PHYTOCHEMICAL ANDIN VITROANTIMICROBIAL SCREENING OF EXTRACTS AND ISOLATION OF BIOACTIVE COMPOUNDS FROM ANACARDIUM OCCIDENTALE (Linn)

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ABSTRACT: The extracts of the leaves, stem-bark and root-bark of Anacardium occidentale Linn were screened for the presence of secondary metabolites and in vitro antimicrobial activity. The Phytochemical screening revealed the presence of tannins, alkaloids, flavonoids, glycosides and steroids. The extracts were tested against Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris and Candida albicans using disk diffusion method. The ethanol extract (F1) and n-hexane extract (F6) of the leaves at 1000µg/ml have showed remarkable zones of inhibition of 14mm and 12mm against P. vulgaris, 13mm and 10mm against S. typhi and12mm and 10mm on S. aureus respectively. The potency was comparable to the standard drug, Levofloxacin. Bioactivity- guided column chromatography of the ethanol extract (F1) led to the isolation of Benzoic acid 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1Hcyclopenta[a]phenanthren-3-ylester.

Key Words: Phytochemical screening, in vitro antimicrobial activity, Bioactivity- guided column chromatography, Anacardium occidentale Linn

INTRODUCTION

Plants synthesize and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins that possess medicinal properties (Kolapo *et al.*, 2009). Over 40% of the drugs are now derived from plants. Historically, medicinal plants were discovered by trial and error. Our ancestors noticed that aches and pains went away when they drank tea made from the bark of willow tree. Later, scientists found that willow bark contains salicylic acid, the active ingredient in aspirin, **1**. This process continues today, botanists and chemists search the plant kingdom for new drugs. For years, the native Pacific yew was burned as trash generated by logging operations in

the Pacific Northwest. In 1975, a substance in its bark, taxol,**2,** was found to reduce the production of cancerous tumors (Goodman and Walsh, 2001).

The World Health Organization (WHO) has defined herbal medicines as "Finished labeledmedicinal products that contain ingredients from aerial or undergroundparts of plant or other plant material or combination thereof, whether in thecrude state or as plant preparations (WHO, 1996)".

In the last few decades, there is a great advance in our understanding of the causes of transmission, treatment and prevention of infectious diseases and this has fostered complacency about infections in a society which is well nourished and has access to vaccines, antibiotics and other drugs (Vahidi *et. al.*, 2002).

With advances in medicinal chemistry, most of today's antibacterial chemicals are semi synthetic modifications of various natural compounds (Nussbaum*et al.*, 2006). These include, the beta-lactamantibacterials, which include the penicillins (produced by fungi in the genus *Penicillium*), the cephalosporins, and the carbapenems. Compounds that are still isolated from living organisms are the aminoglycosides, whereas other antibacterials for example, the sulfonamides, the quinolones, and the oxazolidinonesare produced solely by chemical synthesis. In accordance with this, many antibacterial compounds are classified on the basis of biosynthetic/synthetic origin into natural, semisynthetic, and synthetic products. Another method of classification is based on biological activity; in this classification, antibacterials are divided into two broad groups according to their biological effect on microorganisms: bactericidal agents kill bacteria, and bacteriostatic agents slow down or stall bacterial growth (Nussbaum*et al.*, 2006).

Antibiotics such as ciprofloxacin,**3**, ketoconazole, **4**, amoxicillin,**5**, Ampicillin,**6** chloramphenicol,**7**, gentamicin, **8**and trimethoprim/sulfamethoxazole, **9** have been commonly used to combat different bacterial infections associated with gram-positive and gram-negative bacteria in both developed and developing countries.Thereforeplants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the bioactive compounds associated with the plants (Ali *et. al.*, 2007).

