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Antibacterial Activity of *Anacardium occidentale* Leaf Extracts on Some Microorganisms Associated with Dental Plaque

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Abstract

Dental plaque has become a major problem in Africa and the world and current antibiotics has almost become ineffective for its treatment. Hence there is a need to find alternative ways of treatment for dental plaque. *Anacardium occidentale* is used for this purpose in many parts of Africa. Bacteria were isolated from dental plaque and identified using morphological and biochemical characteristics of the isolates. The study of the activity of *Anacardium occidentale* leaf extracts dissolved in four solvents (Ethanol, Ethyl acetate, Dichloromethane, Hexane) on some bacteria isolated from patients with dental plaque were investigated using the agar well diffusion and the broth dilution method. The bacteria isolated were *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Serratia marcescens*,

Neisseria sicca, *Staphylococcus epidermidis*, *Corynebacterium matruchotii*. The results obtained showed that the ethanolic extracts had the highest antibacterial activity on the test bacteria with zones of inhibition ranging from 2.0mm to 13.0mm, and a minimum inhibitory concentration ranging from 5mg/ml to 10mg/ml. The dichloromethane extracts had the lowest activity with a zone of inhibition ranging from 1.0mm to 4.0mm and a minimum inhibitory concentration ranging from 10mg/ml-20mg/ml while they had no activity on some of the test bacteria. *Anacardium occidentale* leaf can act as a good alternative to standard drugs as a treatment for dental plaque and other infections caused by these bacteria.

Keywords: *Anacardium occidentale*, MIC, MBC, morphology, biochemical, isolates

Introduction

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Plants are sources of medicinal compounds, and have played a dominant role in the treatment of diseases [1]. Over 50% of all modern clinical drugs are of natural product origin [2] and natural products play an important role in the development of new drugs for the effective treatment of a wide range of diseases. Many plant species have pharmacological properties and possess various secondary metabolites like saponins, glycosides, and flavonoids, that are used to combat diseases [3,4,5]. There has been a massive increase in antibiotic resistance over the years that have led to a lot of death and suffering. This has necessitated the production of new drugs that can be effective in treating diseases. Plants are known to produce a variety of substances to protect themselves against different pathogens. It is expected that a lot of plant extracts will be active against those pathogens that are resistant to standard drugs [6]. Resistance of both human and animal pathogens to drugs have been widely encountered in recent years from many parts of the world, mostly in developing countries, due to carelessness in the use of commercial antibiotics for the treatment of infectious diseases [7]. Many people in developing countries use plant herbal extracts in various treatments in any given year [8]. Hence, there has been a lot of research carried out over the years on the antimicrobial activity of crude extract of different leaves, stems,

bark and roots [9,10].

One of the plants used for the treatment of infections is *Anacardium occidentale* (Cashew plant), a member of the genus *Anacardium*, family *Anacardiaceae*. *Anacardium occidentale* is commonly called cashew in English, 'Kashu' in Hausa, 'Okpokpo' and 'Kaju' in Ibo and Yoruba respectively along with various other names in different languages around the world [11,12]. The history of cashew in Nigeria dated back to 15th century. But the planting of cashew for commercial purposes started in Nigeria in the early 1950s with the establishment of first commercial plantations at places like Oghe, Mbala and Oji by the defunct Eastern Nigeria Development Corporation (ENDC) and Eruwa, Iwo and Upper Ogun by the defunct Western Nigeria Development Corporation (WNDC) [13,14, 15]. Today cashew trees are popular all over the country.

Anacardium occidentale is a multipurpose tree whose leaves, stem and bark extracts are utilized for the treatment of diseases such as diarrhea and dysentery [16]. It is also used for the treatment of infections such as diabetes and venereal diseases [16]. It has also been widely reported to be anti-bacterial and anti-inflammatory [17,18]. *Anacardium occidentale* is used for the treatment of various infections caused by various bacteria. One of those infections is dental plaque.

Dental caries is a major infectious disease in the world today. Cariogenic bacteria can interact by various recognized ways. These include co-aggregation and metabolic exchange [19, 20]. Dental plaque causes dental caries and other oral infections. Dental plaque is a diverse community of microorganisms in the form of a biofilm and they exist on the tooth surface and under the gums. The microorganisms present in this biofilm bind tightly to one another, as well as to the solid tooth surface, by means of an extracellular matrix consisting of polymers of both host and microbial origin [21,22].

The dental plaque exhibits an open architecture and is made up of channels that helps to

achieve the flow of nutrients, waste products, and oxygen through the biofilm [23]. Dental plaque biofilms are responsible for many of the diseases common in the oral cavity, including dental caries and periodontitis although they are also present on healthy teeth.

