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Original Research Article

Phytochemical, Antimicrobial and Toxicity Evaluation of *Anacardium occidentale* Linn. Leaf Extracts

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ABSTRACT

Anacardium occidentale is used ethno-medicinally in the treatment of cold, cough, toothaches, gum problems, sore throat, bronchitis, diarrhoea, dysentery, haemorrhoids, diabetes, malaria, rheumatism, arthritis, corns, leprosy and some skin ailments. However, there is dearth of information on their uses scientifically. Thus, this study investigated the phytochemical components, antimicrobial activity and toxicity of *A. occidentale* leaf extracts. The leaf of *A. occidentale* was extracted with methanol and consecutively fractionated using hexane, ethyl acetate, butanol, and water. The methanol extract and fractions were quantitatively screened for phytochemical constituents and tested against selected microorganisms using standard procedures. Acute toxicity tests of the ethyl acetate and aqueous fractions were done on Wistar rats. The kidneys and livers of the rats were histologically examined. *Anacardium occidentale* methanol extract had the highest amount of phenolics (98.30 ± 0.15 gallic acid equivalent (GAE)/g) while the ethyl acetate fraction had the highest amount of anthraquinones and cardiac glycosides. The butanol fraction had highest saponin and tannin contents, while the aqueous fraction had highest steroids, terpenoids and carotenoids contents. The inhibitory activity of the ethyl acetate fraction against *Staphylococcus aureus*, *Bacillus cereus*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* ranged from 15.0 mm to 19.0 mm inhibitory zone diameter at 100 mg/mL. The ethyl acetate and aqueous fractions were not toxic to the rats at 2,900 mg/kg dose. Consequently, *A. occidentale* leaf extracts were rich in phytochemicals, had antimicrobial activity and safe for use.

Keywords: Anacardium occidentale, phytochemical constituents, antimicrobial activity, acute toxicity.

Introduction

Plant secondary metabolites are biologically active, naturally occurring chemical compounds in plants. They are indirectly involved in the growth and adaptation of plants, and are used as medicines and flavourings.[1,2] As an example, salicin was originally extracted from white willow tree before it was synthetically produced as aspirin.[3] Many phytochemicals with physiological capabilities are elements rather than complex organic molecules.[4] Over five thousand estimated entity phytochemicals have been recognized in vegetables, fruits and grains.[5] Moreover, their biosynthetic sources are grouped mainly into three: terpenoids; alkaloids with nitrogen and compounds with sulphur; flavonoids, related phenolics and polyphenolics.[5, 6]
Microorganisms have been positively engaged in food and beverage industries, as well as in biotechnology and genetic engineering. However, a small proportion of microorganisms are pathogenic causing diseases and even death in plants and animals.[7] For instance, *Pseudomonas aeruginosa*, a gram-negative bacterium causes wide spectrum clinical infections such as pneumonia and bacteraemia, a leading cause of nosocomial infections that are associated with high mortality rate, and are often difficult to treat.[8, 9] Also, *Staphylococcus* and *Streptococcus* species cause skin infections, pneumonia, meningitis and devastating sepsis.[10]
Medicinal plants have been used for combating various health problems caused by some microorganisms since the beginning of man's existence. Eighty percent of people globally rely on herbal medicines for some aspect of their primary healthcare.[11] In the same vein, the interest in the study of medicinal plants as pharmacologically active compounds has increased worldwide.[12]
Anacardium occidentale Linn., commonly known as cashew tree belonging to the family Anacardiaceae, is an evergreen, large tree (10 – 12 m tall), with a short and irregularly shaped trunk. Its leathery textured leaves are elliptic to obovate (4 – 22 cm long and 2 – 15 cm broad), with smooth margins and are spirally arranged. The cashew nut, which is a boxing-glove or kidney shaped drupe with a single seed, grows at the end of the cashew apple.[13] An individual tree may produce 200 - 300 fruits per annum and keep on producing for twenty years or more; and may survive up to 50 - 60 years.[14] The immature leaves and shoots of cashew are consumed fresh or cooked; the nuts are popular snacks and food sources, while the apple is processed as jellies, juices, syrups and as intoxicating beverages like wine, brandy, and gin. The apples are also utilized in body care products like lotions, shampoos and anti-aging creams. Moreover, cashew kernels and apples are commonly employed in candy in China, Thailand, and India. The edible oil (similar to olive oil) produced from the kernels is used in cooking.[15-17]
The leaves of *A. occidentale* are utilized in treating gum challenges and toothaches, while the buds and immature leaves are used in treating skin diseases in African herbal medicine and Ayurveda.[13] Also, the matured leaves are used for treating rheumatism or arthritis in Nigeria.[18] In Venezuela, the leaf decoction is used for treating diarrhoea and diabetes, but for amenorrhea and dysentery in Haiti. Its pulverized bark is used for treating diabetes in Colombia, while its

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resin is for cold treatments in Cuba. In Brazil, the fruit juice is used as a diuretic and a therapy for vomiting, diarrhoea, and sore throat, while the gum is applied to fungal infections, corns, and leprosy. Moreover, the leaves are utilized in Brazil for treating eczema, psoriasis, dyspepsia, scrofula, cough, bronchitis, intestinal colic, leishmaniasis, syphilis-related skin disorders, impotence, genital and venereal diseases.[19] Its nut shell oil has anti-diabetic, analgesic and anti-inflammatory effects, and improves healing action on sore of leprosy.[20-23]