JUSTIFICATION

There is global resurgence in the use of herbal preparations and in some developing countries like Nigeria. Several plants are indicated in folk and other indigenous systems of medicine as anti-infective agents. The scientific literature is very rich in reportsof biological activities of many secondary metabolites of plant origin. The scientific evaluation of these plants remains an area of investigations. Growing misuse of antibiotics and chemotherapeutic agents leading to drug resistance is now pushing a considerable amount of people in both developed and developing countries to the use of herbal medicine. The quest for solving these challenges has necessitated a search for new antimicrobial substances from plants (Venkatesan*et al.*, 2009).

RESEARCH PURPOSE

The aim of this research work is to identify and isolate the bioactive compounds present in the stem-bark, root-bark and leaf extracts of *Anacardium occidentale*. The objectives include:

- To screen the extracts for the presence of secondary metabolites (Phytochemical analysis).

- *In vitro* evaluation of antimicrobial activity of the extracts against some selected pathogenic agents and to determine the most active fractions.

- To isolate, identify and characterize the bioactive compound(s) in the most active fractions using ^{1}H, and ^{13}C NMR, and IR spectral data.

PLANT UNDER INVESTIGATION

Anacardium occidentale Linn*(*Anacardiaceae)

Anacardium occidentale ("Kashu", Hausa) is a multipurpose tree of the Amazon that grows up to 15 m high. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground (Paris *et al.*, 1977). Cashew trees are often found growing wild on the drier sandy soils in the central plains of Brazil and are cultivated in many parts of the Amazon rainforest (Fazali *et al.*, 2011). It is a tree native to Brazil that is presently cultivated in many regions of the world. It has a great economic and medicinal value due to its richness in nutrients that constitute 47% fat, 21% protein and 22% carbohydrate, vitamins and all essential amino acids especially thiamine (Ohler,1979).

Chemical constituents of *A. occidentale*

In addition to being delicious, cashew fruit is a rich source of vitamins, minerals, and other essential nutrients. It has up to five times more vitamin C than oranges and contains a high amount of mineral salts. Volatile compounds present in the fruit include esters, terpenes, and carboxylic acids (Bicalho*et al.*, 2001). The bark and

leaves of cashew are a rich source of tannins, a group of plant chemicals with documented biological activity. These tannins, in a study on rat, demonstrated anti-inflammatory and astringent effects, which may be responsible for its effectiveness in treating diarrhoea (Mota*et al.,* 1985). Anacardic acids are found in cashew, with their highest concentration in the nutshells. Several clinical studies have shown that these chemicals curb the darkening effect of aging by inhibiting tyrosinase activity, and that they are toxic to certain cancer cells (Kubo*et al.,* 1994).

MATERIALS AND METHODS

General Procedures

Solvents used are of analytical grade. Column chromatography was performed using silica gel (Lab Tech chemicals 60-120 mesh, pH: 7.0, 10% suspension). Thin layer chromatography was carried out using 20cm x 20cm glass plate coated with 0.5mm silica gel (TLC grade),and bands/spots were viewed under UV light (254-365nm) or by developing in Iodine. All glass wares were first soaked in chromic acid, washed and rinsed very well with water and sterilized in an oven for 24 hours at 110^{o}C before use.Antimicrobial bioassay was carried out using disk diffusion method. The IR spectrum was recorded on FTIR Fourier transform at NARICT ZARIA. ^{1}H-NMR and^{13}C-NMR spectra were recorded using $CDCl_3$ as solvent on Varian ^{unity} nova 400 NMR spectrometer at 400MHz and 100 MHz respectively, Central Analytical Facility, Stellenbosch University South Africa.

Plant materials

Fresh plant materials: stem-bark, root-bark and leaves of *A. occidentale* were collected from Rumfa College Staff Quarters, Kano on 9th March, 2011. They were authenticated by Prof. Bala Sidi Aliyu and Baba Ali Garko of Biological Sciences Department, Bayero University Kano.

