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PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF *TRIBULUS TERRESTRIS* L.

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ABSTRACT

Tribulus terrestris L. is an important shrub which is traditionally used as folk medicine. The aim was to investigate phytochemicals, antioxidant and anti-microbial potential of the shrub that will potentiate its significance in traditional medicine. Ethanol (EAI), chloroform (CAI) and aqueous (AAI) extracts of *T. terrestris* L. leaves were taken to perform these experiments. Phytochemical screening was done qualitatively and quantitatively. Antioxidant effect of the extracts was evaluated by 1, 1 Diphenyl −2- picrylhydrazyl (DPPH) radical scavenging activity. Anti-microbial potential was determined by Agar diffusion method. Phytochemical screening revealed the presence of major phytoconstituents. TPC and TFC were expressed as gallic acid and quercenin equivalents. Among the three extracts, EAI showed the highest TPC and TFC. In DPPH test, IC_{50} value of EAI, CAI and AAI were 25.11±2.57, 35.48±3.29 and 50.11±3.40 µg/ml, respectively. In anti-microbial assay, both the EAI and CAI showed zone of bacterial growth inhibition of the tested organisms as concentration dependent manner whereas AAI did not show any inhibition up to 500 µg/ml concentration.

KEYWORDS: *Tribulus terrestris*, Antioxidant activity, Anti-microbial activity, Phytochemical screening, Radical scavanging assay.

INTRODUCTION

Tribulus terrestris L. is a small shrub under the Zygophyllaceae family, native to tropical and subtropical regions.[1] It is a perennial shrub, softly tomentose and grows up to 3m in height. The leaves are ovate, acuminate, toothed, rarely subtrilobate and 1.9-2.5cm long. The flowers are yellow in colour, peduncle jointed above the middle. The fruits are capsule, densely

pubescent, with conspicuous and horizontally spreading beaks. The stems are stout, branched, 1-2m tall, pubescent. The seeds are 3-5mm; reniform, tubercled or minutely stellate-hairy, 5-8 black or dark brown.[2]

It is extensively used in folk medicine as demulcent, diuretics, anti-diabetic, anthelmintic, astringent, laxative, expectorant, antibacterial, antifungal activities.[3] It is also used as aphrodisiac, sedatives, expectorant, tonic, anti-inflammatory, anthelmintic and analgesics.[4] It is applied in snakebite, leprosy, piles, lumbago, jaundice, ulcer, toothache and liver disorders.[5] It needs only heat and sun and grows even in dry and poor soils. It is quite common in India on road sides and waste places, growing usually after the rains and flowering during winter.

The present study was undertaken to investigate phytochemical constituents, antioxidant and antibacterial effect of ethanol, chloroform and aqueous extracts of *T. terrestris* L. that may unveil the rationality of use of the plant as traditional medicines and potentiality of it in the herbal medicine.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *Tribulus terrestris* L. were collected, cleaned and dried for one week and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

Extract preparation

Approximately 500g of powdered material was placed in a clean, flat-bottomed glass container and soaked in ethanol. Similarly 300g of the powder was soaked in distilled water and in chloroform separately. All the containers with its contents were sealed and kept for 5 days. The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extract then was filtered through Whatmann No.1 filter paper and dried by electric oven at 45^{o}C temperature. The gummy extracts were stored in an air tight container.

Phytochemical screening

Qualitative tests of the EAI, CAI and AAI for the presence of alkaloids, saponins, terpenoids, flavonoids, tannins, steroids, reducing sugars and anthroquinone[6] and determination of phytoconstituents[7] were also carried out.

DPPH radical scavenging activity[8]

The DPPH free radical scavenging activity of the extracts (EAI, CAI and AAI) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Briefly, 0.004% w/v of DPPH radical solution was prepared in methanol and then 900µl of this solution was mixed with 100µl of extract solution (12.5–200µg/ml) and kept in a dark place for thirty minutes. Then absorbance was measured at 517nm where methanol (98%), DPPH solution and ascorbic acid were used as blank, control and standard antioxidant respectively. Scavenging capacity of DPPH radicals (% Inhibition) was measured by the following formula and finally 50% inhibition concentration (IC_{50}) was calculated using Microsoft Excel software.

Inhibition (%) = $(A_0-A_S)/A_0 \times 100$

Where A_0=Absorbance of control group, A_S= Absorbance of sample

Antibacterial Activity

Microbial strains tested

The following strains of Gram positive and negative bacteria were used. Gram positive bacteria: *Sarcina luteae* ATCC# 9341, *Bacillus cereus ATCC#* 14579, *Bacillus megaterium ATCC#* 10778, *Staphylococcus aureus* ATCC# 25923. Gram negative bacteria: *Salmonella typhi* ATCC# 19430, *Escherichia coli* ATCC# 1053, *Shigella dysenteriae* ATCC# 11835, *Vibrio parahemolyticus* ATCC# 17802, *Pseudomonas aeruginosa* ATCC# 27853. Kanamycin was used for this purpose.

