



Phytochemical Screening and Quantitative Estimation of Phytoconstituents of Some Bioactive Ethanolic Extracts

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Abstract

The present study investigates the phytochemical and quantitative composition of bioactive ethanolic extracts from five medicinal plants traditionally used in gastrointestinal disorders: *Plumbago zeylanica* (roots), *Moringa oleifera* (leaves), *Cassia tora* (leaves), *Amaranthus tricolor* (leaves), and *Elephantopus scaber* (whole plant). The primary aim was to evaluate and compare the total phenolic content (TPC), total flavonoid content (TFC), tannin concentration, and saponin content to identify the most potent extract(s) with antioxidant and therapeutic relevance. All plant materials were subjected to ethanol extraction using a maceration technique followed by qualitative phytochemical screening to detect major secondary metabolites including alkaloids, glycosides, terpenoids, flavonoids, tannins, and saponins. Quantitative estimations were carried out using standard spectrophotometric protocols. Among the evaluated samples, *E. scaber* exhibited the highest TPC (115.89 ± 2.84 mg GAE/g) and TFC (89.44 ± 2.01 mg QE/g), suggesting it as a rich source of polyphenolic and flavonoid compounds. Tannin content was also highest in *E. scaber* (47.91 ± 1.65 mg TAE/g), while saponin content peaked at $3.47 \pm 0.14\%$. These phytoconstituents are known for their cytoprotective, anti-inflammatory, and antioxidant properties, which likely contribute to the antiulcer potential observed in subsequent in vivo studies. The findings of this investigation not only validate traditional claims but also provide a scientific rationale for further pharmacological and formulation development. In conclusion, the ethanolic extracts especially that of *Elephantopus scaber* exhibit significant phytochemical richness and hold promise for future development as herbal antiulcer agents or antioxidant supplements.

Keywords: Phytochemical, Screening, Quantitative, Estimation, Bioactive, Ethanolic Extracts.

1. Introduction

Medicinal plants have been an integral part of traditional healthcare systems worldwide, providing a rich source of bioactive compounds with therapeutic potential. The rising interest in natural products is driven by their relative safety, affordability, and historical usage in treating a variety of ailments. Among these, plant-derived polyphenols, flavonoids, tannins, and saponins are known for their significant pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and gastroprotective effects. The evaluation of

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phytoconstituents in medicinal plants is crucial for validating their ethnomedicinal uses and exploring their potential in drug development.¹

Phytochemical screening and quantitative estimation of key plant secondary metabolites not only assist in understanding their therapeutic potential but also guide the standardization and quality control of herbal formulations. Ethanolic extraction is widely preferred due to its efficiency in extracting a broad range of phytoconstituents, particularly polar and semi-polar compounds.²

The present study aims to investigate the phytochemical profile and quantify major phytoconstituents in the ethanolic extracts of five traditionally used medicinal plants: *Plumbago zeylanica* (roots), *Moringa oleifera* (leaves), *Cassia tora* (leaves), *Amaranthus tricolor* (leaves), and *Elephantopus scaber* (whole plant). These plants have been reported in various folk medicine systems for their roles in digestive health, particularly in ulcer management. Through preliminary phytochemical screening and quantitative estimations of total phenolic content (TPC), total flavonoid content (TFC), tannin content, and saponin content, this study endeavors to establish a comparative phytochemical profile of these plant extracts. The findings may contribute to the identification of potent antioxidant-rich botanical resources, thereby facilitating their potential therapeutic applications and formulation into effective natural remedies.

2. MATERIALS AND METHODS

2.1. Chemicals

All the chemicals and reagents used in the present study were of analytical grade and procured from reputed commercial sources. Gallic acid, quercetin, tannic acid, and saponin standard were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and Sigma-Aldrich (St. Louis, MO, USA). Ethanol (95%) used for plant extraction was of analytical grade and purchased from Merck India Pvt. Ltd. (Mumbai, India). Folin-Ciocalteu reagent, aluminium chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), ferric chloride ($FeCl_3$), vanillin, sulfuric acid (H_2SO_4), and other reagents for colorimetric estimation were also sourced from Merck and HiMedia. Distilled water was used throughout all procedures.