The hypotensive and cardio-inhibitory activities of the stem bark extract of *A. occidentale* in *in vitro* experiment was reported by Tchikaya *et al*.[24] and its effectiveness on the pancreas of diabetic rat was demonstrated by Bassey *et al*.[25] and also its cleansing effect on the kidneys of rats by Omotoso *et al*.[26] Aqueous extract of *A. occidentale* stem bark was reported ineffective on the testis of mature Wistar rat,[27] however, there were improvement in the morphological composition of the testes, spermatogenesis, as well as on reproductive hormones with methanol leaf extract on diabetic rats.[28] Acetone extract of *A. occidentale* leaf was active against *Haemonchus contortus* (nematode of sheep) and thus could be useful in the control of helminthes.[29] Additionally, the powder and acetone extracts of the seeds and *Allium sativum* bulbs were effective in preventing *Callosobruchus maculatus* (cowpea bruchid) in stored seeds of *Arachis hypogea*.[30] Interestingly, Ushanandini *et al*.[31] explained the ability of the bark extract in neutralizing enzymatic and pharmacological effects induced by a snake's venom (*Vipera russelii*). Nnamani *et al*.[32] reported the larvicidal effects of the aqueous extracts of nutshell, bark and leaf of cashew on *Anopheles gambiae* and concluded that it has insecticidal property. The inner stem bark extract of *A. occidentale* has be shown to contain tannins, saponins and free reducing sugars,[33] thus, the bark extract has been effectively used in treating cold, nasal congestion, cough, flu and syphilis possibly due to the presence of tannins and flavonoids.[25, 34-36] Also, its bark is utilized in treating toothache the effect of which has been ascribed to its high amount of flavonoids which has been shown to interrupt the membranes of *Streptococcus mutans*.[37] Its apple juice contains antibacterial principles against clinical strains of *S. mutans* and *Staphylococcus aureus*.[38] Moreover, Goncalves and Gobbo[39] reported that the extracts from its apples have valuable substances for cosmetic and antimicrobial applications.

The belief that herbal remedies are harmless may not hold as there are some deficiencies in drug quality control as it concerns the consumption of medicinal plants, and this has led to accidental herbal toxicity.[40] Thus, there is need for more scientific information about the plant. This work focused therefore on evaluating the phytochemical constituent, antimicrobial activity and acute toxicity status of *A. occidentale* leaf extracts.

Materials and Methods

Preparation of plant sample

Anacardium occidentale leaves were collected at Abadina area, University of Ibadan, authenticated by Mr. D. P. O. Esimekhuai and deposited in the University of Ibadan Herbarium (UIH), Ibadan, Nigeria, with voucher specimen number UIH 22489. The leaves were washed with clean water; air dried for fourteen days and ground with a milling machine. The pulverized sample (1 kg) was macerated with 3 litres of methanol (BDH, England) for 48 hours. The methanol extract was decanted, filtered with Whatman filter paper No. 1, concentrated with rotary evaporator (Heidolph Laborota, Germany). The extract was partitioned successively with n-hexane, ethyl acetate, butanol and distilled water; and each fraction was concentrated and freeze dried.

Phytochemical screening

The phytochemical investigation was done on the methanol extract, the fractions and leaf powder of *A. occidentale*. Saponins, tannins, alkaloids, phenolics, anthraquinones, flavonoids, steroids, terpenoids, cardiac glycosides and carotenoids were quantitatively screened for using the methods described by Marcano and Hasenawa,[41] Harborne,[42] and Mayuri.[43]

Saponins

One gram of the plant sample was added to 5 mL of 20% ethanol and placed in a water bath at 55^{o}C for 4 hours. The residue was filtered and washed with 20% ethanol twice. The extract was reduced to 5 mL in the oven. Five millilitres of petroleum ether was added to the concentrated extract inside a separating funnel. The petroleum ether layer was discarded and 3 mL of butanol was added to the residue and washed with 5 mL of 5% sodium chloride. The butanol layer was poured into a weighed Petri dish, evaporated to dryness and the weight of the residue was taken.

Tannins

One gram of the plant sample was extracted with 25 mL of the solvent mixture of 80:20 acetone:10% glacial acetic acid for 5 hours. It was filtered and the absorbance was measured at 500 nm. The absorbance of the reagent blank was also measured. The concentration of tannin was read off against a standard graph (10, 20, 30, 40, 50 mg/100g of tannic acid) taking into consideration the dilution factor.

Alkaloids

One gram of the plant sample (W) was added to 20 mL of 10% acetic acid in ethanol. It was mixed, allowed to stand for 4 hours, and then filtered. The filtrate was evaporated to a quarter of its original volume. One drop of concentrated ammonia was added. The precipitate formed was filtered through a weighed filter paper (W_1). The filter paper was left to dry in the oven at 60^{o}C. The filter paper was weighed after drying to a constant weight (W_2).

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{W} \times 100$$

Phenolics

Two milligrams of the plant extract were mixed with 0.5 mL of Folin-Ciocalteau reagent, 1.5 mL sodium carbonate (20%), and left for 30 minutes at 40^{o}C to develop colour. The absorbance was measured at 765 nm and expressed as Gallic Acid Equivalent per gram sample (GAE/g).

Anthraquinones

One gram of the plant sample was added to 25 mL water and boiled with 10 mL of sulphuric acid. It was filtered while hot, the filtrate was mixed with 5 mL of chloroform and shook for a minute. The chloroform layer was pipetted into another test tube and 1 mL of diluted ammonia was added. The absorbance of the resulting solution was measured at 530 nm.

