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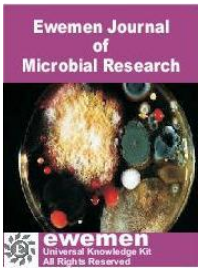

Original Research

PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF TWO MEDICINAL PLANTS (*XIMENIA AMERICANA* AND *ACACIA NILOTICA*)

ADAMU H.M.[1]; USHIE O.A.[2]; *OGAH E.[2]; LONGBAP B.D.[2]; BULUS I.D.[1]

[1]Chemistry Programme, Abubakar Tafawa Balewa University Bauchi, Nigeria
[2]Department of Chemical Science, Federal University, Wukari, Nigeria

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**Corresponding Author's Email:*
uhinyohe@gmail.com

ABSTRACT

The plants *Ximenia americana* L. and *Acacia nilotica* L. are commonly used traditionally for the treatment of malaria and other antimicrobial ailments. The antibacterial activities of the medicinal plants were investigated. Phytochemical screening of the extracts of different parts of the plants revealed the presence of saponins, alkaloids, oils, glycosides, tannins, anthraquinones and steroids. Alkaloids were not detected in *Acacia nilotica*. The antimicrobial studies of acetone and methanol extract were carried out on five pathogenic organisms, namely; *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Shigella dysenteriae* and *Streptococcus pneumonia*. The extracts used showed inhibitory activity on the organisms tested with zones of inhibition from 10 mm to 25 mm. This result confirmed the use of the plants in traditional medicinal treatment of malaria, diarrhoea, dysentery, wound and tuberculosis. The presence of the above secondary metabolites in the plants might be responsible for the activities.

Key words: *Acacia nilotica,* phytochemical screening, *Ximenia Americana.*

INTRODUCTION

The use of plant and plants preparations for the treatment of diseases can be dated back to time immemorial. Plants are able to synthesize different compounds such as carbohydrates, proteins, fats and oil, vitamins, etc. These substances are primary metabolites and are common to all plants. There are other components synthesized by plants either for defense or survival and these are specific to some groups or species of plants. These chemical substances are synthesized by plant in small quantity and are called secondary metabolites. These include alkaloids, tannins, saponins, flavonoids, terpenes, steroids, glycosides, anthraquinones, etc.

Nowadays plants are still used for treatment of diseases especially among the rural dwellers; for example *Caniphera kerstingii* is used as laxative for stomach constipation, dysentery and diarrhoea (Kubmarawa *et al.,* 2007). Also, most micro-organisms have developed resistance to the existing orthodox drugs and as such there is need for alternative drugs (Sofowara, 1982).

Ximenia americana L. and *Acacia nilotica* (L.) Delile are two commonly used plants for the management of ailments related to bacterial infections in Hausa traditional medicine in Nigeria. *Ximenia americana* L. is

called Tsandzara in Margi and Tsaada in Hausa (Gbile, 1980). The plant is widely found in the tropical and temperate regions of the world. The leaves may be thin, alternate, and more or less glossy in the savannah regions or strongly armed with axillaries spins in the cost regions. The leaves may be half-succulent elliptic to narrow elliptic, about 3-7 cm long and 13 cm broad. The petiole is about 3-6 mm long. The fruit is superior ellipsoid about 2.5 cm in diameter. The fruit is yellow and smooth. The seed is about 1.5 cm in diameter (Hopkin and Stanfied, 1966; Hatchinson and Dalziel, 1972). The preparation of branch, leaves, barks, peelings and roots are used for headaches, toothaches, malaria, fever, measles and for the treatment of skin infections. It is also used for diarrhoea remedy. The fruit is also used for the calves suffering from diarrhoea (Kokwaro, 1993).

Acacia nilotica is commonly called by Hausa speaking people of northern Nigeria as Gabaruwa. The wood of this species is hard and reddish in colour and most of the browsers do not eat the leaves. The tree grows to a height of 10 m, with average of 4-7 m height. The leaves consist of 5-11 feather-like pairs of pinnae. It flowers mainly from September-January, but it depends on the rainy season. The seed is dark blackish when mature. The wood of *Acacia nilotica* is hard and is used as firewood. The bark exudes an edible gum and is used medicinally. Other parts of the tree were used to treat eye diseases, or as a tranquillizer and even as an aphrodisiac. A root extract was used in the treatment of tuberculosis, impotent, diarrhoea, haemorrhage, toothaches, dysentery and gonorrhoea. Extracts made from the leaves are used in the treatment of menstrual problems, eye infection. Traditionally, the bark seed of the plant is used for strengthening skin, treatment of wound, dysentery diarrhoea. Ogunleye and Ibitoye (2003), carried out research on the studies of antimicrobial activity and chemical constituents *of Ximenia americana* and found that the extracts was active against the test organism including *E. coli, P. aeruginosa and Candida albicans.* The test also indicates the presence of tannins, flavonoids, alkaloids, saponins, anthraquinones, starch, general glycosides and bitter principles were found to be present in the extracts. In Folk medicine, a number of therapeutic uses *of Ximenia americana* had been reported. Roots are employed as antiseptic for metal disease, fever, jaundice and headache, whereas the leaves are used for treatment of toothache and also as laxative (Sofowara, 1982). Very little work is available in literature on the plant activity of these plants in Mubi area. Hence this study was limited to the *Ximenia americana* and *Acacia nilotica* found in Mubi. Extracts of the two plants are phytochemically screened and tested against pathogenic bacteria isolated from patients in General Hospital Mubi.