Dental plaque biofilms normally consists of various forms of microorganisms such as gram positive and gram negative cocci and bacilli. The gram-positive cocci species involved in the biofilm include *Streptococcus mutans*, *Streptococcus oralis* and *Staphylococcus epidermidis*. The gram-negative rod and filament species include *Actinomyces viscosus*, *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*. These microbial composition of the biofilm are normally able to withstand the oral cavity processes that contribute to the removal of bacteria such as swallowing and chewing. They are also able to survive in the high oxygen concentrations that are present in the oral cavity [24].

Main objectives of this research work are to isolate and identify some bacteria associated with dental plaque, determine the antimicrobial activity of *Anacardium occidentale* leaf extracts on some bacteria isolated from dental plaque, to compare the ability of different solvents in the extraction of the active components of *Anacardium occidentale* that are effective in the treatment of dental plaque and to compare the susceptibility of the isolated bacteria to standard commercial drugs.

Materials and Methods

Plant Collection

The leaves of *Anacardium occidentale* used in this research were obtained from Adewole, Ilorin Kwara State and were authenticated at the Department of Botany University of Ilorin.

Oral Samples Collection

Samples were collected from patients by health officer at Adewole Health Centre Ilorin, Kwara State, Nigeria. The infected area of the tooth was carefully swabbed with sterile swab sticks and

transported immediately to the Laboratory for bacteriological analysis.

Culture Media Preparation

The media used include Nutrient agar, Nutrient broth, Mueller Hinton agar, Blood agar and Chocolate agar. The media were prepared according to manufacturer's specifications. The media were sterilized by autoclaving at 121°C for 15 minutes before use.

Bacteria Isolation

The samples were streak inoculated onto Blood agar and Chocolate agar and incubated at 37°C for 24 hours. After incubation, the colonies were sub-cultured onto petri dishes containing Nutrient agar and incubated at 37°C for 24 hours. Pure colonies were streaked on nutrient agar slopes in McCartney bottles as stock cultures and incubated at 37°C for 24hrs. The bacterial isolates were characterized using morphological and biochemical characteristics which include Gram staining, motility, coagulase, catalase, oxidase, indole, urease production, citrate utilization, methyl red, vogues proskauer, bile solubility and carbohydrate fermentation tests such as mannitol, sucrose, glucose and lactose [25].

Plant Extracts Preparation

The leaves of *Anacardium occidentale* were sun dried for 14 days. They were powdered using mortar and pestle into fine powder. This was then wrapped with aluminium foil for various categories of extraction. Hundred grams (100g) each of the powdered sample were weighed and dispensed into four sterile conical flasks. Four hundred milliliters (400ml) of each of the solvents namely; 95% ethanol, Hexane, Ethyl acetate, Dichloromethane were added to each conical flasks containing plant powder. The conical flasks were covered with cotton wool, wrapped with aluminium foil and left for three days with constant stirring. The extract was filtered through whatman filter paper. The filtrate was concentrated using a rotary evaporator. This was done for each of the extracts.

Antibacterial Susceptibility Test

The antibacterial activities at the different concentrations of the different extracts on the test bacterial isolates were determined by employing the agar well diffusion method [26]. Twenty milliliters of sterile Nutrient agar in petri dishes were seeded with standardized inocula using sterile cotton swabs. Wells of 6.0 mm in diameter were cut out on the seeded plates using sterile cork borer and each of the wells were filled with varying concentrations 5, 10, 15 and 20mg/ml of the plant extracts. The extracts were allowed to diffuse into the medium and the plates were incubated at 37°C for 24 hours. The zones (diameter) of inhibition were measured in millimeter (mm) using a metre rule and a micrometer screw gauge. The zones of inhibition which showed the effects of the antibacterial activity were determined by measuring the zone of inhibition around the wells. Controls were also set up with the different solvents namely; Ethanol, Ethyl acetate, Hexane and Dichloromethane poured into each well with no plant extract.

Determination of Minimum Inhibitory Concentration (MIC)

Previous methods were used for this process [27, 28, 29]. The minimum inhibitory concentrations of the plant extracts were determined using the Broth dilution method. A series of test tubes containing sterile culture medium and various concentrations of each extract were used. The lowest concentration that prevented growth completely was used for the determination of minimum inhibitory concentration (MIC). Different concentrations of 5, 10, 15 and 20mg/ml were drawn from each extract and poured into 9ml sterile broth in each test tube. The test bacterium was introduced into each test tube. These test tubes were incubated at 37°C for 24 hours. The least concentration of the extract that did not show any visible growth or turbidity was taken as the minimum inhibitory concentration [30]

Determination of Minimum Bactericidal Concentration (MBC)

The material from each test tubes used in the minimum inhibitory concentration assay that showed no growth after incubation, were streaked onto a solid nutrient agar plates and then incubated at 37°C for 24hours. The lowest concentration of the extract that showed no growth on the plate after 24 hours was taken as the minimum bactericidal concentration [30].