2.2. Instrumentations

All experimental procedures involved the use of calibrated and well-maintained instruments to ensure precision, reproducibility, and accuracy in the phytochemical screening and quantitative estimations. A UV-Visible Spectrophotometer (Shimadzu UV-1800, Japan) was used for all absorbance-based colorimetric estimations at respective wavelengths. A digital analytical balance (Sartorius, Germany) with a sensitivity of ± 0.1 mg was used for precise weighing of plant extracts and standards. Plant drying and powdering were performed using a hot air oven (Remi Instruments, India) set at 40–45°C and a mechanical grinder (Philips India Ltd.), respectively. Soxhlet extraction was carried out using a standard glass extraction apparatus with 95% ethanol as the solvent, followed by concentration using a rotary evaporator (Heidolph, Germany) under reduced pressure. pH of the solutions and reagents was measured using a digital pH meter (Eutech Instruments, Singapore).

2.3. Plant

2.3.1. Collection of Plant Materials

Fresh plant materials of *Plumbago zeylanica* (roots), *Moringa oleifera* (leaves), *Cassia tora* (leaves), *Amaranthus tricolor* (leaves), and *Elephantopus scaber* (whole plant) were meticulously collected from the local vegetation zones of [District/State, India] during the month of [Month and Year], ensuring botanical integrity and appropriate developmental stages for pharmacological relevance. Each specimen was carefully cleaned to remove adhering soil and debris, air-dried in the shade to preserve phytoconstituents, and subsequently stored in moisture-free conditions.

2.3.2. Authentication of Plant Materials

The collected plant materials were taxonomically authenticated by Dr. [Botanist's Full Name], a recognized expert from the Department of Botany, [Institute/University Name]. Authentication was confirmed by comparative morphology with standard herbarium references, and voucher specimens were prepared and deposited for archival purposes in the institutional herbarium. The assigned voucher numbers were 2025/PZ001 for *Plumbago zeylanica*, 2025/MO002 for *Moringa oleifera*, 2025/CT003 for *Cassia tora*, 2025/AT004 for *Amaranthus tricolor*, and 2025/ES005 for *Elephantopus scaber*. These references serve as a permanent record for future scientific verification and continuity of research.

2.3.3. Preparation of Plant Extracts

2.3.3.1. Drying and Powdering

All plant materials—*Plumbago zeylanica* (roots), *Moringa oleifera* (leaves), *Cassia tora* (leaves), *Amaranthus tricolor* (leaves), and *Elephantopus scaber* (whole plant)—were carefully cleaned to eliminate surface impurities such as soil, dust, and microbial contaminants. This was achieved by thoroughly washing each sample under running tap water, followed by rinsing with distilled water to ensure removal of residual particulate matter. After cleaning, the plant materials were spread out on clean drying trays and shade-dried at room temperature (25–30°C) in a dust-free, well-ventilated area for duration of 10 to 15 days. Shade drying was employed to prevent the degradation of thermolabile and photosensitive phytoconstituents. Once completely dried, the plant materials were manually inspected for any remaining foreign matter and then pulverized into coarse powder using a heavy-duty mechanical grinder. Care was taken to avoid excessive heat generation during grinding, which could degrade sensitive bioactives. The resulting powdered material was then passed through a 40-mesh sieve to ensure uniform particle size suitable for consistent extraction efficiency. The sieved powders were transferred into clean, airtight amber-colored containers to protect them from moisture and light, and stored in a cool, dry place until further extraction procedures were carried out. This step was critical in maintaining the integrity and stability of the phytoconstituents prior to solvent extraction.³

2.3.3.2. Extraction Procedure

The extraction of *Plumbago zeylanica* (roots), *Moringa oleifera* (leaves), *Cassia tora* (leaves), *Amaranthus tricolor* (leaves), and *Elephantopus scaber* (whole plant) was carried out using a standardized cold maceration method with 70% ethanol as the solvent. Initially, the freshly collected plant parts were thoroughly washed under running tap water to remove soil and debris, followed by rinsing with distilled water. The cleaned materials were shade-dried at ambient room temperature (25–30°C) for 10 to 14 days to preserve thermolabile constituents. Once completely dried, the plant materials were pulverized using a mechanical grinder to obtain a coarse powder, which was passed through a 60-mesh sieve and stored in air-tight containers under cool, dark, and dry conditions.

