

Short Communication

Phytochemicals and Antibacterial Evaluation of Root Bark Extract of *Terminalia glaucescens*

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ABSTRACT

The roots bark of *Terminalia glaucescens* was investigated for activity against four bacterial which are two gram-positive and two gram-negative. Preliminary phytochemical screening of the root bark of *Terminalia glaucescens* revealed the presence of steroids, terpenoids, saponins, flavonoids, tannins and cardiac glycoside. The results of antibacterial test of the methanol crude extract and its fractions exhibited a wide range of activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebselle pneumonia* at 200 mg/ml but there was no activity against *Bacillus cereus*. Fractions inhibited the growth of *E. coli*, *S. aureus* and *K. pneumonia*. The fractions had minimum inhibitory concentration (MIC) of 12.5mg/ml and minimum bactericidal concentration (MBC) of 25 mg/ml. The findings indicated that the extract and its fractions from root bark of *T. glaucescens*, contained bioactive components that have antibacterial properties.

INTRODUCTION

Medicinal plants have been in use for the eradication of human diseases since ancient times. In light of their established therapeutic efficiency, the pharmaceutical industries started to use crude extracts of medicinal plants for manufacturing drugs (Ali and Azhar, 2000). The acceptance of traditional medicine as an alternative form of health care and development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Lis-Balchin and Deans, 1996, Maoz and Neeman, 1998, Hammer *et al.*, 1999). Presence of tannins, flavonoid, terpenoid, saponins, steroids, cardiac glycosides, volatile oils, alkaloids, anthraquinones and other phenolics have been reported to have antimicrobial activities (Hostettman and Nakanishi, 1979, Hostettman *et al.*, 1995, Isaac and Chinwe, 2001). *Terminalia glaucescens* is belonging to family Combretaceae, is a tree up to 20 m high. It is commonly found in West Africa especially in Savannah regions. The plant is the most important medicinal species of the genus *Terminalia* (Ndukwe *et al.*, 2005), it is abundant in Nigeria. The plant is commonly called baushe (Hausa).

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The roots bark of the plant has not been thoroughly evaluated for its antibacterial activity and bioactive components. The organisms used in this study are known to causes dysentery, fever, diarrhea, wound, tooth decay, ulcers, typhoid fever and various stomach related problems (Ndukwe *et al.*, 2005). The aim of this study is to evaluating the phytochemicals of n- hexane fraction, ethyl acetate fraction, n - butanol fraction, methanol fraction or methanol extracts of root-bark with a view of authenticating the plant's antibacterial potentials.

MATERIALS AND METHODS

Collection, Identification, Extraction and preparation of plant materials.

The plant materials were collected from Hyera Road of Shaffa District, Hawul Local Government Area, Borno State of Nigeria. The plant was identified and the herbarium (voucher) specimen was prepared and deposited at the Herbarium of Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, by A. M. Umar (Taxonomist). The root bark of the plant was air dried under shed, then pulverized into powder with the aid of pestle and mortar. The powder obtained from the plant was then sieved and stored in polythene bags until required for use (African pharmacopoeia, 1985).

Six hundred gram (600g) of powdered root bark was extracted by Soxhlet extractor used 1500ml of methanol as solvent at temperature of 85 °C, was concentrated using hot air sterilizing cabinet at 60 °C and yield 123.11gram of methanol crude extract. Split method of separation was adopted according to (Abubakar, 2009). The n-hexane was directly added to crude methanol extract and was vigorous stirring before filtration and the filtrate are all n-hexane soluble portion, which is the n-hexane fraction while the residue was allowed to dry and same method was repeated with ethyl acetate, n-butanol and finally the residue obtained is methanol fraction. N-hexane, ethyl acetate, n-butanol, and methanol fractions were obtained and were concentrated at 60 °C in hot air sterilizing cabinet.

Phytochemicals Screening

This was carried out using standard procedure as described by Sofowora (1984), Trease and Evans (2005), El-Olema *et al* (1994), Ganapathi *et al* (2011) and Abubakar (2009).

