ORIGINAL ARTICLE

Evaluation of antibiotic potential of alkaloids of *Tribulus terrestris* L. against some pathogenic microorganisms

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Context: Antibiotic resistance has become a global concern. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. *Tribulus terrestris* (Family: *Zygophyllaceae*) is a well-known medicinal plant and has been used world-wide for the treatment of infectious diseases. Alkaloids are group of naturally occurring chemical compounds, which are known to have antimicrobial properties. **Aim:** The present study is designed to evaluate the antimicrobial potential of alkaloids of *T. terrestris* L. **Materials and Methods:** Alkaloids extracted from different parts (root, stem, leaf and fruits) of *T. terrestris* were screened by Disc diffusion assay against three bacterial strains: gram positive (*Staphylococcus aureus*), gram negative (*Escherichia coli* and *Proteus mirabilis*) and two fungal strains: *Aspergillus flavus* and *Aspergillus niger*. Minimum inhibitory concentration of the extracts was evaluated by micro broth dilution method while minimum bactericidal/fungicidal concentration was determined by sub culturing the relevant samples. Total activity of the extracts against each sensitive test pathogens was also calculated. **Statistical Analysis:** Mean value and standard error mean were calculated for the results of disc diffusion assay and data were analysed by one-way analysis of variance and *P* values were considered significant at $P < 0.05$. **Results:** The alkaloid extracts of *T. terrestris* showed significant antibacterial potential as all test extracts were found active against both the gram positive and negative bacteria, but were inactive against the tested fungi. **Conclusion:** The present study indicates the broad spectrum antibacterial potential of the alkaloids of *T. terrestris*, hence may be exploited for future antibacterial drugs.

Key words: Alkaloids, antimicrobial properties, total activity, *Tribulus terrestris*

INTRODUCTION

Today, world is gradually turning to herbal formulations, which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects and are readily available at affordable prices.[1] In the developing countries synthetic drugs are not only expensive and inadequate for the treatment of diseases, but are also often with adulterations and side-effects, as a result, different remedies evolved in different regions of the world.[2] The World Health Organization has reported that more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic health-care needs. Hence, there is a need to search plants of medicinal value.

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Due to indiscriminate use of antimicrobial drugs, the microorganisms have developed resistance to many antibiotics. This has created immense clinical problem in the treatment of infectious diseases.[3] This situation has risen to an alarming level. Plants used in the traditional medicine contain a vast array of active substances that can be used to treat many human diseases.[4] Plant extracts have been proposed to be used as antimicrobial substances.[5] To determine the potential and to promote the use of herbal medicines, it is essential to intensify the study of medicinal plants that find place in folklore.[6]

Tribulus terrestris L. is a taproot and perennial herb that best grow in dry, loose and sandy soil. It found mostly along with tracks and around habitation, especially, in inland districts. The plant is known all over the world for its medicinal properties. It has been commonly used in Indian folk medicine as a diuretic and in treatment of colicky pains, hypertension and hypercholesterolemia.[7-9] Antimicrobial,[10-12] antihelminthics,[13] anti-insecticidal, anti-molluscicidal and anti-piscidal activities[14,15] of crude extracts of *T. terrestris* have been reported earlier. The present study was carried out to evaluate the antibiotic potential of alkaloids from different parts (root, stem, leaf and fruits) of *T. terrestris*.

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MATERIALS AND METHODS

Plant Material

Different parts of *T. terrestris* (root, stem, leaf and fruits) were collected from different localities of Jaipur, in the month of June, 2008. The plant was identified at Herbarium, Department of Botany, University of Rajasthan, Jaipur. All the parts of *T. terrestris* were separately shade dried and were milled to a fine powder using a grinder.

Preliminary Detection of Alkaloids

Each of the test samples were acidified by 5 ml of 2% HCl at 60°C for 2 h and later cooled and filtered. Formation of the reddish brown colour on the addition of the Wagner's reagent (Prepared by mixing 1.25 g I_2 and 2 g KI in 100 ml of distilled water) individually to 2 ml of the above mentioned solution indicated the presence of alkaloids.