Antibiotic Sensitivity Using Standard Commercial Drugs

This was used to test the susceptibility of the bacteria to commercial antibiotics. Mueller Hinton agar was prepared according to manufacturer's specification and 20ml was dispensed into each petri dish, and allowed to solidify. The test bacteria were introduced into the petri dishes. The antibiotics used include both gram positive and gram negative. Antibiotic discs were placed on the media containing the test bacteria and incubated at 37°C for 24 hours. The zones of inhibition which showed the effects of the antibacterial activity were determined around the antibiotic discs.

Results and discussion

In Table 1, the diameters of the wells were subtracted from total to get the resultant bacterial zones of inhibition. The result showed that the ethanol extracts of *Anacardium occidentale* at 20 mg/ml inhibited the growth of *Fusobacterium nucleatum*, *Neisseria sicca* and *Serratia marcescens* by 9.0, 13.0 and 8.0 mm respectively. Ethyl acetate extracts of *Anacardium occidentale* at 20 mg/ml inhibited the growth of *Staphylococcus aureus* and *Fusobacterium nucleatum* by 9.5 mm each. *Serratia marcescens* was resistant to dichloromethane extracts of *Anacardium occidentale* at 15.0 mg/ml. The results obtained revealed that that the higher the concentration of the extracts, the higher the activity against the test bacteria.

Table 2 shows the Minimum Inhibitory Concentration (MIC) of the extracts that were active against the test bacteria. Ethanol extracts of *Anacardium occidentale* showed the lowest MIC value of 5.0 mg/ml against *Fusobacterium nucleatum*, *Staphylococcus epidermidis* and *Serratia marcescens*. Ethyl acetate extracts of *Anacardium occidentale* showed MIC against *Fusobacterium nucleatum* and *Neisseria sicca* at 5.0 mg/ml and 10.0 mg/ml respectively, while dichloromethane extracts of *Anacardium occidentale* expressed its MIC against *Fusobacterium nucleatum* and *Neisseria sicca* at 10.0 mg/ml.

In Table 3, Ethanol extracts of *Anacardium occidentale* exerted its bactericidal activity against *Fusobacterium nucleatum*, *Staphylococcus epidermidis* and *Serratia marcescens*.

Table 1: Antibacterial susceptibility to plant extracts

		Zone of Inhibition (mm)						
Extracts		mg/ml	<i>Fusobacterium nucleatum</i>	<i>Aggregatibacter actinomycetemcomitans</i>	<i>Staphylococcus epidermidis</i>	<i>Neisseria sicca</i>	<i>Serratia marcescens</i>	<i>Corynebacterium matruchotii</i>
ANA ETH	+	5	3.5	2.5	2.0	5.0	2.5	3.5
		10	6.0	3.5	3.5	8.0	4.0	4.5
		15	7.0	5.5	5.0	11.5	6.0	6.5
		20	9.0	7.0	7.5	13.0	8.0	8.0
ANA ACE	+	5	3.0	1.0	2.5	R	2.0	2.5
		10	4.5	2.5	4.5	1.5	3.5	3.5
		15	6.0	3.5	6.0	2.5	5.0	4.0
		20	9.5	5.5	9.5	4.0	7.0	5.5
ANA DCM	+	5	1.5	R	R	R	R	R
		10	2.5	R	R	2.5	R	1.0
		15	3.0	2.0	2.0	2.0	R	2.0
		20	4.0	3.5	3.0	3.5	2.5	3.5
ANA HEX	+	5	R	3.0	R	R	R	R
		10	3.5	6.5	2.0	2.5	R	R
		15	5.5	9.0	2.5	4.0	1.0	1.5
		20	7.0	12.5	4.0	5.0	3.5	3.0

Legend: Ana - *Anacardium occidentale*, R –Resistant, ETH – Ethanol, HEX – Hexane, ACE - Ethyl acetate, DCM -Dichloromethane

Ethyl acetate extracts of *Anacardium occidentale* showed that the growth of *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* and *Staphylococcus epidermidis* were static at concentration of 15.0 mg/ml.

This study shows that the growth of *Neisseria sicca* was only static against ethyl acetate, dichloromethane and hexane extracts of *Anacardium occidentale*. This implies that these extracts could not wipe out 99.9% of *Neisseria sicca*.