Extraction

The plant materials were air dried and ground using mortar and pestle. 200g of each was percolated with 96% ethanol (700cm^3) for two weeks (Fatope *et al.*, 1993). The percolates were evaporated to dryness using a rotary evaporator (R110) at 40^{0}C. The crude residue from each was weighed, labeled as $F_1$and kept in a deep freezer.

Fractionation of crude (ethanol) extracts:

The residue, F_1 was macerated four times with 20cm^3 of n-Hexane in each case and the soluble fraction evaporated to afford n-Hexane fraction, F_2. The insoluble residue was further macerated three times with 20cm^3 of chloroform in each case to obtain the chloroform soluble fraction F_3 and evaporated to dryness. The chloroform insoluble residue was macerated four times with 20cm^3 ethyl acetate in each case. The ethyl acetate soluble fraction F_4 was also evaporated to dryness. While the insoluble residue was further macerated four times with 20cm^3 of methanol in each case and the soluble fraction, F_5, was evaporated to dryness. The flow chart of the fractionation process, the weight, texture and color of fractions are shown in scheme 1 and Table 1 respectively.

Phytochemical Analysis

The fractions were subjected to phytochemical screening, to determine the classes of secondary metabolites present in the plant materials. These include alkaloids, saponins, carbohydrates, tannins, resins, flavonoids and steroids (Table 2).**3.5.1 Test for Alkaloids**

Each fraction (0.5g) was stirred with 1% HCl (5cm^3) on steam bath. The solution was cooled and filtered. The filtrate 1cm^3 was treated with 2-3 drops of dragendoffs reagent. Formation of an orange red precipitate/turbidity with dragendoffs reagent indicates the presence of alkaloids (Trease and Evans, 1989).

Test for Flavonoids

Each fraction (2.0g) was dissolved in 50% methanol (2cm^3) by heating. Magnesium metal (0.01g) and concentrated hydrochloric acid 50% (5-6 drops) were added. Appearance of a red color indicates the presence offlavonoids (Sofowora, 1984)

Test for Reducing Sugars.

Each fraction (1.0g) was taken and diluted with distilled water (2cm^3), Fehling's solutions (A and B) were added and the mixture warmed. A brick-red precipitate at the bottom of the tube indicates the presence of reducing sugars (Brain and Turner, 1975).

Test for Steroids (Salkowski's Test)

Concentrated Sulphuric acid (2cm^3) was added to each fraction (2cm^3). Appearance of effervescence after which a clear reddish brown color appeared at the interface confirms the presence of steroids (Harbone, 1998).

Test for Tannins

Each fraction (2cm^3) was treated with 5% $FeCl_3$ (3 drops). A dark black colored precipitates in a very dark solution, which turns green-black to blue-black coloration on dilution indicates the presence of tannins (Harbone, 1998).

Test for Saponins

Each fraction ($2cm^3$) was vigorously shaken with distilled water and allowed to stand for a while. A persistent frothing indicates the presence of saponins (Sofowora, 1984)

ANTIMICROBIAL BIOASSAY

Preparation of Test Solution and Disc Concentration

The extracts were dissolved using dimethyl sulphoxide (DMSO).The stock solutions were prepared by dissolving 100mg of each extract in $1cm^3$ of the solvent. For each fraction,concentrations of 1000µg/disc, 500µg/disc, 200µg/disc, and 100µg/disc were prepared separately in sterilized Bijour bottles containing 50 sterile improvised Whatman No. 1 filter paper discs that have absorbance potency of $0.01cm^3$ in each case.The filter papers weresaturated/impregnated with plant extract of desired concentration. The serial dilution was as follows:

1. Concentration of 1000µg/disc: - From the stock solution, $0.5cm^3$ was taken into a Bijour bottle containing the discs and labeled 1000µg/disc.
2. Concentration of 500µg/disc: - $0.5cm^3$ of DMSO was added into the remaining stock solution making $1cm^3$; $0.5cm^3$ was taken into another Bijour bottle containing the discs and was labeled 500µg/disc.
3. Concentration of 200µg/disc: - $0.5cm^3$ of DMSO further added to the remaining stock solution, making the volume $1cm^3$ again, from which $0.4cm^3$ was taken and diluted with $0.1cm^3$ of DMSO to make the volume to $0.5cm^3$ and transferred into another Bijour bottle containing the discs.

