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Phytochemical and Antimicrobial Evaluation of *Tribulus terrestris* L. (Zygophyllaceae) Growing in Nigeria

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Abstract: The methanolic leaf extract of *Tribulus* terrestris L. (Zygophyllaceae) growing in Nigeria was subjected to preliminary phytochemical screening and *in vitro a*ntimicrobial tests. The phytochemical tests was conducted using standard methods of analyses and the extract revealed the presence of alkaloids, tannins, saponins and cardiac glycosides. The antimicrobial activity of the plant extract was assayed using the agar plate disc diffusion and nutrient broth dilution techniques. Test micro-organisms were *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*; all the organisms were laboratory isolates. The extract inhibited all the test organisms at various concentrations. It showed a minimum inhibitory concentration of 3.125 mg mL^{-1} against *Salmonella typhi* while against *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Candida albicans* was 6.250 mg mL^{-1}. The minimal bactericidal concentration against *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Candida albicans* was found to be 1.563 mg mL^{-1} while against *Salmonella typhi* was 3.125 mg mL^{-1}. This study laid credence to the use of this plant as a remedy for stomachic and urinary tract infections in folk medicine the world over, whose causative agents are some of the organisms used in this study.

Key words: Antibacterial, evaluation, phytochemical, *Tribulus terrestris*, zygophyllaceae

INTRODUCTION

The traditional medical methods, especially the use of medicinal plants still play a major role in the developing countries of Africa south of the Sahara and more so, the use of herbal remedy have risen in the developed countries in the last decades (Kianbakht and Jahaniani, 2003). However, over 80 % of the World's population use plant as their primary source of medication (Cordell, 2000) and in view of the fact that antibiotics are sometimes associated with adverse side effects to the host including hypersensitivity, immuno-suppressive and allergic reactions, it is of interest to develop alternative antimicrobial drugs such as medicinal plants for the treatment of infectious diseases (Clark, 1996; Usman *et al*., 2005). Therefore, plants continue to be rich sources of crude drug for therapeutic purposes.

T. terrestris is an annual plant belonging to the family Zygophyllaceae, found widely distributed in warm region of Asia, Africa, Europe, America and Australia. It is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithontriptic and urinary tract anti-infections (Ody, 2000). The antimicrobial effects of *Tribulus terrestris* from other countries have been reported; Yemeni sp. showed no detectable activity against any of the reference bacteria (Awadh *et al*., 2001). However, Turkish and Iranian sp. (fruits, stems plus leaves and roots) showed activity against all test bacteria (Abbasoglu and Tosun, 1994; Kianbakht and Jahaniani, 2003) while exclusive activity against *E. coli* and *S. aureus* was shown by the fruit and leaf of Indian sp. (Williamson, 2002). In view of the fact that antibacterial study of the Nigerian *T. terrestris* has not been extensively studied, it is therefore imperative to assay this species against most of the reference bacteria earlier studied.

MATERIALS AND METHODS

Plant material: The leaves of *Tribulus terrestris* L. was collected in April 2006, from "*Bakin Rafi*" Ward, Misau Town, Misau-Bauchi State, Nigeria. The herbarium specimen (Voucher No. 13/0221) was identified by Dr. S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri-Nigeria where a herbarium specimen was deposited.

Extraction and preparation of plant extract: The leaves of *T. terrestris* was air-dried at room temperature for 5 days and then pulverized with mortar and pestle.

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Two hundred grams of the powered leaves was exhaustively extracted with 85% methanol in water using cool maceration technique. The extract was concentrated *in vacuo* and a greenish-brown gummy mass which weighed 30.8 g (15.4 % w w^{-1}) was obtained and then coded "MTT"-Methanolic *T. terrestris* extract. The extracts was then stored aseptically at room temperature until required for further research.

Phytochemical screening: The crude methanolic leaf extract was tested phytochemically for the presence of its constituents utilizing standard methods of analyses (Sofowora, 1993; Trease and Evans, 2002).

Test organisms: *Staphylococcus aureus* is the only Gram-positive bacterium used; others are Gram-negative organisms *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Salmonella typhi*. The only fungus utilized is *Candida albicans*. Most of the isolates were obtained from the Department of Veterinary Microbiology and Parasitology, University of Maiduguri except *Staphylococcus aureus* and *Candida albicans* which were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital, Maiduguri-Nigeria.