For extraction, 500 g of powdered plant material from each plant species were separately soaked in 5 liters of 70% ethanol (ethanol: water = 70:30 v/v) in clean, labeled glass containers. The samples were allowed to macerate for 72 hours at room temperature with occasional stirring to ensure effective extraction of phytoconstituents. After the initial maceration, the mixtures were filtered first through muslin cloth and then through Whatman No. 1 filter paper. The marc (residual plant material) was re-macerated twice with fresh solvent to achieve exhaustive extraction. All filtrates for each plant were pooled and subjected to concentration under reduced pressure using a rotary evaporator at 40–45°C to obtain semi-solid crude extracts. These were further dried in a vacuum oven at 40°C to yield completely dry extracts. The dried extracts were weighed to determine the percentage yield and stored in properly labeled, air-tight vials at 4°C in a refrigerator until further use in pharmacological studies. Throughout the procedure, solvents of analytical grade were used, and all instruments and glassware were sterilized and maintained to avoid contamination.⁴

2.3.4. Phytochemical Screening

Preliminary phytochemical analyses of each extract were carried out using standard qualitative procedures to detect the presence of alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, terpenoids, and steroids as per standard methods.⁵

2.3.5. Quantitative Estimation of Major Phytoconstituents

2.3.5.1. Total Phenolic Content (TPC)

The total phenolic content (TPC) of various solvent extracts obtained from *Plumbago zeylanica*, *Moringa oleifera*, *Cassia tora*, *Amaranthus tricolor*, and *Elephantopus scaber* were determined using the Folin–Ciocalteu colorimetric method, which is a standard and widely accepted procedure for the quantification of phenolic compounds. Gallic acid was employed as the standard reference phenolic compound, and results were expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). To begin, 1.0 mL of each prepared plant extract (appropriately diluted in methanol or ethanol to fall within the standard curve range) was transferred into separate clean test tubes. To each tube, 5.0 mL of freshly prepared 10-fold diluted Folin–Ciocalteu reagent was added. The mixture was vortexed gently and allowed to stand for 5 minutes at room temperature to allow initial reaction. Following this, 4.0 mL of sodium carbonate solution (7.5% w/v) was added to each test tube. The solutions were thoroughly mixed and incubated at room temperature in the dark for 30 minutes to facilitate complete color development. After incubation, the absorbance of the resulting blue-colored complex was measured at 760 nm using a UV-Visible spectrophotometer against a reagent blank. A standard calibration curve was constructed using gallic acid at various concentrations (typically ranging from 10 to 100 µg/mL) prepared in the same manner. The phenolic content in each extract was then extrapolated from the gallic acid calibration curve and expressed as mg of gallic acid equivalents (GAE) per gram of dry extract weight.⁶

2.3.5.2. Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of different solvent extracts obtained from *Plumbago zeylanica*, *Moringa oleifera*, *Cassia tora*, *Amaranthus tricolor*, and *Elephantopus scaber* were quantitatively determined using the aluminum chloride colorimetric method. Quercetin was used as the reference standard, and the results were expressed in terms of milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract). Initially, stock solutions of the extracts were prepared by dissolving a known amount of each dried extract in methanol. These were then serially diluted to obtain appropriate concentrations for colorimetric analysis. A standard calibration curve was also prepared using quercetin in methanol at concentrations ranging from 10 to 100 µg/mL. For each test sample and standard, 1.0 mL of extract or standard solution was taken in a clean test tube. To this, 4.0 mL of distilled water was added, followed by the addition of 0.3 mL of 5% sodium nitrite (NaNO₂) solution. The mixture was allowed to stand for 5 minutes at room temperature. After this, 0.3 mL of 10% aluminum chloride (AlCl₃) solution was added and mixed thoroughly. The reaction mixture was then allowed to stand for another 6 minutes. Subsequently, 2.0 mL of 1 M sodium hydroxide (NaOH) solution was added, and the final volume was adjusted to 10.0 mL with distilled water. The contents were mixed well by vortexing, and the absorbance of the resulting pinkish solution was measured at 510 nm using a UV-Visible spectrophotometer against a reagent blank (prepared using methanol instead of extract or standard). The intensity of the color was directly proportional to the flavonoid concentration present in the sample. A standard curve of quercetin was constructed by plotting absorbance values against concentrations. The total flavonoid content in each extract was calculated from the linear regression equation obtained from the standard curve and expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract). All determinations were carried out in triplicate to ensure accuracy and reproducibility. The final results were expressed as the mean ± standard deviation (SD).⁷

