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Phytochemical and Antimicrobial Investigation of the Aqueous and Methanolic Extracts of *Ximenia americana*

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The root, stem bark and leaf of *Ximenia americana* from the Olacaceae family which is used as herbal remedies for the cure of many ailments by natives in the Northern part of Nigeria was studied. The aqueous and methanolic extracts of the afore mentioned parts of the plant were subjected to phytochemical screening and antibacterial evaluation. From the tests carried out, it was observed that the extracts contained carbohydrates in the form of sugars and soluble starch, cardiac glycosides, saponins, tannins and flavonoids while alkaloids were absent in all the extracts. Anthraquinones were present in all the extracts except the leaf extracts. The extracts were tested against five bacteria in which they inhibited the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* while *Shigella flexneri* was inhibited by only the methanolic leaf, aqueous bark and aqueous leaf extracts. *Salmonella typhi* and *Escherichia coli* were not affected by these extracts. The Minimum Inhibitory Concentration (MIC) was only evident for the methanolic extracts at 1.25×10^{4} μg mL^{-1} (1:4) against *Staphylococcus aureus* while the Minimum Bactericidal Concentration (MBC) of these extracts on *Staphylococcus aureus* was obtained at 2.50×10^{4} μg mL^{-1} (1:2).

Key words: Phytochemical, antimicrobial, *Ximenia americana*, microorganism, bactericidal, Nigeria

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INTRODUCTION

Phytochemicals are sometimes referred to as phytonutrients and these terms are often used interchangeably. Most broadly defined, they could be said to be any chemical or nutrient derived from a plant source. However, in common usage they have a more limited definition. They are usually used to refer to compounds found in plants which are not required for normal functioning of the body but which nonetheless have a beneficial effect on health or an active role in the amelioration of disease. Thus, they differ from what are traditionally termed nutrients in that they are not a necessity for normal metabolism and their absence will not result in a deficiency disease at least not on the timescale normally attributed to such phenomena. A minority claim that many of the diseases afflicting the people of industrialized nations are the result of those people's lack of phytonutrients in their diet. What is beyond dispute is that phytonutrients have many and various salubrious functions in the body. For example, they may promote the function of the immune system, act directly against bacteria or viruses, reduce inflammation, or be associated with the treatment and/or prevention of cancer, cardiovascular disease or any other malady affecting the health or well-being of an individual (http://dictionary.laborlawtaik.com/).

For a long period of time, plants have been a valuable source of natural products for maintaining human health and according to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs.

The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. A large number of plants indeed were used to combat different diseases and known to possess antimicrobial activity (Arora and Kaur, 1999).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, the resistance to these drugs by microorganisms has increased (Cohen, 1992). In general, bacterial have the genetic ability to transmit and acquire resistance to drugs, which are, utilized as therapeutic agents. Such a fact is cause for concern because of the number of patients in hospitals who have suppressed immunity and due to new bacterial strains, which are multi resistant (Bisset, 1994).

The plant *Ximenia americana* has been in use for centuries in many countries where it is used for may herbal preparations. In Nigeria, it is extensively used among the Hausa/Fulani communities as herbal remedies in treating malaria, leprotic ulcer and skin infections of mixed origin (Ogunleye and Ibitoye, 2003). Although Ogunleye and Ibitoye (2003) reported on the chemical constituents of the aqueous and antimicrobial activities of ethanolic extract of the leaf, there has been no documented scientific evidence on the stem bark and root of the plant.

This study is an attempt to determine some phytochemical components of the aqueous and methanolic (stem bark, roots and leaf) extracts and to compare their antimicrobial activity of the plant on some selected pathogenic microbes isolated from patients.

MATERIALS AND METHODS

Plant materials: The plant samples (leaf, stem bark and roots) of *Ximenia americana* were collected from Shika in Zaria, Nigeria. It was identified at the herbarium, Biological Sciences Department, Ahmadu Bello University, Nigeria.

Test organism: Five test organisms were clinical isolates obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. These organism were; *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Shigella flexneri* and *Klebsiella pneumoniae*.

Preparation of leaf, stem bark and root: The leaf, stem bark and roots were dried in a well ventilated room. The dried materials were ground into fine powder using a mortar and pestle. The powder obtained from each sample material was used to prepare the extracts.

Extraction: To 100 g of the powder samples (roots, stem bark and leaf), 300 mL of distilled water was added. A second set was prepared with 100 g of each powdered samples in 300 mL of 100% methanol. The six mixtures were left to stand for 24 h.