MATERIALS AND METHODS

Materials

All reagents use were of analytical grade and unless otherwise specified were according to manufacturer's specification and direction for use.

Extraction of the bioactive agent

Successive maceration was performed using acetone and methanol as solvent at ambient temperature. Briefly, the stem-bark (or leaf) was soaking 100 g of the extract in 250 mL of acetone for four days. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation to dryness in a water bath and weighed. The procedure was repeated on the residue using the following solvents methanol. The extracts were store in a desiccator.

Phytochemical screening

Qualitative analyses of the crude extracts were carried out by methods described by Brain and Turner (1975), Odebiyi and Sofowara (1978), Trease and Evan (2000) and Ushie *et al*. (2013), to identify the presence of secondary metabolites, which include alkaloid, anthraquinones, flavonoids tannins, saponins, steroids and glycosides.

Test for saponins

2 mL of the aqueous extracts in a test tube was vigorously shaken for two minutes. Frothing observed in the three extracts tested indicated the presence of saponins.

Test for tannins

To each extract 2 drops of 5% $FeCl_3$ were added. A greenish precipitate indicated the presence of tannins in the extracts.

Test for flavonoids

To 3 mL of the extract was added 1 mL of 10% of NaOH. A yellow coloration showed the presence of flavonoids in the extract.

Test for anthraquinones

About 1 mL of the extract was shaken with 2 mL of benzene and 4 mL of 10% NH_3 solution was shaken and the presence of a pink colour in the ammonia solution (lower layer) phase indicates the presence of anthraquinones.

Test for steroids

Salkowski's test: To 1 mL of the extracts 5 drops of concentrated H_2SO_4 was added. A red colouration was observed in each extract showing the presence ofsteroids.

Test for alkaloids

To 1mL of each extract 2 drops of Hager's reagent were added. A reddish brown precipitate observed indicates the presence of alkaloids in each extract.

Test for glycosides

A small portion of each of the plant extracts was placed in two separate test tubes of 0.1 M H_2SO_4 was added to one and distilled water (5.0 cm^3) added to the other. The test tubes were heated for 45 minutes in a water bath. The cooled solutions were made alkaline with a solution of 2 M NaOH. Fehling solutions (5.0 cm^3) A and B (ratio1:1) was added to the two test tubes and were allowed to stand for 3 minutes. The solution of the extracts in distilled water serves as control. A brick - red precipitated was observed in the extracts showing the presence of glycosides.

Antimicrobial assay

Clinical isolates and standard culture

Clinical isolates of *Pseudomonas aeruginosa, E. Coli, Staphylococcus aureus, Shigella dysenteriae* and *Streptococcus pneumonias* were collected from the General Hospital Mubi, Adamawa State. The agar ditch diffusion method similar to that described by Hufford *et al.,* (1975) was employed. The extracts were dissolved in 5 mL of water to produce 0.5% dilution to give 0.5%. The positive and negative controls were amoxicillin and water, respectively. The positive control was of equivalent dilution as the extract.

The prepared nutrient agar (Oxoid) contained 2.8 g nutrient agar in 100 mL distilled water. The media were sterilized by autoclaving at 121°C for 20 minutes. Bacterial suspensions were cultured in nutrient agar in petri - dishes. Wells (6 mm in diameter) were punched in the middle of the agar using a sterile borer and filled with 0.l mL of the test extract dilutions and control drug. The plates were incubated for 24 hours at 37°C and the diameters of any resulting zones of inhibition were measured in millimeter and recorded.

