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Research Article

Phytochemical Screening and Inhibitory Activities of *Anacardium occidentale* Leave Extracts against Some Clinically Important Bacterial Isolates

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ABSTRACT
The antibacterial activity of various extracts of *Anacardium occidentale* (cashew) against clinically important bacterial isolates of *Klebsiella spp, E. coli, Salmonella typhi* and *Staphylococcus aureus* were investigated using the Agar diffusion method. The result obtained showed that acetonitrilic extract gave the widest zone of inhibition against one of the four test organisms at the concentration of 25mg/ml. However, *Staphylococcus aureus* was more sensitive to the extract. The phytochemical screening indicated the presence of flavonoids, tannins, glycosides, terpenoids etc., in the extracts which confirm its inhibitory activities against the test organisms. This therefore, supports the traditional medicinal use of *Anacardium occidentale* in the treatment of bacterial infections in Nigeria.

Keywords: *Anacardium occidentale* (cashew), antibacterial activities, phyochemicals, bacterial infections, inhibitory activities

INTRODUCTION

Klebsiella spp. is a typhical example of opportunistic pathogens of human which mainly attack individuals whose immune system has been compromised and suffer from severe underlying diseases like chronic pulmonary obstruction. *Klebsiella* is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in liver diseases, diabetes mellitus and chronic alcoholics[1] and showing characteristic radiographic abnormalities[2] due to a severe suppurative infection which has a high fatality rate if untreated.

Escherichia coli are a bacterium that is commonly found in the gut of humans and other warm-blooded animals. National Center for Emerging and Zoonotic Infectious Diseases[3] reported that most strains of *E. coli* are harmless, however some few to contaminated food[4]. Symptoms of disease include abdominal cramps, pains, bloody diarrhoea and nausea. Fever and vomiting may also occur. Most individuals recover within 2 weeks, even though in a few cases the disease may become very dangerous.

Salmonella typhi is an obligate parasite that has no known natural reservoir outside of humans. Typhoid fever is a life-threatening illness caused by the bacterium *Salmonella typhi* and the bacteria is commonly found in developing countries as a result of poor hygiene or consumption of aquatic animals from polluted bodies of water.

Staphylococcus aureus causes a variety of pyogenic (pus-forming) infections and toxinoses (microbial toxins) in humans. Staphylococcus aureus causes superficial skin lesions such as pimples or boils and more serious infections such as osteomyelities and endocarditis. It is an important community-acquired infections, nosocomial infections of surgical wounds and is the most common cause of hospital acquired infection including surgical wounds and *S. aureus* in hospitals are becoming increasingly resistant to antibiotics. Mustapha[5] stated that lately, problems with microorganisms that are unaffected by drugs, side effects of orthodox drugs, and developing diseases where no medicines are obtainable, have inspired an awareness and curiosity in plants once again as a significant source of novel medicines.

Medicinal plants are plants which contain secondary metabolites that provides health-promoting characteristics, temporary alleviate from symptomatic problems or has curative and biological properties. Secondary metabolites are bioactive substances present in plants that are responsible for curative or biological activities. The plants have been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from plant sources[6].

Anacardium occidentale L. (Family Anacardiaceae), *Anacardium occidentale* L. (Family Anacardiaceae), is a multipurpose tree of the tropics which attains a height of about 10-15m. They grow on relatively dry soil in nature but in cultivation grow well in the tropical rain forest. The cashew tree produces many products and resources. The leave, bark, and the apple are explored medicinally to treat variety of diseases in Nigeria. The tree is a native plant of

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Nigeria commonly called Kànjùù in Hausa. The leaves, stems and bark extracts are used extensively for the treatment of diarrhea, dysentery and colonic pain[7]. It has also been reported to possess anti-ulcerogenic, anti-diabetic and anti- inflammatory properties[8]. The ethanolic extracts of cashew nuts revealed the presence of various phytochemical compounds such as phenolic, triterpenoids, carbohydrate, xantoprotein and flavenoids[9]. Murphy and Sivajamban[10] reported that the liquid obtained from the shell of cashew nut has wide commercial applications, bio-medicinal properties.

The medicinal properties of phytochemicals present in cashew nut have cytotoxic activity against several tumour cell limes, anti-diabetic, anti-inflammatory and analgesic effects[11-12]. The plants have been reported to possess anti-diabetic, anti-ulcerogenic, anti-inflammatory and bacterial activities[8, 13].

Omojasola and Awe[14] reported the antimicrobial activity of the leaf extract of *Anacardium occidentale* and *Gossypium hirsutum* against *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Stapphylococcus aureus* and *Pseudomonas auroginosa*.

Based on the fact that the plant *Anacardium occidentale* is very useful, as found by above mentioned literatures and there is a need to find out more about the potentiality of this plant as an antibacterial agent. The present study is, therefore, designed to study the phytochemical constituents and inhibitory activities of *Anacadium occidentale* leave extracts against some clinically important bacterial isolates.