After the time elapsed each suspension was then filtered using Whatman's filter paper. The filtrates were then evaporated to dryness in a water bath at 40°C and the weight of the dried samples taken.

Phytochemical screening: Tests and reagent preparations were as described by standard method in Sofowora (1993) and Evans (2000).

Preparation of stock solution of extracts for microbal analysis: A 50% (w/v) stock solution of each extract was prepared by dissolving the extracts in their appropriate solvents in a ratio of 1:2 (water extract in water, methanolic extract in 10% methanol).

Microbial tests of plant extracts: From the nutrient agar, slants containing a pure culture of the organisms, a wire loop, was used to suspend the bacterium in 3 mL of sterile saline until the suspension matches the opacity of McFarland number 7 standards (2.1×10^9 micro organism mL^{-1}).

Disks of 5 mm in diameter were made by perforating Whatman's filter paper with a perforator. The disks were autoclaved at 121°C for 15 min in six separate glass containers, one for each extract.

The disks were soaked in 1 mL of stock solution (50%w/v) of each extract for 48 h for maximum absorption. They were then decanted and dried in an oven for 30 min at 40°C.

The nutrient agar was prepared according to manufacturer's specification, which stated that 28 g of nutrient agar is to be dissolved in 1000 mL of distilled water. It was boiled, autoclaved and dispensed into sterile Petri dishes in preparation for antimicrobial test. The disks method technique was used (Cheesbrough, 1984).

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts was determined by the macro broth dilution method (Cheesbrough, 1984).

Statistical analysis: The statistical analysis was conducted by using t-test on a statistical software package (SPSS).

RESULTS

The results in Table 1 indicate that the phytochemical constituents present in the extracts were Carbohydrates in the form of sugars and soluble starch except for aqueous leaf extracts. Glycosides such as saponins, cardiac glycosides and anthraquinone were all present in all the extracts except for leaf extracts that containing no anthraquinone. Similar observation on the leaf extracts has been reported by Ogunleye and Ibitoye (2003), flavonoids and tannins were also found to be present in all the extracts while Alkaloids were absent in all the extracts.

From the result in Table 2 inhibitory activity of extracts (methanolic root) was more pronounced on *Klebsiella pneumoniae* whereas it shows no activity against *Escherichia coli*, *Salmonella typhi* and *Shigella flexneri*. The methanolic root extract showed highly significant ($p<0.05$) activity on *Klebsiella pneumoniae* when compared with leaf extracts and methanolic bark extract. It was obvious that the effect of methanolic root extract was significantly ($p<0.05$) high on *Staphylococcus aureus* when compared with other extracts (Table 3).

There was no significant ($p>0.05$) difference between the activity of methanolic leaf, aqueous bark and aqueous leaf on *Shigella flexneri*. The zones of inhibitions of these extract were significantly ($p<0.05$) lower than all the other extracts on the microorganism used except for water leaf of *Klebsiella pneumoniae*.

Table 1: Some of the aqueous and methanolic extracts of root, stem bark and leaf of *Ximenia americana*

	Extracts					
Test	MR	MB	ML	WR	WB	WL
Glycosides						
General test	+	+	+	+	+	+
Borntrager's test	+	+	-	+	+	-
Cardiac Glycosides	+	+	+	+	+	+
Saponin Glycosides	+	+	+	+	+	+
Cape aloes	-	-	+	-	-	-
Carbohydrates						
Molish test	+	+	+	+	+	+
Barfoed tests	-	-	-	-	-	-
Fehling's test	+	+	+	+	+	+
Combined reducing sugar	+	+	+	+	+	+
Resorcinol test	-	-	-	-	-	-
Pentoses	+	+	+	+	+	-
Soluble starch I	+	+	+	+	+	-
Soluble starch II	+	+	+	+	+	-
Tannins						
Lead acetate	+	+	+	+	+	+
Iron III chloride	+	+	+	+	+	+
Alkaloids						
Mayer's test	-	-	-	-	-	-
Wagner's test	-	-	-	-	-	-
Dragendorf test	-	-	-	-	-	-
Flavonoids						
NaOH test	+	+	+	+	+	+
H_2SO_4 test	+	+	+	+	+	+
Shinoda test	+	+	+	+	+	+

MR = Methanolic root, WR = Water root, MB = Methanolic bark, WB = Water bark, ML = Methanolic leaf, WL = Water leaf; +: Present, -: Absent

Table 2: Effect of different extracts on *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Shigella flexneri*

