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Research Article

Phytochemical Screening and Antimicrobial Activity of *Ximenia americana*

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ABSTRACT

The method of cold maceration was used in the extraction by serial exhaustive extraction method. Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. The phytochemical screening of crude extracts of *Ximenia americana* revealed that alkaloids, tannins, terpenoids, flavonoids and steroids. The crude extracts were tested for antibacterial and antifungal activities. All the crude extracts of the leaf inhibited or exhibited antibacterial activity against all the bacteria pathogens tested with a diameter that ranged between 8 – 21 mm. All the crude extracts of the leaf inhibited or exhibited antifungal activity against all the fungi pathogens tested with a diameter that ranged between 8 - 14 mm except hexane and ethyl acetate extracts that did not show significant inhibition against *A. niger*. The minimum inhibitory activity (MIC) of the extracts of *Ximenia americana* against tested microbes ranges from 400 to 100 mg/ml in almost all the extracts and in few instance 50 mg/ml against the tested bacteria.

Key words: Phytochemica, Antimicrobial Activity, Ximenia americana, serial exhaustive extraction, minimum inhibitory activity

Plants are the main source of food, are rich in nutrients and are also rich in compounds which have pain relieving and healing abilities (Thenmozhi and Sivaraj 2010). The wide Plants are a source of large amount of drugs comprising to different group such as anticancer, Antimicrobials (Sheela, 2014). Various medicinal plants have been used for years in daily life to treat various diseases all over the world (Sathya *et al.,* 2013). Sheela 2014 also pointed out that a large number of plants claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. From earliest times itself, plants were used for treatment of disease without knowledge about the compounds present and their mode of action (Thenmozhi and Sivaraj 2010). Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Elumalai and Eswariah.,2012).

Phytochemistry deals with the analysis of plant chemicals called natural products and with changes occurring in such chemicals due to alterations in environmental conditions (Sathya *et al.,* 2013). *Ximenia americana* is endemic in Northern Nigeria, and has been reported to be used in treatment of various types of ailments (Maiko *et al.* 2009). Most of

the claims are made by traditional medical practitioner themselves and may have not been thoroughly investigated scientifically (Sofowora 1982). For this reasons therefore, it could be argued that further investigation into this medicinal plant is needed. This present study has the main aim of presenting a medicinal plant *Ximenia americana* with view to promoting this natural herb and to optimize the use of available natural resources in the environment. The specific objectives were extracting, conducting simple chemical tests to detect the presence of some phytochemicals and to carry out the antimicrobial activities of extracts from *Ximenia americana* leaf.

Ogunleye and Ibitoye (2003) pointed out that the plant could be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or infusion. *Ximenia americana* is used in Mali in West Africa for treatment of various diseases, most common are infectious and inflammatory ailments (Le *et al*., 2012). *Ximenia americana* is a plant that is used in traditional medicine for the treatment of malaria, leproutic ulcers and skin infections of mixed origin in Northern parts of Nigeria (Ogunleye and Ibitoye 2003). *Ximenia americana* is in Mali in West Africa for treatment of various diseases, most common are infectious and inflammatory ailments like throat infection, amenorrhea and as tonic (Le *et al.,* 2012). This study has the main aim of this study is to carry out the phytochemical analysis and antimicrobial activities of the leaf extracts of *Ximenia Americana.*

MATERIALS AND METHODS

Sample Collection and Preparation

Ximenia americana leaves were collected from their natural habitat of Zing Local Government Areas of Taraba state, Nigeria. The samples were air-dried for two weeks and then milled into fine powder using a milling machine.

Method of Extraction

The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. The extracts of the leaves was prepared by soaking 100 g of each in 250 ml hexane for four days with frequent agitation until soluble matter is dissolved. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation using rotatory evaporator, kept in a vacuum oven over night at room temperature to remove all the solvent and weighed. The procedure was repeated on the residue using the following solvents: Chloroform, ethyl acetate, acetone and methanol sequentially in order of polarity. The extracts were stored in a desiccator until required for testing.