Susceptibility tests: The susceptibility tests were performed according to the method earlier described by Sidney *et al.* (1978), Vollekova *et al.* (2001) with little modification by (Usman *et al.*, 2005). The tests were carried out using a stock concentration of 100 mg mL^{-1} prepared by dissolving 1 g of the crude extract into 10 mL of sterile distilled water. The dilution ratio for gram-positive bacteria and Gram-negative bacteria are 1:1000 and 1:5000, respectively using peptone water (Usman *et al.*, 2005) while for *C. albicans*, sabouraud dextrose broth was used which was incubated for 48 h. One millilitre of the diluted cultures was inoculated into 19 mL sterile molten nutrient agar (48 °C) and poured into sterile petri dishes. Similarly, 1 mL of the diluted fungal suspensions was poured unto sterile sabourand dextrose agar plates and the excess sucked up with sterile Pasteur pipette. These were gently swirled and allowed to solidify. Afterwards, discs (5 mm diameter) impregnated with the crude extract 5 mg $disc^{-1}$ at a concentration of 100 mg ml^{-1} were aseptically mounted on inoculated agar and incubated for 24 h at 25 and 37°C for fungal and bacterial strains, respectively. Moreover, filter paper discs (5 mm diameter) containing standard antibiotics; Amoxiclive (30 μg), Levoxine (5 μg), Ofloxacin (5 μg) and Peflotab (5 μg) were used as positive controls. The inhibition zones were recorded in millimetres as the diameter of growth free zones around discs using a transparent metre rule. Each extract and standard antibiotics were independently tested in triplicate and results presented as mean±SEM. Diameters of zones of inhibition ≥10 mm exhibited by plant extracts were considered active (Zwadyk, 1972; Usman *et al.*, 2005).

Minimum Inhibitory Concentration (MIC): MIC was defined as the lowest concentration where no visible turbidity was observed as described earlier by (Vollekova *et al.*, 2001) with some modification by (Usman *et al.*, 2005). In this test, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 100 mg mL^{-1} in sterile distilled water and then serially diluted (two-fold) to a working concentrations ranging from 0.098 mg mL^{-1} to 50 mg mL^{-1} using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 24 h incubation at 37 °C, the tubes were then observed for the presence of turbidity. The lowest concentrations where no turbidity was observed was determined and noted as the minimum inhibitory concentration.

Minimum Bactericidal Concentration (MBC): The MBC was determined from broth dilution test resulting from the MIC tube by sub-culturing to antimicrobial free agar as described earlier (Vollekova *et al.*, 2001; Usman *et al.*, 2005). The lowest concentration of the extract which shows no growth was recorded as the minimum bactericidal concentration.

Table 1: Phytochemical analysis of the methanolic leaf extracts of *Tribulus terrestris* L

S/No	Constituents/ Test	Results
1.	Alkaloids	
	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
2.	Carbohydrates	
	Molisch's test	+
	Barfoed's test	+
	Fehling (reducing sugar) test	+
	Fehling(combine reducing sugar) test	+
3.	Cardiac glycosides	
	Legal's test	+
	Keller-killiani's test	+
4.	Flavonoids	
	Shinoda's test	-
	Lead acetate test	+
	NaOH test	-
	$FeCl_3$ test	+
5.	Saponins	
	Frothing test	+
6.	Steroidal *nucleus*	
	Salkowski test	-
	Liebermann-Burchard's test	-
7.	Tannins	
	$FeCl_3$ test	+
	Lead acetate test	+

Key: + = Present, = Absent

RESULTS AND DISCUSSION

The search for new antimicrobial agents is an important line of research because of the resistance acquired by several pathogenic micro-organisms. The phytochemical screening of the crude methanolic leaf extracts of *T. terrestris* revealed the presence of alkaloids, cardiac glycosides, saponins and tannins as shown in Table 1. These classes of compounds are known to show curative activity against several pathogens and therefore could explain its wide usage traditionally for the treatment of wide array of illnesses (Hassan *et al*., 2004; Usman *et al*., 2005).

The *in vitro* antimicrobial screening presented in Table 2 showed the susceptibility test against Grams-positive and negative organisms and a fungal species. The extract exhibited considerable amount of inhibition against all the test organisms; the highest being against *Staphylococcus aureus* (18.5±0.2 mm) and the lowest was against *E. coli* (10.5±0.9 mm). Meanwhile, comparison with the reference antibiotics especially Levoxine, showed that the extract exhibited much higher activity against *E. coli* and *S. aureus*. The zone of inhibition produced by most antibiotic discs against some of the reference bacteria were found to be higher (though of different concentration)compared to that of the extract although others with no significant difference. However, it was suggested that plant extracts exhibiting diameters of zones of inhibition ≥10 mm were considered active (Zwadyk, 1972; Usman *et al*., 2005).

