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Short Communication

Environmental Applications and Bio-Profiling of *Tribulus Terrestris:* an Ecofriendly Approach

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Abstract

The antibacterial and antioxidant activity of different parts of *Tribulus terrestris*, such as leaves and roots, was carried out using methanol and n-hexane as solvents. The leaves of plant have greater extraction yield than roots. The antibacterial activity was checked against both gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*). Antibacterial activity was performed by disk inhibition method. Leaves show greater antibacterial activity (29 mm and 30 mm) against both *S. aureus* and *E. coli*, whereas roots show (16 mm and 21 mm) against both species respectively. Antioxidant activity was carried out by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging free-radical assay. Leaf extract showed greater antioxidant activity (73%) than that of roots (52%). Total phenolic content (TPC) and total flavonoid content (TFC) were determined by spectrophotometer analysis. The results indicate that leaf extract contains higher phenolic and flavonoid contents (723 mg Gallic acid equivalents (GAE)/g and 476 mg Quercetin equivalents (QE)/g as compared to roots extract (235mg GAE/g and 93 mg QE/g).

Keywords: *Tribulus terrestris*, antibacterial, antioxidant, total phenolic content, total flavonoid content

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Fig. 1. *Tribulus terrestris* plant with flowers and seeds.

Introduction

Tribulus terrestris is an annual plant widely distributed in the world and usually grows in a dry climate location where only a few other plants can survive. It can grow more upwards in the shade and is recognized among the taller plants [1]. *Tribulus terrestris* is common in sandy soils of India, Pakistan and Sri Lanka. *Tribulus terrestris* (Fig. 1) is recognized by different names such as Puncture Clover, Bhakra and Gokhru [2].

This plant shows significant enhancement of sperm concentration and motility and is used as an anti-infertility drug [3]. *Tribulus terrestris* is used in treatment of diabetes [4], heart disease [5], liver disease [6], and kidney disease [7]. It also shows anti-inflammatory [8], antibacterial [9], anti-oxidant [10-13] and antitumoral properties [14, 15].

The drugs extracted from plants are cost-effective and less toxic, with few side effects. Due to this reason, various phytochemicals isolated from plants are being used in the pharmaceutical industry, the most important of which are alkaloids, flavonoids, and phenolic compounds [16-18]. Extensive research on the role of free radicals has been in process and there are various activities in which free radicals are produced in our environment. Higher concentrations can damage all macromolecules and eventually cause cell death. Free radicals have applications in a variety of ways and for curing multiple diseases like atherosclerosis, cancer and heart disease. The phytochemicals possess a multiple of bioactive properties, including antioxidant potential due to phenolic compounds. It is a basic need to understand the mechanism of action, therapeutic dose, bioavailability and bio-efficacy of theses phytochemicals [12, 19-23].

The antioxidant activity of *Tribulus terrestris* is due to its phenolics, flavonoids and flavone constituents. Antioxidant-based drugs are used in the treatment of diabetes, stroke and cancer diseases. The objective of this research is to evaluate phytochemical profiles of *tribulus terrestris* to correlate possible antibacterial as well as antioxidant properties of plants. Rifampicin was used as a standard, which is a broad-spectrum antibiotic with trade name rifadin and is effective in treating various bacterial infections like tuberculosis, leprosy, etc. [24].

Material and Methods

The sample of *Tribulus terrestris* was collected from the botanical garden at the University of Agriculture, Faisalabad, dried and washed for many times with distilled water to remove dust particles and stored with identification number TT-UOL-BOT-Herb-14. The dried parts of plant were ground to fine powder and stored at room temperature in a screw-top container. The powdered sample (200g) was soaked in methanol and n-hexane with a sample:solvent ratio of 1:5. The samples were kept in a sealed container for seven days with occasional stirring and shaking. The resulting mixtures were then filtered and concentrated by evaporation. The final obtained extracts from leaves, roots of *Tribulus terrestris* and standard (Rifampicin) in methanol were labeled as TTM-L, TTM-R and TTM-S, whereas TTH-L, TTH-R and TTH-S for n-hexane which were then subjected to antibacterial and antioxidant activities.

