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Evaluation of the Biological Activity of Stem Bark of *Tribulus tresstris* (zygophyllaceae)

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Abstract

Tribulus tresstris is a widely grown annual plant used traditionally for treating infertility, low sex drive, and erectile dysfunction. The present study aimed at determining the cytotoxic effect, antimicrobial and antioxidant activity of the crude extracts of the stem bark of *Tribulus tresstris*. Phytochemical screening was carried out using standard protocol. Cytotoxicity and antimicrobial activity were investigated using Brine shrimp Lethality test (BSLT) and Agar diffusion method respectively. Antioxidant activity was evaluated using the 2,2-diphenyl-l-picry hydrazyl radical (DPPH) free-radical scavenging activity, and hydrogen peroxide (H_2O_2) assays. Phytochemical analysis revealed the presence of alkaloids, anthraquinones, combined anthraquinone, glycosides, cyanogenic glycosides, reducing sugars, saponins, tannins, phloba tannins, cardiac glycosides, steroids, triterpenes and flavonoids. The result of the cytotoxicity test gave LC_{50} values of 20.67, 25.07, and 128.56 µg/ml for the n-butanol, ethyl acetate and n-hexane extracts. Also, the antimicrobial activity results indicated that the extracts exhibited high level of activity against all selected microbes with the highest zone of inhibition (33mm) against s*taphylococcus aureus*. However, the antioxidant activity of the extracts showed that the crude extracts exhibited strong radical scavenging activity against all tested free radical when compared with the standards butyrate hydroxyl anisole (BHA), ascorbic acid and alpha-tocopherol. The findings from this study provide scientific evidence that the plant possesses bioactive compounds with good antimicrobial and antioxidant agents.

Keywords: *Tribulus tresstris,* Phytocompounds, Cytotoxicity, Antimicrobial, Antioxidant

1.0 Introduction

The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in some developing countries, plants are the main medicinal source to treat infectious diseases. Plant extracts represent a continuous effort to find new compounds with the potential to act against multi-resistant bacteria (Olasehinde *et al.*, 2012). Thus, plants continue to be a rich source of therapeutic agents. The active principles of many drugs are found in plants and are produced as secondary metabolites. The remarkable contribution of plants to the drug industry is possible, because of the large number of phytochemical and biological studies all over the world (Kpadonou, *et al.*, 2019). Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and toxicity of the currently available commercial antibiotics. Therefore, it is of great interest to investigate these medicinal plants to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. *Tribulus terrestris*, also known as "Puncture Vine", "Caltrop", "Yellow Vine" and "Goat head" is a flowering plant which belongs to

the Zygophyllaceae family. It is a prostrate, annual herb native to warm temperate and tropical regions of the whole World, in Southern Europe, Southern Asia, throughout Africa and including Nigeria. (Abimbade, 2009). It was widely used in ancient Chinese medicines as well as the ancient Indian art of healing called Ayurveda. It forms a major constituent of Ayurvedic tonics and is known by its Sanskrit name, 'Gokshura' in India. *T. terrestri*s is known to enhance sex drive and its aphrodisiac properties came to light in Eastern Europe in the 1970s. In Nigeria, *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithontriptic and urinary anti-infectives (Teijo, Y. 2004 ; Todar, K. 2008; Usman *et al*.,2013). *T. terrstris* is a non-irritant diuretic and urinary antiseptic. It is used in case of urinary stone and incontinence of urine and it is used in various herbal formulas to treat headaches, eye problems such as itching, conjunctivitis and weak vision, and nervousness. It is also used to treat high blood pressure and rib pain (Adzu *et al*., 2001). The active components in the plant are called steroidal saponins of furastanol type, which are of two types: furastanol glycosides and spirostanol glycosides. These saponins are extremely useful in the treatment of nervousness, high blood pressure and rib pain. The plant contains sterols: beta sitosterols that are known to protect the prostrate from swelling and cancer when combined with steroidal saponins (Abubakar *et al.,* 2006). *Tribulus terrestris* is most commonly used in the treatment of impotence and spermaturia or premature ejaculation in herbal medicine. It increases the level of the luteinizing hormone in the body and boosts the synthesis of testicular male sex hormone. It is also used in the treatment of gout, premenstrual syndrome (PMS), low libido and has a positive effect on the bone marrow activity for red blood cell production. It is also known to augment the immune system (Ahn, 2017; Ayse *et al.*, 2021 ; Ajao, *et al*., 2022). Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction. The aim of this research work was to carry out preliminary phytochemical screening of the extract and fractions and to investigate their antioxidant, antimicrobial, and cytotoxic effects.

