



Antimicrobial Properties of Stem Bark Extracts of *Ximenia Americana*

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Abstract

Ximenia americana is endemic in Northern Nigeria, and has been reported to be used in treatment of various types of ailments. In the present study, the antimicrobial properties of the stem bark extracts of *Ximenia americana* were screened against *Escherichia coli*, *P.aeruginosa*, *Staphylococcus aureus*, *P. vulgaris*, *Candida albicans*, *B. subtilis* using the disc diffusion method. The result revealed the methanolic and water extract showed significant ($P<0.05$) broad spectrum activity on the growth of many of the test organisms (*E. coli*, and *P. vulgaris*, *S.aureus*, *P. aeruginosa* and *B. subtilis*), while the butanolic extract had little activity. Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, cardiac glycosides, terpenoids and tannins. The study supports the traditional usage of this plant by herbalist as remedy in curing microbial infections.

Keyword: *Ximenia americana*, *Antimicrobial activity*, Microorganisms

1. Introduction

Traditional medicine is well recognized in Africa and many rural communities rely on the use of ecofriendly and easily available plants in the treatment of a wide variety of ailments. *Ximenia americana* (Olacaceae) which is also known as “wild olive” in English, is a shrub like plant found in abundance in the West African region. It flowers usually in the second part of the dry season, producing cream – white to greenish yellow flowers. The fruits are green but turn golden-yellow or red. The fruit when eaten is very refreshing and has almond acid taste. In Northern Nigeria, the plant is found in the Savannah areas. It is extensively used among the Hausa/Fulani as herbal remedies in treatment of malaria, leproutic ulcers and skin infections of mixed origin (Ogunleye and Ibitoye, 2003). The leaves have been reported to have antibacterial activity (Ogunleye and Ibitoye, 2003) while leaves have been reported to be used in the treatment of fever, tuberculosis, stiffness, Onchocerciasis tooth decay and wounds (Abonnier, 2004). Abonnier, (2004) have reported the roots to be used in treatment of leprosy, syphilis, dysentery, and wounds. The stem bark has been reported to have antitypanosomal activity (Maikai *et. al.*, 2008). The stem bark and the leaves also are used in treating headaches and mumps. The present study is aimed at investigating the antimicrobial activity of the stem bark extracts of *Ximenia americana*.

2. Materials and methods

2.1 Collection of plant Material

The stem bark of *Ximenia americana* was collected from Afaka village 35km to Kaduna (11° 10' N, 7° 38' E) in the month of August 2006 and taken to Department of Biological Sciences herbarium, Ahmadu Bello University Zaria for

identification the voucher No. 1612 was deposited. The stem bark was dried at room temperature before crushing into powder using an electric blender and then stored in air tight container and kept at 4^oc until needed.

2.2 Solvent extraction

The powdered material (200g) was shaken in 300ml of acetone: water (2:1) and further extracted according to Latte (1999) to obtain fractions of different polarities. The extracts included petroleum ether extract (PEE), chloroform extract (CFE), butanol extract (BE), methanol extract (ME) and water extract (WE). Each extract was evaporated to dryness using a hot air oven set at 35^oC. The dried extracts were weighed and then stored in air tight container and kept at 4^oc until needed.

2.3 Phytochemical screening of extracts

Phytochemical analyses were done using standard procedures (Harborne 1973; Trease and Evans, 1989; Sofowora, 1993).

2.4 Organisms

The species of bacterial organisms were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Bacillus subtilis*, *Proteus vulgaris* and *Candida albicans*. They were clinical isolates obtained from Ahmadu Bello University Teaching Hospital, Shika Zaria. The cultures of bacteria were maintained on nutrient agar slants at 4^oC, re-identified by biochemical tests (Cheesburgh, 1982) and sub cultured into nutrient broth for 24h prior to testing.

2.5 Antimicrobial screening

The PEE, BE, ME, and WE extracts (100mg) were dissolved in 1ml of (DMSO, Merck, Germany) and diluted to a final concentration of 20mg/ml; other concentrations were made (15, 10 and 5mg/ml) by serial dilutions. The solutions were sterilized by filtration through a 0.45µm membrane filter. Sterile 9mm discs were impregnated with 50µl of extract and placed on the surface of agar plates inoculated with a microbial culture. Each extract was tested in triplicate. Ciprofloxacin (40µg/disc) served as positive control for the bacteria, whereas amphotericin B (25µg/disc) was control for *C. albicans*. The plates were incubated at 37^oC for 24hours. Antimicrobial activity was recorded if the zone of inhibition was greater than 9mm.

2.6 Statistics

One way analysis of variance (ANOVA) was used to test for level of significance.