MATERIALS AND METHODS

Plant collection

The leave samples of *Anacardium occidentale* was collected in the month of June from Nasarawa State University Keffi campus. The fresh and healthy leaves were separated instantly and packed in a polyethylene bag. The samples were transported to the laboratory until processing.

Preparation of samples

The whole leaves samples of *Anacardium occidentale* tree were washed with natural water twice to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried under sun for 10 days. The dry leave samples were grounded using mortar and pestle for 1 hour and a fine powdered sample was obtained. The whole leave powder samples of *Anacardium occidentale* were packed in a sealed plastic bottle until extraction.

Extraction procedure

Twenty five grams (25) of the dried powder sample was mixed with 250ml 2- propanol, ethyl acetate and acetonitrile in a 500ml beaker respectively, stirred and then transferred into 500ml conical flask, tightly closed and left for 48 hours under room temperature, it was then filtered and the respective extracts were transferred into a round bottom conical flask and placed on water bath for evaporation at 48^0C.

Reconstitution of extracts

The crude extract obtained was weighed and dissolved in distilled water for both the phytochemical and inhibitory activity studies.

Phytochemical Screening

Phytochemical screening for qualitative detection of flavonoids, alkaloids, tannins, saponins, glycosides, terpenoids, reducing sugar and volatile oils was performed on the crude extract as described by Talukdar and colleagues[15] with modifications.

Flavonoids

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange colour for flavones

Alkaloids

2ml of extract was measured in a test tube to which picric acid solution was added. An orange colouration was observed for alkaloids.

Tannins

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color was observed for gallic tannins and green color for catecholic tannins.

Saponins

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth was observed for saponins.

Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. A brick red precipitate was observed for Glycosides.

Terpenoids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated sulphuric acid solution was added slowly and red violet color was observed for terpenoid.

Reducing Sugars

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate was observed for reducing sugars.

Volatile oils

2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate was observed for volatile oils

Source of microorganisms

The organisms used were *Klebsiella spp*, *Escherichia Coli*, *Salmonella spp* and *Staphylococcus aureus.* The organisms were obtained from the Microbiology Unit of Laboratory Science Department, Federal Medical Centre, Keffi.

Preparation of culture media

Table 1: The results of the phytochemical screening of different *Annacadium occidentale* leave extracts

Phytoconstituents	Ethyl acetatic extract	2-propanolic extract	Acetonitrilic extract
Flavonoids	+	+	+
Alkaloids	-	-	-
Tannins	+	+	+
Saponins	±	+	-
Glycosides	±	+	+
Terpenoids	+	+	+
Reducing Sugar	+	-	-
Volatile Oils	±	-	+

-Absent, + Present, ±Weakly Present

Table 2: Inhibitory activities of Ethyl acetate leave extract of *Annacadium occidentale* against some clinically important bacterial isolates

S.No.	Organism	Zones of inhibition (mm)/Concentration (mg/ml)						
		25	12.5	6.25	3.125	1.5625	NS	C(4mg/ml)
1	*Klebsiella spp*	10	8	-	-	-	-	40
2	*E. coli*	13	10	-	-	-	-	36
3	*Salmonella typhi*	9	8	-	-	-	-	38
4	*S. aureus*	9	9	-	-	-	-	30

- No inhibition; NS: Normal saline; C: Ciprofloxain

Table 3: Inhibitory activities of 2-propanol leave extract of *Annacadium occidentale* against some clinically important bacterial isolates

S.No.	Organism	Zones of inhibition (mm)/Concentration (mg/ml)						
		25	12.5	6.25	3.125	1.5625	NS	C(4mg/ml)
1	*Klebsiella spp*	12	10	10	10	9	-	40
2	*E. coli*	14	11	11	9	-	-	36
3	*Salmonella typhi*	13	12	10	9	9	-	38
4	*S. aureus*	12	12	10	10	9	-	30

- No inhibition; NS: Normal saline; C: Ciprofloxain

Table 4: Inhibitory activities of Acetonitrile leave extract of *Annacadium occidentale* against some clinically important bacterial isolates

S.No.	Organism	Zones of inhibition (mm)/Concentration (mg/ml)						
		25	12.5	6.25	3.125	1.5625	NS	C(4mg/ml)
1	*Klebsiella spp*	14	12	11	10	9	-	40
2	*E. coli*	16	14	11	9	-	-	36
3	*Salmonella typhi*	16	13	12	10	9	-	38
4	*S. aureus*	18	14	12	10	-	-	30

- No inhibition; NS: Normal saline; C: Ciprofloxain

The media used in this work was Muella-Hinton agar. The media were prepared according to manufacturer's direction. The media were sterilized by autoclaving at 121^0C for 15 minutes before use.