RESULTS AND DISCUSSION

Phytochemical screening of the *Ximenia americana* (Table 1) revealed that saponins, flavonoids, glycosides, tannins, anthraquinones and steroids were present in methanol extract and acetone of the leaf. Alkaloids was present in the methanol extracts was absent in acetone extract. It result also revealed that chemical constituents in stem bark were all present in acetone extract. Alkaloids, anthraquinone and steroids were not detected in methanol extract of stem bark. Anthraquinone and steroids that were absent in water extracts. The findings agreed with work of Ogunleye and Ibitoye (2003), that found the presence of tannin, flavonoids, alkaloids, saponins and anthraquinone in *Ximenia americana*. The study further reveals that *Ximenia americana* contain steroids which was not reported by Ogunleye and Ibitoye (2003). This therefore could be used as anti-inflammatory agent as indicated by Olanyi et al (1993).

The Phytochemical screening of *Acacia nilotica* (Table 2) revealed the presence of saponins, flavonoids, glycosides, tannins and steroids in the acetone leaf extract while alkaloids and anthraquinones were absent. Of the metabolite screened for, only alkaloids were not found in methanol extracts of leaves of *Acacia nilotica.* Alkaloid was absent in the acetone and methanol extracts of the stem bark, and anthraquinones and steroids were also absent in the methanol extracts of the stem bark. Alkaloids and steroids were also absent in the water extract of stem bark of *Acacia nilotica*.

In the antimicrobial sensitivity test, the results (Table 3), showed that all the organisms tested were sensitive to all plant extracts for both *Ximenia americana* and *Acacia nilotica* except *Shigella dysenteriae* which was not sensitive to only methanol stem bark extract of *X. americana*. The highest zone of inhibition by *A. nilotica* was by methanol stem bark extract against *Streptococcus pneumoniae* with a ZID of 24 mm against the positive control of 17 mm, and by the acetone seed bark extract against *S. aureus* with a ZID of 24 mm against the positive control ZID of 23 mm. The least activity was the acetone and methanol extracts of the

seed and stem barks against *Shigella dysenteriae* with a ZID of 12 and 10 mm, respectively while the organism was not sensitive to the control.

For *Ximenia americana* the highest ZID was 25 mm against S. aureus which was exhibited by the methanol extract of the leaf and acetone extract of the stem bark. The ZID of the positive control was 24 mm. The least ZID of 11 mm was exhibited by the methanol stem bark against E. coli, against a control ZID of 28 mm. All microorganisms tested were not sensitive to water used as negative control. The antimicrobial activities *of Ximenia americana* and *Acacia nilotica* were in agreement with similar research on *Ximenia americana* conducted by Ogunleye and Ibitoye (2003), which showed that it was active against *P. aeruginosa*. The result suggests that the plants could be a good therapeutic agent for dysentery, fever and as antiseptic as observed by Sofowara (1982).

CONCLUSION

These plant extracts showed good antimicrobial activities against *P. aeruginosa, S. aureus, E, coli, S. dysenteriae* and *S. pneumoniae.* The activities exhibited could be attributed to the presence of bioactive secondary metabolites like saponins, tannins, anthraquinones and steroids. The find justified the ethnomedicinal use as antiseptic or antibacterial and therefore, the plant could be a good therapeutic agent for dysentery, fever and as antiseptic as observed by Sofowara (1982). Further should be conducted to identify the bioactive compounds responsible for the antibacterial activities.

Table 1: Result of phytochemical screening of extracts of *X. Americana*

Metabolites	Leaves		Stem bark		
	A	M	A	M	W
Alkaloids	-	+	+	-	+
Saponins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Glycosides	+	+	+	+	+
Tannins	+	+	+	+	+
Anthraquinones	+	+	+	-	-
Steroids	+	+	+	-	-

Key: A= acetone extract, M = methanol extract, W = water extract.

Table 2: Result of Phytochemical screening of extracts of *A. nilotica*

Metabolites	**Leaves**		**Stem bark**		
	A	M	A	M	W
Alkaloids	-	-	-	-	-
Saponins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Glycosides	+	+	+	+	+
Tannins	+	+	+	+	+
Anthraquinones	-	+	+	-	+
Steroids	+	+	+	+	+

Table 3: Zone of inhibition diameter (ZID) of extracts of *X. americana* and *A. nilotica*

	X. americana			**Control**		***Acacia nilotica***		
Test organisms	Leaf	Stem bark				Seed bark	Stem bark	
	M	A	M	DC	WC	A	A	M
P. aeruginosa	16	17	12	23	NS	17	16	17
S. aureus	25	25	13	24	NS	24	23	20
E. coli	14	16	11	28	NS	20	15	22
S. dysenteriae	19	12	NS	NS	NS	12	10	10
S. pneumoniae	21	17	12	17	NS	18	18	24

Key: A = Acetone extract, M = methanol extract, NS = not sensitive, DC = Drug as positive control (Amoxycilin 250mg), WC = Water as negative control

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