Micro-Organisms	MR	MB	ML	WR	WB	WL
Staphylococcus aureus	15.0 ± 1.4^{a}	11.5 ± 0.7^{b}	12.5 ± 2.1^{ab}	13.5 ± 0.7^{a}	14.5 ± 0.5^{a}	13.0 ± 1.4^{ab}
Salmonella typhi	0	0	0	0	0	0
Klebsiella pneumoniae	17.0 ± 0.1^{a}	11.5 ± 0.7^{b}	12.0 ± 1.4^{b}	14.5 ± 3.8^{ab}	14.0 ± 2.8^{ab}	4.2 ± 0.7^{c}
Escherichia coli	0	0	0	0	0	0
Shigella flexneri	0	0	7.8 ± 1.3^{a}	0	6.6 ± 1.1^{a}	6.0 ± 1.0^{a}

MR = Methanolic root, WR = Water root, MB = Methanolic bark, WB = Water bark, ML = Methanolic leaf, WL = Water leaf, Means with different superscripts in a row differ significantly $p<0.05$; values are means of triplicates determinations

Table 3: Effect of different extracts on Minimum Inhibitory Concentration (MIC) of the extracts

	Serial dilutions	Concentration g mL^{-1}	*Staphylococcus aureus*	*Klebsiella pneumoniae pneumoniaei*	*Shigella flexneri*
MR	1:2	0.2500	-	+	X
	1:4	0.1250	*	+	X
	1:8	0.0625	+	++	X
	1:16	0.0313	++	++	X
	1:32	0.0156	+++	+++	X
MB	1:2	0.2500	-	+	X
	1:4	0.1250	*	+	X
	1:8	0.0625	+	++	X
	1:16	0.0313	++	++	X
	1:32	0.0156	+++	+++	X
ML	1:2	0.2500	-	+	+
	1:4	0.1250	*	+	++
	1:8	0.0625	+	++	++
	1:16	0.0313	++	++	++
	1:32	0.0156	+++	+++	+++
WR	1:2	0.2500	+	++	X
	1:4	0.1250	+	+	X
	1:8	0.0625	++	++	X
	1:16	0.0313	++	++	X
	1:32	0.0156	+++	++	++
WB	1:2	0.2500	+	+	+
	1:4	0.1250	+	+	++
	1:8	0.0625	++	++	++
	1:16	0.0313	++	++	+++
	1:2	0.2500	+	+	+
	1:8	0.0625	++	++	++
	1:16	0.0313	++	++	++
	1:32	0.0156	+++	+++	+++
	1:32	0.0156	+++	+++	+++
WL	1:2	0.2500	+	+	+
	1:4	0.1250	+	+	++
	1:8	0.0625	++	++	++
	1:16	0.0313	++	++	++
	1:32	0.0156	++	++	++

MR = Methanolic root, WR = Water root, MB = Methanolic bark, WB = Water bark, ML = Methanolic leaf, WL = Water leaf, No growth + turbid * MIC ++ slightly turbid, +++ highly turbid x MIC not determined

Table 4: Effects of different extracts on Minimum Bacteriocidal Concentration (MBC) of the extracts

	MR		MB		ML	
Micro organism	1:2	1:4	1:2	1:4	1:2	1:4
Staphylococcus aureus	-	+	-	+	-	+

MR = Methanolic root MB = Methanolic bark ML = Methanolic leaf; +: Present, -: Absent

DISCUSSION

The present study was designed to obtain preliminary information on phytochemical and antimicrobial effect of *Ximenia americana* leaf, stem bark and root on certain pathogenic microorganisms. The level of anti microbial action of all the extracts on different microorganism may be partially ascribed to their chemical components. Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods and diarrhea and as antidote in heavy metal poisoning. Flavonoids are naturally occurring phenols, which possess numerous biological activities including anti-inflammatory, antiallegic, antithrombitic antibacterial, antifungal and vasoprotective effects. The observed antimicrobial activity against the tested organisms could be due to the presence of tannins and flavonoids in the extract as these have previously been reported to possess antimicrobial activities (Erah *et al*., 1996; Wild and Fasel, 1969).

Furthermore the components have different mechanisms of action on microorganisms, which include their ability to inhibit enzymes produced by the bacteria and substrate deprivation. In this study the most promising extract is methanolic extract, however *Salmonella typhi*, *Escherichia coli* and *Shigella flexneri* were not affected by this extract. This could be to the fact that they are gram negative bacterial, the observed resistance of *E. coli* probably could be due to cell membrane permeability or due to other genetic factors.