Antibacterial assay

Bacterial cultures used in this research were obtained from School of Medical Laboratory Science Department of microbiology, Usmanu Danfodiyo University, Sokoto. Bacterial isolates used in this study include; *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Bacillus cereus*. All the cultures were grown on nutrient agar medium.

The plant extract & fractions (methanol extract, methanol fraction, ethyl acetate fraction, n-hexane fraction and n-butanol fraction) were tested for antibacterial activity by agar well diffusion assay. Concentration of 200mg/ml was prepared from each fractions and the extract. The concentration was used for antibacterial analysis using agar well incorporation method (Pelezer *et al*, 1993) plates of nutrients agar were prepared and allowed to solidify in petridishes, each plates were then seeded with test bacterium (standard size inoculums, 0.5 Mcfarland std). Five wells were made onto each of the agar plate with a sterile 6mm diameter cork borer. Each of the five wells were filled with a given concentration of methanol extract, methanol fraction, ethyl acetate fraction, n-butanol fraction and n-hexane fraction and gentamicin was used as control. The plates were then incubated at 37 °C for 24 hours. The diameters of zones of inhibition were measured using vernier caliper and the value for each organism was recorded (WHO, 1997).

Minimum Inhibitory Concentration (MIC)

The method used in this was as described by (Irobi 1992), A 0.5 Mcfarland standard broth culture of the test organism, *E. coli*, *S. aureus*, and *K. pneumoniae* were prepared. Eight (8) test tubes were sterilized and set up on a test tube rack. Concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml were prepared from the fractions. Test tube 6, 7 and 8 were set up as controls containing the un-inoculated culture media, extract free growth medium and the test organisms without extract. Test tubes 1 – 5 were inoculated with the test organism.

The mixtures were incubated at 37 °C for 24 hours. Turbidity was checked to determine MIC and recorded. The minimum inhibitory concentration of the fractions was regarded as the lowest concentration that shows no visible turbidity (growth of the test organism) (WHO, 1997).

Minimum Bactericidal Concentration (MBC)

To determine the MBC a wire loop full of the organisms were sub-cultured from the test tubes showing no turbidity onto sterile nutrient agar plate. The plates were incubated at 37 °C for 24 hours. The MBC is the plates showing 0 – 5 colony forming unit (Richard, *et al*, 2004).

Statistical Analysis

Results are expressed as mean ± standard error. The data obtained during zone of inhibition was subjected to one way analysis of variance (ANOVA), (triplet comparison) using least significant difference and t-test. The level of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemicals Screening.

The phytochemical screening of the root bark of *T. glaucescens* revealed the presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids, but alkaloids and anthraquinones are not detected as shown in Table 1. The presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids in plants part is an indication that the plant is of pharmacological importance (Hostettmann and Marston, 1995).

Table 1. Phytochemicals Screening.

S.No.	Phytochemicals	Methanol Extract
1.	Tannins	++
2.	Alkaloid	ND
3.	Flavonoid	++
4.	Terpenoid	++
5.	Saponins	++
6.	Cardiac glycoside	+
7.	Anthraquinones	ND
8.	Steroid	++

Key: ND = not detected, ++ = present, + = trace amount

Anti-bacterial activity

The antibacterial activities of the methanol extract and its fractions of the root bark of *T. glaucescens* was tested against two gram positives and two gram negatives bacteria such as *S. aureus*, *B. cereus* and *E. coli*, *K. pneumoniae* respectively as shown in table 2. Methanol extract, n-hexane fraction, ethyl acetate fraction, n-butanol fraction and methanol fraction were active against or showed significant ($p < 0.05$) inhibitions of *S. aureus*, *E. coli*, and *K. pneumoniae* but *B. cereus* was resistance to the extract, fractions and even the standard control (gentamicin) no doubt it has reported by (Richard *et al* 2004) that *B. cereus* is resistance to gentamicin, penicillin and other antibiotics. The plant extract and its fractions inhibited the growth of *S. aureus*, *E. coli*, and *K. pneumoniae* to a high degree and large zone of inhibition