Antibacterial Activity

Antibacterial activity was carried out against *E. coli* and *S. aureous* by disk inhibition method. Nutrient agar (30 g) was mixed well in 1 liter distilled water and then sterilized for 20 minutes by autoclaving at 123ºC. The sterilized media was transferred to already sterilized petri plates. Then small filter paper discs were placed on media having 100 µg extracts of *Tribulus terrestris*. The petri plates were incubated for 24 hours at 37ºC for bacteria growth. Methanol and n-hexane extracts of plants inhibited the growth of bacteria due to their antibacterial activity, which resulted in the formation of clear spots. These clear spots were measured in millimeters with the help of a zone reader [25, 26].

Antioxidant Activity

The antioxidant activity of plant extract was carried out by DPPH assay [27]. The plant extract was added to 0.1mM methanolic and n-hexane solutions of DPPH and incubated for 30 minutes at 37ºC. The absorbance of resulting samples and standard (ascorbic acid) was taken, and scavenging was calculated by using the relationship:

$$\text{DPPH radical scavenging activity (\%)} = 100 \times (A_{blank} - A_{sample}) / A_{blank}$$

…where A_{blank} is absorbance of reaction mixture without test compound and A_{sample} is absorbance of test sample [28, 29].

TPC has been calculated using Folin-Ciocalteu reagent [30, 31]. In this method, 2 ml extract of each

part (i.e., roots and leaves in methanol and n-hexane) were kept in separate test tubes having 10 ml distilled water, and then 1 ml Folin-Ciocalteu reagent was added. The samples were incubated for 90 minutes and absorbance was taken at 765 nm. The amount of TPC was calculated as Gallic acid equivalents, i.e., mg GAE/g.

Aluminium chloride hexahydrate is used as a reagent for analysis of TFC [32, 33]. The reagent is prepared by dissolving 0.073g of aluminium chloride hexahydrate in 30 ml methanol and n-hexane. 2 ml of reagent was taken in separate test tubes and 2 ml of plant extracts from TTM-L and TTM-R and 2ml from TTH-L and TTH-R is added in each test tube and then incubated the sample for 30 minutes. The absorbance was taken at 430 nm. Quercetin was used as standard and the amount of TFC was calculated as quercetin equivalent antioxidant g/100g.

Gas Chromatography Mass Spectrometry Analysis

GC-MS analysis was performed using GC (Perkin Elmer) interfaced to a mass spectrometer equipped with Elite-5MS. Carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed in split mode. The oven temperature was programmed from 110ºC, with an increase of 10ºC/min up to 200ºC, and then it was increased at the rate of 5ºC/min to 280ºC. Injector temperature was 250ºC. Total GC running time was 26 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software Turbo mass 5.2 was used for analysis. The spectrums of the unknown components were compared with the spectrum of the known components stored in the database of the National Institute Standard and Technology (NIST), version-year 2005 [34].

Statistical Analysis

All the experiments were conducted in triplicate in order to get the results in terms of mean±SD. The variation among means was considered significant for probability (p)< 0.05) at 95% confidence interval.

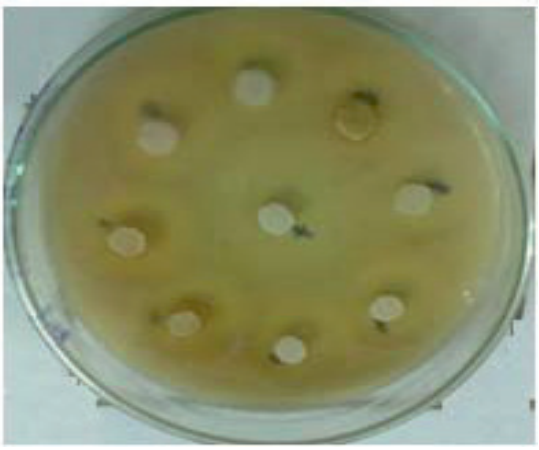

Fig. 2. Antibacterial activity of leaves extracts of *Tribulus terrestris* in methanol against *E. coli.*

