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Phytochemical evaluation of various parts of *Tribulus terrestris* collected from Jaipur, Rajasthan

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Abstract
The present study investigates the phytochemical composition of different parts (root, stem, leaves, and fruits) of *Tribulus terrestris* collected from Jaipur, Rajasthan. The plant materials were processed, aqueous extracts were prepared using sonication, and preliminary phytochemical screening was carried out using standard qualitative methods. The extractive values varied significantly among plant parts, with leaves showing the highest yield (81±5.76 mg/g dry weight), followed by stem (52±4.18 mg/g), root (46±2.38 mg/g), and fruits (32±2.36 mg/g), indicating differential accumulation of bioactive compounds. Phytochemical analysis revealed the presence of diverse secondary metabolites, including carbohydrates, proteins, flavonoids, alkaloids, tannins, phytosterols, saponins, and terpenoids, with distinct distribution patterns across plant parts. Flavonoids and phytosterols were found to be universally present, while carbohydrates were detected only in leaves and fruits, and terpenoids were restricted to root and stem extracts. Proteins were present in all parts, with higher intensity observed in the root and stem. The variation in phytochemical composition suggests tissue-specific metabolic activity and functional specialization. The presence of these bioactive compounds demonstrates the potential of *Tribulus terrestris* as a valuable source of natural phytochemicals for applications in pharmacology, antioxidant studies, and green synthesis of nanoparticles.

Keywords: *Tribulus terrestris*, phytochemical screening, phytochemical composition, aqueous extracts, secondary metabolites, flavonoids, phytosterols, plant parts, bioactive compounds, green synthesis

Introduction

Medicinal plants have long been recognized as valuable sources of bioactive compounds with therapeutic potential. They play a crucial role in traditional as well as modern medicine due to the presence of diverse secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids. These phytochemicals are known to exhibit a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. Recently, there has been growing scientific interest in exploring plant-based compounds as safer and eco-friendly alternatives to synthetic drugs.

Tribulus terrestris, a well-known medicinal plant belonging to the family Zygophyllaceae, is widely distributed in arid and semi-arid regions, including Rajasthan, India. It has been extensively used in traditional systems of medicine such as Ayurveda for the treatment of various ailments, including urinary disorders, inflammation, and reproductive health issues (Chhatre *et al.*, 2014; Patel *et al.*, 2016) [1, 3]. The plant is rich in pharmacologically active constituents such as steroidal saponins, flavonoids, alkaloids, and glycosides, which contribute to its therapeutic efficacy. Different parts of the plant, including roots, stems, leaves, and fruits, are known to possess varying concentrations of these bioactive compounds, leading to differences in their biological activities.

Phytochemical screening is an essential step in identifying the presence of these secondary metabolites and understanding their distribution within different plant parts. Such studies provide valuable insights into the chemical composition and potential applications of plant extracts in pharmaceuticals, nutraceuticals, and nanotechnology (Evans, 2009; Tiwari *et al.*, 2011) [2, 4]. Therefore, the present study aims to evaluate the phytochemical profile of root, stem, leaves, and fruits of *Tribulus terrestris* using standard qualitative methods in order to assess their potential as sources of bioactive compounds for further scientific and industrial applications.

Materials and Methods

Collection of plant parts and their processing

Roots, stems, leaves, and fruits of *Tribulus terrestris* were collected from the local area of Jaipur, Rajasthan. The healthy parts were taken and washed with running tap water to remove dust and dirt and then with distilled water to remove ionic impurities. The plant materials were dried in shade and ground to make coarse powder and stored in the refrigerator for further use.

Fig 1: The collected plant materials.

Extraction of the plant parts

Plant parts were extracted in distilled water. The extraction was done on a sonicator. 1 gram of dried plant material was dipped into 10 ml of distilled water and extracted on a sonicator for 10 minutes at 40°C. The extracted material was filtered using Whatmann's filter paper no. 1, and the filtrate was used for further use.