2.0 Materials and Method.

Plant Collection and Identification

The plant - Tribulus terrestris was collected from Mokwa local government area of Niger state, Nigeria, identified by Mr. Musa Namadi of Herbarium Section, Department of Biological Science Ahmadu Bello University, Zaria. Voucher specimen (no. 3130) was deposited in the herbarium for future reference.

Plant Preparation and Extraction

The stem bark was air dried at room temperature and pulverized using wood mill machine. The pulverized samples were weighed and kept in polythene bag at room temperature until required for the extraction. The pulverized sample (200g) were extracted by percolation using 70% methanol in water. The extract was concentrated using a rotary evaporator to obtain the crude extract. 20.8g weighed crude extract were suspended in distilled water and partitioned with n-hexane (250 ml, ethyl acetate (250 ml) and n- butanol (250 ml) to get the n -hexane, ethyl acetate and n- butanol fractions respectively.

Phytochemical Analysis

Phytochemical analyses were carried out on the crude extract using the method prescribed by Sofowora (2008).

Brine shrimp Cytotoxicity test

Brine shrimp eggs were hatched in a beaker containing 250ml of sea water. The beaker was placed beside a window for light and proper ventilation at room temperature. After 48 hours, the brine shrimp larvae were collected by dropping pipette from the lightened side. Solvent was evaporated from 0.2 g of each extract sample and then 2 drops of DMSO was added, and the mixture was made up to 2 ml with distilled water. From this 2 ml of 1000, 100 and 10 µg ml, respectively were

prepared. The control was prepared by diluting 2 drops of DMSO to 2 ml of distilled water. Ten (10) shrimp larvae were added to each of the prepared solutions in triplicate including the control. The number of the surviving larvae after 25 h was recorded and the LC50 was computed using Finney probit analysis (Garba and Salihu, 2008).

Microorganisms and Media

Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pneumonia were used for the in-vitro anti-microbial assay. All the microorganisms were collected from laboratory stock of Microbiology Department, Ahmadu Bello University Zaria, Nigeria. Muller Hatton Agar was used in the assay. Levoxin, Ofloxaxin and Peflotab were used as standard drug, each solvent of extraction was used in dissolving the corresponding extract/drug, and as negative control in the studies.

Antimicrobial test using Agar diffusion method

The agar diffusion method was used to determine the antimicrobial activity of the crude extracts of the plant. Solutions of 1.05 µg/ml, 0.5 µg/ml, and 0.25 µg/ml concentrations were prepared for each extract using the solvents of extraction. 35 g of Muller Hilton Agar (MHA) was dissolved in 11 L of distilled water and autoclaved at 121°C for 15 minutes. It was then cooled and poured into sterile petri-dishes to solidify. A sterile cock borer was used to make holes on each seeded agar plate for each concentration prepared for the plant samples and the control. The surface of the plates was inoculated evenly with the isolate using sterile cotton sward stick, after which 0.5 ml of the extract solutions and the control were introduced into the holes and incubated. The diameters of zones of inhibition were recorded after 48 h using a transparent ruler.

Antioxidant Activity

The antioxidant activity was determined using the 2.2-diphenyl-l-picry hydrazyl radical (DPPH) free - radical scavenging activity and hydrogen peroxide (H2O2) in accordance to method described by Kpadonou D. et al.,(2019).