Results and Discussion

Extraction of leaves and roots was carried out in methanol and n-hexane and results indicate higher extraction yield from leaves than that of roots in methanol. The extracts from roots and leaves (TTM-L and TTM-R) were subjected to antibacterial activity against *E. coli* and *S. aureus,* taking Rifampicin as standard. Highest antibacterial activity was shown by TTM-L, which inhibits the growth of 29 mm against *S. aureus* and 30 mm against *E. coli*. TTM-R inhibits 16 mm growth against *S. aureus*, in contrast with 21 mm against *E. coli*. The results were compared with Rifampicin standard TTM-S, which shows 33 mm inhibition against gram positive bacteria *S. aureus*, whereas 37 mm inhibition against gram negative bacteria *E. coli* has a higher value than both extracts (TTM-L and TTM-R) of Tribulus *terrestris* (Table 1). The antibacterial activity of TTH-L, TTH-R and TTH-S is less than that of methanolic extracts (Fig. 2).

Antioxidant activity of leaf and root extracts of *Tribulus terrestris* was performed by the DPPH scavenging free radicals assay. Methanolic leaf extract (TTM-L) shows highest antioxidant activity, which is 73%, whereas roots extract (TTM-R) shows 52% antioxidant activity as shown in Fig. 3. Ascorbic acid was taken as standard, which showed higher antioxidant

Table 1. Diameter zone of inhibition (mm) of leaf and root extracts against *S. aureus* and *E. coli,* and radical scavenging activity, TPC and TFC of *Tribulus Terrestris* extracts.

	TTM-L	TTM-R	TTM-S	TTH-L	TTH-R	TTH-S
S. aureus	29±2^{d}	16±1.5^{d}	33±2.0^{b}	10±1.5^{d}	6±2.0^{c}	13±1.5^{b}
E. coli	30±2.5^{d}	21±2.0^{d}	37±2.0^{b}	9±2.0^{d}	8±1.5^{c}	17±1.5^{b}
Antioxidant activity (%)	73±3.0^{c}	52±3^{c}	91±3.0^{a}	31±3.0^{c}	24±2.0^{b}	42±2.0^{a}
TPC	723±2.5^{a}	235±2.5^{a}	--	221±3.5^{a}	83±1.5^{a}	--
TFC	476±3.0^{b}	93±1.8^{b}	--	184±2.0^{b}	79±2.0^{a}	--

The superscript in column showing significant differences among parameters (P<0.05)

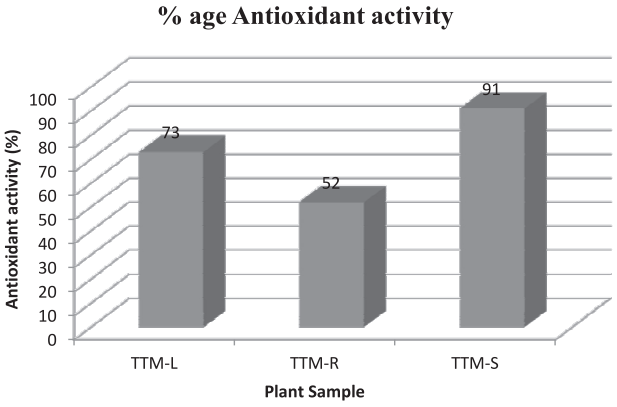

Fig. 3. Antioxidant activity of leaf and root extracts of *Tribulus terrestris* in methanol.

activity (91%) than all extracts studied. The variation in the antioxidant activity of leaves and roots is due to variation in the ability of these parts to dissolve in methanol. Leaves dissolve easily in methanol and their extraction yield as well as antibacterial and antioxidant activities is higher than that of roots extract. The antioxidant activity of TTH-L, TTH-R and TTH-S is less than that of methanolic extracts.