Table 2: Minimum inhibitory concentration of the extracts

Test organisms	Minimum Inhibitory Concentrations (mg/ml)			
	ANA ETH	+ ANA ACE	+ ANA + DCM	ANA + HEX
<i>Fusobacterium nucleatum</i>	5	5	10	10
<i>Aggregatibacter actinomycetemcomitans</i>	10	15	15	10
<i>Staphylococcus epidermidis</i>	5	10	15	10
<i>Neisseria sicca</i>	10	10	10	10
<i>Serratia marcescens</i>	5	10	20	15
<i>Corynebacterium matruchotii</i>	10	10	10	15

Legend: Ana –*Anacardium occidentale*, HEX –Hexane, ETH – Ethanol, ACE -Ethyl acetate, DCM –Dichloromethane.

The antibiotic sensitivity of Standard Drugs to test bacteria is shown in Table 4.

Staphylococcus epidermidis was susceptible to septrin, ciprofloxacin, perfloxacin, gentamycin and erythromycin by 11.5, 29.0, 15.0, 24.0 and 22.0 respectively, while *Corynebacterium matruchotii* was resistant

Table 3: Minimum bactericidal concentration of the extracts.

Test organisms	Minimum Bactericidal Concentration(mg/ml)			
	ANA+ETH	ANA+ACE	ANA+DCM	ANA+HEX
<i>Fusobacterium nucleatum</i>	10	15	20	15
<i>Aggregatibacter actinomycetemcomitans</i>	15	15	BS	20
<i>Staphylococcus epidermidis</i>	10	15	15	20
<i>Neisseria sicca</i>	15	BS	BS	BS
<i>Serratia marcescens</i>	15	BS	20	BS
<i>Corynebacterium matruchotii</i>	25	15	BS	BS

Legend: Ana - *Anacardium occidentale*, BS –Bacteriostatic, ETH- Ethanol, HEX –Hexane, ACE - Ethyl acetate, DCM -Dichloromethane

to septrin, perfloxacin, amoxicillin and gentamycin. *Fusobacterium nucleatum*, *Neisseria sicca*, *Serratia marcescens* and *Aggregatibacter actinomycetomitans* were susceptible to gentamycin by 16.0, 9.5, 10.5 and 6.0 mm respectively. *Neisseria sicca*, *Serratia marcescens* and *Aggregatibacter actinomycetomitans* were resistant to erythromycin and amoxicillin. The result showed that the bacteria were resistant to many of the antibiotics used.

Table 4: Antibacterial sensitivity to standard drugs

Test organisms	Zone of Inhibition (mm)										
	SXT 30µg	CPX 10µg	PEF 10µg	AM 30µg	CN 10µg	APX 30µg	Z 20µg	R 25µg	S 30µg	E 10µg	
<i>Staphylococcus epidermidis</i>	11.5	29.0	15.0	R	24.0	R	R	22.0	21.0	12.0	
<i>Corynebacterium matruchotii</i>	R	12.5	R	R	R	9.0	1.0	R	R	R	
(Gram negative)	Zone of Inhibition (mm)										
	E 11.5µg	CN 10µg	DA 10µg	AMC 20µg	FEP 30µg	SXT 23.75µg					
<i>Fusobacterium nucleatum</i>	6.0	16.0	R	8.0	8.0	R					
<i>Neisseria sicca</i>	R	9.5	6.0	R	R	R					
<i>Serratia marcescens</i>	R	10.5	9.5	R	R	8.0					
<i>Aggregatibacter actinomycetemcomitans</i>	R	6.0	7.0	R	R	R					

Legend: R – Resistant, SXT- septrin, CPX - ciprofloxacin, PEF – perfloxacin, AM – CN–gentamycin, APX – ampiclox, Z – zinacef, R – rocephin, S – streptomycin, E – erythromycin, DA – clindamycin, AMC – amoxicillin, FEP – cefepime, SXT - sulphamethoxazole

Morphological and biochemical characteristics were used to identify the isolated bacteria. The antibacterial activity of *Anacardium occidentale* extracts dissolved in different solvents was

compared and the extracts with the highest antibacterial activity on the test bacteria were determined. The antibacterial activity of the plant extracts on the test bacteria were also compared with that of commercial antibiotics. The zone of inhibition was observed around indicated the antibacterial activity of the plant extracts on the test bacteria.