5.0 g of DPPH was dissolved in 100 ml methanol to give a 1 M solution. The extract stock solutions were prepared by dissolving about 0.1 g of each extract in ml of the solvents of extraction. Test solutions of concentrations 0.5, 0.25, and 0.125 mg/ml, respectively were prepared by diluting appropriate volumes of the stock solutions. 2 ml of 1 M DDPH was added to 0.5 ml of each of the test solutions. The mixture was shaken and left to stand for 10 minutes, and the decrease in absorption at 517 nm of the solutions was measured against that of control, and percentage inhibition was calculated. The same procedure was carried out using Butyrate Hydroxyl Anisole (BHA), Vitamin C or α-Tocopherol as standards. The antioxidant activity was calculated from equation 1 as the percentage inhibition of DPPH.

$$Inhibition = \frac{(A_{DPPH} - A_S)}{A_{DPPH}} \times 100\% \qquad 1$$

A_{DPPH} and A_S are the respective absorbances of the DPPH and test solutions.

3.0 Results and Discussion

Table 1: Phytochemical constituents of the crude extracts of *Tribulus terrestris*

Secondary metabolites	*n*-hexane	Ethyl acetate	*n*-butanol
Alkaloids	–	–	–
Carbohydrates	+	+	+
Glycosides	+	+	+
Free Anthraquinone	–	–	–
Combined Anthraquinone	–	–	–
Reducing sugar	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phlobatanins	+	–	+
Cadiac glycosides	+	+	+
Steroids	+	+	+
Triterpenes	+	–	+
Flavonoids	+	+	+

Where: + = present; – = negative

Table 2: Result of Brine Shrimp Lethality Assay of the crude extracts of *Tribulus Terrestris*

Sample	BST LC_{50} (µg/ml) *
n-Hexane fraction	128.56 (119.9-23.7)
Ethyl Acetate fraction	43.65 (115-25.5)
n-butanol extract	21.7 (78.9-12.8)

*High to Low 95% confidence interval BST = Brine Shrimp Lethality Test

Table 3: Results of antimicrobial assay of methanol extract and fractions of *Tribulus terrestris*

Plant extracts	**Concentration (µg/ml)**	**Zone of inhibition (mm)**				
		S. aureus	*E. coli*	*P. aureginosa*	*S. pneumonia*	*C. candida*
n-hexane	1.0	24	18	14	16	14
	0.5	18	14	12	12	10
	0.25	16	10	10	10	08
	Control	Ni	Ni	Ni	Ni	Ni
Ethyl Acetate	1.0	28	20	31	24	29
	0.5	16	14	15	10	12
	0.25	10	10	12	10	09
	Control	Ni	Ni	Ni	Ni	Ni
n-butanol	1.0	33	27	28	30	30
	0.5	17	13	16	14	12
	0.25	10	10	12	10	10
	Control	Ni	Ni	Ni	Ni	Ni
Levoxin	1.0	31	27	28	30	30
	0.5	17	13	16	14	12
	0.25	10	10	12	10	10
	Control	Ni	Ni	Ni	Ni	Ni
Ofloxaxin	1.0	31	27	28	32	30
	0.5	17	13	16	14	12
	0.25	10	10	12	10	10
	Control	Ni	Ni	Ni	Ni	Ni
Peflotab	1.0	32	27	28	30	31
	0.5	17	13	16	14	12
	0.25	10	10	12	10	10
	Control	Ni	Ni	Ni	Ni	Ni

KEY: *S.a = Staphylococcus aureus, E.coli = Escherichia coli, Ps.a = Pseudomonas aeruginosa, Strep=Streptococcus pneumonia, C.a= Candida albicans,* NI = no inhibition, Control = solvent of extraction.