Fig 2: Plant extracts.

Phytochemical evaluation of the plant extracts

The preliminary phytochemical analysis of *Tribulus terrestris* was performed to detect the presence of major classes of secondary metabolites in different plant parts (aerial roots, stem, and leaves). The plant materials were extracted using four different solvents. Methanol, ethanol, acetone, and water to compare the solubility and distribution of bioactive compounds. Standard phytochemical tests were employed as follows:

Carbohydrates (Fehling's Test): 0.5 mg of extract was treated with 2 ml of Fehling's solution and boiled in a water bath. The formation of a brick-red precipitate confirmed the presence of reducing sugars.

Proteins (Ninhydrin Test): To 0.5 mg of extract, 2 drops of freshly prepared 0.2% ninhydrin reagent were added and heated. Development of a pink or purple color indicated the presence of proteins, peptides, or amino acids.

Tannins (Gelatin + NaCl Test): 0.1 g of extract was boiled in 10 ml water, filtered, and mixed with 2 ml of 1% gelatin solution containing NaCl. The formation of a white precipitate indicated the presence of tannins and phenolic compounds.

Alkaloids (Mayer's Test): Extracts were treated with Mayer's reagent (Potassium mercuric iodide solution). The appearance of a cream-colored precipitate confirmed the presence of alkaloids.

Flavonoids (Lead Acetate Test): 1 ml of extract was treated with 10% lead acetate solution. Formation of a yellow precipitate indicated the presence of flavonoids.

Phytosterols (Salkowski's Test): Extracts were dissolved in 2 ml of acetic anhydride, and concentrated sulfuric acid was added along the side of the test tube. The appearance of characteristic color changes indicated the presence of phytosterols.

Saponins (Froth Test): 0.5 mg of extract was shaken with 5% sodium bicarbonate solution and allowed to stand for 3 minutes. Persistent honeycomb-like frothing confirmed the presence of saponins.

Phenols (Ferric Chloride Test): Extracts were treated with freshly prepared ferric chloride solution. The development of a violet to dark coloration indicated the presence of phenolic compounds.

Glycosides (NaOH Test): Extracts were dissolved in water and treated with aqueous NaOH solution. The appearance of a yellow color indicated the presence of glycosides.

Results

Extraction of the selected plant parts

The extraction efficiency of different plant parts was evaluated based on their extractive values, color, and physical nature. Among the selected plant parts, the leaf extract exhibited the highest extractive value of 81 ± 5.76 mg/g dry weight, indicating a higher abundance of extractable phytochemicals. The leaf extract was green in color and obtained in powdered form, suggesting the presence of chlorophyll and other bioactive constituents. The stem extract showed a moderate extractive value of 52 ± 4.18 mg/g dry weight with a brown-colored powder, followed by the root extract with an extractive value of 46 ± 2.38 mg/g dry weight, also appearing as a brown powder. The fruit extract recorded the lowest extractive value of 32±2.36 mg/g dry weight and was light brown in color.

Overall, all plant parts yielded solid powdered extracts, confirming efficient solvent removal and extract stability. The variation in extractive values among different plant parts reflects differences in their phytochemical composition and metabolite accumulation. The higher extractive value observed in leaves correlates with their rich phytochemical profile and supports their effective role in the green synthesis of nanoparticles and biological activity studies.

Table 1: Extractive values of different parts of *T. terrestris*.

Plant part	Extractive value (mg/g.dw)	Extract color	Extract nature
Root	46±2.38	Brown	Powder
Stem	52±4.18	Brown	Power
Leaves	81±5.76	Green	Powder
Fruits	32±2.36	Light brown	Powder

*Experiments were conducted in triplicates. Values are represented as mean ± SD.

Determination of the phytochemicals in the extract

Preliminary phytochemical screening was carried out to qualitatively assess the presence of major bioactive compounds in different extracts of the selected plant parts, namely root, stem, leaves, and fruits. The results revealed notable variations in the phytochemical composition among the different plant parts, indicating differential distribution of secondary metabolites.