Flavonoids

One gram of the plant sample was extracted with 10 mL of 80% methanol. It was allowed to stand for 2 hours, after which it was filtered into a weighed Petri dish, and it was dried at 40^{o}C in the oven. The Petri dish was weighed when the sample has dried to a constant weight.

Steroids

One hundred milliliters of water was added to 5 g of the plant sample. 0.1 M ammonium hydroxide was added to adjust the pH to 9.1, then 2 mL petroleum ether, 3 mL acetic anhydride and conc. H_2S0_4 were added one after the other. The absorbance was read at 420 nm.

Terpenoids

One gram of the plant sample was dissolved in 10 mL petroleum ether. It was allowed to extract for 15 min, after which it was filtered and the absorbance was read at 420 nm.

Cardiac glycosides

One gram of the plant sample was extracted with 40 mL water and placed in the oven at 100^{o}C for 15 min. One millilitres of the extract and 5 mL water were added to 2 mL glacial acetic acid. A drop of

$FeCl_3$ and 1 mL conc. H_2S0_4 were added. The absorbance of the resulting solution was measured at 410 nm.

Carotenoids
One gram of the plant sample was mixed with 20 mL acetone. It was left for an hour and then filtered. Ten millilitres water was added to the filtrate. The filtrate was poured into a separating funnel and 5 mL petroleum ether was added and left for some minutes to separate. The lower layer was discarded; the absorbance was measured at 440 nm and read off a standard graph.

Antimicrobial Activity Screening
Collection of the test organisms
Two Gram positive bacteria: *Staphylococcus aureus* (NCIB 8588), *Bacillus cereus* (NCIB 6349); four Gram negative bacteria: *Serratia marcescens* (NCIB 1377), *Klebsiella pneumoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), *Proteus vulgaris* (NCIB 67); and six fungal isolates: *Penicillium camemberti*, *Trichophyton mentagrophytes*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Trichoderma* sp., and *Cladosporium herbarum* were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Media preparation
Dissolution and autoclaving of the nutrient media were according to the manufacturers' instructions. Thirty-eight grams of Muller Hinton Agar (Sigma Aldrich) and 48 g of Malt Extract Agar (Sigma Aldrich) were separately dissolved in 1 litre of distilled water. Each was heated to boiling, of which 20 mL was dispensed into MacCartney bottle, autoclaved at 121^{o}C, 15 psi (1 kg/cm^3) for 15 minutes and kept in a molten state. One gram of each plant extract was measured and dissolved in 10 mL of each of the solvent used to extract (methanol, n-hexane, ethyl acetate, and water). Thereafter, 5 mL from each extract was aseptically transferred into a Bijou bottle containing 5 mL of each solvent and were successively diluted five times and the resulting concentrations were: 100, 50, 25, 12.5, 6.25 and 3.125 mg/mL.

Antibacterial screening
Agar well diffusion technique was used for antibacterial screening where the broth culture of each test organism (0.1 mL) was added to 20 mL sterile molten Muller Hinton Agar which was cooled to 44^{o}C. The bottle was gently rotated to mix the microbe with the medium, poured into a properly labeled sterilized glass Petri dish (120 x 20 mm) and allowed to set. Seven wells were bored on the seeded medium using a sterile cork borer (8 mm). The wells were about 0.5 mm to the edge of the plate and labeled. Each well was filled with the appropriate plant extract concentration (3 - 6 µL) using sterile Pasteur pipette. The culture plates were incubated at 35^{o}C for 24 hours and care was taken not to stock-pile the culture plates. Susceptibility of each test organism to the plant extract was examined, measured with a pair of divider and a ruler.[44-46]

Antifungal screening
Ditch plate method was used. The prepared Malt Extract Agar was poured into a plate (120 x 20 mm), allowed to solidify, and then a trough (5 x 9 mm) was cut out of the agar. A loop full of each test organism (0.1 mL) was streaked outwards from the ditch on the agar surface and each extract (15 - 20 µL) was carefully run into the ditch, about three-quarters full. Each plate accommodated three test organisms but same concentration of the plant extract. The plate that served as the control had its ditch filled with the solvent used to extract the plant. The zone of inhibition was measured after 72 hours of incubation at 30^{o}C.[47-49]

Toxicity investigation
Ethical approval for animal use (UI-ACUREC/App/2016/033) was obtained from the University of Ibadan animal care and use research ethics committee. Seventy (70) Wistar male rats (15 - 80 g) were purchased from the Central Animal House, University of Ibadan, grouped into fourteen cages (five per group) and given food and water *ad libitum* for fourteen days to acclimatize them prior to experimentation. Different doses (10, 100, 1000, 1600, 2900 and 5000 mg/kg) of *A. occidentale* leaf aqueous and ethyl acetate fractions were prepared using 20% Dimethyl sulphoxide (DMSO) as a dissolvent. The plant fractions were orally administered to the rats using syringes with cannulas. Only 20% DMSO was orally given to five rats as control I, while the untreated five rats as control II. The rats were fasted and observed for 24 hours to monitor their behaviour and also for any mortality. All the rats were sacrificed by quick cervical dislocation on the third day with their kidneys and livers harvested and preserved in 10% formalin for histological assessment.[50]