Table 4a: Minimum inhibitory concentration of the methanol extract of stem back of *T. terrestis*

Concentration (µg/ml)									
Organisms	0.098	0.195	0.390	0.780	1.563	3.125	6.250	12.500	25.000
S. a	+	+	+	+	+	+	+	-	-
E.c	+	+	+	+	+	+	+	-	-
Ps. A	+	+	+	+	+	+	B	B	-
S. p	+	+	+	+	+	+	+	-	-
C. a	+	+	+	+	+	+	+	-	-

Table 4b: Minimum bacterial concentration of the methanol extracts of stem back of *T. terrestis*

Concentration in µg/ml									
Organisms	0.098	0.195	0.390	0.780	1.563	3.125	6.250	12.500	25.000
S. a	+	+	+	+	B	-	+	-	-
E.c	+	+	+	+	B	-	+	-	-
Ps. A	+	+	+	+	B	B	B	-	-
S. p	+	+	+	+	+	+	-	-	-
C. a	+	+	+	+	B	+	-	-	-

KEY: + = present of inhibition, - = absent of inhibition**,** *S. a = Staphylococcus aureus, E. coli = Escherichia coli, Ps. a = Pseudomonas aeruginosa, Strep=Streptococcus pneumonia, C. a= Candida albicans,* NI = no inhibition β= MIC values

Antioxidant Activity

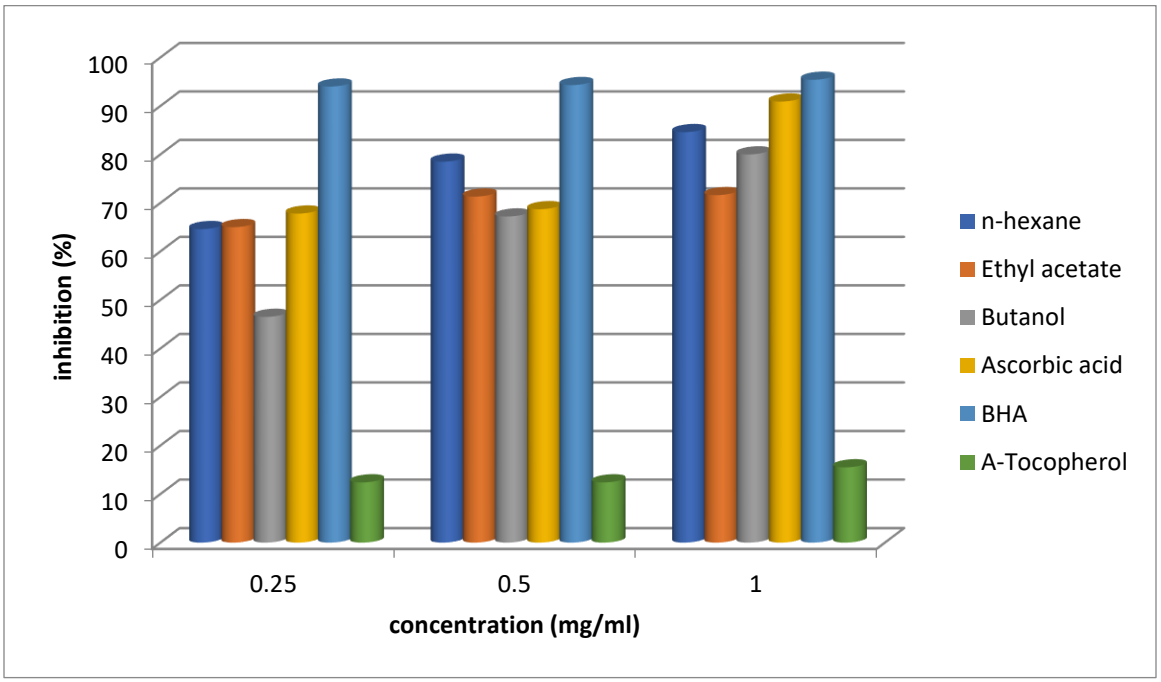

Figure 1: Antioxidant activity of *T. terrestis* extracts against DPPH free radicals