Preparation of Stock solution

Stock solution (50mg/ml) of the plant extract was prepared by dissolving 100mg of each extract in 2ml of sterile normal saline.

Preparation of Control

Positive control (4mg/ml) was prepared by dissolving 200mg of ciprofloxacin in 50ml sterile normal saline of ciprofloxacin and normal saline as a negative control. The same concentration of the control was used throughout the research.

Preparation of Inoculum

Inoculums were prepared by direct colony suspension where a small volume of sterile normal saline was poured into a McCartney bottle to which general colonies of the test organisms taken directly from the plate, was emulsified and the suspension adjusted to match the 0.5 McFarland standard which has similar appearance of an overnight broth culture by adding normal saline.

Preparation of McFarland Turbidity Standard

McFarland standards are used as turbidity standards in the preparation of bacterial suspensions so that the number of bacteria will be within a given range. 0.5 McFarland standard is prepared by mixing 0.5 ml of 1.175% barium chloride dehydrate ($BaCl_2 \cdot 2H_2O$), with 99.5 ml of 1% sulphuric acid solution (H_2SO_4) to form a barium sulphate precipitate, which causes turbidity in the solution. A small volume of the turbid solution was transferred to McCartney bottle of the same type that was used to prepare the test and of control inocula. This was stored in a sterile-dark room temperature. Exactly 0.5 McFarland gives an equivalent approximate density of bacteria $1x10^{-8}$ cfu[16].

Serial Double Dilutions of the Plant Extracts

Serial double dilutions of the plant extracts were carried out by adding 1ml of normal saline (stock solution) at each serial dilution. Five concentrations were prepared from the stock solution in the order of 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.5625mg/ml were prepared respectively in five different McCartney bottle and left in a sterile environment.

Determination of Antibacterial Activity

The antibacterial activity of the leaf extracts was determined using cup-plate diffusion method following the known procedure. A pasteur pipette was used to add 0.1ml of the suspension to an already prepared medium. A sterile swab stick was used to spread by streaking the organisms all over the surface of the medium and allowed to dry for about 5 minutes. Wells of 5mm in diameter and 6mm in length were made in the Mueller-Hinton agar using sterile cork borer.

Exactly 0.1ml of each concentration (plant extracts, positive and negative controls) was introduced into each well on the medium and was allowed to stand in a sterile environment for about one hour for proper diffusion. It was thereafter incubated at 37°C for 24hrs. The sensitive bacteria grew everywhere except in areas around the holes in the medium. Then, antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition.

RESULTS AND DISCUSSIONS

The phytochemical analysis of plant extracts using ethyl acetate, 2-propanol and acetonitrile was shown in Table 1. From the phytochemical analysis flavonids, tannins, glycosides and terpenoids were found in *Anacardium occidentale* in the solvents such as ethyl acetate, 2-propanol and acetonitrile. The ethyl acetatic extract of *Anacardium occidentale* showed the presence of reducing sugar and saponins were found in presence of ethyl acetatic and 2-propanolic extracts. Volatile oils were observed in ethyl acetatic and acetonitrilic extracts of *Anacardium occidentale*. The ethyl acetate, 2-propanol and acetonitrile all extract of *Anacardium occidentale* showed the absences of alkaloids.

The result of this work indicates that the extracts of *Anacardium occidentale* have antibacterial properties when the extracts were tested on *Klebsiella spp, Escherichia Coli, Salmonella typhi* and *Staphylococcus aureus* was shown in Table 2, 3 and 4. The widest zone of inhibition was obtained with *Staphylococcus aureus* while *Salmonella typhi* showed a little zone of inhibition on ethyl acetatic extract shown in table 2. The difference in the zone of inhibition may be directly related to the susceptibility of each test organism to the cashew extracts. The factors responsible for this high susceptibility of *S. aureus* to the extract may be attributed to the solvent of extraction and secondary plant metabolites presence in the plant. Moreover, the positive control in each of the test organism was sensitive, given an average value of 25 mm in the entire tested organism.

From this work, it is certain that the solvent of extraction affected the degree of antibacterial activity of the extracts. It was observed that the acetonitrilic extract of *Anacardium occidentale* gave the widest zone of inhibition (18mm) at the concentration of 25mg/ml and the least was observed on ethyl acetatic extract shown in table 4 and 4 respectively. This inhibitory activity of acetonitrilic extract showed how the extract liberates the active component required for antibacterial activity. It is worth mentioning that the antibacterial activity of this plant is depended on the concentration of the extract and also the solvent of extraction.

CONCLUSION

It can be concluded from this study that the leave extract of *Anacardium occidentale* L. contains phytochemicals which confer medicinal properties on the plant and could be responsible for its antibacterial property.

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