Results and Discussion

Phytochemical constituents of Anacardium occidentale leaf extracts
The phytochemical screening result shows that *A. occidentale* leaf is rich in phytochemical constituents (Table 1). The organic solvents extracted seven of the phytochemicals better than water.
Saponins have been reported to reduce the danger of atherosclerosis and coronary problems because they are useful in binding cholesterol and reduce its quantity in the body.[51,52] Moreover, saponins are essential for the activity of cardiac glycosides; facilitate the absorption of foods and medicine; and also have anticancer activity.[53,54] The butanol fraction of *A. occidentale* had the highest saponins content, followed by the hexane fraction, methanol extract, ethyl acetate fraction, and aqueous fraction, while the powdered leaf had the least saponin content.
The pharmacological applications of alkaloids are many including; antimalarials, anesthetics, analgesics and CNS stimulants. Although some plants containing alkaloids do not feature strongly in herbal medicine, because they are extremely toxic. However, they have always been important in homoepathy where the dose-rate is so low making them harmless and in allopathic systems where the dose is also strictly controlled.[54,55] *Anacardium occidentale* leaf powder had the highest alkaloid content, followed by the methanol extract, aqueous fraction, ethyl acetate fraction, and hexane fraction, while the least alkaloids content was observed in the butanol fraction. Interestingly, phenols have gained considerable attention as protecting factors against cancer and heart diseases because of their antioxidant potency.[56-58] Phenolics was highest in the methanol extract of *A. occidentale* followed by butanol fraction, hexane fraction, ethyl acetate fraction, and aqueous fraction but the leaf powder had the least phenolic content.
The usefulness of anthraquinones having anti-inflammatory, neuro-protective and anti-atherogenesis activities have been emphasized.[59- 62] They act as potent antioxidants with similar capacity to reduce the effect of free radical-induced oxidative stress.[62] Anthraquinones in *A. occidentale* was highest in the ethyl acetate fraction, followed by the methanol extract, aqueous fraction, butanol fraction, and hexane fraction, while the leaf powder had the least value.
Flavonoids are significant in ethnomedicine displaying anti-allergic, anti-inflammatory, anti-tumour, anti-cancer, and antioxidants activities.[63] Flavonoids was highest in the leaf powder of *A. occidentale*, followed by the methanol extract, ethyl acetate fraction, aqueous fraction, hexane fraction, and butanol fraction.
Cardiac glycosides are essentially steroids with an intrinsic capacity to afford a definitive action mostly on the cardiac muscle when injected into man or animal. Small amounts of steroidal glycosides would cause needed stimulation on a diseased heart while too much dose may cause death.[64] Steroids were highest in the aqueous fraction of *A. occidentale*, then methanol extract, butanol fraction, ethyl acetate fraction, and hexane fraction, while the leaf powder gave the least value. Also, the ethyl acetate fraction of *A. occidentale* had the highest content of cardiac glycosides. It was followed by the aqueous fraction, methanol extract, butanol fraction, and hexane fraction. Noticeably, the methanol extracts, all the fractions and the leaf powder of *A. occidentale* were rich in steroids, but low in cardiac glycosides.
Terpenes are main components of numerous essential oils that are used in medicine and aromatherapy. They have sedative and anti-inflammatory properties, and are also useful in treating ovarian and breast cancers.[65] The aqueous fraction of *A. occidentale* had the

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highest terpenoid content, followed by the butanol fraction, ethyl acetate fraction, hexane fraction, methanol extract, and the leaf powder.

Carotenoids are useful in preventing macular degeneration, treating prostate problems, asthma, and certain cancers.[66,67] The aqueous fraction of *A. occidentale* had the highest content of carotenoids, followed by the butanol fraction, methanol extract, ethyl acetate fraction, hexane fraction, and the leaf powder. The leaf of *A. occidentale* has little quantity of carotenoids compared to the contents of other phytochemicals in the leaf.

Tannins are notable for wound healing. Agedah *et al*.[68] and Ashok and Upadhyaya[69] reported that *A. occidentale* leaf is used in treating wounds, healing burns, stopping bleeding and for the treatment of infections. The latter added that while concurrently healing the wound internally, it forms a protective layer over it to prevent further infection. Tannin content was highest in the butanol fraction, followed by ethyl acetate fraction, hexane fraction, methanol extract, aqueous fraction, and was least in the leaf powder of *A. occidentale*. The presence of tannins in the leaf extract supported the work of Razalia *et al*.[70] who reported that tannins were isolated from the ethanol leaf extract of *A. occidentale*. However, the findings in this study contradict the work of Abulude *et al*.[71] who stated the absence of tannins in alcohol leaf extract of *A. occidentale*.

The high content of saponins and flavonoids in this study slightly supported the works of Ezeigbo *et al*.[72] and Abubakar *et al*.[73] They reported that the anti-diarrhoeal activity of *A. occidentale* leaf might be due to the presence of flavonoids and saponins. It also agreed with Ranjith *et al*.[74] who reported the presence of flavonoids, saponins and terpenoids in *A. occidentale* leaf powder.

Saponins, tannins, alkaloids, phenolics were higher in value for the methanol extract than the aqueous fraction, thus agreeing with the work of Ojezele and Agunbiade[75] who reported that tannins, polyphenols, alkaloids and saponins had higher values in the ethanol extract than the water extract of *A. occidentale* leaf. Moreover, Belonwu *et al*.[62] reported highest concentrations of alkaloids and phenolics in the leaf extract of *A. occidentale* than in the stem bark, fruit and root extracts.