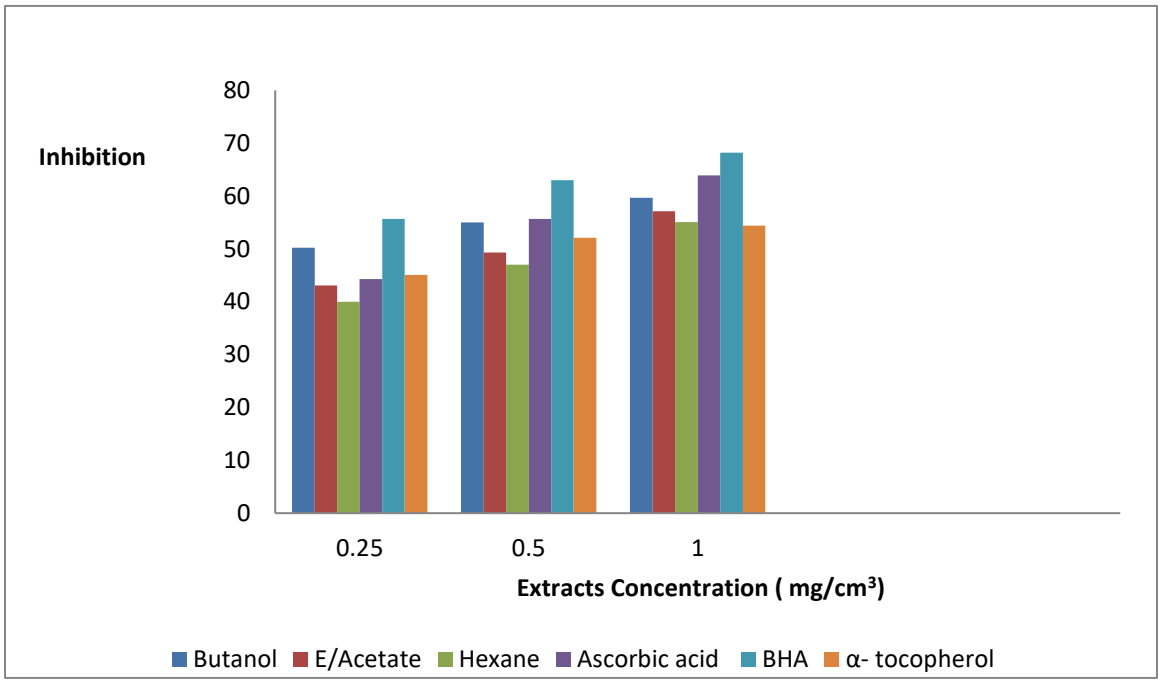

Figure 2: Antioxidant activity of *T. terrestis* extracts against H_2O_2

The results of phytochemical screening presented i n table 1 showed the presence of reducing sugars, carbohydrate, glycosides, cardiac glycosides, flavo noids, steroids, tannins, phlobatannins, saponins, and phenols in the extract with exception of anthraquinone and alkaloids in both extracts. Similar observations were made by Usman *et al.* (2007) on *T. terrestis*. The presence of these phytochemicals in the methanol extract have been reported to be responsible for the medicinal properties displayed by many medicinal plants (Flodden *et al.*, 2006; Garba and Salihu, 2008). These phytochemicals have been reported to have antimicrobial properties due to their abilities to form complexes with nucleophilic amino acids of the microorganism thereby leading to the loss of function of proteins (Stern et *al.*, 1996). Dixon *et al*. (2018) reported high antimicrobial effects of saponins and flavonoids detected as they are synthesized by plants to safeguard themselves against microbial infections. This is an indication that this plant could possess antimicrobial properties.

High cytotoxicity activities were recorded in the crude extracts from highly polar solvent extracts to non-polar solvent extracts (butanol, ethyl acetate and n-Hexane) (table 2). The highest activities were recorded for butanol extracts (BST LC_{50} 21.07 µg/ml) and the lowest activities were recorded for the hexane extract (BST LC_{50} 128.56µg/ml). The result of brine shrimp lethality test (BST) (Table 2) agrees with those of the phytochemical test where themajority of the phytochemicals were present in the crude extracts of the plant (table1 and 2). High toxicity to brine shrimp larvae has been reported i n another species of *Tribulus* species (Usman *et al*., 2010). In antimicrobial assays, zone diameters of inhibition of 0-20mm, 21- 25mm and 26-35mm correspond to low, moderate and high activities respectively (Amnabhavi *et al.*, 1984 ; Usman, *et al* 2013).). The result of the antimicrobial screening of the crude extracts (Table 3-4) showed that most of the crude extracts with high activities against the shrimp larvae also had high activities against the test microorganisms. For instance, butanol extract with LC_{50} 21.07 µg/ml showed high activity against all the test microbes with maximum zones of inhibition of 31 & 33 mm against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, (Tables 3). However, moderate activities were generally recorded in the n-hexane extracts (BST

LC_{50}128.56) which is in conformity with moderate activity in BST (Table 2).

