark greenish-brown in color with a characteristic herbal odor, slightly bitter taste, and a semi-solid sticky consistency (Table 4.2).

Table 4.2. Organoleptic characteristics of the ethanolic extract

Parameter	Observation
Colour	Dark greenish-brown
Odour	Characteristic herbal aroma
Taste	Slightly bitter
Texture	Semi-solid, sticky

4.2.2 Moisture Content

The moisture content of the ethanolic extract was determined by the loss-on-drying method. The extract showed a moisture content of $6.5 \pm 0.2\%$, indicating adequate drying and suitability for storage (Table 4.3).

Table 4.3. Moisture content of the ethanolic extract

Parameter	Observation (Mean $\pm$ SD)
Moisture content (%)	6.5 ± 0.2

4.2.3 Determination of pH

The pH of the 1% ethanolic extract solution was 6.8 ± 0.1 , indicating that the extract possesses a near-neutral nature (Table 4.4).

Table 4.4. pH of the ethanolic extract

Parameter	Observation (Mean $\pm$ SD)
pH	6.8 ± 0.1

4.2.4 Determination of Ash Values

The ash values of the ethanolic extract were determined to evaluate its inorganic residue content. The total ash, acid-insoluble ash, and water-soluble ash values were found to be $7.25 \pm 0.12\%$, $1.45 \pm 0.05\%$, and $5.80 \pm 0.08\%$, respectively (Table 4.5).

Table 4.5. Ash values of the ethanolic extract

Parameter	Observation (Mean $\pm$ SD)
Total ash (%)	7.25 ± 0.12
Acid-insoluble ash (%)	1.45 ± 0.05
Water-soluble ash (%)	5.80 ± 0.08

4.2.5 Extractive Value

The ethanolic extract exhibited an extractive value of $18.65 \pm 0.15\%$, indicating efficient extraction of ethanol-soluble constituents from the plant material (Table 4.6).

Table 4.6. Extractive value of the ethanolic extract

Solvent	Extractive Value (%) (Mean $\pm$ SD)
Ethanol	18.65 ± 0.15

4.2.6 Solubility Studies

The ethanolic extract showed different solubility characteristics in various solvents. It was soluble in ethanol and methanol, sparingly soluble in distilled water and acetone, and insoluble in chloroform (Table 4.7).

Table 4.7. Solubility profile of the ethanolic extract

Solvent	Solubility
Distilled water	Sparingly soluble
Ethanol	Soluble
Methanol	Soluble
Acetone	Sparingly soluble
Chloroform	Insoluble

4.3 Fourier Transform Infrared (FTIR) Analysis

The FTIR spectrum of the ethanolic extract revealed characteristic absorption peaks corresponding to hydroxyl, aliphatic, carbonyl, aromatic, and ether functional groups. These absorption bands indicate the presence of several phytochemical classes, including phenolics, flavonoids, terpenoids, and glycosides (Table 4.8).

Table 4.8. FTIR spectral analysis of the ethanolic extract

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FTIR Peak (cm ⁻¹)	Functional Group	Probable Phytoconstituent
3400-3200	O-H stretching	Phenols, alcohols
2925-2850	C-H stretching	Alkanes, terpenoids
1735	C=O stretching	Esters, aldehydes, ketones
1630-1600	C=C stretching	Aromatic compounds
1380-1370	C-H bending	Methyl groups
1240-1220	C-O stretching	Phenolic ethers
1050-1030	C-O-C stretching	Glycosides
820-750	Aromatic C-H bending	Aromatic rings

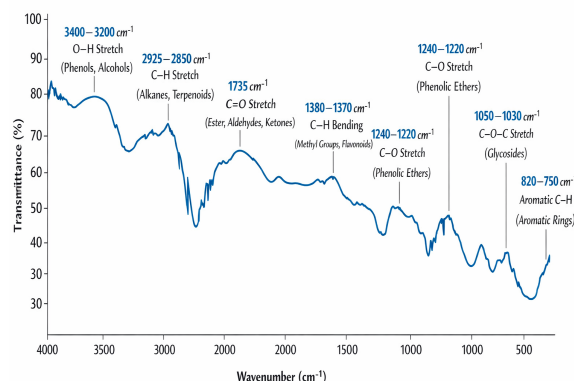


Figure 1. Fourier Transform Infrared (FTIR) spectrum of the ethanolic leaf extract of *Elephantopus scaber* Linn. indicating the characteristic functional groups present in the extract

4.4 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extract demonstrated the presence of several bioactive secondary metabolites. Positive results were observed for alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, steroids, terpenoids, carbohydrates, proteins, amino acids, anthraquinones, coumarins, and fixed oils (Table 4.9).

Table 4.9. Preliminary phytochemical screening of the ethanolic extract

S. No.	Phytochemical Test	Result
1	Liebermann-Burchard test (Steroids)	+
2	Salkowski test (Terpenoids)	+
3	Foam test (Saponins)	+
4	Mayer's test (Alkaloids)	+
5	Dragendorff's test (Alkaloids)	+
6	Wagner's test (Alkaloids)	+
7	Keller-Killiani test (Glycosides)	+
8	Gelatin test (Tannins)	+
9	Ferric chloride test (Phenolics)	+
10	Shinoda test (Flavonoids)	+
11	Alkaline reagent test (Flavonoids)	+
12	Biuret test (Proteins)	+
13	Ninhydrin test (Amino acids)	+
14	Molisch's test (Carbohydrates)	+
15	Fehling's test (Reducing sugars)	+
16	Borntrager's test (Anthraquinones)	+
17	UV fluorescence test (Coumarins)	+
18	Sudan III/IV staining test (Fixed oils and fats)	+
19	Hydrodistillation test (Essential oils)	+

Note: (+) = Present; (–) = Absent.

Conclusion

The present study successfully established the pharmacognostic and physicochemical profile of the ethanolic leaf extract of *Elephantopus scaber*. The Soxhlet extraction method using 95% ethanol produced a satisfactory extraction yield, indicating the effectiveness of ethanol in extracting bioactive constituents from the plant material. The prepared extract exhibited acceptable organoleptic characteristics, low moisture content, near-neutral pH, appropriate ash values, and good extractive value, confirming its quality, purity, and suitability for further pharmacological investigations.

The solubility profile demonstrated that the extract was readily soluble in polar organic solvents, reflecting the predominance of polar and moderately polar phytoconstituents. FTIR analysis further confirmed the presence of characteristic functional groups, including hydroxyl, carbonyl, aromatic, and ether groups, suggesting the occurrence of phenolics, flavonoids, terpenoids, glycosides, and other bioactive compounds. Preliminary phytochemical

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screening supported these findings by revealing the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, steroids, terpenoids, carbohydrates, proteins, amino acids, and other secondary metabolites.

Overall, the findings indicate that the ethanolic extract of *Elephantopus scaber* leaves possesses a rich phytochemical composition and satisfactory physicochemical characteristics, providing a reliable basis for its standardization and quality control. The study also supports the traditional medicinal importance of *Elephantopus scaber* and demonstrates that the prepared extract is suitable for further biological and pharmacological investigations. These results provide a scientific foundation for future studies aimed at isolating active constituents, elucidating their mechanisms of action, and exploring the therapeutic potential of *Elephantopus scaber* in the management of various diseases.

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Conflict of Interest

The authors declare that there are **no conflicts of interest** regarding the publication of this research paper. The authors alone are responsible for the content and writing of this manuscript.

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