Extraction of Alkaloids

Alkaloids were extracted from different parts (root, stem, leaf and fruits) of *T. terrestris* by the Method of Harborne, 1984[16] after preliminary detection of alkaloids. Finely powdered samples (100 g) were extracted with 10% acetic acid in ethanol (500 ml) for 4 h. The extracts were concentrated and were made alkaline by NH_4OH. Precipitates thus obtained were collected by centrifugation, washed with 1% NH_4OH, filtered, dried in *vaccuo* and weighed. Extracts thus, obtained were stored in glass vials at 4°C for further use.

Selected Test Microorganisms

Pathogenic microorganisms selected for the study include three bacterial strains: gram positive (*Staphylococcus aureus* MTCC 87) and gram negative (*Escherichia coli* MTCC 46 and *Proteus mirabilis* MTCC 1425) and 2 fungal strains: *Aspergillus flavus* MTCC 277 and *Aspergillus niger* MTCC 282. They were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on "Muller-Hinton Agar Medium" while fungal strains were kept on "Sabouraud Dextrose Agar Medium".

Antimicrobial Screening

Disc diffusion assay[17] was performed for antimicrobial screening of the alkaloids extracts. Agar medium base plates (MHA for bacteria and SDA for fungi) were prepared for the test. Standard size of microbial inoculums (1×10^8 CFU/ml for bacteria and 1×10^7 CFU/ml for fungi) was used with 1 mg/disc concentration of both the test extracts and standards (streptomycin for bacteria and itraconazole for fungi). Each extract was tested in the triplicate. Antimicrobial activity was determined by measuring zone of inhibition (IZ) in mm. Activity Index (AI) for each extract was also calculated by using the following formula:

AI = IZ of the extract/IZ of the standard.

Minimum Inhibitory Concentration

MIC was determined for each plant extract showing activity against test pathogens in disc diffusion assay. Microbroth dilution method[18] was followed for determination of MIC values.

Minimum Bactericidal/Fungicidal Concentration

MBC/MFC was determined by subculturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC/MFC.

Total Activity

TA for each active extract was also calculated, which is the volume at which the test extract can be diluted without losing the ability to kill microorganisms.[19] It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract.

TA = Amount extracted from 1 g plant material/MIC of the extract.

Statistical Analysis

Mean value and standard error mean were calculated for the results of disc diffusion assay and data were analysed by one-way analysis of variance and *P* values were considered significant at $P < 0.05$.

RESULTS

All the parts (root, stem, leaf and fruits) of *T. terrestris* showed positive results for the presence of alkaloids in the preliminary detection test. The alkaloid content estimated in each gram of dried plant material was recorded [Table 1]. Content of alkaloids were recorded maximum in leaves (23.9 mg/g.d.w) whereas recorded minimum in Fruit (3.6 mg/g.d.w).

Alkaloids extracts were then screened for antimicrobial activity against the selected test pathogenic microorganisms [Table 2]. Results indicated that all the four extracts demonstrated significant antibacterial activity and found active against all the tested bacteria while the extracts showed no activity against the tested fungi. Maximum activity against *E. coli* was observed for leaf (IZ 12.5 mm ± 0.278 and AI 0.62 ± 0.001), against *S. aureus*

Table 1: Quantitative estimation of alkaloids of *Tribulus terrestris*

Part	Alkaloids (mg/g.d.w)
Root	8
Stem	19.78
Leaf	23.9
Fruit	3.6

for root (IZ 10.3 mm ± 0.343 and AI 0.41 ± 0.003) and against *P. mirabilis* for fruit (IZ 20 mm ± 0.234 and AI 0.83 ± 0.004).

MIC and MBC values were evaluated for the active extracts [Table 3]. The range of MIC and MBC of extracts recorded was 0.019-0.625 mg/ml and 0.019-1.25 mg/ml, respectively. Most of the alkaloid extracts showed MIC values less than 0.5 mg/ml indicate strong antibacterial efficacy against the inhibited bacteria. All the extracts were found bactericidal against one or more test bacteria as their MIC and MBC values were recorded same against the tested bacteria. Same values of MIC and MBC against *E. coli* were recorded for leaf (0.156 mg/ml) and fruit (0.312 mg/ml), against *S. aureus* for root (0.312 mg/ml) and fruit (0.312 mg/ml), against *P. mirabilis* for stem (0.078 mg/ml) and fruit (0.019 mg/ml).