3. Results

The results of phytochemical screening of stem bark solvent extracts of *X. americana* are presented in Table 1. The three solvent extracts revealed the presence of flavonoids, saponins, tannins and terpenoids. However, alkaloids, pylobatanins and cardiac glycosides were absent in the water extracts. The antimicrobial screening of the extracts showed that the activity of methanolic and water extracts appeared to be more significant (P <0.05) broad spectrum effect against the growth of *E.coli*, *P. aeruginosa*, *P. vulgaris*, *S. aureus* and *B.subtilis* than the butanolic extract at 10, 15 and 20mg/ml concentrations. The results indicate that the organisms were susceptible at higher concentration of the extract (20mg/ml). The extract also showed some activity against *C.albicans* at 20mg/ml while ciprofloxacin the standard drug for bacteria, failed to inhibit the growth of *C. albicans*. In general the bacteria were found to be more sensitive to the extract.

4. Discussion

Microbial infection involving microorganism's poses a very serious public health problem all over the world especially in resource poor African countries. To provide scientific support on the use of *X. americana* traditionally in the treatment of infections we tested the different solvent extracts on some common pathogenic organisms. Extracts of *X. americana* which was tested for antibacterial activity was found to have broad spectrum effect on the growth of many of the test organisms, *E. coli*, and *P. vulgaris*, *S.aureus*, *P. aeruginosa* and *B. subtilis* . The results tend to agree with similar work (Msonthi, 1986; Vlietinck *et al.*, 1995; Akiniyi *et al.*, 1996; Ogunleye and Ibitoye, 2003; Rabe and Van Staden, 1997; Buwa and Stada, 2006; Mohanasundari *et al.*, 2007).

The reasons for the differential sensitivity pattern between gram-positive and gram- negative bacterial strains could be due to the outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impenetrable to antimicrobial agents (Nikaido and Va'ara, 1985), while the gram positive bacteria is more susceptible having only an outer peptidoglycan, which is not an effective permeability barrier (Scherer and Gerhardt, 1971). *Ximenia americana* exerted more broad spectrum inhibitory activity against the microorganisms. Our results showed that there was some little activity of the extract on *C. albicans*, a fungus at a higher concentration. This also tends to agree with similar work (Balakrishna *et al.*, 2000) who reported that alcoholic extracts of *Solanum trilobatum* were effective on fungi at higher concentrations. Though the extract concentration was high (20mg/ml) when compared to the concentration of the antibiotic (Ciproflaxacin) which was 40µg. This could be explained that the extracts were still in

their crude form made of complex composition of chemicals compared to the standard drug which was a pure compound. Further purification of the extracts could lead to isolation of purer compounds with increased microbial activity. The medicinal value of plants lies in some chemical substances produced by these plants, these chemicals called "secondary metabolites" include; alkaloids, flavonoids, tannins and phenolic compounds (Cowan, 1999). The mechanisms of action of the chemical constituents of the extracts are difficult to speculate at this point, however, it is known that many antibacterial agents exhibit their actions either by inhibiting the synthesis of the cell wall, nucleic acid, or blocking the synthesis of proteins. Flavonoids have been reported (Dixon *et al.*, 1983; Hostettman *et al.*, 1995; Tsuchiya *et al.*, 1996) to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Tannins have the ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins and also complex with polysaccharide (Ya *et al.*, 1988; Scalbert, 1991). Terpenoids have also been (Tassou *et al.*, 1995; Taylor *et al.*, 1996) reported to be active against bacteria, the mechanism of action involve membrane disruption by the lipophilic compounds. The results of the present study support the use of *X. americana* as having antibacterial properties that can be used as agent in new drugs for the therapy of infectious diseases caused by pathogens. The phytochemicals can further be isolated and undergo further pharmacological evaluation.

4.1 Conclusion

This study justifies the claimed uses of the *X. americana* in the traditional system of managing various infectious diseases caused by microorganisms. The herbalists simply use water as the medium of extracting the active principles.

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Table 1. Phytochemical screening of stem bark extracts of *X. americana*

Extract	Alkaloids	Antraquinones	Cardiac glycosides	Flavonoids	Pylobatannins	Saponnins	Tannins	Terpenoids
Butanol extract (BE)	+	-	+	++	+	++	++	++
Methanolic extract (ME)	++	+	++	++	++	+++	+++	+++
water extract (WE)	-	+	-	+++	-	++	++	++

+++ - highly present, ++ - moderately present, + - faintly present, - absent

Table 2. Antimicrobial activity of stem bark extracts of *X. americana*

Extract/drug	Extract conc. (mg/ml)	Zone of inhibition (mm)					
		Escherichia coli	Pseudomonas aeruginosa	Proteus vulgaris	Staphylococcus aureus	Candia albicans	Bacillus subtilis
Butanol extract (BE)							
	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	-	10	5	8	-	10
	20	7	12	7	11	-	14
Methanol extract (ME)							
	5	-	-	-	-	-	-
	10	6	10	11	9	-	8
	15	10	15	15	13	-	9
	20	18	17	18	15	8	14
	5	-	-	-	-	-	-
Water extract (WE)							
	10	9	7	10	8	-	10
	15	14	12	14	14	-	13
	20	19	17	19	20	12	19
	40µg/disc	37	40	38	38	0	40
Ciprofloxacin							
n	25 µg/disc	0	0	0	0	28	0
Amphotericin							
n							

- no activity

Values greater than 9mm indicate activity