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Phytochemical Screening of Some Primary and Secondary Metabolites of Medicinal Plant *Tribulus terrestris* in Jaipur District of Rajasthan, India

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ABSTRACT

Many herbs are used as medicines, in which, *Tribulus terrestris* is an important medicinal herb, which belongs to family Zygophyllaceae. The effectiveness of *Tribulus terrestris* extracts in a variety of applications is proved by many studies. In this pharmacological research different primary and secondary metabolites were examined in aqueous and ethanolic extracts of different plant parts. The Present paper deals with the pharmacognostical studies on *Tribulus terrestris* plant. In this study, primary and secondary metabolites of *Tribulus terrestris* were evaluated from different plant parts. Results showed that highest amount of alkaloids, flavonoids and total soluble sugar are present in leaves while proteins and total phenolic content are more in fruits and roots are rich in tannins and total lipids.

Figures : 02 References : 37 Tables : 02
KEY WORDS : Flavonoids, Phytochemical, *Tribulus terrestris*, Zygophyllaceae.

Introduction

Pharmaceuticals are largely derived from a variety of chemical compounds present in plants. Around 35,000 plant species are utilized in traditional medicine worldwide by different cultures because they contain physiologically active compounds that have medicinal advantages[34]. Microorganisms are essential in the biosynthesis of these secondary metabolites, as they assist in their production, survival and reproduction. These microbes participate in various biochemical pathways that facilitate the production of bioactive compounds, which support the development of organisms and their interactions within the ecosystem[37].

These metabolites often possess pesticidal and antimicrobial properties, in addition to acting as biocontrol agents and biosurfactants. They present valuable opportunities for promoting crop health and unlocking the untapped potential of specific plants for agricultural studies[5, 8]. These metabolites either are used in their natural state or processed to isolate these compounds for the production of pharmaceutical drugs [12]. These plants often exhibit therapeutic or pharmacological effects on both humans and animals. Around 119 species were identified with commercial and therapeutic value belong to the families Asteraceae, Amaranthaceae, Poaceae, and Zygophyllaceae [7].

These Pharmaceutically important plants and their secondary metabolites exhibit a range of biological and pharmacological effects. *Tribulus terrestris* is one such plant, recognized for its diverse therapeutic properties and medicinal value in different health conditions [24]. It thrives in diverse climates and has been traditionally used in many cultures for its medicinal properties. Tropically adapted genus *Tribulus* is originally native to areas including Africa, Southern Asia, Southern Europe, Northern Australia and New Zealand [25]. This plant is commonly known as puncture vine, holds a prominent place in traditional medicine systems, especially in Ayurveda, where it is utilized for a variety of health benefits[33]. This plant is a small, silky, and hairy herb that grows in a prostrate manner, reaching heights between 10 and 60 cm[4]. It is commonly seen on roadsides, lawns, overgrazed pastures, abandoned areas and cultivated lands[36]. The full above ground portion of *Tribulus terrestris* is used for medicinal

TABLE-1: Showing total primary and secondary metabolite content in different plant parts of *Tribulus terrestris*

Plant part	Soluble sugars (mg/g.dw)	Protein (mg/g.dw)	Lipids (mg/g.dw)	Phenolic content (µg/g.dwCAE)	Flavonoids (µg/g.dwQE)	Alkaloids (µg/g.dwAE)	Tannins (µg/g. dwGAE)
Root	3.731	6.04	1.912	126.71	23	122.66	92.142
Stem	2.89772	5.51	1.266	103.85	38.625	51	57.85
Leaves	5.01027	9.91	1.671	215.28	95.5	162.66	19.28
Fruits	2.24635	11.1	1.824	226.71	73	97.66	50.71

purposes. It is characterized by an absence of a strong odor or taste, which enhances its adaptability for different therapeutic uses[22]. Because of its therapeutic qualities and possible health advantages, *T. terrestris* fruit is very important in medicine [14].