Antimicrobial activity of Anacardium occidentale leaf extracts

The antibacterial activity of *A. occidentale* leaf extracts is shown in Table 2. The susceptibility of each test microorganism to the plant extracts was shown by clear zones of growth inhibition, thus showing the relative activity of the test plant extracts against the organisms. The highest zone of inhibition was from the highest concentration of the plant extracts (100 mg/mL). None of the extraction solvents (methanol, hexane, ethyl acetate, and water) showed zone of inhibition.

Table 1: Phytochemical composition of *Anacardium occidentale* methanol leaf extract, fractions and leaf powder

Parameters	Leaf extract and fractions					
	M	**H**	**E**	**B**	**A**	**K**
Saponins (mg/100 g)	1625.00 ± 8.66^{c}	1703.33 ± 6.01^{b}	1455.00 ± 10.41^{d}	1750.00 ± 7.64^{a}	691.67 ± 10.14^{e}	438.33 ± 6.01^{f}
Tannins (mg/100 g)	26.67 ± 1.67^{d}	151.67 ± 4.41^{c}	635.00 ± 8.66^{b}	860.00 ± 10.41^{a}	23.33 ± 1.67^{d}	18.33 ± 1.67^{d}
Alkaloids (mg/100 g)	683.33 ± 9.28^{b}	216.67 ± 4.41^{e}	258.33 ± 7.26^{d}	91.67 ± 1.67^{f}	326.67 ± 6.01^{c}	1105.00 ± 10.41^{a}
Phenolics (GAE/g)	98.30 ± 0.15^{a}	67.27 ± 0.15^{c}	48.70 ± 0.12^{d}	76.63 ± 0.12^{b}	36.43 ± 0.12^{e}	21.43 ± 0.18^{f}
Anthraquinones (mg/100 g)	523.33 ± 6.01^{b}	380.00 ± 7.64^{e}	598.33 ± 4.41^{a}	413.33 ± 4.41^{d}	461.67 ± 4.41^{c}	93.33 ± 1.67^{f}
Flavonoids (mg/100 g)	366.67 ± 6.01^{b}	175.00 ± 7.64^{c}	345.00 ± 7.64^{b}	155.00 ± 7.64^{c}	176.67 ± 9.28^{c}	2196.67 ± 8.82^{a}
Steroids (mg/100 g)	446.67 ± 7.26^{b}	366.67 ± 6.01^{c}	385.00 ± 7.64^{c}	426.67 ± 9.28^{b}	528.33 ± 6.01^{a}	261.67 ± 6.01^{d}
Terpenoids (mg/100 g)	345.00 ± 10.41^{e}	393.33 ± 6.01^{d}	421.67 ± 4.41^{c}	521.67 ± 7.26^{b}	581.67 ± 6.01^{a}	115.00 ± 5.00^{f}
Cardiac glycosides (mg/100 g)	25.00 ± 2.87^{c}	11.67 ± 1.67^{e}	43.33 ± 4.41^{a}	16.67 ± 1.67^{d}	30.00 ± 2.89^{b}	ND
Carotenoids (μg/100 g)	1935.00 ± 7.64^{c}	925.00 ± 7.64^{e}	1848.33 ± 9.28^{d}	2076.67 ± 6.01^{b}	2126.67 ± 7.26^{a}	711.67 ± 4.41^{f}

n = 3; Values = Means ± S.E., Superscripts (a – f = highest to least) are level of significance ($p < 0.05$) for rows, M = Methanol extract of *A. occidentale*, H = Hexane fraction of *A. occidentale*, E = Ethyl acetate fraction of *A. occidentale*, B = Butanol fraction of *A. occidentale*, A = Aqueous fraction of *A. occidentale*, K = powdered leaf of *A. occidentale*. ND = Not detected. *1000 μg = 1 mg.

Table 2: Antibacterial activity of *Anacardium occidentale* leaf extracts

Microorganism	E/W concentration (mg/mL)					
	3.125	**6.25**	**12.5**	**25**	**50**	**100**
	Zones of inhibition (mm)					
Staphylococcus aureus (NCIB 8588)	-	-	E (11)	E (12)	E (14)	E (19)
Serratia marcescens (NCIB 1377)	-	-	E (9)	E (12)	E (14)	E (18)
Bacillus cereus (NCIB 6349)	-	-	E (9)	E (11) W (10)	E (15) W (13)	E (19) W (15)
Klebsiella pneumoniae (NCIB 418)	-	-	E (9)	E (12) W (11)	E (13) W (13)	E (18) W (15)
Proteus vulgaris (NCIB 67)	-	-	E (9)	E (11)	E (13)	E (18)
Pseudomonas aeruginosa (NCIB 950)	-	E (9)	E (10)	E (12)	E (13)	E (15)

E = ethyl acetate fraction, W = aqueous fraction, NCIB = National Collection of Industrial Bacteria, Scotland. - = No inhibition.