4. Concentration of 100µg/disc: - 0.2cm^3 of the stock solution was taken and diluted with 0.3cm^3 of DMSO. 0.5cm^3solution was then taken into a Bijour bottle containing the discs and labeled 100µg/disc.

Source of Bacteria and Fungus

The cultures of gram positive organisms,*Streptococcus pyogenes, Staphylococcus aureus*, and gram negative organisms,*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, and Pseudomonas aeruginosa* were used. The fungal culture used was *Candida albicans.* The test microorganisms were clinical isolates obtained from Microbiology Department of Aminu Kano Teaching Hospital, Kano, which were further identified and confirmed in the Microbiology laboratory of the Department of Biological Sciences, Bayero University,Kano.

Inoculums Standardization

As described by standard sensitivity test (National Committee for Clinical Laboratory Standards, NCCLS), loops of the confirmed isolates were introduced in Peptone water in separate sterilized bottles and kept overnight in an incubator (37^0 C). Few colonies of the overnight growth of the isolates to be tested were dispersed in sterile normal saline to form a turbid culture suspension that match 0.5 McFarland turbidity (NCCLS, 2000).

Preparation of Media

Nutrient Agar(28g) was dissolved in 1liter of distilled water, as per manufacturer's instructions. The conical flask containing the Nutrient Agar solution was Autoclaved for 15minutes at 121^0C. 20-25cm^3 of the sterilized Nutrient Agar was transferred into Petri-dishes under septic condition. The Petri-dishes were allowed to cool and solidify.

Control Antibiotics

Control antibiotics were obtained from a pharmaceutical store to serve as positive controls. Levofloxacin was selected as a reference standard for the antibacterial, while Ketoconazole was selected as a reference standard for theantifungus.

In vitro Antimicrobial Susceptibility Test (AST)

Antibacterial activities of the extracts were determined using Disc Diffusion method of Antimicrobial Susceptibility Test (AST) as described by (NCCLS, 2000). Standardized inocula of the isolates were swabbed onto the surface of the solidified and oven-dried Nutrient Agar in separate petri-dishes under sterilized environment. The four prepared discs of the different concentrations of the extracts were then placed onto the surface of the inoculated media at intervals in a clockwise direction. The positive control discs were placed at the center of the bacteria and fungi inoculated media, respectively. The plates were incubated for 24 hours at 37^0C for bacteria cultures and for 72 hours at 25^oC for fungal culture, after which the antimicrobial activity was observed by measuring the width of the clear inhibition zone around the discs and the values obtained recorded (Table 3).

Column Chromatography

Column chromatography of the leaf ethanol extract*of Anacardium occidentale* F1 was carried out. Silica gel [(60-120mesh) (500g)] was loaded in a column (58.5inch length, 1.2inch id) in slurry of n-hexane. The silica gel was washed several times with n-hexane and later with chloroform to remove oily materials. The silica gel was then removed from the column and allowed to dry overnight. The ethanol extract (25g) was mixed thoroughly with 50g of silica in a beaker using spatula until the mixture becomes

homogenous. The mixture was then carefully loaded on to the column that was already packed. Additional silica gel (10g) was added on top to serve as a protective layer.

The column was eluted using solvents and solvent mixtures of increasing polarity, in the following order with n-hexane (100%; 2 liters), n-hexane: chloroform (1:1; 2liters), chloroform (100%; 2 liters), chloroform:ethyl acetate (1:1; 2 liters), ethyl acetate (100%; 2 liters), ethyl acetate: methanol (1:1; 2 liters), methanol (100%; 2 liters), giving 131 fractions.The eluents were collected in fractions of 100cm^3 each in beakers, evaporated to dryness and analyzed on TLC(Sharma and Achaya, 1988).Similar fractions were pooled together and the weight and appearances of the fractions are as shown in Table 4.