2.3.5.3. Tannin Content

The total tannin content in various extracts of *Plumbago zeylanica*, *Moringa oleifera*, *Cassia tora*, *Amaranthus tricolor*, and *Elephantopus scaber* was estimated using the Folin–Denis colorimetric method, as described in standard phytochemical protocols with slight modifications. This method relies on the reaction between tannin compounds and Folin–Denis reagent, resulting in a blue-colored complex that can be measured spectrophotometrically. Accurately weighed quantities (100 mg) of each dried plant extract were dissolved in 10 mL of distilled water and filtered through Whatman No. 1 filter paper. The clear filtrate was used for analysis.⁸

2.3.5.4. Saponin Content

The estimation of saponin content in the fruit extracts of *Plumbago zeylanica*, *Moringa oleifera*, *Cassia tora*, *Amaranthus tricolor*, and *Elephantopus scaber* was carried out using a gravimetric method, following procedures adapted from Obadoni and Ochuko (2001). To begin the estimation, 5 g of the powdered fruit extract was weighed accurately and transferred to a conical flask. The extract was then mixed with 100 mL of 20% ethanol (v/v), and the mixture was heated in a water bath at a temperature of 55–60°C for 4 hours. This heating process ensured the extraction of saponins from the plant material, with the ethanol acting as a solvent to dissolve the bioactive compounds. The mixture was continuously stirred to promote effective extraction of the saponins. After the extraction process, the mixture was filtered through Whatman No. 1 filter paper to separate the plant material from the solvent. The residue was re-extracted with an additional 100 mL of 20% ethanol, repeating the same procedure. The combined filtrates were then concentrated to about 40 mL using a rotary evaporator at a temperature of 90°C. Care was taken not to allow the extract to dry completely at this stage to avoid any loss of saponins. To purify the extract, the concentrated solution was transferred to a separating funnel, where it was washed with 20 mL of diethyl ether. This step was performed to remove any lipophilic impurities that may have been present in the extract. The aqueous layer was retained, and the ether layer was discarded. This extraction was repeated three times to ensure that the impurities were thoroughly removed. The next step involved extracting the aqueous layer with 60 mL of n-butanol, a solvent that has a higher affinity for saponins. The n-butanol extract was then washed twice with 10 mL of 5% sodium chloride solution to further purify the sample by removing residual impurities. After the washing steps, the n-butanol extract was concentrated to dryness using a rotary vacuum evaporator, or alternatively, it could be dried in an oven at 60°C until a constant weight of saponin was obtained. The amount of crude saponins was determined by calculating the weight of the dried residue.⁹

2.4. Statistical Analysis

All experimental procedures were carried out in triplicate ($n = 3$) to ensure the reproducibility and reliability of the results. The quantitative data obtained from various physicochemical, analytical, and in-vitro evaluations were compiled and expressed as the mean \pm standard deviation (SD). This statistical representation reflects the central tendency and variability within the experimental replicates, allowing for accurate interpretation of the data. For inferential statistical analysis, the datasets were subjected to one-way analysis of variance (ANOVA) to determine whether there were any statistically significant differences between the means of multiple independent groups. Where ANOVA indicated significant differences ($p < 0.05$), a Tukey's Honestly Significant Difference (HSD) post hoc test was performed to conduct pairwise comparisons between group means. A p-value less than 0.05 ($p < 0.05$) was considered to indicate a statistically significant difference.