Determination of antimicrobial activity[9]

The antibacterial tests were performed using agar well diffusion method. Agar plates were prepared by using sterile Mueller-Hinton (MH) agar. Bacterial cultures of standardized cultures were prepared by adding the seed culture in the autoclaved agar medium followed by pouring into petriplates. The wells were made with 8 mm sterile cork borer. 50µl of each extract (125, 250, 500µg/ml) was added in the pre labeled wells together with ethanol, chloroform and water as negative control and kanamycin as positive control. The plates were observed for the presence of inhibition of bacterial growth and that was indicated by clear zone of inhibition of bacterial growth around the wells. The size of zone of inhibition was measured in millimeters (mm).

RESULTS

Phytochemical screening

The preliminary phytochemical screening of the extracts (EAI, CAI and AAI) revealed the presence of secondary metabolites such as alkaloids, saponins, terpenoids, flavonoids, tannins, steroids, reducing sugars and anthroquinone (Table 1).

Total phenol content (TPC)

Phenolics are important classes of phytochemicals that possess significant antioxidant and subsequently biological functions. Quantitatively, phenolics have measured in terms of total phenolic contents by using Folin-ciocalto reagent test and expressed as gallic acid equivalent. EAI, among the three extracts have shown highest (212.36±8.23 mg/g gallic acid) TPC. CAI and AAI exhibited 190.40±9.47 and 90.23±5.78 mg/g gallic acid of TPC respectively at 200µg/ml concentration. TPC of the extracts varied significantly ($p < 0.01$) with each other (Table 2).

Total flavonoid content (TFC)

Total flavonoid content (TFC) of the three extracts was measured as quercetin equivalents. EAI presented the highest TFC (85.69±8.12) followed by CAI (72.37±5.89) and AAI (55.10±5.08) at 200µg/ml concentration. The flavonoid content of the three extracts was significantly different from each other ($p < 0.01$) (Table 2).

DPPH radical scavenging assay

In this assay ascorbic acid (AA) is used as a standard antioxidant. Here, all the three extracts of *Tribulus terrestris* L. have shown inhibition of the DPPH radicals as concentration dependent manner. Scavenging activity is also expressed as median inhibition concentration (IC_{50}) value. Here, less IC_{50} value of the extracts indicates more antioxidant power.

Among the three extracts of *T. terrestris* L. the crude ethanol extract (EAI) showed maximum inhibition (47.12±3.69 to 92.08±6.23%) in respect to dose ranging from 12.5 to 200µg/ml) and minimum IC_{50} value (25.11±2.57 µg/ml). CAI showed moderate inhibition (24.36±2.17 t0 84.20±6.12%) in the same dose. AAI showed least Inhibition (21.45±2.45 to 83.20±6.78%) in the same dose with the highest IC_{50} value of 50.11±3.40µg/ml. The standard AA showed least value of IC_{50} (12.58±2.13 µg/ml) indicating the highest antioxidant potential (Table 3).

Antimicrobial Activity

The antimicrobial activity of each extract was monitored in three different concentrations such as 125, 250 and 500 µg/ml. The activity of each extract was then compared against the standard drug kanamycin at 50 µg/ml dose. The samples were run in triplicate to get a complete picture of effectiveness of extracts against each experimented microorganism. The effectiveness of the extracts was measured in the form of zone of growth inhibition.

The extracts were tested against Gram positive and Gram negative strains of bacteria. Table 4 represents the antimicrobial activity (zone of inhibition) of the three extracts and the standard kanamycin against respective microbial strain. Kanamycin showed maximum inhibition against the organisms. Among the three extracts of *T. terrestris* L. EAI showed maximum zone of inhibition followed by CAI. On the other hand, AAI had no potential to inhibit growth of any of the organisms up to 500µg/ml dose.

DISCUSSION

In recent years, there has been an increasing interest in finding natural antioxidants from medicinal plants.[10] Antioxidants are the prominent bioactive compounds that can effectively protect cells of different organs from damage by opposing the activities of the free radicals.[11] Recently, interest has considerably increased in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenesis. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. Hence, studies on natural antioxidants have gained increasingly greater importance.[12] The antimicrobial effects of plant materials are believed due to secondary products present in the plant, although it is usually not attributed to a single compound, but to a combination of metabolites.[13]

It has been unveiled that *Tribulus terrestris* L. contains many biologically active compounds such as phenols, tannins, alkaloids, flavanoids glycosides, proteins, amino acids, sesquiterpenes, steroids, sterols, terpenoids, terpenes, carbohydrate, β– sitosterol, gallic acid, p-coumaric acid, quercetin-3- O-beta glucopyranoside etc.[14] So, the anti- microbial effect of the extracts is due to any of these compounds. However, further analysis is necessary to separate these compounds and to find out the most active compound having antimicrobial effect as well as to clarify the actual mode of microbial growth inhibition of that compound.