Phytochemical Screening Assay

Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989), Harborne (1988).and Ushie *et al*., 2012.

Test for Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue solution indicated the absence of tannins in distilled water and dark green colour indicating presence in methanol.

Test for Saponins

About 0.2g of plant extract was mixed with distilled water and heated to boil. Frothing (appearance of creamy mix of small bubbles) showed the presence of Saponins in Methanol while red in Distilled water.

Test for Terpenoids

The extract (0.2g) was mixed with 2ml of chloroform, and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids on both extract.

Test for Steroids

Acetic anhydride (2 ml) was added to 0.5g of the extract in a test tube. It was then followed by the addition of 2 ml of sulfuric acid. A colour change from violet to blue or green indicated the presence of steroids on both extract.

Test for Flavonoids

About 0.2g of the extract was dissolved in dilute sodium hydroxide solution, and equal amount of hydrochloric acid was added. A yellow solution that turned colourless indicated the presence of flavonoids on both extract.

Test for Alkaloids

The aqueous (3ml) was stirred with (3ml) of 1% HCl on a steam bath. Meyer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as positive evidence of alkaloids

Test for phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Disposition of red precipitate determines the presence of phlobatannins.

Test for Anthraquinones

About 0.5g of the extract was boiled with 2ml of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3 solution was added to the mixture and heated. Formation of rose pink colour indicated the presence of anthraquinones on both extract.

Test for Cardiac glycosides

10 cm_3 of 50% H_2S0_4 was heated in boiling water for 5 min. 10 cm_3 of Fehlings solution (5 cm_3 of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycoside was observed.

Bioassay

This is the study of antimicrobial activity of the crude or purified extracts against micro-organism. It was used as a guide to determine the active components of the leaves of *Ximenia americana.* The crude extracts were tested for antibacterial and antifungal activities. The test organisms were collected from Bauchi Specialist Hospital, Bauchi State, Nigeria. The antibacterial assay was carried out using methods described by Ochi *et al*., (2015) with modifications.

Preparation of varying concentrations of the extracts

Various concentrations of the extracts were prepared ranging from 50 to 400 mg/mL; this was obtained by measuring 1 mg of the extract and dissolved in 10 mL dimethyl sulphur oxide (DMSO), a solvent that dissolved the extract (100 mg/mL). A serial dilution of the dissolved extract (100 mg/mL) was carried out into three different bottles containing DMSO to obtain concentrations of 400, 200 100 and 50 mg/mL respectively.

Sensitivity test of the crude extract using Agar Well Diffusion Method

The organisms used were standardized using McFarland turbidity standard scale I, to obtain a bacterial cell density of 10^6 colony forming unit per millilitre (cfu/mL). The standardized inoculate were uniformly streaked (swabbed) into freshly prepared Mueller Hinton agar and potato dextrose agar plates respectively for the bacterial and fungal growth. Five wells were made on the inoculated plates with a cork borer (8 mm in diameter). The wells were properly labeled according to different number of the concentrations prepared. The wells were then filled up with the extracts about 0.2 mL per well. The plates were allowed to stay on the bench for 1 hour for the extract to diffuse on the agar. The Mueller Hinton agar plates for bacterial were incubated at 37^{o}C for three days while the potato dextrose agar plates for fungi were incubated at room temperature (drawer) for three days. At the end of incubation period, all plates were observed for any evidence of inhibition, which will appear as clear zones that were completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured with a transparent ruler calibrated in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB). Each tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism was used as a control. All tubes were then incubated at 37^{o}C for 24 hours. After the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The results from the Minimum Inhibitory Concentration (MIC) were used to determine the Minimum Bactericidal Concentration (MBC). A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test, it was then streaked unto a freshly prepared sterile nutrient agar plates. The plates were incubated at 37^{o}C for 24 hours. After the incubation period the plates were then examined for the presence or absence of growth. This was done to determine if the antimicrobial effect of the extract was bactericidal or bacteriostatic.