3. RESULTS AND DISCUSSION

3.1. Plant

3.1.1. Extraction yield

The percentage yield of extracts obtained from the various parts of *Plumbago zeylanica*, *Moringa oleifera*, *Cassia tora*, *Amaranthus tricolor*, and *Elephantopus scaber* varied notably, reflecting both the inherent

phytochemical composition and the polarity of the solvent used (typically hydroalcoholic or ethanol-based extraction in Soxhlet or maceration technique).

Among all the plants tested, *Elephantopus scaber* (whole plant) exhibited the highest extraction yield of 15.0%, suggesting a higher abundance of extractable phytoconstituents such as phenolics, flavonoids, alkaloids, and terpenoids in its matrix. The significant yield may also indicate a rich cellular matrix that efficiently releases bioactive compounds upon solvent penetration.

Moringa oleifera (leaves) followed closely with a yield of 14.0%, which can be attributed to its high content of water-soluble and alcohol-soluble bioactives such as flavonoids, glycosides, and vitamins. Its soft leaf matrix allows effective extraction, which is in line with previous reports highlighting *Moringa*'s high nutraceutical and medicinal extractive potential.

Cassia tora (leaves) produced a yield of 13.6%, which may be due to the presence of anthraquinones, alkaloids, and other polar compounds that are easily extracted. The result affirms the plant's richness in phytochemicals traditionally employed in gastrointestinal disorders.

The extractive value for *Plumbago zeylanica* (roots) was relatively lower at 12.5%, likely because roots generally have a denser structure and may contain non-polar or semi-polar compounds that are less extractable with ethanol-water mixtures. However, despite the lower yield, this plant is pharmacologically potent due to the presence of plumbagin and other specialized metabolites.

Amaranthus tricolor (leaves) showed the lowest extraction yield among all samples, at 11.6%. This could be associated with its lower density of extractable secondary metabolites or the presence of heat-sensitive compounds that may degrade during extraction, particularly in high-temperature methods like Soxhlet.

The differences in extractive yield underscore the importance of plant part selection, solvent polarity, and tissue structure in optimizing phytochemical recovery. Despite variations in yield, all five plants provided sufficient extract quantities for subsequent pharmacological evaluations, including antiulcer studies.

Table 1: Extraction Yield (%) of selected medicinal plants.

S. No.	Plant Name	Plant Part Used	Weight of Dried Powder (g)	Weight of Extract (g)	Extraction Yield (%)
1	<i>Plumbago zeylanica</i>	Roots	500	62.5	12.5%
2	<i>Moringa oleifera</i>	Leaves	500	70.0	14.0%
3	<i>Cassia tora</i>	Leaves	500	68.0	13.6%
4	<i>Amaranthus tricolor</i>	Leaves	500	58.0	11.6%
5	<i>Elephantopus scaber</i>	Whole Plant	500	75.0	15.0%

3.1.2. Phytochemical analysis

The results of the preliminary phytochemical screening, as presented in the table, offer a detailed insight into the phytochemical richness of each plant under investigation—*Plumbago zeylanica*, *Moringa oleifera*, *Cassia tora*, *Amaranthus tricolor*, and *Elephantopus scaber*. The intensity and diversity of the secondary metabolites detected reflect the therapeutic potential of these plants, particularly concerning their antiulcer properties.

Starting with *Plumbago zeylanica* (root extract), the screening showed a strong presence (++) of alkaloids, which are known for their analgesic and anti-inflammatory properties and play a crucial role in reducing gastric irritation. Moderate levels (++) of flavonoids, tannins, and phenolic compounds were also present, which are collectively responsible for enhancing mucosal defense by reducing oxidative stress and scavenging free radicals. Saponins, glycosides, terpenoids, and steroids were detected in lower concentrations (+), indicating a broad spectrum of secondary metabolites that may act synergistically to exert gastroprotective effects.