Table 1: Results of preliminary phytochemical screening of three solvent extracts of *Tribulus terrestris* L.

Phytochemicals	Extracts		
	EAI	CAI	AAI
Alkaloids	+	+	+
Saponins	+	-	+
Terpenoids	+	-	+
Flavonoids	+	+	+
Tannins	+	-	+
Steroids	+	-	+
Reducing sugars	+	+	+
Anthroquinone	-	-	-

Table 2: Total phenol content, total flavonoid content and extraction yield of EAI, CAI and AAI.

		Extracts	
Phytoconstituents			
	EAI	**CAI**	**AAI**
Total phenolic contents (mg gallic acid equivalent/g)	121.23±6.25	87.21±7.26	48.78±3.27
	175.69±7.12	115.26±8.25	75.36±4.36
	212.36±8.23	190.40±9.47	90.23±5.78
Total flavonoid contents (mg quercetin equivalent/g)	32.45±3.47	24.36±2.78	17.35±1.27
	62.72±5.23	50.10±3.45	43.58±3.56
	85.69±8.12a	72.37±5.89a	55.10±5.08
Extraction yield (%)	3.05±0.14	1.66±0.25	4.33±0.10

Table 3: Antioxidant capacity of EAI, CAI and AAI of *Tribulus terrestris* L.

Sample	Concentration (µg/ml)	% Inhibition
	12.5	43.25±3.10
	25	66.75±4.12
AAI	50	78.23±3.20
	100	86.12±4.26
	200	94.23±5.12
	12.5	35.35±2.45
	25	47.12±3.69
EAI	50	68.47±4.12
	100	84.10±6.58
	200	92.08±6.23
	12.5	24.36±2.17
	25	37.20±3.47
CAI	50	59.28±3.10
	100	75.12±3.72
	200	84.20±6.12
	12.5	21.45±2.45
	25	32.34±3.45
AAI	50	51.07±4.56

	100	76.41±5.78
	200	83.20±6.78

Table 4: Antimicrobial activities of three *Tribulus terrestris* L. extracts against gram positive and gram negative bacteria.

Sample	Concentration (µg/ml)	Zone of inhibition (mm)				
		EAI	CAI	AAI	Control (Solvent)	Kanamycin 50 µg/ml
***Sarcina luteae* (ATCC#9341,+ve)**	125	10.20±0.25	8.23±0.12	-	-	
	250	14.50±0.30	12.58±0.37	-	-	30±2.15
	500	20.23±0.15	16.42±0.45	-	-	
***Bacillus cereus* (*ATCC*#14579, +ve)**	125	11.42±0.52	9.50±0.24	-	-	
	250	16.33±0.62	14.40±0.36	-	-	32±3.10
	500	23.25±0.45	19.36±0.60	-	-	
***Bacillus megaterium* (*ATCC*#10778,+ve)**	125	8.55±0.20	6.78±0.27	-	-	
	250	10.20±0.35	8.56±0.21	-	-	25±2.40
	500	15.56±0.45	13.25±0.22	-	-	
***Staphylococcus aureus* (ATCC#25923, +ve)**	125	11.07±0.64	10.39±0.69	-	-	
	250	15.12±1.32	13.20±0.78	-	-	28±3.55
	500	22.21±1.56	20.58±1.04	-	-	
***Salmonella paratyphi* (ATCC#19430, -ve)**	125	9.15±0.56	7.25±0.37	-	-	
	250	14.32±0.15	12.36±0.12	-	-	26±3.10
	500	20.05±1.26	18.25±0.39	-	-	
***Escherichia coli* (ATCC#1053, -ve)**	125	12.70±1.03	10.65±0.28	-	-	
	250	17.64±0.62	14.23±0.69	-	-	32±2.08
	500	23.36±1.62	19.30±1.15	-	-	
***Shigella dysenteriae* (ATCC#11835, -ve)**	125	9.14±0.78	7.65±0.57	-	-	
	250	14.15±0.65	9.45±0.39	-	-	29±3.10
	500	18.25±0.73	15.74±0.48	-	-	
***Vibrio parahemolyticus* (ATCC#17802, -ve)**	125	12.15±0.15	8.45±0.23	-	-	
	250	17.23±0.71	13.10±0.47	-	-	31±2.65
	500	22.20±0.64	20.22±0.37	-	-	
***Pseudomonas aeruginosa* (ATCC#27853, -ve)**	125	12.09±0.32	10.45±0.78	-	-	
	250	16.78±1.10	15.54±1.07	-	-	33±3.45
	500	24.34±1.46	21.23±1.49	-	-	

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