This herb is rich in several bioactive compounds, with steroidal saponins being the primary active constituents, though it also contains flavonoids, alkaloids, phytosterols, inorganic nitrates, amino acids carbohydrates, resins and tannins. The primary substances thought to be in charge of the herb's therapeutic qualities are the steroidal saponins. Flavonoids provide antioxidant benefits, while alkaloids offer other pharmaceutical properties[29, 33]. These constituents are significant for the plant's diverse pharmacological potential. These compounds contribute to the therapeutic value of *T. terrestris* in treating various health conditions [30]. It was found that flavonoid levels were about 1.5 times greater than those of saponins[2]. Fruits of the plant are rich in flavonoids. Research on the leaves has revealed additional significant flavonoids, such as dinatin and its 7-glucuronide, diosmetin and its 7-glucuronide, along with pedaltin and pedalin. These compounds play significant role to enhance medicinal properties of plant [32].

Key components such as saponins, alkaloids, phenols, tannins and flavonoid were also detected by phytochemical analysis of *T. terrestris* seed extract. The extract displayed cytotoxicity against cancer cells and exhibited strong antioxidant activity [17]. The plant has been shown to have a positive impact on kidney tissues and function, due to the presence of various pharmaceutically important chemical constituents[1]. Steroid saponins are thought to be the main source of the plant's therapeutic benefits. Many of its medicinal actions are thought to be caused by these chemicals[18]. These steroidal saponins are unique to the species and define its chemical composition[15]. Additionally, the chemical-laden extracts from these plants are used in nanotechnology to create nanoparticles. A variety of precursor salts, such as gold, silver, cobalt, copper, and zinc, could be utilized for their synthesis [11]. It is revealed that nanoparticles synthesized *by T. terrestris* leaf extract by using copper oxide nanoparticle method is a simple and cost-effective method[23]. The genus *Fagonia* another important medicinal plant of family Zygophyllaceae has demonstrated significant therapeutic potential, especially anti-cancer effects, due to having flavonoids, alkaloids, and terpenoids[19].

This plant specially leaves and seeds have been utilized for treating dyspepsia, kidney stones, bladder stones, anorexia, asthma, tuberculosis, hemoptysis, heart conditions, anemia, ulcers, gonorrhea, skin disorders, inflammation, hemorrhages and many more. Due to these therapeutic properties, the plant is widely used in ayurvedic practices as a medicinal remedy and tonic [26].

Materials and Methods

Collection of Plant material: Fresh, mature samples of the different parts of the selected plant were gathered from Neendad village, Jaipur. Plant was identified by previous literature findings. The samples were prepared and stored for herbarium records. Following this, they were shaded to dry in preparation for further metabolite screening.

Determination of primary metabolites

Total soluble sugars : It was determined using phenol sulphuric acid method [21]. A 50 mg portion of dried plant material was ground with 20 ml of 80% ethanol and left to soak overnight. After 15 minutes of centrifuging the mixture at 1200 rpm, the supernatant was collected.

TABLE-2 : Showing % Free radical scavenging activity at concentrations in different parts of *Tribulus terrestris*

Plant part	% Free radical scavenging activity at concentrations (mg/L)						
	20	40	60	80	100	Regression	IC50 mg/L
Root	31.18669	31.83792	33.50217	34.44284	36.17945	Y=0.063x+29.653	322.96
Stem	29.01592	32.9233	34.73227	36.46889	38.35022	Y=0.1111x+27.634	201.31
Leaves	35.89001	38.78437	41.24457	43.70478	45.51375	Y=0.1208x+33.777	134.29
Fruits	37.98842	40.52098	41.8958	43.05355	44.42836	Y=0.0771x+36.954	169.208

The concentrated solution was subsequently diluted to a final volume of 50 ml. The phenol-H, $SO_{„}$ reagent was applied to determine the carbohydrate content, and a standard glucose regression curve was established to calibrate the measurements. By plotting the absorbance against known glucose concentrations, the carbohydrate levels in the test samples were determined using Beer's Law.

Total proteins : It was assessed according to Lowry's method [16]. This method is commonly employed to quantify the total protein content in a sample. This was accomplished by combining 1 milliliter of the sample with 0.5 milliliters of 0.1 N NaOH and 5 milliliters of alkaline copper reagent. For half an hour, the mixture was left to incubate at room temperature. It was followed by the addition of 0.5 ml of Folin-Ciocalteau reagent, and then put to incubate at 27-28 degree for about 10 min. At 660 nm absorbance was measured, with a blank used as the negative control.