The antioxidant potential of most reported plants is due to the phenolic compounds present in these plants [35-40]. In phenolic compounds, larger groups of bioactive compounds are present, which impart them various biological properties such as antioxidants, antifeedants, pigmentation contributors, etc. [41-46] and also protect plant pigments from UV light. Previous study [21] focuses on evaluating TPC from the family Asteraceae. The maximum phenolic contents were found in hydro-alcoholic extract (691.6 mg/g GAE) in comparison with aqueous extract. Sytykiewicz et al. [47] showed that methanolic extract from walnut leaves characterized by the highest anticandidal activity.

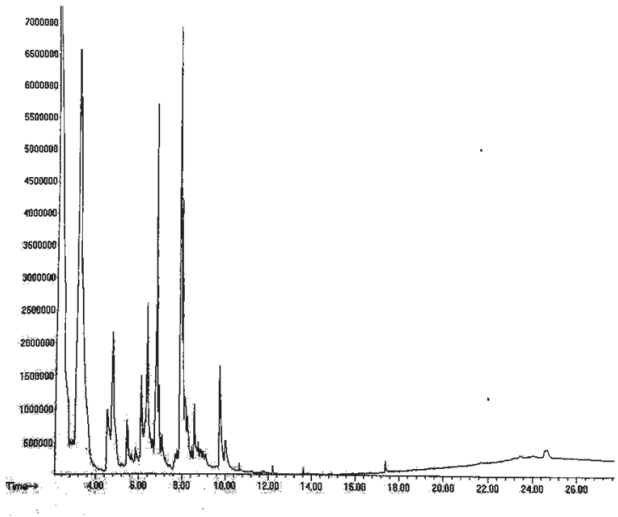

Fig. 4. Mass chromatogram of *Tribulus terrestris*.

The compounds present in the ethanolic extract of *Tribulus terrestris* were identified by GC-MS analysis. Mass spectrum exhibiting the peak identities of the compound (Fig. 4). Ten compounds were identified in this extract. n-Hexadecanoic acid, 2-Cyclopentene-1-undecanoic acid, Didodecyl phthalate, squalene, 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione (3.10%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 4-Cyclopropylcarbonyloxytridecane, Z-10-Tetradecen-1-ol acetate, Methoxyacetic acid, 1-Nonadecanol.

Highest phenolic content (723 mg GAE/g) was shown by methanolic extract of leaves (TTM-L) whereas methanolic extract of roots (TTM-R) gives less (235 mg GAE/g) phenolic contents. Similarly, the flavonoids contents of leaves extract are higher (476 mg QE/g) than that of roots extract (93 mg QE/g). TPC and TFC values (Table 1) of TTH-L, TTH-R and TTH-S are also less than that of methanolic extracts. Another study [48] exhibited that *Tribulus terrestris* has excellent antioxidant and free-radical-scavenging properties, so it can improve human sperm motility and viability. On these grounds, it is concluded that the extract from different part of the plant can be used as a safe therapeutic alternative for the management of motility dysfunction. Phytochemical studies [49, 50] were carried out on aerial parts of plants and it was found out that it possesses potent antioxidant activity. Phenolic contents derived from plants are strongly associated with antioxidant activity [51].

Conclusions

In present research work, different parts of *Tribulus terrestris* were compared for biological activities and phenolic composition. The results for antibacterial and antioxidant activity of different parts of *Tribulus terrestris* such as leaves and roots reveal that leaves of plant have grater extraction yield than roots. TTM-L shows greater antibacterial activity against both *S. aureus* and *E. coli* as compared to TTM-R. The results indicate that leaves extract contains higher phenolic and flavonoid contents as compared to roots extract. Furthermore, the leaves of *Tribulus terrestris* have high extraction yield and show better antibacterial and antioxidant activity than any other part. TPC, TFC, antibacterial and antioxidant activities of TTH-L, TTH-R AND TTH-S are less than that of corresponding methanolic extracts.

Conflict of Interest

The authors declare no conflict of interest.

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