The antimicrobial activities of the solvent extracts were compared with those of reference standards drugs. The antimicrobial activities of the butanol and ethyl acetate extracts are similar to those of the reference standards (Levoxine, Ofloxaxin and Peflotab) with zones of inhibition of 31, 31 and 32 mm respectively against *staphylococcus aureus* and *Pseudomonas aeruginosa* at the concentration of 1.0 mg/ml (Table 3). The moderate antimicrobial activities recorded in different solvent extracts of the stem bark of the plant against *Staphylococcus aurenus, P. aeruginosa, S. pneumonia* and *C. albicans* suggest that this plant may be potential source of compounds that may be employed in the treatment of respiratory tract infection, urinary tract infection, and other diseases caused by the test organisms. These results are comparable with observations made by Usman *et al.,* (2007) and Usman, *et al* (2013). From the result of MIC and MBC presented in Tables 4a & 4b respectively, it was observed that the broadest activity of the extracts against most Gram-negative organism was 6.250 mg/mL^{-1} as MIC while the MBC of 1.563 $mg/mL^{-1}$was noted. The Gram-positive bacterial assayed *Staphylococcus aureus* appreciably exhibited some level of bactericidal and bacteriostatic effect. Hence indicating possible high potency of the crude extract which could thus serve as a lead to pharmaceuticals.

Antioxidant activities of all the crude extracts (Fig 1) were compared with those ascorbic acid, butylated hydroxyl anisole (BHA) and α-tocopherol which were the reference standards. It was observed that n-hexane extract had higher activity than the ethyl acetate and methanol extracts at concentration of 1.0mg/ml. The scavenging activity of n-hexane, butanol and ethyl acetate extracts were 86 %, 78 % and 70 %, respectively. The radicals scavenging ability of all the extracts were closer to that of BHA and higher than ascorbic acid and α-tocopherol (see Fig 1 and 2). The study shows that the extracts have the proton donating ability and could serve as free–radical inhibitors. The ability to scavenge free radicals is an important property in order to minimize oxidative damage to living cells (Bray, 1999). The synthetic drugs available e.g. butylated hydroxyl anisole and butylated hydroxyl toluene have been found to be toxic to human systems and therefore cause liver damage. They are also promoters of carcinogenesis and by the general consumers rejection of synthetic food additives, there is need for their replacement with natural antioxidants (Cow-chin *et al* 1994; Gulcin *et al.,* 2002; Kpadonou *et al*., 2019). This plant may be found useful as antitumor, anticancer or antimicrobial agent because of its strong antioxidant activity.

4.0 Conclusion

The stem back of *Tribulus tresstris* was investigated for its cytotoxic effect, antimicrobial and antioxidant activities with the following findings. Phytochemical analysis revealed the presence of various secondary metabolites that have been reported to have antimicrobial properties. The crude extracts possess high cytotoxicity activity from highly polar solvent to non-polar solvent extracts. The extracts exhibited high antimicrobial activity against all selected microbes with the highest zone of inhibition against s*taphylococcus aureus*. The antioxidant activity of the extracts showed that the hexane extracts exhibited strong radical scavenging activity against all tested free radical when compared with the standards antioxidants. It can be concluded that the crude extract of the stem back of *T. terrestis* exhibited high antimicrobial and antioxidants activity. It may also be suggested that these inhibitory activities may be attributed to the presence of phytochemicals found in the extract. This findings from this study provide scientific evidence that the plant possesses bioactive compounds with good antimicrobial and antioxidant agents.

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