Determination of Lipid : An altered method[10] was used to estimate the total lipid content. For this process, 5 grams of ground plant material were mixed in 50 ml of methanol and chloroform in 1:2 ratio. After being well combined, the mixture was put for about 3-4 days. Now the mixture was filtered and at 1000 g. it was subjected to centrifugation. A layer of methanol was visible which was separated by using a pipette, now for the evaporation of chloroform gentle heating was done. The substance which was left was collected as the crude lipid.

Quantification of secondary metabolites

Estimation of total phenolic content

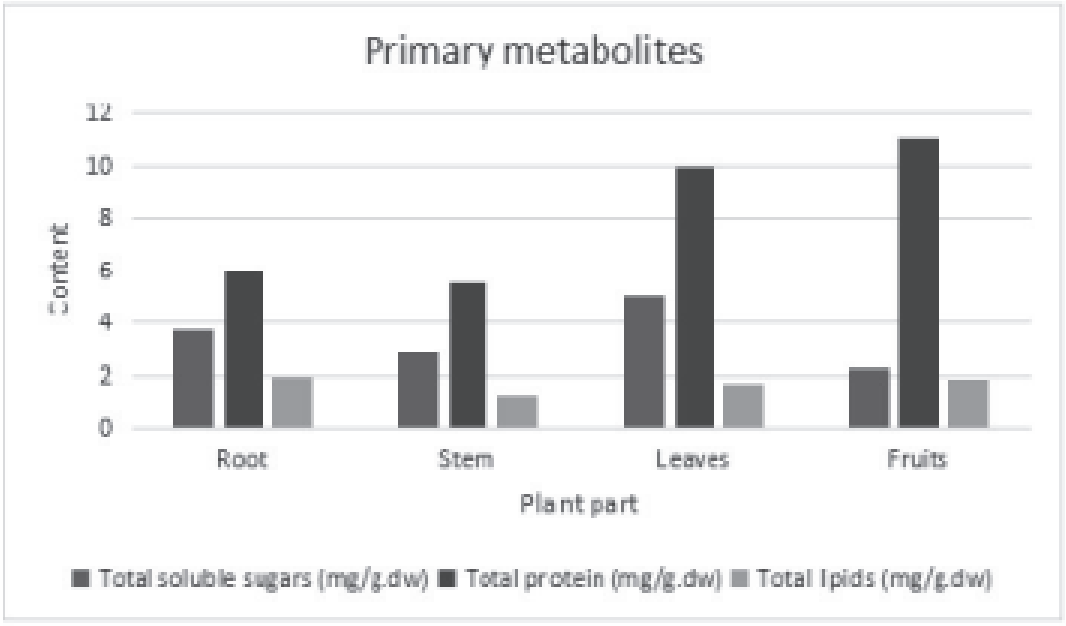
To determine the phenolic content a spectrophotometric method[31] was used given by Singleton. Using a volumetric flask, 1 milliliter of the extract was combined with 9 milliliters of distilled water to make the reaction solution. The solution was then thoroughly agitated after adding 1 milliliter of Folin-Ciocalteu phenol reagent. 10 ml. of a 7% sodium carbonate ($NaCO_3$) solution was added after five minutes. Distilled water was used to modify the final volume. For the calibration curve, standard Gallic acid solutions were prepared with different concentrations following the same procedure. At room temperature, the samples and standards were left to incubate for ninety minutes. Next, using a UV/Visible spectrophotometer, the absorbance of the standards and test samples was measured at 550 nm in comparison to a reagent blank. Total phenolic content in the extract was determined by comparing its absorbance with that of the Gallic acid standards.

Determination of tannin Content : The tannin was quantified by using a most common method[28]. To measure the tannin content, 0.1 ml of the plant sample extract was mixed with 7.5 ml of distilled water. After adding 1 ml of 35% Na, $CO\mathit{f}$ solution and 0.5 ml of Folin-Ciocalteu phenol reagent, the volume was increased to 10 ml using distilled water. The mixture left at room temperature for 30 minutes after shaken thoroughly. Standard solutions of different concentrations of gallic acid were prepared. A blank was used as a reference when measuring absorbance at 725 nanometre. Now, the tannin concentration was calculated.