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The ethyl acetate fraction showed the highest zone of inhibition at 100 mg/mL against *Staphylococcus aureus*, *Serratia marcescens*, *Bacillus cereus*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. The antibacterial activity of *A. occidentale* supported the report of Ayepola and Ishola[76] that *A. occidentale* extracts have been efficiently applied for treating bacterial infections. The methanol extract of *A. occidentale* leaf was inactive against *S. aureus*, *P. aeruginosa*, and *A. niger*, but Akash *et al*.[77] reported activities of petroleum ether and ethanol extracts of *A. occidentale* leaf against same microorganisms. This however contradicted Chaithra *et al*.[78] findings that methanol leaf extract of *A. occidentale* was more effective in inhibiting bacterial isolates than its methanol bark extract. The methanol extract and water fraction of *A. occidentale* leaf were not active against *S. aureus* and *P. aeruginosa*, but was contrary to the activities reported on the ethanol and aqueous extracts of *A. occidentale* bark by Arekemase *et al*.[46] Likewise, the methanol extract of *A. occidentale* leaf was inactive against *S. aureus*, *P. aeruginosa* and *K. pneumoniae*; which contradicted the activities of the ethanol extract of *A. occidentale* seed coat against same microbes as reported by Vijayakumar and Kalaichelvan.[79] Additionally, the methanol extract of *A. occidentale* leaf was inactive against *B. cereus*; which was similar to the report of Akinpelu[34] for methanol extract of *A. occidentale* bark. However, Akinpelu[34] reported inhibition against *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, and *S. aureus*. Contrarily, the methanol extract of *A. occidentale* leaf was inactive against *S. aureus*, *B. cereus* and *S. marcescens*. Rajesh *et al*.[19] reported zones of inhibition for the methanol leaf extract of *A. occidentale* against the microorganisms.

The ethyl acetate fraction of *A. occidentale* leaf did not show antifungal activity against *A. flavus* and this contradicted the report of Rajesh *et al*.[80] that had ethyl acetate fraction of *A. occidentale* to inhibit *A. flavus*. The activity of the ethyl acetate fraction of *A. occidentale* leaf however supported the report of Chabi *et al*.[81] that the ethyl acetate fraction of *A. occidentale* leaf showed bacteriostatic and bactericidal effects on nineteen strains of bacteria.

The inhibition of *B. cereus* and *P. aeruginosa* by *A. occidentale* leaf extract supported the reports of Mackeen *et al*.[82] It also agreed with Tan and Chan[83] who reported the inhibition of *A. occidentale* leaf against *P. aeruginosa*. The antibacterial activity of *A. occidentale* supported the work of Aderiye *et al*.[84] who reported that the phenolic compounds such as cardols, triterpenoids, cardanols, methylcardols, xantoprotein and anacardic acids in *A. occidentale* extracts were responsible for cell wall inhibition in bacteria.

Concalves *et al*.[85] gave credence to the antimicrobial action of *A. occidentale* resulting from its saponins; while Aderiye *et al*.[84] claimed that flavonoids and tannins were responsible for the anti-microbial activity in *A. occidentale* extracts. In this study, *A. occidentale* leaf extracts had higher saponin content (methanol extract > hexane fraction > ethyl acetate fraction > water fraction > control), than tannins (hexane fraction > ethyl acetate fraction > control). Moreover, it was practically observed that the ethyl acetate fraction showed better and broad-spectrum antimicrobial activity than the hexane fraction. This is suggestive that the antimicrobial potency is actually much more of tannins than saponins.

It was observed that the methanol extract, and fractions of *A. occidentale* leaf extract, and the control solvents did not show any antifungal activity on all the tested molds: *Trichophyton mentagrophytes*, *Cladosporium herbarum*, *Trichoderma species*, *Penicillium camemberti*, *Aspergillus flavus* and *Rhizopus stolonifer*.

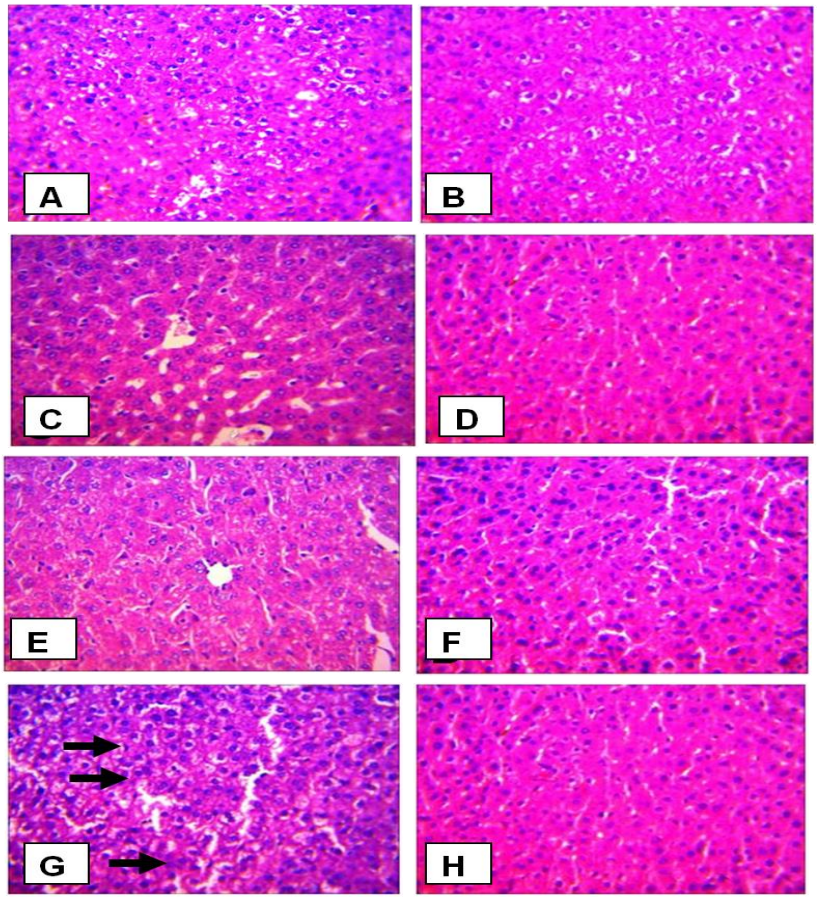

Plate 1: Liver photomicrographs (x400) of rats fed with *Anacardium occidentale* leaf ethyl acetate fraction (10 – 5000 mg/kg)
A = Rat fed with 10 mg/kg; B = Rat fed with 100 mg/kg; C = Rat fed with 1000 mg/kg; D = Rat fed with 20 % Dimethyl sulphoxide (control I); E = Rat fed with 1,600 mg/kg; F = Rat fed with 2,900 mg/kg; G = Rat fed with 5,000 mg/kg: there is a severe diffuse vacuolar degeneration of hepatocytes; H = Rat without dose (control II). All (except G) show no visible lesions.