Isolation and purification of compound AO1-1-23

The fraction AO1-1-23(0.055g) was loaded on preparative TLC and developed in chloroform: ethyl acetate (4:1) solvent system. Two bands were observed at 365nm, the top white solid AO1-1-23A (0.48g R_f=0.59) and the bottom a brownish substance AO1-1-23B (0.5g R_f=0.15). AO-1-23 was obtained as a white solid.Different spectroscopic methods were used to elucidate thestructure of isolated compound AO-1-23. Among the spectroscopictechniques IR, ^{1}H-NMR, and ^{13}C-NMRwere carried out. The IR spectrum was recorded on FTIR Fourier transform at NARICT ZARIA. ^{1}H-NMR and^{13}C-NMRspectra were recorded using $CDCl_3$ as solvent on Varian ^{unity} nova 400 NMRspectrometer at 400MHz and 100 MHz respectively, Central Analytical Facility, StellenboschUniversity South Africa.

The IR absorption spectrum showed absorption peaks at 1729.24cm^{-1} (C=O, C=C stretching.); 2923.22cm^{-1} (aliphatic C-H Stretching);1456.3cm^{-1} (Aromatic ring

absorption); 1367.58cm^{-1} (C-H bending), 1193.798cm^{-1}(cycloalkanecm^{-1}); 993.37 and 721.4cm^{-1} (C=O finger band) and 451.36 cm^{-1}. The ^{1}H-NMR showed aromatic group (ppm) H-2`=H-3`=H-4`=H-5`=H-6`=8.10; steroidal nucleus signals (ppm), H-1 to 29 = 0.79 to 1.9; H-3=4.47(m) and H-6=5.18(t). The $^{13}$C-NMR showed aromatic group (ppm), C1`=151.12, C2`=C6`=122.72, C3`=C4`=C5`=109.49; steroidal signal (ppm) C1 to C29=12.01 to 56.85 (see Table 6); C3=80.7, C5=139.88, C6=121.79 and Carbonyl carbon=173.86.

RESULTS

PERCOLATION AND MACERATION OF PLANT PARTS OF *AOCCIDENTALE*

Air dried leaves, stem-bark and root-bark of *A. occidentale*percolated with ethanol yielded a crude ethanol extracts each. The maceration of the crude extracts with solvents of different polarity yielded several fractions as shown in Table 1.

PHYTOCHEMICAL SCREENING OF THE PLANT FRACTIONS

The phytochemical screening of the various fractions of the plant materials, revealed the presence of alkaloids, steroids, tannins, Flavonoids and glycosides (Table 2). The phytochemical compounds are known to be biologically active and thus aid the antimicrobial activities of *A. occidentale.* Phytochemicals exert antimicrobial activity through different modes of action; tannins for example act by hydrogen deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Alkaloids have been associated with medicinal uses for centuries and other possible roles have not been examined. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and

reduction of human cancer cell lines (Nobori *et al.*, 1994; Akinpelu *et al.*, 2008). Flavonoids also exhibit a wide range of biological activities such as antimicrobial, anti-inflammatory, analgesic, and cytostatic, hypoglycemic and antioxidant properties (Hodek *et al.*, 2002). They have the ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy-radicals which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA (Ferguson, 2001). Saponins are considered a key ingredient in Chinese medicine and are responsible for most of the observed biological activity (Liu and Henkel, 2002). They are known to produce inhibitory effect on inflammation (Just *et al.*, 1998).