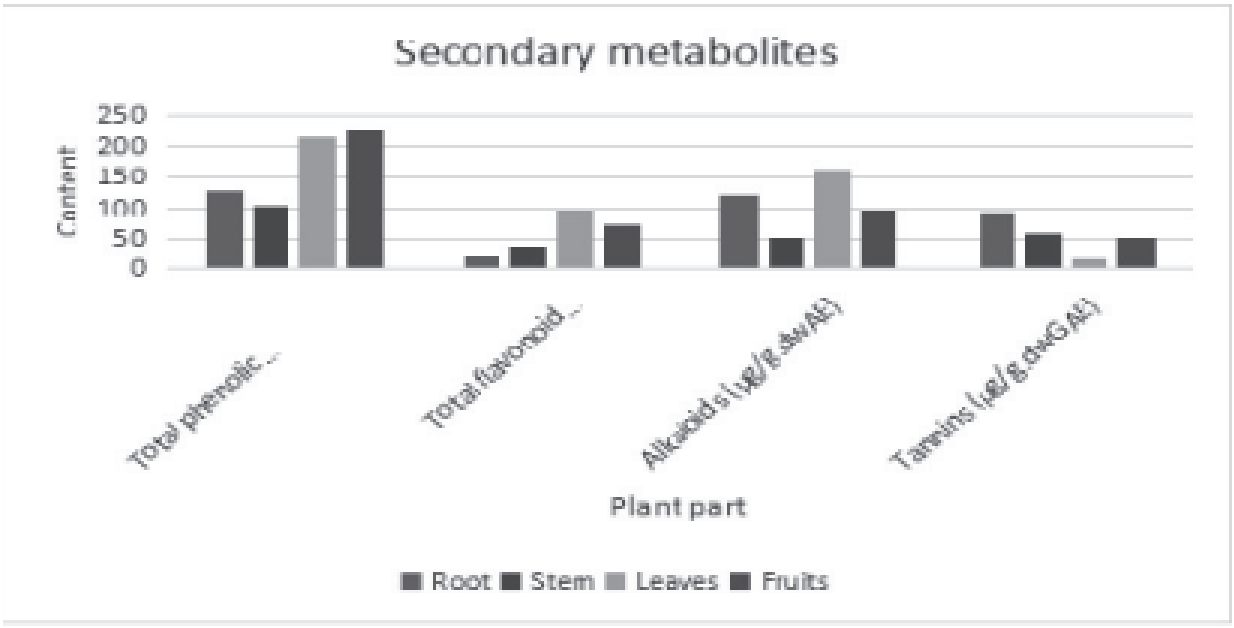
Determination of Alkaloids : The extract of selected plant was prepared by dissolving 1 mg of the sample in dimethyl sulfoxide (DMSO), followed by the addition of 1 ml HCl (2N), and then filtered it. This filtrate was moved to a separating funnel, where five milliliters of phosphate buffer and bromocresol green solution were added. By using varying volumes of chloroform (1, 2, 3, and 4 ml) the mixture was then vigorously shaken. Afterward, this final volume was made up in a 10 ml

volumetric flask with chloroform. Standard solutions of atropine were made in varying concentrations. At 470 nm, the absorbance of standard and test samples was taken. Now, the alkaloids quantity was quantified [9, 27].

Calculating the Total Flavonoid Content : Using the aluminum chloride colorimetric method, the total flavonoid content was measured. Firstly, 5% sodium nitrite in 0.30 ml quantity was added into the flask, followed by 0.3 ml of 10% aluminum chloride. After 5 minute incubation, 2 ml (1M) sodium hydroxide was mixed and the final volume was adjusted. Standard solutions of quercetin were prepared in the same way. At 510 nm, the absorbance of the test and reference solutions was determined [6, 13, 35].



(A)



(B)

Fig. 1: : Quantitative determination of metabolites in different plant parts of genus *Tribulus* collected from Jaipur district (A) Primary metabolites (B) Secondary metabolites