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Toxicity of Anacardium occidentale leaf extracts

The photomicrographs of the livers and kidneys for the toxicity evaluation of *A. occidentale* leaf fractions in rats are shown in plates 1– 4. The ethyl acetate fraction of *A. occidentale* leaf was not toxic to the rats at 1,000 mg/kg body weight. This was similar to Okonkwo *et al*.[33] findings that the administration of methanol stem bark extract of *A. occidentale* did not significantly ($p < 0.05$) depress the function of hepatocytes in Wistar rats. However, Tédong *et al*.[86] reported some signs of acute toxicity in hexane extract of *A. occidentale* leaf in mice as asthenia, anorexia, diarrhoea, and syncope, while the sub-chronic study produced a reduction in food intake, weight gain, and behavioural effects. The ethyl acetate fraction of *A. occidentale* leaf was not toxic to the albino rats at 2,900 mg/kg body weight. Konan and Bacchi[87] corroborated the report that there were no signs of acute toxicity or apparent changes in the organs when Swiss mice were treated with ethanol extract of *A. occidentale* leaf up to 2,000 mg/kg body weight.

None of the rats died during the experiment, showing that the ethyl acetate and aqueous fractions of *A. occidentale* leaves were not toxic. Moreover, the photomicrographs showed no sign of toxicity to the rats' kidneys and livers even at 5,000 mg/kg, except for the ethyl acetate fraction as indicated in the livers. However, Dare *et al*.[88] reported low birth weight and low crown-rump length effect of the leaf extract when given to pregnant rats, thus warned expectant mothers to avoid the extract.

Conclusion

The leaf extracts of *A. occidentale* contain saponins, tannins, alkaloids, phenolics, anthraquinones, flavonoids, steroids, terpenoids, cardiac glycosides and carotenoids as each phytochemical was optimally extracted. The leaf has antibacterial activity, but without antifungal activity. The leaf is not toxic and safe at 2,900 mg/kg. Thus, this work might be useful in the development of *A. occidentale* leaf as antibacterial agents.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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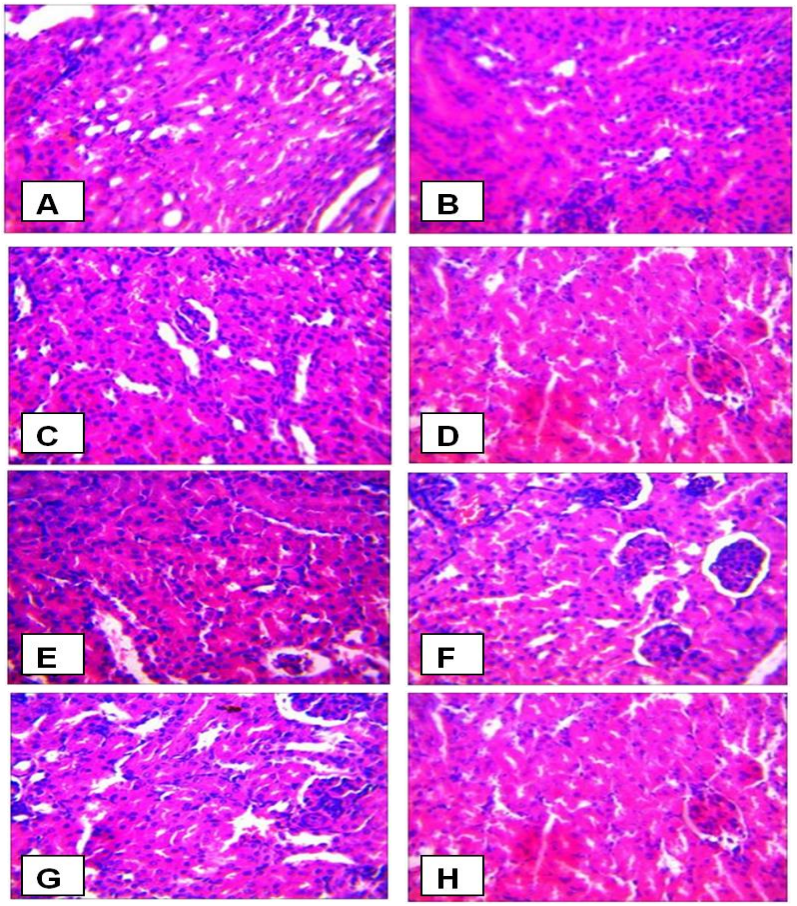

Plate 2: Kidney photomicrographs (x400) of rats fed with *Anacardium occidentale* leaf ethyl acetate fraction (10 – 5000 mg/kg)
A = Rat fed with 10 mg/kg; B = Rat fed with 100 mg/kg; C = Rat fed with 1000 mg/kg; D = Rat fed with 20 % Dimethyl sulphoxide (control I); E = Rat fed with 1,600 mg/kg; F = Rat fed with 2,900 mg/kg; G = Rat fed with 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

