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Phytochemical Screening and Antioxidant Effect of *Elephantopus scaber* L. Found in Ranchi

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Abstract

The importance of medicinal plants in drug discovery is attributed to their varied pharmacognostic, phytochemical, and pharmacological characteristics. Comprehensive studies have demonstrated the biological efficacy of phytochemicals from several chemical categories, presenting possible remedies for various diseases. Plant extracts are widely used around the world for their biologically active compounds and offer protection against various diseases due to their Pharmacognostic properties. The present study was conducted to examine the phytochemical constituents, and antioxidant potential of various extracts of *Elephantopus scaber* L. collected from Ranchi. Preliminary Qualitative test was conducted to find out the presence of bioactive compounds followed by some quantitative tests like Total Phenolic Contents and Total Flavonoid Contents in the plant extract. DPPH method was employed for investigating antioxidant activity. HPLC analysis was also conducted to see the dominant bioactive compounds found in various extracts of *E. scaber* L. The results of the qualitative phytochemical analysis showed that Ethanolic, Petroleum ether, chloroformic, Acetone and distilled water extract of *Elecphantopus scaber* consist of alkaloids, terpenoids, anthocyanide, phlobatanin, glycosides, carbohydrates, proteins, phenol, terpenoids, favonoids, tannins, and saponins. The total phenol and favonoid content of *E. scaber* showed that the aceone extract of Leaf of *E. scaber* had a significantly higher total phenolic content (45.07 ± 0.10 mg of GAE/g) and the favonoid content (32.22 ± 0.30 mg of quercetin) than other extracts. The content of total phenolic and favonoids was more in Chloroform extract as compared to other extracts of *Elecphantopus scaber*.

***Key words*:** *Elephantopus scaber*, Bioactive constituents, TPC, TFC, Radical scavenging

Since ancient times, humans have used plants for medicine, highlighting their importance in modern drug discovery. The therapeutic effects of plants come from phytochemicals, which can be identified through screening methods to develop new drugs. With rising resistance to synthetic drugs and fewer new antimicrobials, there's a growing need for affordable plant-based treatments, especially in developing countries where infections cause high mortality [1-2]. Phytochemicals also help combat oxidative stress caused by reactive oxygen species (ROS), which damage biomolecules and contribute to diseases like diabetes, inflammation, and aging [3]. Antioxidants in plants—such as polyphenols and vitamins—neutralize ROS and support the body's defense, which weakens with age. *Elephantopus scaber L.* is a little herbaceous plant that belongs to the family Asteraceae within the order Asterales, classified under the subclass Asteridae. *Elephantopus scaber* L., commonly referred to as Prickly-leaved Elephant's Foot in English, is a terrestrial, scabrescent, aromatic, erect, and stiff perennial herb growing up to 60 cm in height. Its taxonomy and distribution have been widely documented in various floras and herbarium collections [4]. It produces small purplish flowers in compound clusters and a dry, ribbed fruit with a bristly pappus for dispersal, while its robust rootstock and subterranean rhizome allow it to thrive in grasslands and wastelands, surviving dormant periods [5]. The plant's extensive use in traditional medicine is rooted in its rich phytochemical profile, with key compounds like sesquiterpene lactones (e.g., elephantopin) and flavonoids serving as the etiological basis for its anti-inflammatory, antioxidant, and anti-cancer effects. Historically and in modern applications, various parts of the plant are used to address a wide range of ailments, including rheumatism, skin conditions, respiratory and digestive issues, and cardiovascular problems, with ongoing scientific research actively exploring its potential as an anti-diabetic and hepatoprotective agent and as a source of novel therapeutic compounds [6]. It is a significant plant in traditional and folk medicine across tropical regions of the world, including India, Southeast Asia, Africa, and Australia. Its wide range of medicinal uses is supported by the presence of a diverse array of phytochemicals. The main aim of this research study is to analyze the phytochemicals and antimicrobial along with the antioxidant activity of *Elephantopus scaber* L.

Present study involves two primary objectives: first, to perform a qualitative and quantitative analysis of the plant's various phytochemicals (bioactive compounds) such as alkaloids, flavonoids, terpenoids, saponins, phenols, and tannins, using different solvent extracts; and second, to evaluate the plant's antioxidant properties by its ability to neutralize harmful free radicals. This evaluation would typically be conducted through several standard *in vitro* assays, including

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the DPPH Radical Scavenging Assay, which measures the plant's ability to fade the deep violet color of a stable free radical, the FRAP (Ferric Reducing Antioxidant Power) Assay, which quantifies the reduction of ferric to ferrous ions resulting in an intense blue color, and the Total Antioxidant Capacity (TAC) Assay, which measures the reduction of Mo(VI) to Mo(V). The overall goal is to create a detailed profile of the plant's compounds and their antioxidant capacity, providing scientific evidence to support its traditional medicinal use and potentially leading to the discovery of new therapeutic compounds.

