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Evaluation of phytochemical compounds and antimicrobial activity of leaves and fruits *Tribulus terrestris*

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ABSTRACT

Antimicrobial activity of methanolic and aqueous extracts from fruits and leaves of Tribulus terrestris were examined against seven pathogenic bacteria Staphylococcus Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, aureus, Staphylococcus saprophyticus, Enterococcus faecalis and Enterobacter cloacae using disc diffusion method. Extracts from the leaves and fruits of the plant showed antimicrobial activity against most tested microorganisms. The most active extract was methanolic extract from the fruits against P. vulgaris. Minimum inhibitory concentration for aqueous and methanolic extracts of leaves ranged from 15.0–60.0 mg/ ml and 5.0–20.0 mg/ml and aqueous and methanolic extracts of fruits ranged from 10.0–40.0 mg/ ml and 5.0–15.0 mg/ml respectively Phytochemical analysis was showed the presence of flavonoids, alkaloids, saponins, tannins and carbohydrates.

Key words: Antimicrobial, aqueous, methanolic, pathogenic, minimum inhibitory concentration

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INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in same chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [1]. Plant derived substances have recently become of great interest owing to their versatile applications [2]. It has been estimated that 14-8% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants [3].

In the last few years, the search continues for safe and effective antimicrobial agents with which can be treated a wide variety of bacterial infections. This need has been heightened recently by the emergence of many antimicrobial-resistant organisms [4]. This worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. [5]

Plants are rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids, and flavanoids which have been proved invitro to have anti microbial properties. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [6].

Tribulus terrestris (Puncture Vine, Caltrop, Yellow Vine and Goat head) is a flowering plant of the Zygophyllaceae family, native to warm temperature and tropical regions of the old world in Southern Europe, Southern Asia, Africa and Northern Australia. It can thrive even in desert climates and poor soil [6]. In Iraq *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithontriptic and urinary antiinfectives [7, 8].

The present study was done to screen the phytochemical compounds and antimicrobial activity of aqueous and methanolic extract of fruits and leaves of *Tribulus terrestris* against seven pathogenic bacteria which belongs to two group gram positive and gram negative. These are *Staphyllococcus aureus, Staphyllococcus saprophyticus, Escherichia coli, Enterococcus faecalis, Enterococcus cloacae, Proteus vulgaris,* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Plant Material

The fresh leaves and fruits of *T. terrestris* were collected from Ghaziabad near railway station. Leaves were simple, pinnate and opposite. Fruits are five angled or winged spinous tuberculate woody schizocarp.

Preparation of Plant Extract

Aqueous Extract

One hundred gram of air dried fine powder of fruits and leaves of *T. terrestris* were infused in double distilled water (900ml) until complete exhaustion. The extract was filtered using Whatman filter paper No.1 and the filtrate was evaporated in vacuum rotatory evaporator at 45^{o}C [9].

Methanolic Extract

One hundred of air dried fine powder of fruits and leaves of *T. terrestris* were infused in 85% methanol (900 ml) until complete exhaustion. After that infusion was filtered with four layered muslin cloth and the filtrate was then centrifuge at 10,000 rpm for 20 minutes to remove fine particles. Supernatant was allowed to evaporation vacuum rotatory evaporator at 45^{o}C (Kandil *et al*, 1994) and then 5 ml (5%) DMSO was added to concentrate the one-tenth of the original volume and stored at 4^{o}C in sterile airtight bottles.

Microbial culture

Plant extracts were assayed for antimicrobial activity against seven species of bacteria was used as test microorganisms. Seven bacterial strains *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 10741, *Enterobacter cloacae* ATCC 10699, *Proteus vulgaris* ATCC 12454, *Klebsiella pneumoniae* ATCC 15380, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus saprophyticus* ATCC 35552 were used in our study. Microbial cultures were preserved at -20^{o}C in microcentrifuge tube having 40% sterile glycerol.