Table1: Weight, texture and appearance of the different fractions of *Anacardium occidentale*

Plant parts	Fractions	Weight (g)	Texture	Appearance
Leaves	F_1	40.50	Gummy	Dark green
	F_2	5.00	Gummy	Dark green
	F_3	4.50	Oily	Dark green
	F_4	5.00	Gummy	Dark green
	F_5	6.50	Gummy	Dark green
Stem-bark	F_1	42.61	Gummy	Reddish brown
	F_2	2.50	Gummy	Light brown
	F_3	1.50	Gummy	Light brown
	F_4	2.00	Gummy	Reddish brown
	F_5	1.57	Gummy	Reddish brown

Root-bark	F_1	21.30	Gummy	Reddish brown
	F_2	1.00	Gummy	Light brown
	F_3	2.35	Gummy	Reddish brown
	F_4	4.85	Gummy	Reddish brown
	F_5	4.80	Gummy	Reddish brown

F_1 – Ethanol extract
F_2 - n-Hexane fraction
F_3 – Chloroform fraction
F_4 – Ethyl acetate fraction
F_5 – Methanol fraction

Table 2: Result of Phytochemical screening of the various fractions of *Anacardium occidentale*

Plant parts	**Fractions**	**Phytochemical**					
Tannins	**Alkaloids**	**Saponins**	**Glycosides**	**Steroids**	**Carbohydrates**		
Leaves	F_1	+	+	-	+	+	-
	F_2	+	+	-	+	+	-
	F_3	-	+	-	+	-	-
	F_4	+	+	-	-	+	-
	F_5	+	+	-	-	-	-
Stem-bark	F_1	+	+	-	+	+	-
	F_2	+	+	-	+	+	-
	F_3	+	+	-	+	-	-
	F_4	+	+	-	-	+	-

	F_5	+	+	-	-	+	-
Root-bark	F_1	+	+	-	+	+	-
	F_2	+	+	-	+	-	-
	F_3	+	+	-	+	+	-
	F_4	-	+	-	-	+	-
	F_5	-	+	-	-	+	-

+ = Present; - = Absent

4.3 Screening the plant fractions for biological activity

The antimicrobial activities of the fractions (leaves, stem-bark and root-bark) of *A. occidentale* were investigated against some microbial isolates and found to possess bioactivity against some of the tested microorganisms. The crude extract and n-hexane fractions of the leaves at 1000µg/disc shows a remarkable zone of inhibition against the tested microorganisms. The extracts of stem-bark and root-bark showed less susceptibility to the test organisms. The leaf extracts of *A. occidentale*at 1000µg/discwere

found to be as active as levofloxacin and ketoconazole antibiotics when tested against the microorganisms (see Table 3).

Table 3: Antimicrobial activity of leaf extracts of *Anacardium occidentale* against some selected human Pathogens

Plant parts	Fractions	Concentration (µg/disc)	[a]Zones of inhibition (mm) against microorganisms[b]							
			1	2	3	4	5	6	7	8[b]
Leaves	F_1	1000	++[a]	++	++	+++	+++	+++	+++	++
		500	+	+	++	++	++	++	++	+
		200	+	+	++	++	+	++	+	-
		100	+	-	-	++	+	+	+	-

		1000	-	++	++	++	++	++	++	++
		500	-	-	++	++	++	++	++	+
	F_2	200	-	-	+	+	+	+	+	-
		100	-	-	-	+	+	+	-	-
		1000	++	++	++	+++	++	-	-	-
		500	++	++	++	++	++	-	-	-
	F_3	200	++	+	+	++	+	-	-	-
		100	+	-	+	++	+	-	-	-
		1000	-	+++	++	++	++	-	-	-
		500	-	++	++	-	+	-	-	-
	F_4	200	-	++	+	-	+	-	-	-
		100	-	+	+	-	+	-	-	-
		1000	+	++	++	++	++	-	++	++
		500	-	+	+	++	+	-	+	++
		200	-	+	-	++	+	-	-	+
	F_5	100	-	+	-	-	+	-	-	+
		5.00	+++	+++	+++	+++	+++	+++	+++	+++
	F_1	1000	-	++	+	-	+	-	-	-
Stem-bark		500	-	++	-	-	+	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	F_2	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	F_3	1000	+	+	-	-	+	-	-	-
		500	+	+	-	-	-	-	-	-
		200	+	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	F_4	1000	-	+	-	-	+	-	-	-
		500	-	+	-	-	+	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	F_5	1000	+	++	+	-	+	-	-	-
		500	-	+	+	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
Reference Standard		5.00	++++++++++++++++++++							+++