MATERIALS AND METHODS

Plant collection

Fresh samples of *Elephantopus scaber* were collected in August-Septembers 2024 from forests of Nagri, Ranchi. The plant sample was preserved on herbarium sheet and was identified by Botanical Survey of India, Kolkata with voucher number CAL0000267132 was given and deposited in BSI herbarium for future reference. The plants were dried in the shade at room temperature 30 °C for 30 days. Plant parts were grounded to fine powder with the assistance of mortar and pestle. 100 g of sample powder was saturated each in 1000mL Ethanol, chloroform, Petroleum ether, Acetone and Distilled water. After 10 days, the extracts were filtered by Whatman filter paper. This process was applied triple time and the extracts were combined and then it was concentrated utilizing a rotary type of evaporator. The obtained extracts were then packaged and stored at in the refrigerator for maintaining temperature.

Qualitative phytochemical screening [7]

1. *Test for Saponin*
2ml of extract was added into the test tube and shaken vigorously to see the presence of Foams.

2. *Test for anthocyanide*
1ml of extract was added into the test tube and 5ml of dil. HCL was added to it.

3. *Test for protein*
1ml of extract was added into the test tube and 5-6 drops of millions reagent was added to the sample.

4. *Test for flavonoid*
1 ml of extract was added into the test tube and was diluted with 2ml of distilled water and 3ml of 10% lead acetate was added into it.

5. *Test for carbohydrate*
1 ml of extract was added into the test tube and 5ml benedict reagent was added to it.

6. *Test for phlobatamin*
1 ml of extract was added into the test tube and few drops of dil HCL was added to it to see the red precipitate.

7. *Test for glycosides*
5 ml of extract was added into the test tube and 2ml of Gallic acetic acid was added then, a drop of ferric chloride was added to the test tube.

8. *Test for phenol*
2 ml of extract was added into the test tube and 5% of Ferric Chloride(0.5ml) solution was added to it.

9. *Test for tanin*
2ml of extract was added into the test tube and 5% of Ferric Chloride(0.5ml) solution was added to it.

10. *Test for steroids*
2ml of extract was added into the test tube and 2ml of chloroform then, 3ml of conc. H_2So_4 by interior walls of test tube was added.

11. *Test for terpenoid*
2ml of extract was added into the test tube and 2ml of chloroform then, 3ml of conc. H_2So_4 by interior walls of test tube was added.

12. *Test for alkaloid*
2ml of extract was added into the test tube and 2ml of Dragendorff's reagent was added to it.

Total phenolic content assay [7]

For the phenolic analysis of *Elephantopus scaber* extracts, 0.5 mL of the various extract was combined with 4.5 mL of distilled water. Then, 0.2 mL of Folin–Ciocalteu reagent and 0.5 mL of saturated Na_2CO_3 solution were added, followed by 4.3 mL of distilled water. The mixture was incubated in the dark at room temperature for one hour. Absorbance was measured at 725 nm, and the results were expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g).

Flavonoid content assay [7]

For flavonoid determination, 0.5 mL of the extract was mixed with 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 415 nm. Flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry sample (mg QE/g).

DPPH free-radical scavenging assay

The antioxidant activity of *Elephantopus scaber* extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free-radical assay, following standard procedures. A 25 mg portion of the dried crude extract was dissolved in respective sample to prepare a 50 mL stock solution, from which test concentrations of 50, 100, and 150 µg/mL were made. Ascorbic acid served as the standard.

For each concentration, 5 mL of the respective extract solution was mixed with 1 mL of 0.001 M DPPH solution in a test tube. A control sample consisted of 5 mL methanol and 1 mL DPPH. All mixtures were incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Each experiment was conducted in triplicate. The percentage of DPPH radical inhibition was calculated using the standard formula:

$$\% \text{ Scavanged DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Qualitative phytochemical screening

Phytochemical screening of *Elephantopus scaber* L. extracts (ethanolic, acetone, chloroform, petroleum ether, and aqueous) revealed the presence of proteins, carbohydrates,

glycosides, alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, and phlobatannins. These compounds exhibit various bioactivities, including antioxidant, antimicrobial, antiviral, antitumor, CNS, and immune-modulatory effects.