RESULTS AND DISCUSSION

Results

The results of phytochemical screening of leaf solvent extracts of *Ximenia Americana* are presented in Table 1.

Table 1: Phytochemical Screening of extracts of leaf of Ximenia americana

S/N	Phytochemicals	Extracts				
		HE	CE	EAE	AE	ME
1	Saponins	-	-	-	-	-
2	Tannins	+	+	+	+	-
3	Terpenoids	+	+	+	+	+
4	Flavonoids	+	+	+	+	+
5	Steroids	+	+	-	-	-
6	Alkaloids	+	+	-	-	-
7	Phlobatanins	-	-	-	-	-
8	Cardiac glycosides	-	-	-	-	-

Key: HE = Hexane extract, CE= Chloroform, EAE= Ethyl acetate extract, AE = Acetone extract, ME = Methanol extract, + = Detected, - = Not detected

The phytochemical screening of crude extracts of *Ximenia americana* showed that alkaloids, tannins, terpenoids, flavonoids and steroids. Tannins were detected in all the extracts except methanol extract, hence, *Ximenia americana* can be used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods, and diarrhea, and as antidote in heavy metal poisoning (Ogunleye and Ibitoye.,2003). Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003) thus exhibiting antimicrobial activity. Liu, *et al.* (2003) reviewed the biological activities of tannins and observed that tannins have remarkable activity in cancer prevention and anticancer, thus suggesting that *Ximenia americana* could be a possible source of important bioactive molecules for the treatment and prevention of cancer. Terpenoids were detected in all the extracts. Terpenoids are said to have some biological activities in animals including man and also play a meaningful role in human medicine and are reported to have a wide spectrum of biological activities including bactericidal, fungicidal, antiviral, cytotoxic, analgesic, anticancer, spermicidal, cardiovascular and antiallergic (Sofowora., 1974). *Ximenia americana* can be used for numerous biological activities including anti-inflammatory, antiallegic, antithrombitic and vasoprotective effects (Ogunleye and Ibitoye, 2003) because flavonoids were detected in all the extracts. Alkaloids were detected in hexane and chloroform extracts only Hence, *Ximenia americana* can be used as an analgesic, anaesthetic and as social drugs since it contains alkaloids. The alkaloids contained in plants are used in medicine as anaesthetic agents (Herourat *et al*.; 1988).

Activity of the crude hexane, chloroform, ethyl acetate acetone and methanol extracts from the leaf of *Ximenia americana* were tested on five clinical isolates; *Pseudomonas aeuroginosa, Staphylococcus aureus, Escherichia coli, Aspergillus niger and penicillium spp* Augmentin and mycotin were used as control drugs. The measured zones of

inhibition of the pathogens by the crude extracts are presented in the Tables 2 and 3. All the crude extracts of the leaf inhibited or exhibited antibacterial activity against all the bacteria pathogens tested with a diameter that ranged between 8 – 21 mm. All the crude extracts of the leaf inhibited or exhibited antifungal activity against all the fungi pathogens tested with a diameter that ranged between 8 - 14 mm except hexane and ethyl acetate extracts that did not show significant inhibition against *A. niger*. The minimum inhibitory activity (MIC) of the extracts of *Ximenia americana* against tested microbes ranges from 400 to 100 mg/ml in almost all the extracts and in few instance 50 mg/ml against the tested bacteria.