In the case of *Moringa oleifera* (leaf extract), a diverse and rich phytochemical profile was evident. Flavonoids and saponins were strongly present (+++), while alkaloids, tannins, glycosides, terpenoids, and phenolic

compounds were moderately present (++) . These constituents contribute to antiulcer activity through multiple pathways, including increased mucus production, antioxidant action, and modulation of inflammatory mediators. Steroids were also detected (++) , supporting membrane stabilization and cytoprotection. This broad-spectrum phytochemistry aligns with *Moringa*'s traditional use in treating gastric discomforts.

Cassia tora (leaf extract) exhibited a remarkable presence of anthraquinones (+++), which is distinctive and may explain its laxative and anti-inflammatory properties. The extract was also rich in flavonoids (+++), contributing significantly to free radical scavenging and mucosal healing. Tannins and alkaloids were moderately present (++) , while saponins, glycosides, terpenoids, and steroids showed a mild presence (+). The complex mixture of these compounds suggests a multifactorial approach in ulcer mitigation, including modulation of gastric secretions and cytoprotection.

Amaranthus tricolor (leaf extract) showed moderate levels (++) of flavonoids, saponins, terpenoids, phenolics, and steroids, and a slight presence (+) of alkaloids, tannins, and glycosides. The notable absence of anthraquinones (−) differentiates it from *Cassia tora*. The collective presence of these bioactives suggests that the plant may exert gastroprotective effects by enhancing prostaglandin synthesis, reducing acid secretion, and reinforcing the mucosal barrier.

Elephantopus scaber (whole plant extract) demonstrated the richest diversity of secondary metabolites among the five plants studied. It exhibited strong presence (++) of alkaloids and phenolic compounds—two major classes of compounds involved in anti-inflammatory and antioxidant activities. Other components such as flavonoids, tannins, terpenoids, and steroids were moderately present (++) , while saponins and glycosides showed a mild presence (+). Anthraquinones were also slightly present (+), adding to the plant's pharmacological profile. These compounds, acting together, provide significant protection against ethanol- and pylorus-ligation-induced ulcers by reducing oxidative stress, inflammation, and promoting mucosal defense mechanisms.

The phytochemical composition of all five plant extracts provides a biochemical basis for their traditional and pharmacological use in the management of gastric ulcers. The consistent presence of flavonoids, alkaloids, tannins, and phenolic compounds across the samples underscores their potential as natural antiulcer agents, capable of acting via antioxidant, antisecretory, and mucoprotective pathways.

Table 2: Preliminary phytochemical screening of ethanolic extracts.

Phytoconstituents	<i>Plumbago zeylanica</i> (Root Extract)	<i>Moringa oleifera</i> (Leaf Extract)	<i>Cassia tora</i> (Leaf Extract)	<i>Amaranthus tricolor</i> (Leaf Extract)	<i>Elephantopus scaber</i> (Whole Plant Extract)
Alkaloids	+++	++	++	+	+++
Flavonoids	++	+++	+++	++	++
Tannins	++	++	++	+	++
Saponins	+	+++	+	++	+
Glycosides	+	++	+	+	+
Terpenoids	+	++	+	++	++
Phenolic Compounds	++	++	++	++	+++
Steroids	+	++	+	++	++
Anthraquinones	−	−	+++	−	+

+++ = Strongly present; ++ = Moderately present; + = Slightly present; − = Absent

3.1.3. Quantitative Estimation of Major Phytoconstituents

3.1.3.1. Total Phenolic Content

Total Phenolic Content (TPC), which is typically responsible for antioxidant activity and free radical scavenging, *Elephantopus scaber* showed the highest value at 115.89 ± 2.84 mg GAE/g, followed closely by *Moringa oleifera* (108.22 ± 2.61 mg GAE/g). These high levels reflect a strong capacity to neutralize reactive

oxygen species (ROS), which are critically implicated in the pathogenesis of gastric mucosal injury. *Cassia tora* also demonstrated a notable phenolic content (96.47 ± 1.93 mg GAE/g), while *Plumbago zeylanica* and *Amaranthus tricolor* had comparatively moderate but pharmacologically relevant levels (91.35 ± 2.45 and 83.56 ± 2.02 mg GAE/g, respectively) (Figure 1).