TA was also calculated and tabulated [Table 4]. Significant values of TA were recorded against *P. mirabilis* for stem, fruit and leaf alkaloid (253.58 ml/g, 189.47 ml/g and 153.20 ml/g, respectively) whereas, maximum TA values calculated against *E. coli* and *S. aureus* for leaf alkaloid (153.20 ml/g and 38.24 ml/g, respectively).

DISCUSSION

T. terrestris have been reported to have alkaloids, flavonoids and other constituents like fatty acids, tannins and potassium salts.[20,21] Literature indicates that crude extracts from different parts of *T. terrestris* have previously been studied for their antibacterial and antifungal activity;[22-24] but, still meager work has been carried out as far as the antimicrobial activity of alkaloid is concerned. Most of the research has been restricted on determination of IZ of crude extracts without calculating AI, MIC, MBC/MFC and TA. Determination of MIC and MBC/MFC has now become an inevitable step in antimicrobial studies in order to establish their antimicrobial activity so as to explore them at industrial level for production of drugs, which could replace the existing ones. Hence, most of the studies carried out so far could only reveal their antimicrobial activities, but are not helpful for establishing them as antibiotic.

Table 2: Inhibition zone and Activity index of alkaloids of *Tribulus terrestris*

Part	Test microorganisms									
	Escherichia coli		*Staphylococcus aureus*		*Proteus mirabilis*		*Aspergillus flavus*		*Aspergillus niger*	
	IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI
Root	9±0.577	0.45±0.003	10.3±0.343	0.41±0.003	9±0.577	0.37±0.007	-	-	-	-
Stem	10±0.333	0.5±0.013	9.5±0.577	0.38±0.007	16±0.333	0.66±0.001	-	-	-	-
Leaf	12.5±0.278	0.62±0.001	8±0.882	0.32±0.007	13±0.167	0.54±0.001	-	-	-	-
Fruit	11.3±0.123	0.56±0.001	10±0.333	0.4±0.029	20±0.234	0.83±0.004	-	-	-	-
Standard	20		25		24		15		10	

± – Standard error mean; - – No inhibition; Standards – Streptomycin for bacteria and itraconazole for fungi; IZ – Inhibition zone; AI – Activity index

Table 3: Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration of alkaloids of *Tribulus terrestris*

Part	Test microorganisms									
	Escherichia coli		*Staphylococcus aureus*		*Proteus mirabilis*		*Aspergillus flavus*		*Aspergillus niger*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
Root	0.312	0.625	0.312	0.312	0.625	1.25	-	-	-	-
Stem	0.312	0.625	0.625	1.25	0.078	0.078	-	-	-	-
Leaf	0.156	0.156	0.625	1.25	0.156	0.312	-	-	-	-
Fruit	0.312	0.312	0.312	0.312	0.019	0.019	-	-	-	-

All figures are in mg/ml unit; - – Not determined since there was no activity; MIC – Minimum inhibitory concentration; MBC – Minimum bactericidal concentration; MFC – Minimum fungicidal concentration

Table 4: Total activity of alkaloids of *Tribulus terrestris*

Part	TA (ml/g)				
	Test microorganisms				
	Escherichia coli	*Staphylococcus aureus*	*Proteus mirabilis*	*Aspergillus flavus*	*Aspergillus niger*
Root	25.64	25.64	12.8	-	-
Stem	63.39	31.64	253.58	-	-
Leaf	153.20	38.24	153.20	-	-
Fruit	11.53	11.53	189.47	-	-

- – Not determined since there was no activity; TA – Total activity

Result of the present study indicated that the alkaloid extracts of all the parts of *T. terrestris* have activity against both gram-positive (*S. aureus*) and gram-negative (*E. coli* and *P. mirabilis*) bacteria indicative of the presence of broad spectrum antibiotic compounds. The results of the antibacterial activity of the study were in agreement with the findings of previous studies.[25,26] Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and provide biochemical tools for the study of infectious diseases.

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