From the results of the MIC and MBC presented in Table 3 and 4, respectively; it was observed that the broadest activity of the extract against most Grams-negative organisms was 6.250 mg mL^{-1} as MIC while the MBC of 1.563 mg mL^{-1} was noted similarly. The only Gram-positive bacterium assayed - *S. aureus* appreciably exhibited some level of bactericidal and bacteriostatic effects. Hence, indicative of finding pure active principle(s) from extract with the possible high potency than the crude extract which could serve as a lead to the pharmaceuticals. *In view* of the fact that prevalence of *S. aureus* resistant strains to conventional antibiotics has increased to high levels in some hospitals (Shalit *et al*., 1989) and that *S. aureus* is a pyogenic bacterium known

Table 2: Antimicrobial susceptibility tests of methanolic leaf extracts of *Tribulus terrestris* L

		Inhibition Zone (mm)					
Extract or standards	Conc $disc^{-1}$	P. e	E.c	S.a	S.t	K.s	C.a
MTT	5 mg	17.5± 0.2	11.8±0.9	18.5±0.2	13.5±0.2	12.5±0.2	17.5±0.2
LEV	5 µg	23.0±0.0	*	20.0±0.0	30.0±0.0	*	NT
OFL	5 µg	21.0±0.0	35.0±0.0	*	*	42.0±0.0	NT
PEF	5µg	*	31.0±0.0	26.0±0.0	38.0±0.0	28.0±0.0	NT
Sterile distilled H_2O	-	-	-	-	-	-	-

Key: MTT = Methanolic T. terrestris extract; P.e = *Pseudomonas aeruginosa*; E.c = *Escherichia coli*; S.a = *Staphylococcus aureus*; S.t = *Salmonella typhi*; K.s = *Klebsiella*; C.a = *Candida albicans*; – = No activity; * = No disc; NT = Not Tested; LEV = Levoxine; OFL = Ofloxacin; PEF = Peflotab

Table 3: Minimum inhibitory concentration of the methanolic leaf extracts of *Tribulus terrestris* L

	Concentration (mg mL^{-1})									
Organisms	0.098	0.195	0.390	0.780	1.563	3.125	6.250	12.500	25.000	50.000
P.e	+	+	+	+	+	+	β	-	-	-
E.c	+	+	+	+	+	+	β	-	-	-
S.a	+	+	+	+	+	+	+	β	-	-
S.t	+	+	+	+	+	β	-	-	-	-
K.s	+	+	+	+	+	+	β	-	-	-
C.a	+	+	+	+	+	+	β	-	-	-

Key: P.e = *Pseudomonas aeruginosa*; E.c = *Escherichia coli*; S.a = *Staphylococcus aureus*; S.t = *Salmonella typhi*; K.s = *Klebsiella* sp.; C.a = *Candida albicans*; + =Turbidity observed; – = No turbidity observed; β = MIC value

Table 4: Minimum bactericidal concentrations of the Methanolic leaf extracts of *Tribulus terrestris* L.

	Concentration (mg mL^{-1})									
Organisms	0.098	0.195	0.390	0.780	1.563	3.125	6.250	12.500	25.000	50.000
P.e	+	+	+	+	β	-	-	-	-	-
E.c	+	+	+	+	β	-	-	-	-	-
S.a	+	+	+	+	+	β	-	-	-	-
S.t	+	+	+	+	+	+	β	-	-	-
K.s	+	+	+	+	β	-	-	-	-	-
C.a	+	+	+	+	β	-	-	-	-	-

Key: P.e = *Pseudomonas aeruginosa*; E.c = *Escherichia coli*; S.a = *Staphylococcus aureus*; S.t = *Salmonella typhi*; K.s = *Klebsiella* sp.; C.a = *Candida albicans*; + = Growth = No growth; β = MBC value

to play a significant role in invasive skin diseases including superficial and deep follicular lesion (Srinivasan *et al*., 2001; Usman *et al*., 2005) the extract could serve as a remedy to such resistance. The extract also showed some level activity against *E.coli* which is the common cause of urinary tract infection and accounts for approximately 90 % of first urinary tract infections in young women (Brooks *et al*., 2002).

CONCLUSION

From the reports of the antibacterial study of *T. terrestris* from other countries, it is therefore, imperative to conclude that the activity of *T. terrestris* growing in Nigeria against most of the reference bacteria earlier studied could be said to be similar to that of the species found in Turkey and Iran; although this species is found from different flora and fauna from the those earlier reported. This study therefore, laid credence to the traditional use of this plant as a remedy for stomach ache and urinary tract infections as practised ethno medically the world over.

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