Carbohydrates, analyzed using Fehling's method, were detected only in the leaf and fruit extracts, as indicated by the formation of a red precipitate, whereas the root and stem extracts remained blue, confirming the absence of reducing sugars. The presence of carbohydrates in leaves and fruits suggests higher metabolic activity and storage of energy-rich compounds in these tissues, which may contribute to their reducing potential during green synthesis of nanoparticles.

Proteins, assessed using the Ninhydrin test, were present in all plant parts, though with variations in color intensity. The dark purple coloration observed in root and stem extracts

indicates a relatively higher protein content compared to leaves and fruits, which exhibit brown to yellow coloration. Proteins play a crucial role in nanoparticle synthesis by acting as stabilizing and capping agents through their amino and carboxyl functional groups, which prevent nanoparticle aggregation.

Tannins were detected in the stem and leaf extracts, as evidenced by the formation of a green precipitate upon addition of gelatin and sodium chloride, while root and fruit extracts showed no precipitate, indicating their absence. Tannins are polyphenolic compounds known for their strong metal-chelating ability and antioxidant properties, which facilitate effective reduction of metal ions and stabilization of nanoparticles.

Alkaloids, identified using Mayer's reagent, were present in root, stem, and fruit extracts but absent in leaves. The formation of red coloration in positive samples confirms the presence of nitrogen-containing alkaloids, which are known to interact with metal ions and participate in nucleation and growth control of nanoparticles due to their electron-donating nature.

Flavonoids, detected by the $FeCl_3$ test, were present in all plant parts, as indicated by the disappearance of yellow coloration. Flavonoids are well-recognized for their strong antioxidant and reducing properties, attributed to hydroxyl and carbonyl groups, making them key contributors to the green synthesis of nanoparticles. Their universal presence across all plant parts suggests a consistent reducing capability.

Phytosterols, confirmed by the Liebermann–Burchard test, were detected in all extracts, showing brown to dark purple coloration. Phytosterols contribute to nanoparticle stabilization by forming hydrophobic interactions and surface coatings that enhance colloidal stability and biocompatibility.

Saponins, analyzed using the Sofowora frothing test, were absent in the root extract but present in stem, leaf, and fruit extracts, as evidenced by stable froth formation. Saponins possess surfactant properties that can reduce surface tension, enhance dispersion, and improve stability of nanoparticles in aqueous systems.

Terpenoids, identified using the Salkowski test, were present in root and stem extracts, as indicated by ring formation, but absent in leaves and fruits. Terpenoids are known for their strong reducing power and ability to influence particle morphology and growth kinetics during nanoparticle synthesis.

Phytochemical screening confirms the presence of a wide range of bioactive compounds, including carbohydrates, proteins, flavonoids, alkaloids, tannins, phytosterols, saponins, and terpenoids, with varying distribution among plant parts. These phytochemicals collectively contribute to the reduction, stabilization, and capping of metal ions during green synthesis of nanoparticles, thereby influencing particle size, morphology, crystallinity, and stability. The observed variation in phytochemical composition among different plant parts may explain differences in nanoparticle characteristics obtained using root, stem, leaf, and fruit extracts.

Table 2: Preliminary Phytochemical Screening of different extracts of the selected plant parts showing the presence and absence of various phytochemicals.