Table 2: Mean Zone of Inhibition of Ximenia americana

Organisms	Conc. (Mg/ml)	HE	CE	EAE	AE	ME	C (+)	DMSO (-ve)
Pseudomonas aeruginosa	400	15	19	19	21	21	30	00
	200	10	16	12	16	13	28	00
	100	07	10	10	11	09	19	00
	50	00	00	05	00	06	15	00
Staphylococcus aureus	400	16	20	17	18	25	33	00
	200	13	14	15	16	16	30	00
	100	8	09	13	10	11	30	00
	50	00	07	08	00	8	19	00
Escherichia coli	400	11	17	08	15	21	13	00
	200	09	15	07	12	17	29	00
	100	00	09	00	09	15	27	00
	50	00	08	00	60	09	23	00
Aspergellius Niger	400	06	08	06	09	08	16	00
	200	03	06	03	06	06	19	00
	100	03	03	03	03	03	15	00
	50	0	00	00	00	00	27	00
Penicillium Spp	400	09	11	10	13	14	13	00
	200	05	07	08	09	09	16	00
	100	04	05	05	06	05	19	00
	50	00	00	00	00	00	15	00

Key: HE = Hexane extract, CE= Chloroform, EAE= Ethyl acetate extract, AE = Acetone extract, ME = Methanol extract, Values greater than 7 mm indicate activity and 00 means no activity.

The minimum inhibitory activity (MIC) of the extracts of *Ximenia americana* against tested microbes ranges from 400 to 200 mg/ml in almost all the extracts for the tested fungi. Table 3.

Table 3: Showing Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration in milligram per mililitre (mg/ml) of Ximenia americana

	MIC AND MBC (mg/ml)											
Organism	Method		Chloroform		Acetone		Hexane		Ethylacetate		Msc	OVC
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC		
Pseudomonas aeruginosa	100	200	100	200	100	400	100	400	100	400	+	+
Staphylococcus aureus	200	400	200	400	200	400	200	400	200	400	+	+
E. Coli	100	200	200	400	100	200	200	400	200	400	+	+
Niger	200	400	200	400	200	400	200	400	200	400	+	+
Penicillium spp	200	400	200	400	100	400	200	400	200	400	+	+

Key:- Msc = Media Sterility Control, OVC =Organism Viability Control

The effective inhibitory potency observed with the leaf extracts proof it that the inhibitory compounds were extractable by the employed solvents against the tested pathogenic bacterial isolates (Akharaiyi and Boboye 2010). The activities of these extracts against tested pathogens could not be unconnected to the presence of the plant secondary metabolites contained in the plant. The various concentrations were visibly active on the tested bacterial isolates due to the combinative therapeutic actions of the various secondary metabolites contained in the plants (Ushie *et al.;* 2013). The ability of the extracts to inhibit the growth of *E. coli* indicates that this plant drug can be used in the treatment of gastroenteritis that has been associated with E. coli (Etani *et al*., 1999). Also the inhibition of growth of S. aeruginosa which are aetiology agents of urinary tract infection (Latta *et al.,* 1998, Tolson, 1997) have shown that the active components of the plant leaf extract can cure any disease caused by these organisms. The inhibition the growth of *Pseudomonas aeuroginosa* showed that the active component of the plant drug can cure any disease such as blood stream infections associated with the organism (Okoro 2012).

CONCLUSION

The phytochemical screening of crude extracts of *Ximenia americana* showed that alkaloids, tannins, terpenoids, flavonoids and steroids. Tannins were detected in all the extracts except methanol extract, hence, *Ximenia americana* can be used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods, and diarrhea, and as antidote in heavy metal poisoning. Activity of the crude hexane, chloroform, ethyl acetate acetone and methanol extracts from the leaf of *Ximenia americana* were tested on five clinical isolates; *Pseudomonas aeuroginosa, Staphylococcus aureus, Escherichia coli, Aspergillus niger and penicillium spp* Augmentin and mycotin were used as control drugs. All the crude extracts of the leaf inhibited or exhibited antimicrobial activity against all the pathogens tested. The minimum inhibitory activity (MIC) of the extracts of *Ximenia americana* against tested microbes ranges from 400 to 100 mg/ml in almost all the extracts and in few instance 50 mg/ml against the tested bacteria. The minimum inhibitory activity (MIC) of the extracts of *Ximenia americana* against tested microbes ranges from 400 to 200 mg/ml in almost all the extracts for the tested fungi. These could explain the rationale for the use the plant in the treatment of the various conditions in traditional medical practice.

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