Plant part	Fraction	Concentration µg/disc	[a]Zone of inhibition (mm) against microorganism[b]							
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8[b]**

Root-bark	F_1	1000	-	++	-	-	++	-	-	++
		500	-	++	-	-	+	-	-	+
		200	-	+	-	-	-	-	-	+
		100	-	-	-	-	-	-	-	-
	F_2	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	F_3	1000	-	++	-	-	+	-	-	-
		500	-	+	-	-	+	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	F_4	1000	-	++	-	-	++	-	-	-
		500	-	+	-	-	+	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	F_5	1000	-	-	-	-	+	-	-	+
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	Reference Standard	5.00	+++	+++	+++	+++	+++	+++	+++	+++

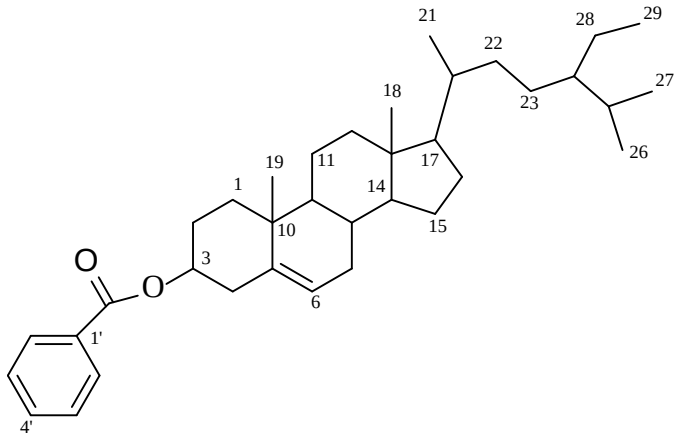
[a]Zones of inhibition (mm); 0-6 = -, 7-9 = +, 10-12 = ++, 12-25 = +++

[b]Microorganisms (1) *Streptococcus pyogens*, (2) *Klebsiella pneumonia*, (3) *Staphylococcusaureus*, (4) *Proteus Vulgaris*, (5) *Salmonella typhi*, (6) *Pseudomonas aeruginosa*, (7*) Escherichiacoli* (8) *Candida albicans* Reference Standard: Levofloxacin (1-7) and Ketoconazole(8)

Column chromatography of the ethanol extract of *A. occidentale* leaves.

The column chromatography of ethanol leaf extracts yielded 131 fractions. The eluted fractions were combined based on their TLC patterns and retention time (Table 4). Fractions 23-26 eluted with n-Hexane: chloroform (1:1) were combined based on their TLC and the compound AO-1-23 was obtained as a white solid {R_f value = 0.59, solvent system ethyl acetate: chloroform (4:1)}. Compound AO-1-23 was characterized as Benzoic acid 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*cyclopenta[*a*]phenanthren-3-yl ester, **24,** based on spectroscopy, mainly ^{13}C, ^{1}H NMR, and IR.The ^{13}C NMR (Appendix I and