Screening of Antimicrobial Activity

Extract were tested against the strains for their inhibitory activity by 'Disc diffusion method'. Nutrient broth for inoculum preparation and MHA (Muller-Hinton agar) media were used for screening the antimicrobial activity. The impregnated discs are then placed onto the surface of a suitable solid agar medium like Mueller Hinton [10], Trypton soy agar [11] or Nutrient agar [12]. The media has been pre-inoculated with test organisms. The standard inoculum size is of 1-2 x 10^{8} CFU/ml of bacteria for inoculating diffusion plates [13] which is equal to McFarland 0.5 turbidity standard. After that standard disc along with test disc placed on the plate incubate the plate at 37^{o}C for 24 hrs. Each test was carried out in triplicate with controls. Microbial growth was determined by measuring the diameter zone of inhibition with the help of scale.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the tube dilution techniques. Varying amounts of the extract in the following concentration 5% to 100% were prepared using single dilution method. An 8 ml of the nutrient broth was pipetted into the various test tubes and sterilized at 121^{o}C for 15 minutes, they were allowed to set. Later each tube was inoculated with an overnight standard inoculum size is of 1-2 x 10^{8} CFU/ml of *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis* and *Enterobacter cloacae* which is equal to McFarland 0.5 turbidity standard and then transferred into the test tube containing the extract. The test tubes were incubated at 37^{o}C for 24 hours. The least concentration of the plant extract that does not permit any visible growth or turbidity of the inoculated test

organisms in broth culture were taken as the minimum inhibitory concentration in each case. Control experiment with plant extract and another tube with no plant extract were also performed [14].

Phytochemical Analysis:
Phytochemical analysis to screen the plants for the presence of alkaloids, saponins, flavonoids and carbohydrates, was performed according to the method described by Sofowora (1993) and Evans (1998) [14, 15].

Test for Alkanoids:
Few drops of the manager's reagent, Drangendorff's reagent, Wanger's reagent or Hanger's reagent and 10% tannic acid solution were added. The presence of precipitate in at least 3 or all of the above reagents was indicated the presence of alkaloids [15].

Test for Carbohydrates:
A few drops of Molisch's reagent were added to 2ml of each of the water extract in two tubes. A small quantity of concentrated sulphuric acid was then added and allowed to form a lower layer. A purple ring at the interface of the liquids indicates the presence of carbohydrates. Each mixture was then shaken and allowed to stand for 2 minutes and diluted with 5ml of water. A purple precipitate also showed the presence of carbohydrates [15].

Test for Tannins (Ferric chloride test):
A portion of the water extract was diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution was added. A blue or green colour was indicated the presence of tannins [15].

Test for Saponins:
A small quantity of the ethanolic extract was boiled. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of the distilled water in a test tube. The test tube was corked and shaken vigorously for about 30 seconds then it was allowed to stand for half an hour. A honeycomb forth was an indicator of the presence of saponins [14].

Test for Flavonoids (Shinoda test):
Four pieces of magnesium fillings was added in ethanolic extract followed by few drops of concentrated hydrochloric acid. A pink or red colour was indicated the presence of flavonoid [16].

RESULTS AND DISCUSSION

Antibacterial Activity of Extracts
The extracts of leaves and fruits of *Tribulus terrestris* was showed antibacterial activity with diameters of zone of inhibition ranging from 8-23 mm. Ampicilin, streptomycin and chloramphenicol discs were used as positive control produced inhibition zones. Distilled water and methanol were used as negative controls not produced zones of inhibition. The antibacterial activity of leaves and fruits extracts of *Tribulus terrestris* is shown in Table 1. The investigation was made on methanolic extract of fruit have highest activity against *P. vulgaris* (23.0 mm), *Staphylococcus aureus* (19.0 mm) and *E. faecalis* (16.0 mm) and least inhibition zone of aqueous extract of leaves was observed against *E. faecalis* (8.0 mm) and *E. cloacae* (9.0 mm).

Table 1: Antibacterial activity of aqueous and methanolic extracts of *Tribulus terrestris* by disc diffusion test

Test Microorganisms	Inhibition Zone in (mm)							
				Aqueous Extract		Methanolic Extract		
	Amp	Strp	Chl	leaf	fruit	leaf	fruit	control
E. coli	-	+	+	13	16	11	14	0
E. faecalis	+	-	+	8	15	13	16	0
E. cloacae	-	-	-	9	14	12	12	0
P. vulgaris	+	+	+	17	19	16	23	0
K. pneumoniae	+	+	+	10	17	11	13	0
S. aureus	+	+	+	12	16	10	19	0
S. saprophyticus	+	+	+	11	12	14	12	0

(-) resistance (+) sensitive, Amp- ampicillin, Strp- streptomycin, Chl- chloramphanicol

Minimum Inhibitory Concentration

The extracts of fruits and leaves were shown considerably good antibacterial activity for each test organisms were selected to determine. Values of MICs were dependent on the bacterial species. MIC values of aqueous and methanolic extract of leaves were 15-60 mg/ml and 5.0-20 mg/ml respectively against bacterial species. Aqueous and methanolic extracts of fruits were shown 10.0-40.0 mg/ml and 5.0-15.0 mg/ml against test bacterial species. MIC values are shown in Table 2.