Table 1 Phytochemical screening of various extracts of *Elephantopus scaber* L. (+ indicates presence)

S. No.	Phytochemical constituents	Ethanol extract	Acetone extract	Chloroform extract	Petroleum ether extract	Distilled water extract
1.	Alkaloid	+	+	+	+	+
2.	saponin	+	+	+	+	+
3.	Anthocyanide	+	+	+	+	+
4.	Protein	+	+	+	+	+
5.	Flavonoid	+	+	+	+	+
6.	Carbohydrate	+	+	+	+	+
7.	Phlobatamin	+	+	+	+	+
8.	Glycosides	+	+	+	+	+
9.	Phenol	+	+	+	+	+
10.	Tanin	+	+	+	+	+
11.	Terpenoid	+	+	+	+	+

The data depicted in (Table 1), revealed that the phytochemical screening of *Elephantopus scaber* L., presents the highly improbable finding that all eleven tested phytochemicals including alkaloids, saponins, anthocyanides, proteins, flavonoids, carbohydrates, phlobatamins, glycosides, phenols, tannins, and terpenoids are present in every single one of the five different solvent extracts (ethanol, acetone, chloroform, petroleum ether, and distilled water). This uniform distribution across extracts of widely varying polarities, from highly polar water to non-polar petroleum ether, is scientifically anomalous, as different classes of compounds have distinct solubility characteristics [8]. A realistic phytochemical analysis would show a selective presence of compounds; for instance, polar compounds like glycosides would be more soluble in polar solvents, while non-polar terpenoids would be preferentially extracted by non-polar solvents. The consistent positive results (+), therefore, strongly suggest potential methodological flaws, such as cross-contamination or errors in the screening assays, and do not represent a biologically plausible profile for the plant's phytochemical composition [9].

Total phenolic and flavonoid content

Phenolic and flavonoid contents in natural products are key indicators for quantitatively assessing extracts and determining their biological potency, as they play a vital role in various physiological processes. In this study, the quantity of total phenolic and favonoid content of ethanol, chloroform, petroleum ether, acetone and distil water extract of *Elephantopus scaber* is determined as shown in (Table 2). *Elephantopus scaber* showed that the acetone extract of leaf of *Elephantopus scaber* had a significantly higher total phenolic content (45.07 ± 0.10 mg of GAE/g) and the favonoid content (32.22 ± 0.30 mg of quercetin) than other extracts [10].

Table 2 Total phenolics content, total favonoids content of the various extract of *Elephantopus scaber* L.

Extracts	Plant sample	Phenol content (mg GAE/g extract)	Flavonoid content (mg quercetin/g extract)
Acetone extract	Leaf	34 ± 0.30	42 ± 0.26
	Root	36 ± 0.54	38 ± 0.76
	Inflorescence	33 ± 0.30	29 ± 0.67
Ethanol extract	Leaf	30 ± 0.45	34 ± 0.66
	Root	29 ± 0.54	23 ± 0.54
	Inflorescence	28 ± 0.65	29 ± 0.87
Chloroform extract	Leaf	32 ± 0.07	32 ± 0.07
	Root	31 ± 0.44	31 ± 0.44
	Inflorescence	27 ± 0.76	27 ± 0.76
Petroleum ether extract	Leaf	34 ± 0.66	34 ± 0.66
	Root	23 ± 0.54	23 ± 0.54
	Inflorescence	29 ± 0.87	29 ± 0.87
Distilled water extract	Leaf	14 ± 0.22	14 ± 0.22
	Root	13 ± 0.22	13 ± 0.22
	Inflorescence	12 ± 0.12	12 ± 0.12

The qualitative phytochemical screening of various solvent extracts of *Elephantopus scaber L.* revealed the presence of a wide array of secondary metabolites, including proteins, carbohydrates, glycosides, alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, and phlobatannins, which are responsible for its diverse medicinal properties [11]. However, the observation that all eleven tested phytochemicals were uniformly present across all five solvent extracts (ethanol, acetone, chloroform, petroleum ether, and distilled water) is scientifically anomalous and suggests potential methodological inconsistencies that warrant further investigation [12]. The subsequent quantitative analysis of total phenolic and flavonoid content indicated that the acetone extracts generally exhibited the highest concentrations, with the acetone root extract having the most phenolic compounds and the acetone leaf extract having the most flavonoids [13]. This high concentration of antioxidant-related compounds in the acetone extracts correlated with the plant's antioxidant potential. While the initial interpretation of the antioxidant activity data in the original text was flawed, the results of the DPPH radical scavenging assay show that the chloroform leaf extract had the highest potency, as indicated by its lowest IC50 value (1.49).

This finding confirms that *Elephantopus scaber* possesses significant antioxidant properties, which are likely due to its rich and varied phytochemical composition [14]. This suggests that further research should focus on isolating and characterizing the specific compounds in the most potent extracts to better understand their therapeutic potential.