Table 6) has shown recognizable signals at 139.88 and 121.79 ppm,which are assigned C5 and C6 double bonds respectively. The value at 18.37 ppm corresponds to angular carbonatom (C19). Spectra show thirty six (36) carbon signal including sixmethyls, eleven methylenes, ten methine,two quaternarycarbons, 1 carbonyl carbon at 173.86, C3 at 80.7 and the aromatic carbons at C1`=151.12, C2`=C6`=122.72, C3`=C4`=C5`=109.49.The ^{1}H NMR (Appendix II)showedH-3 appeared at δ 4.47 and H-6 at δ 5.18.Angular methyl proton at 0.79, 0.83 and 1.02 corresponds to C18 and C19 proton respectively while, protons of the aromatic carbons showed a signal at 8.10ppm. The observed IR absorption bands (Appendix III) are 1729.24cm^{-1}, that is a characteristic of carbonyl carbon and carbon double bond stretching; 2923.22cm^{-1} showed aliphatic C-H Stretching; 1456.3cm^{-1} Aromatic ring absorption; 1367.58cm^{-1} C-H bending, 1193.798cm^{-1} cycloalkane; 993.37 and 721.4 cm^{-1} carbonyl finger band and 451.36 cm^{-1}. Compound AO-1-23 was tested against the 8 microorganisms at three different concentrations and the result is presented in Table 5.



Benzoic acid 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*cyclopenta[*a*]phenanthren-3-ylester

Table 4: Pooled fractions obtain from column chromatography of ethanol leaf extract of*A. occidentale*

Eluent	Pooled fraction	Pooled number	Weight(g)	Appearance
n-hexane/chloroform 1:1	20-22	AO1-20	0.06	Pale yellow
,,	23-26	AO1-23	0.055	Colorless
,,	27-28	AO1-27	0.001	Colorless
,,	29-34	AO1-29	0.086	Colorless
Chloroform 100%	35-37	AO1-35	0.0355	Colorless

,,	38-39	AO1-38	0.04	Pale yellow
,,	40-43	AO1-40	0.109	Pale yellow
,,	44-47	AO1-44	0.108	Yellow
,,	48-49	AO1-48	0.175	Light green
Chloroform/ ethyl acetate 1:1	50	AO1-50	0.256	Dark green
,,	51	AO1-51	0.216	,,
,,	52	AO1-52	0.311	,,
,,	53	AO1-53	0.274	,,
,,	54-55	AO1-54	0.135	,,
,,	56-59	AO1-56	0.129	,,
,,	60-71	AO1-60	0.306	,,
Ethyl acetate 100%	72-88	AO1-72	1.705	,,
Ethyl acetate/methanol 1:1	89-102	AO1-89	15.583	,,
Methanol 100%	103-131	AO1-103	0.43	Brown

Table 5: Results of zone of inhibition (mm) of compound AO-1-23

Code	Concentration **(µg/disc)**	Diameter of zone of inhibition(mm)							
		1	2	3	4	5	6	7	8
Compound AO-1-23	50	++	-	++	-	-	-	-	-
	30	+	-	+	-	-	-	-	-
	10	-	-	-	-	-	-	-	-

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The leaves, stem-bark and root-bark of *A. occidentale* Linn were selected on the basis of its wide range of uses and tested against *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris* and *Candida albicans* using disk diffusion method. The crude extract and n-hexane fraction of the leaves at a concentration of 1000µg/ml showed a remarkable

zone of inhibition of 14mm and 12mm against *P. vulgaris*, 13mm and 10mm against *S. typhi* and12mm and 10mm against *S. aureus* respectively. Bioactivity guided column chromatography on the crude extract of the leaves (AO1-1) led to the isolation of Benzoic acid 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*cyclopenta[*a*]phenanthren-3-ylester.

Conclusion

Anacardium occidentale is used among many tribes in the West Africa and Asia to prepare decoctions for the treatment of different ailments ranging from diarrhea, malaria, poisoning, toothache, and eye and ear infections among others. The results supported the traditional usefulness of *A. occidentale* as a medical remedy for some ailments.

Recommendation

In view of the above, the leaves of A. occidentale should be further investigated for the possible isolation of compounds that have strong activity against the tested microorganisms used in this research. However, it will lead to the formulation of new drugs that could be accessible and affordable and also to the development of the folkloric herbalists to meet the modern age.

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