exhibited as shown in table 2. It means that the *T. glaucescens* can be used as a broad spectrum anti-bacterial agent. Ndukwe *et al.*, (2005) had reported that saponins, steroid and aglycones present in plant extract or fraction have varied uses as anti-ulcerogenic, anti-inflammatory, anti-edematous and analgesic. The activities of the extracts and the fractions from the root bark against *S. aureus*, which is the potential causative organism of the tooth decay and wound infections agreed with previous work (Rotimi and Bartlett, 1988) reported *T. glaucescens* as phytotherapeutic agent for dental hygiene and wound treatment. The observed antibacterial effects corroborate its traditional uses of the plant. The plant root bark is used traditionally in treatment of wound, fever, typhoid fever, dysentery, diarrhea, tooth decay, ulcers and various stomach related problems (oral communication with traditional healer). In this work the extract and fractions of the plant's root bark inhibited the growth of *E. coli* and *K. pneumoniae* to a high degree. These two bacteria are responsible for various stomach related illnesses, *K. pneumoniae* is the causative organism of pneumonia and typhoid fever, a systemic infection associated with the consumption of contaminated food while, *E. coli* is responsible for a number of food related illnesses that manifest themselves in form of diarrhea, dysentery, fever etc.

Table 2. Zone of Inhibition at Concentration of 200mg/ml.

Extract / Fraction	<i>S. aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Bacillus cereus</i>
Methanol extract	14.25±0.28	16.10±0.08	21.03±0.13	nil
n-hexane fraction	13.43±0.16	23.00±0.82	16.00±0.05	nil
Ethyl acetate fraction	12.35±0.13	17.11±0.98	21.12±0.18	nil
n-butanol fraction	17.40±0.18	21.98±0.11	22.09±0.32	nil
Methanol fraction	12.11±0.19	11.24±0.56	17.12±0.55	nil

Table 3. Minimum Inhibitory Concentration (MIC).

Fractions	Minimum Inhibitory Concentration (MIC)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>
n-hexane fraction	50mg/ml	25mg/ml	50mg/ml
Ethyl acetate fraction	25mg/ml	12.5mg/ml	12.5mg/ml
n-butanol fraction	12.5mg/ml	25mg/ml	25mg/ml
Methanol fraction	25mg/ml	25mg/ml	25mg/ml

Table 4. Minimum Bactericidal Concentration (MBC).

Fractions	Minimum Bactericidal Concentration (MBC)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>
n-hexane fraction	50mg/ml	100mg/ml	50mg/ml
Ethyl acetate fraction	50mg/ml	25mg/ml	25mg/ml
n-butanol fraction	25mg/ml	50mg/ml	50mg/ml
Methanol fraction	25mg/ml	25mg/ml	50mg/ml

Minimum Inhibitory Concentration (MIC)

The MIC results of the fractions were presented in table 3. The fractions inhibited and prevented their total growth of *S. aureus*, *E. coli*, and *K. pneumoniae* at MIC of 12.5mg/ml. The MBC results of the fractions shown in table 4, the fractions finally prevented the growth and killed the organisms completely at MBC of 25mg/ml. The findings in this work have justified the potent use of this plant in ethno medicinal treatment of oral infections of diarrhea, dysentery, fever, typhoid etc. which are caused by some of these organisms used in this study. However, this result strongly suggested that the antibacterial activity of the

plant may be due to the presence of steroid, phenolics, saponins, tannins.

CONCLUSION

The findings in this work have justified the traditional use and the potent use of this plant in ethno medicinal treatment of oral infection of dysentery, fever, diarrhea, wound, tooth decay, malaria, ulcers, typhoid fever and various stomach related problems which are caused by some of these organisms used in this study. Work is still on in the isolate and characterization of the bioactive compounds in this plant.

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