Antioxidant activity

The results of the antioxidant activity of Pet Ether, Chloroform and Acetone leaves and root extracts of *Elephantopus scaber* are shown in (Table 3). The results revealed that root extracts in Petroleum ether as well as leaves extracts in acetone shows High IC50 value and % radical scavenging activity (RSA).

The (Table 3) details the DPPH radical scavenging activity of various extracts of *Elephantopus scaber L.*, highlighting their antioxidant potential. The activity is measured at three different concentrations (25 µL, 50 µL, and 75 µL) for extracts from the leaves and roots using petroleum ether, chloroform, and acetone as solvents [15]. The data shows a clear dose-dependent increase in scavenging activity for all samples; as the concentration of the extract increases, so does the percentage of radical scavenging. The chloroform leaf extract exhibits the highest scavenging activity at 75 µL, with 91.2%, closely followed by the petroleum ether root extract (91%) and the acetone leaf extract (90.6%) at the same concentration. In contrast, the petroleum ether leaf extract showed the lowest scavenging activity across all concentrations, with its highest value being 81.4% at 75 µL. This demonstrates that the specific solvent used for extraction and the plant part significantly influence the antioxidant capacity of the resulting extract [16-18].

Table 3 % radical scavenging activity for various extract of *Elephantopus scaber* L.

Sample	Conc.	UV absorbance at 517nm	Antioxidant: DPPH scavenging activity (%)	Percentage (%)
Elephantopus scaber leaves Pet Ether extract	25µL	0.479	1.164 - 0.479 ÷ 1.164 × 100	58
	50µL	0.338	1.164 - 0.338 ÷ 1.164 × 100	70
	75µL	0.216	1.164 - 0.216 ÷ 1.164 × 100	81.4
Elephantopus scaber leaves Acetone extract	25µL	0.199	1.164 - 0.199 ÷ 1.164 × 100	82.9
	50µL	0.138	1.164 - 0.138 ÷ 1.164 × 100	88
	75µL	0.109	1.164 - 0.109 ÷ 1.164 × 100	90.6
Elephantopus scaber leaves Chloroform extract	25µL	0.204	1.164 - 0.204 ÷ 1.164 × 100	82.5
	50µL	0.130	1.164 - 0.130 ÷ 1.164 × 100	88.8
	75µL	0.102	1.164 - 0.102 ÷ 1.164 × 100	91.2
Elephantopus scaber roots pet ether extract	25µL	0.202	1.164 - 0.202 ÷ 1.164 × 100	82.6
	50µL	0.135	1.164 - 0.135 ÷ 1.164 × 100	88.4
	75µL	0.104	1.164 - 0.104 ÷ 1.164 × 100	91

Her, the IC50 for leaves extract of *Elephantopus scaber* in petroleum ether is 1.61 whereases the IC 50 for leaves extract of *Elephantopus scaber* in chloroform and acetone extract is 1.49 and 1.64 respectively. IC50 for root extract of *Elephantopus scaber* in petroleum ether is 1.63. From the above analysis it shows the root extracts in Petroleum ether of *Elephantopus scaber* and leaves extract in acetone exhibits high IC50 value and high % RSA than that of other samples [19].

CONCLUSION

The phytochemical investigation of *Elephantopus scaber* L. extracts revealed the presence of a wide range of secondary metabolites, including alkaloids, saponins, flavonoids, terpenoids, and phenols. These findings align with the plant's traditional medicinal use and suggest a potential for various bioactivities, such as antioxidant, antimicrobial, and immune-modulatory effects. A notable observation from the qualitative screening was the apparently uniform presence of all tested phytochemicals across five extracts of varying polarity (ethanol, acetone, chloroform, petroleum ether, and distilled water). This result is scientifically improbable, as different compounds exhibit distinct solubility characteristics. This finding suggests a need for a more detailed and rigorous analysis to accurately profile the plant's chemical composition. Further quantitative analysis indicated that the acetone extract of *E. scaber* leaves possessed a particularly high content of both total phenolic and total flavonoid compounds. The presence of these compounds is often directly correlated with antioxidant potential. Evaluation of the antioxidant activity using the DPPH radical scavenging assay provided quantitative evidence of the plant's free radical scavenging ability. The results showed that the chloroform leaf extract demonstrated the lowest IC50 value (1.49), indicating it possessed the highest antioxidant potency among the tested samples. It is important to note that a lower IC50 value signifies a more potent antioxidant activity, as it requires a smaller concentration of the extract to achieve 50% radical inhibition. Overall, the study confirms that *E. scaber* contains a variety of bioactive compounds and exhibits significant antioxidant properties, warranting further research to isolate and characterize the specific compounds responsible for these effects.

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