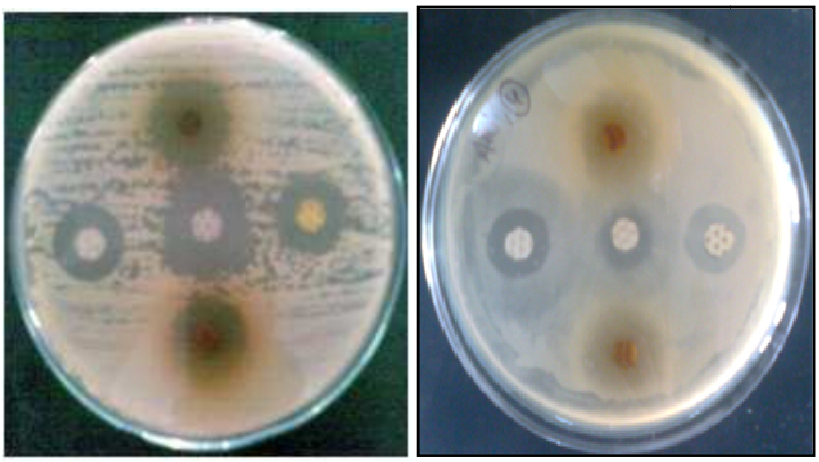

Figure 1: Aqueous and methanolic extracts of *T. terrestris showing inhibition zone*

The evaluation of the invitro antimicrobial activity of ether, chloroform and ethanol extracts of *Tribulus subramanyamii* Linn against standard bacterial and fungal strains by using disc diffusion method. The MIC value of extracts against gram positive bacteria were ranging between 300 to 500 µg, gram negative bacteria were >600µg [17]. The present study result was found to be quite different from those reported by Saied kianbakht and Fereshteh jahaniani and and Firas A. AI- Bayati and Hassan F.AI- Mola. [17,18]. The MIC value of the methanolic extracts of fruits and stems plus leaves against all bacteria was 2 mg/mL and the MIC value of roots against *S. aureus*, *E. faecalis* and *E. coli* was 4 mg/mL and the MIC value of roots against *P. aeruginosa* was 2 mg/mL [7]. The present study was indicated the activity of plant against gram positive and gram negative bacteria. The leaves and fruits of plants were shown antimicrobial activity against all reference bacteria. The highest activity was shown in methanolic extract of fruit against *P. vulgaris* which is a gram negative bacterium that is known to cause urinary tract infections and wound infection.

Table 2: Minimum inhibitory concentration of extracts of *T. terrestris* by broth dilution method

S. No.	Test Microorganisms	MIC of Aqueous extract mg/ml		MIC of Methanolic Extract mg/ml	
		Leaves	Fruits	Leaves	Fruits
1.	*E. coli*	20.0	25.0	15.0	10.0
2.	*E. faecalis*	60.0	40.0	20.0	15.0
3.	*E. cloacae*	60.0	15.0	15.0	10.0
4.	*P. vulgaris*	15.0	10.0	5.0	5.0
5.	*K. pneumoniae*	25.0	15.0	10..0	5.0
6.	*S. aureus*	25.0	20.0	20.0	15.0
7.	*S. saprophyticus*	25.0	35.0	15.0	15.0

Phytochemical Analysis

The phytochemical analysis of aqueous and methanolic extracts of *Tribulus terrestris* leaves and fruits was investigated**.** The investigation showed the presence of flavonoids, alkaloids, saponins, tanins and carbohydrates in the leaves and fruits extract. The presences of photochemical are shown in Table 3.

Table 3: Phytochemical analysis of leaves and fruits extracts of *Tribulus terrestris*

Components	Aqueous extract		Methanolic extract	
	leaves	fruits	leaves	fruits
Flavanoids	-	+	-	+
Alkaloids	+	+	_	+
Saponins	+	+	+	+
Tanins	-	+	_	+
Carbohydrates	+	-	+	-

CONCLUSION

In conclusion, fruits and leaves from *T. terrestris* possessed inhibitory activity against the tested bacteria. Aqueous as well as methanolic extracts of fruits showed almost comparable antibacterial activity, which support their traditional use against infectious diseases. Since, Indian *T. terrestris* showed activity against all tested bacteria but the highest was against *P. vulgaris*, the use of plant as a urinary antiinfective is validated. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tool for the study of infectious diseases. The presence of most general phytochemicals might be responsible for their therapeutic effects. It further reflects a hope for the development of many more novel chemotherapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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