The results obtained showed that the ethanol extract had the highest zone of inhibition against majority of the test bacteria, and were active against all the test bacteria at all the concentrations used. The ethanol extract was most active against *Neisseria sicca* with a zone of inhibition of 13.0 mm at the highest concentration of 20.0 mg/ml. The ethanol extract was also least active against *Aggregatibacter actinomycetemcomitans*, with a zone of inhibition of 7.0 mm at the same concentration. This may be due to the ability of the ethanol to extract more of the active ingredients than the other solvents used being a polar solvent.

The ethyl acetate extract was found to be active against all the bacteria at all concentrations except against *Neisseria sicca* at a concentration of 5 mg/ml. The inability of the extracts to be active against some of the bacteria may be due to the inability of the solvent to extract the active ingredient of the plant material that is effective against the bacteria. The hexane extracts of *Anacardium occidentale* had the highest activity against *Aggregatibacter actinomycetemcomitans* with a zone of inhibition of 12.5 mm. They were not active against *Neisseria sicca*, *Corynebacterium matruchotii* at lower concentration of 5mg/ml.

The dichloromethane extracts of *Anacardium occidentale* had the lowest activity on the majority of the test bacteria at all concentrations used and they were also not active against *Serratiamarcescens*, *Neisseria sicca*, at low concentration of 5 mg/ml, 10mg/ml. But they were active at higher concentration of 20 mg/ml, except on *Serratia marcescens* where they are not active at 15mg/ml. These findings showed that the higher the concentration, the higher the sensitivities of the test bacteria to the *Anacardium occidentale* leaf extracts. This is evidenced by the increased size of the bacterial growth inhibition zones, thus exhibiting concentration

dependent activity and these results are in conformity with [31]. Previous studies also showed that the higher the concentrations of plant extracts of *Vernonia amygdalia*, the larger the diameter of the bacterial growth inhibition zones [32].

The results showed that the ethanol extracts had the lowest minimum inhibitory concentrations. This indicated that the ethanol extracts has the highest activity on the test bacteria. The lowest MIC was 5 mg/ml, while the highest was 20 mg/ml. The results equally showed that the extracts were bacteriostatic against many test bacteria at the concentrations used, and were not able to exert bactericidal action at that same concentration. The ethanol extracts had the lowest minimum bactericidal concentration on all the test microorganisms with the lowest being on *Fusobacterium nucleatum* and *Staphylococcus epidermidis* at 10 mg/ml.

The antibacterial activities of the extracts increased as the concentration increased in this work. This does not differ from previous research findings which found that the tannins isolated from medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance in future [33]. The potentials of a drug to be effective against infections depend on the active components present in it. The results obtained from this research tend to confirm the efficacy of the extracts of the leaves of *Anacardium occidentale* as traditional remedies against bacteria that cause dental infections. The comparison of the activity of the plant extracts with conventional antibiotics showed that conventional antibiotics were more active than the plant extracts, although some of the bacteria such as *Aggregatibacter actinomycetemcomitans*, *Neisseria sicca*, *Corynebacterium matruchotii* were resistant to most of the antibiotics used. The antibacterial activity of the plant extracts could also be enhanced if the active components are purified.

It is therefore recommended that further studies should be conducted in order to analyze and purify the active ingredients of *Anacardium occidentale* that are capable of inhibiting the growth of the bacteria associated with dental plaque. *Anacardium occidentale* should be used as an

alternative to standard drugs in the treatment of dental plaque, dental caries, and other dental infections. Plants produce and contain a wide range of bioactive constituents such as alkaloids, tannins and flavonoids and provide excellent leads for the drug development [34].

Conclusions

The study is aimed at investigating the viability of cashew (*Anacardium occidentale*) leaf extracts in the treatment of dental plaque, dental caries, and other dental infections caused by the bacteria. The study was also used to determine the best solvent for the extraction of the active compounds of *Anacardium occidentale* leaf that are effective against the test bacteria. This study determined that *Anacardium occidentale* leaf extracts was active against all the test bacteria at various concentrations, although some of the bacteria utilized for this study were resistant to the extracts at low concentrations. The effectiveness of the plant extracts were compared with that of commercial antibiotics and the results showed that many of the organisms were resistant to the commercial antibiotics used, although in the case of those antibiotics that were active, they had higher activity than the plant extracts. This could be attributed to the fact that the commercial antibiotics were better purified than the plant extracts. Thus, *Anacardium occidentale* may be effective in the treatment of diseases caused by the bacteria used in this research and may contribute to the improvement of health care delivery in Nigeria and in the world generally, if the active chemical compounds capable of inhibiting the growth of the test bacteria are analyzed and formulated into dosage forms for use. Further studies need to be carried out to bring out the potentials of this plant in managing dental plaque, dental caries, and other diseases.

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