The findings that the methanolic extracts have bactericidal activities over the concentration of 2.5×10^{4}-1.25×10^{4} μg mL^{-1} on *Staphylococcus aureus* (Table 4) are also similar to the susceptibility of that microbe to different plant extracts reported by several researcher (Arora and Kaur, 1999; Okemo *et al*., 2001; Digraki, 1999; Madamombe and Afolayan, 2003).

However, gram-positive bacteria were found to be more susceptible than gram-negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope (Hawkey, 1998; Gould and Booker, 2000)

The methanolic root extract showed slightly better killing action than the aqueous extract, which means that the methanolic extract could be used more. But it needs further investigations to distinguish its components and their individual antimicrobial effect. The low antimicrobial action of the aqueous extracts could be ascribed to low level of the anionic components such as thiocynate, nitrate, chloride and sulphates beside other water soluble components, which are naturally occurring in most plant materials (Darout *et al*., 2000).

It can be concluded that the extracts of methanolic roots, stem bark and leaves have bactericidal activities over the concentration of 2.5×10^{4}-1.25×10^{4} μg mL^{-1} and that the presence of carbohydrates, glycosides, flavonoids and tannins in the different extracts are responsible for their anti bacterial activity. This findings have validated the use of *Ximenia america* in control of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Shigella flexneri*, which have been implicated in the cure of food poisoning boils, skin infections, ulcers, eczema, diarrhea and pneumonia. It seems important to recommend that, further studies using isolated constituents instead of whole extract must be done in a bid to produce a drug with a fixed dosage of administration. Toxicological studies are also necessary in order to increase the safety of the drug for intake. Efficacy of the plant can be increased by combining with other components probably from other plants

REFERENCES

Arora, D. and J. Kaur, 1999. Antimicrobial activity of spices. Int. J. Antimicrob. Agents, 12: 257-262.

Bisset, N.M., 1994. Herbal Drugs and Phytopharmaceuticals. CRC Press, London, pp: 106-108.

Cheesbrough, M., 1984. Medical Laboratory Manual for Tropical Countries (Vol. 2), Microbiology Butterworth and Co. Ltd., Britain, pp: 198-200.

Cohen, M.L., 1992. Epidermiology of drug resistance: Implications of a post-microbial era. Science, 257: 1050-1055.

Darout, I., A. Cristy, N. Skaug and P. Egeberg, 2000. Identification and quantification of some potentially antimicrobial anionic components in Miswak extract. Indian J. Pharm., 32: 11-14.

Digraki, M., M. Alma Pharmaceut, A. Ilcim and S. Sen 1999. Antibacterial and antifungal effects of various commercial plants extract. Biology, 37: 216-220.

Erah, P.O., G.E. Osuide and E.K.I. Omogbai, 1996. Hypoglycaemic effect of the extract of *Solenostemon monostachys* (P. Beauv) leaves. J. West Africa Pharm., 10: 21-27.

Evans, W.C., 2000. Trease and Evans Pharmacology, 14th Edn., WB Saunders Company Ltd., pp: 224-293.

Gould, D. and C. Booker, 2000. Applied microbiology for nurses. Aardvark Editorial, Mcndham, Suffolk., pp: 75-94.

Hawkey, B.M., 1998. The origins and molecular basis of antibiotic resistance. BMJ., 317: 657-660.

Madamombe, I. and A. Afolayan, 2003. Evaluation of antimicrobial activity of extracts from South African Usnea barbata. Pharm. Biol., 41: 199-202.

Ogunleye, D.S. and S.F. Ibitoye, 2003. Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. Department of Pharmaceutical and Medical Chemistry. Faculty of Pharmacy. Olabisi Onabanjo University, Nigeria, 2: 239-241.

Okemo, P., W. Mwatha, S. Chhabra and W. Fabry, 2001. The kill kinetics of *Azadirachta indica* A. juss. (Meliaceae) extracts on *Staphylococcus aureus*, *E. coli*, *Psedomonas aeruginosa* and *Candida albicans*. African J. Sci. Techol., 2: 113-118.

Phytochemical: Meaning and uses (http://dictionary. laborlawtaik.com/Sofowora, A., 1993. Medical Plants and Traditional Medicine in Africa 2nd Edn., Spectrum Books Ltd. Ibadan/Lagos, pp: 69-70, 119: 150-153.

Wild, C. and J. Fasel, 1969. Effect of a flavonoid on the capillary resistance of the rectal mucosa in hepatic cirrhosis. Am. J. Proc., 20: 60-62.