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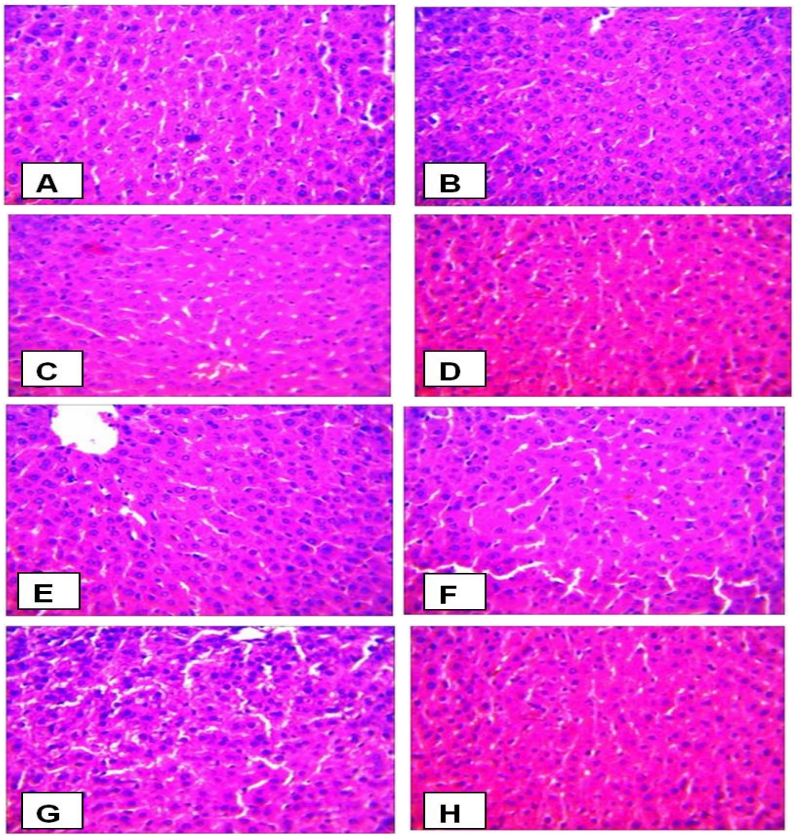

Plate 3: Liver photomicrographs (x400) of rats fed with *Anacardium occidentale* leaf aqueous fraction (10 – 5000 mg/kg)
A = Rat fed with 10 mg/kg; B = Rat fed with 100 mg/kg; C = Rat fed with 1000 mg/kg; D = Rat fed with 20 % Dimethyl sulphoxide (control I); E = Rat fed with 1,600 mg/kg; F = Rat fed with 2,900 mg/kg; G = Rat fed with 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

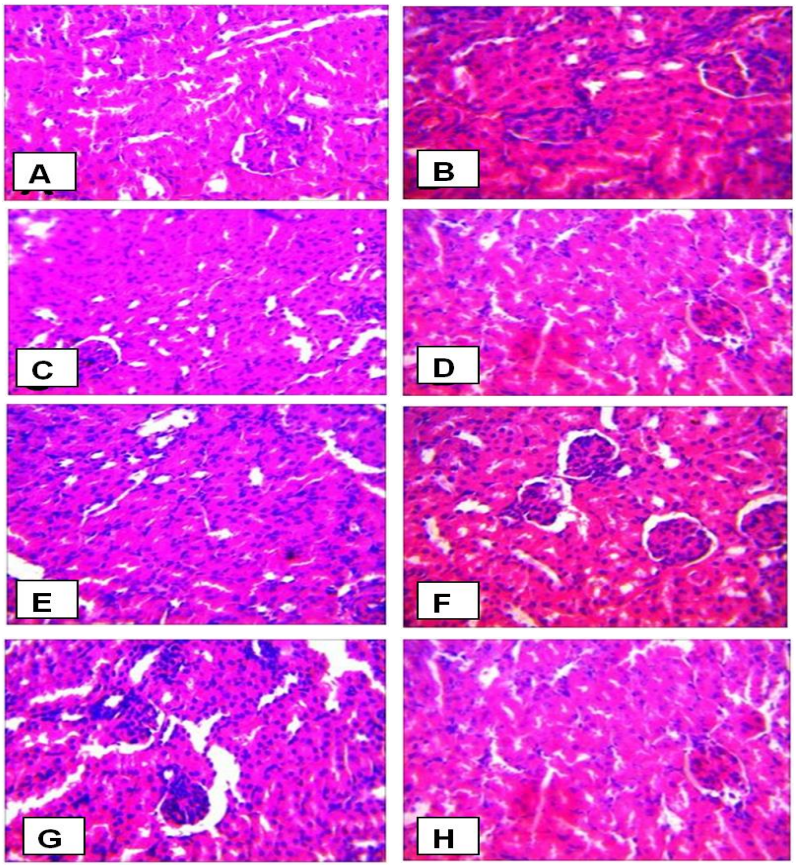

Plate 4: Kidney photomicrographs (x400) of rats fed with *Anacardium occidentale* leaf aqueous fraction (10 – 5000 mg/kg)
A = Rat fed with 10 mg/kg; B = Rat fed with 100 mg/kg; C = Rat fed with 1000 mg/kg; D = Rat fed with 20 % Dimethyl sulphoxide (control I); E = Rat fed with 1,600 mg/kg; F = Rat fed with 2,900 mg/kg; G = Rat fed with 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

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