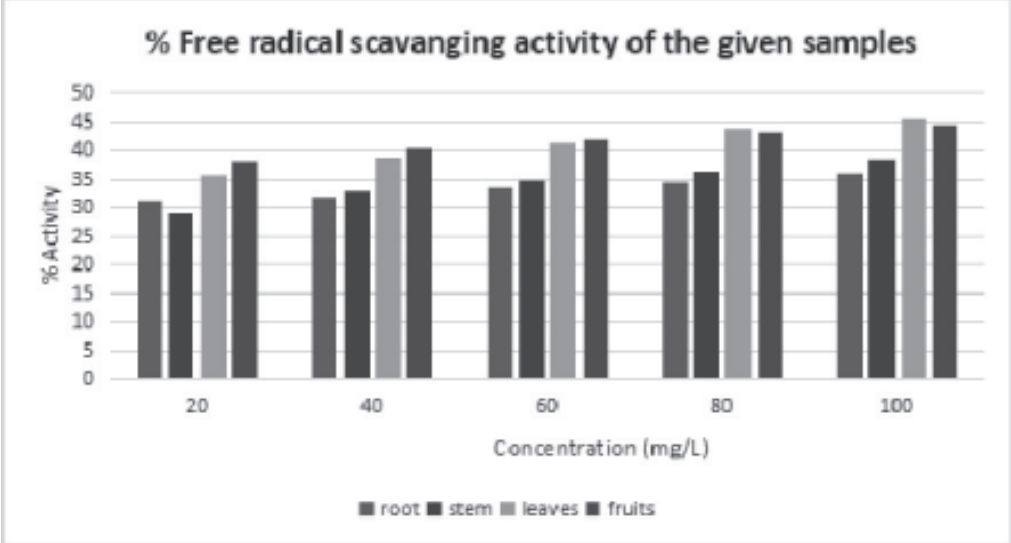


Fig. 2: Showing % free radical scavenging activity at different concentrations in different part of plant in genus *Tribulus*

Determination of free radical scavenging activity

It was measured using Blois's DPPH method[3]. For this, taken the different concentrations of the plant extract, dissolved in methanol, and were combined with a DPPH methanol solution. After shaking the mixture, it was left for half an hour in dark at room temp. At 515 nm, the absorbance was measured.

Results and Discussion

Taking consideration of all findings of wide-ranging medicinal value of this family Zygophyllaceae, it is significant to research on its genus *Tribulus*, which have various therapeutic properties. The findings revealed that different aerial parts and roots of *Tribulus terrestris* contained a variety of primary and secondary metabolites. Current study revealed the presence of important phytochemicals which are well known for showing medicinal properties. Primary metabolites and secondary metabolites are formed and stored in different plant parts. The results from the quantitative analysis of the primary and secondary metabolites in the selected plant are summarized in Tables 1 and 2. These findings are also illustrated graphically in Figures 1 and 2, respectively.

Total soluble sugars (TSS), proteins and lipids are primary metabolites which were determined in the present study. Total soluble sugars are maximum amount 5.01 (mg/g.dw) in leaves, proteins are present in maximum amount 11.1 (mg/g.dw) in fruit and lipids are present in maximum amount 1.91 (mg/g.dw) in root. Results revealed that the used plant parts are rich in these primary metabolites which are precursors for various secondary metabolites. So, these plant parts were also found to be rich in secondary metabolites (total phenols, flavonoids, alkaloids, tannins). In our research finding highest amount of alkaloids and flavonoids are present in leaves while total phenolic content are maximum in fruits and roots are rich in tannins. Therefore plant serves as a good source in pharmaceuticals.

Conclusion

From the current study's findings, it can be concluded that the selected plant is a valuable source of pharmaceutically significant phytochemicals. Thus, it seems likely that the various bioactive compounds were detected in the plant *Tribulus terrestris*. The present results showed that the various extracts of root, stem, leaves and fruit exhibited a rich amount of these metabolites. This proves that this plant could be used as a natural source for human welfare. This research showed that highest amount of alkaloids, flavanoids and total soluble sugars are present in leaves while proteins and total phenolic content are maximum in fruits. Likewise, roots are rich in tannins and total lipids. The chemical composition and biological activity of ***Tribulus terrestris*** is highly influenced by various factors, including the conditions under which it grows including soil quality, climate, environmental conditions and timing of harvesting. All of these factors are crucial in shaping the plant's chemical composition and influencing the potency of its bioactive compounds[20]. So also in our opinion Research on the genus *Tribulus* highlights significant biochemical variations across species due to differences in bioactive compounds like saponins and flavonoids. Geographic location, Climate and soil, are some environmental factors that can further influence the plant's chemical composition. Additionally, harvesting

time and processing methods play a role in the variability of its medicinal properties.

Future perspectives

This paper would be a valuable reference for future studies on the genus *Tribulus* and other similar plants, providing valuable insights into their pharmacological and phytochemical properties. Further research is required to clarify the pathophysiological mechanisms of *Tribulus terrestris* and explore its potential for treating various diseases.

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