Phytochemical test and method	Plant part	observation	Result
Total carbohydrates (Fehling method)	Root	blue	-ve
	Stem	blue	-ve
	Leaves	Red	+ve
	Fruits	Red	+ve
Protein (Ninhydrin)	Root	Dark purple	+ve
	Stem	Dark purple	+ve
	Leaves	brown	+ve
	Fruits	Yellow	+ve
Tannins (Gelatin + NaCl)	Root	Transparent (no ppt)	-ve
	Stem	Green ppt	+ve
	Leaves	Green ppt	+ve
	Fruits	Transparent (No ppt)	-ve
Alkaloids (Mayer's method)	Root	Red	+ve
	Stem	Red	+ve
	Leaves	yellow	-ve
	Fruits	Red	+ve
Flavonoids ($FeCl_3$ test)	Root	Yellow to disappear	+ve
	Stem	Yellow to disappear	+ve
	Leaves	Yellow to disappear	+ve
	Fruits	Yellow to disappear	+ve
Phytosterols (Libermann Burchard's test)	Root	Brown	+ve
	Stem	Dark purple	+ve
	Leaves	brown	+ve
	Fruits	brown	+ve

Saponins (Sofowora)	Root	No frothing	-ve
	Stem	frothing	+ve
	Leaves	frothing	+ve
	Fruits	frothing	+ve
Terpenoids (Salkowski)	Root	Ring +	+ve
	Stem	Ring +	+ve
	Leaves	No ring -	-ve
	Fruits	No ring -	-ve

*+ve shows the presence of phytochemicals, while -ve shows their absence.

Discussion

The present study reveals significant variation in the phytochemical composition of different parts (root, stem, leaves, and fruits) of *Tribulus terrestris*, indicating tissue-specific distribution of bioactive compounds. The extractive values showed that leaves possessed the highest yield, followed by stem, root, and fruits, suggesting a higher concentration of soluble phytochemicals in leaf tissues. This observation is consistent with earlier reports that leaves are metabolically more active and serve as primary sites for the synthesis and accumulation of secondary metabolites such as flavonoids and phenolic compounds (Tiwari *et al.*, 2011) [4]. The green coloration of leaf extract further supports the presence of chlorophyll and associated bioactive constituents.

Preliminary phytochemical screening demonstrated the presence of multiple classes of secondary metabolites with distinct distribution patterns across plant parts. Flavonoids and phytosterols were detected in all extracts, indicating their fundamental role in plant defense and metabolic functions. These compounds are well known for their antioxidant, antimicrobial, and anti-inflammatory properties (Patel *et al.*, 2016) [3]. Carbohydrates were found only in leaves and fruits, suggesting their role in energy storage and metabolic activity, particularly in photosynthetic and reproductive tissues. Proteins were present in all plant parts, with higher intensity in root and stem extracts, which may contribute to enzymatic activities and also play a role in stabilization processes during biochemical interactions (Evans, 2009) [2].

The presence of tannins in stems and leaves highlights their role in plant defense mechanisms due to their ability to precipitate proteins and inhibit microbial growth. Alkaloids, detected in roots, stems, and fruits, are known for their pharmacological activities and ability to interact with biological systems through nitrogen-containing functional groups. Saponins, present in most parts except roots, are surface-active agents that contribute to membrane permeability and biological activity. Interestingly, terpenoids were restricted to root and stem extracts, suggesting their involvement in structural and protective functions in these plant parts. Overall, the observed variation in phytochemical composition reflects the functional specialization of plant tissues and supports the potential of *Tribulus terrestris* as a rich source of bioactive compounds for applications in pharmacology, nutraceuticals, and green nanotechnology.

Conclusion

The present study highlights the phytochemical richness of different parts (root, stem, leaves, and fruits) of *Tribulus terrestris* and demonstrates significant variation in the distribution of bioactive compounds among them. The highest extractive value observed in leaves indicates their greater potential as a source of phytochemicals, while other parts also contribute distinct classes of secondary metabolites. Preliminary screening confirmed the presence of important compounds such as flavonoids, alkaloids, tannins, phytosterols, saponins, proteins, and terpenoids, each exhibiting tissue-specific occurrences. This variation reflects the functional specialization of plant parts and their metabolic diversity. Overall, the findings establish *Tribulus terrestris* as a valuable reservoir of bioactive constituents with potential applications in pharmacology, nutraceuticals, and green nanotechnology and provide a scientific basis for further detailed phytochemical and biological investigations.

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