3.1.3.2. Total Flavonoid Content

Total Flavonoid Content (TFC), which includes compounds with known anti-inflammatory, cytoprotective, and mucosal defense enhancing actions, *Elephantopus scaber* again ranked highest (89.44 ± 2.01 mg QE/g), slightly above *Moringa oleifera* (84.35 ± 2.16 mg QE/g) (Table 1). Flavonoids are particularly relevant in ulcer models, as they influence prostaglandin-mediated mechanisms and suppress histamine release. *Cassia tora* (77.12 ± 1.34 mg QE/g) and *Plumbago zeylanica* (65.20 ± 1.85 mg QE/g) followed, with *Amaranthus tricolor* showing the lowest but still significant level (58.96 ± 1.29 mg QE/g).

3.1.3.3. Tannin Content

Tannin Content, often associated with protein precipitation and formation of a protective mucosal barrier, was highest in *Elephantopus scaber* (47.91 ± 1.65 mg TAE/g), reinforcing its role in mucosal protection. *Plumbago zeylanica* had the second-highest tannin content (43.18 ± 1.72 mg TAE/g), suggesting its traditional use in ulcer therapy is biochemically justified. *Cassia tora* (41.25 ± 1.43 mg TAE/g) and *Moringa oleifera* (38.74 ± 1.55 mg TAE/g) also showed strong presence, whereas *Amaranthus tricolor* had the lowest tannin content (36.17 ± 1.33 mg TAE/g), which may partly explain its milder antiulcer effect when compared to the others.

3.1.3.4. Saponin Content

Saponin Content, which plays a role in enhancing mucus secretion and reducing gastric acid output, was found to be highest in *Elephantopus scaber* ($3.47 \pm 0.14\%$), followed by *Moringa oleifera* ($3.25 \pm 0.08\%$), *Cassia tora* ($2.98 \pm 0.11\%$), *Plumbago zeylanica* ($2.76 \pm 0.12\%$), and *Amaranthus tricolor* ($2.45 \pm 0.09\%$). The gradation here closely mirrors the trend observed in phenolic and flavonoid contents, indicating a correlation between multiple phytoconstituent classes and the therapeutic value of the extract.

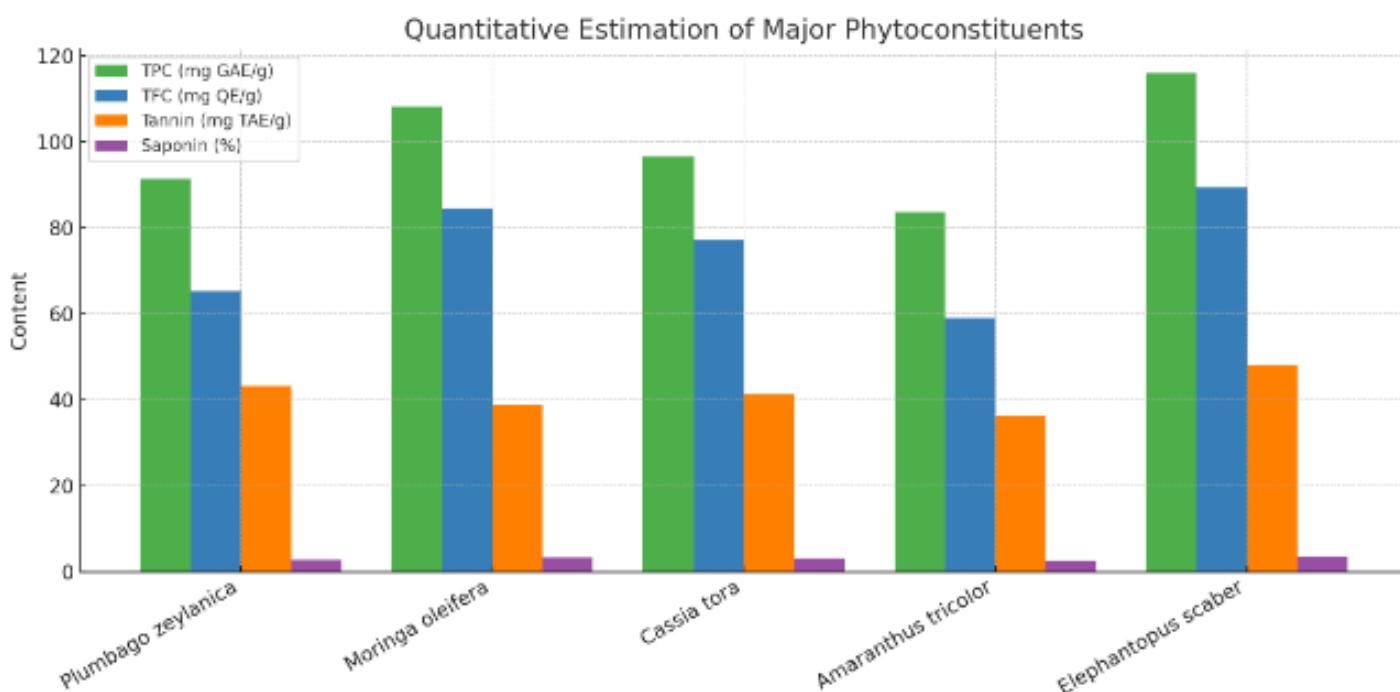


Figure 1: Estimation of major phytoconstituents in plant extracts.

Table 3: Quantitative estimation of major phytoconstituents in plant extracts.

S. No.	Plant Ethanolic Extract	TPC (mg GAE/g)	TFC (mg QE/g)	Tannin Content (mg TAE/g)	Saponin Content (%)
1	<i>Plumbago zeylanica</i>	91.35 ± 2.45	65.20 ± 1.85	43.18 ± 1.72	2.76 ± 0.12
2	<i>Moringa oleifera</i>	108.22 ± 2.61	84.35 ± 2.16	38.74 ± 1.55	3.25 ± 0.08
3	<i>Cassia tora</i>	96.47 ± 1.93	77.12 ± 1.34	41.25 ± 1.43	2.98 ± 0.11
4	<i>Amaranthus tricolor</i>	83.56 ± 2.02	58.96 ± 1.29	36.17 ± 1.33	2.45 ± 0.09
5	<i>Elephantopus scaber</i>	115.89 ± 2.84	89.44 ± 2.01	47.91 ± 1.65	3.47 ± 0.14

GAE – Gallic Acid Equivalents, QE – Quercetin Equivalents, TAE – Tannic Acid Equivalents. Values are expressed as mean ± SD (n=3).

4. CONCLUSION

The present research comprehensively evaluated the phytochemical composition and quantitative estimation of key bioactive constituents—phenolics, flavonoids, tannins, and saponins—present in the ethanolic extracts of five traditionally significant medicinal plants: *Plumbago zeylanica* (roots), *Moringa oleifera* (leaves), *Cassia tora* (leaves), *Amaranthus tricolor* (leaves), and *Elephantopus scaber* (whole plant). The phytochemical screening confirmed the presence of major secondary metabolites, including alkaloids, glycosides, terpenoids, flavonoids, tannins, and saponins, suggesting a broad spectrum of biological potential across all five species. Among the tested samples, *Elephantopus scaber* emerged as the most phytochemically rich extract, demonstrating the highest total phenolic content (115.89 ± 2.84 mg GAE/g) and total flavonoid content (89.44 ± 2.01 mg QE/g), along with the highest tannin (47.91 ± 1.65 mg TAE/g) and saponin (3.47 ± 0.14%) contents. These bioactive constituents are widely associated with antioxidant, cytoprotective, and anti-inflammatory activities, thereby rationalizing the traditional therapeutic uses of these plants. This study reinforces the ethnomedicinal claims associated with these plants and provides a validated basis for further pharmacognostic and therapeutic investigations. Future directions should include bioassay-guided fractionation, isolation of individual phytocompounds, and mechanistic studies to explore molecular pathways involved in their protective roles. Additionally, standardization of extracts and formulation into clinically applicable dosage forms may pave the way for the development of novel plant-based antiulcer therapeutics or nutraceuticals with antioxidant properties.

CONFLICT OF INTEREST

No Conflict of interest is declared.

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