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Identification of antimicrobial properties of cashew, *Anacardium occidentale* L. (Family Anacardiaceae)

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ABSTRACT: The antimicrobial capabilities of plant extract derived from the leaves of the cashew plant, *Anacardium occidentale* L. (Family Anacardiaceae), on two common human pathogens of clinical importance, *Escherichia coli* and *Staphylococcus aureus* were investigated. All test organisms were identified to be sensitive to the plant extract, although zones of inhibition were about 50% of those observed using the commercial antibiotic, ampicillin. In terms of sensitivity, *S. aureus,* a Gram +ve organism, without an outer membrane in its cell wall was more sensitive relative to *E. coli*, a Gram –ve organism, which possesses an outer membrane in its cell wall. The degree of sensitivity could probably be due to this outer membrane which when present prevents a substantial amount of the extract from making contact with the cell. The antimicrobial properties of *A. occidentale* are derived from the presence of a polyphenol, anacardic acid and other compounds, tatrols and tanins. Therefore, it is suggestive of the fact that extracts could be used as an antibiotic especially in poor communities. @ JASEM

Medicinal plants are of great importance to the health of individuals and communities. It was the advent of antibiotics in the 1950s that led to the decline of the use of plant derivatives as antimicrobials (Marjorie, 1999). Medicinal plants contain physiologically active components which over the years have been exploited in the traditional medical practices for the treatment of various ailments Adebanjo, *et al.*, 1983). A relatively small percentage of less than 10% of all the plants on earth but percentage is believed to serve as sources of medicine (Marjorie, 1999). Medicinal plant history dates back to the fifth century AD., with Hippocrates and Discorides (with his famous, *De materia medica* (Croom and Walker, 1995). Cashew, *Anacardium occidentale* L. (Family Anacardiaceae), is a multipurpose tree of the tropics which attains a height of about 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. According to Duke (2001), the bark and leaves of *A. occidentale* are used medicinally; the shell oil around the nut is used medicinally and for industrial applications in the plastic and resin industries for its phenol content; the nut has international appeal and market value as a food source, especially in the beverage industry. The aim of this work was to test the antimicrobial properties of the leaves of the cashew plant, *A. occidentale,* using two common pathogens, *Escherichia coli* and *Staphyloccocus aureus.*

MATERIALS AND METHODS

Live specimens authenticated at the University of Port Harcourt herbarium were used for this study. These live specimens, leaves of *A. occidentale,* were rinsed with sterile water and sun dried. The dried leaves were ground into fine particles using a clean mortar and pestle and sieved using a 2mm sieve. The powder was collected after sieving. All glassware as well as other materials used were washed with detergent and rinsed thoroughly with distilled water (Cheesbrough, 2000). The glassware were further placed in racks to dry and autoclaved at 121^{o}C for 15 mins to kill off all contaminants.

The leaf extract was obtained using the 'ethanol extraction' technique in which 20g of the powdered plant material was soaked in a conical flask containing 100ml of 98% ethanol. This mixture was allowed to stand for 24h at room temperature with periodic stirring. At the end of this period, the mixture was filtered using a muslin cloth. The filtrate was then evaporated in a water bath at 78^{o}C, and the extract collected. This extract was stored in a refrigerator at 4^{o}C until time of use.

Medium and test organisms used: The medium used is the general purpose medium, Nutrient agar. Stock cultures of *E. coli* and *S. aureus* were obtained from the Microbiology Department of the University of Port Harcourt Teaching Hospital, Alakahia, Port Harcourt, Rivers State. These organisms were maintained in nutrient agar slants at 4^{o}C in the refrigerator.

Antimicrobial discs from the extract were prepared by perforating paper discs of 10mm diameter with the aid of a hollow metal and sterilized in the autoclave at 121^{o}C for 15min. The discs were thereafter impregnated with 0.1 ml of the aqueous plant extract and dried in three respective formats i.e. direct impregnation, sun-dried and oven-dried.

Antimicrobial susceptibility testing: The antimicrobial capability of the extract on the selected pathogens of clinical importance was determined by the disc diffusion technique (Oboh and Abulu, 1997). The nutrient agar plates were inoculated with the test organisms each with a sterile wire loop. The plates were allowed to dry for 15 min in an incubator. The discs impregnated with the extract were transferred to the plates with the aid of flamed but cooled forceps and were sufficiently spaced on the medium. The plates were then inoculated at 37^{o}C for 24h to observe the zones of inhibition produced by the plant extract.

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Antibiotic sensitivity testing: This was carried out on all the test organisms using the Kirby-Bauner's paper disc diffusion technique (Prescott *et al*., 1996).

Determination of bacteriostatic/bacteriocidal properties: Samples collected from the zones of inhibition were inoculated on pure nutrient agar plates of *E. coli* and *S. aureus* respectively. The plates were then incubated for 24h at 37^{o}C and growth observed.

RESULTS AND DISCUSSION

The results are presented in Tables 1- 4. Zones of inhibition were observed 24h after inoculation at a steady temperature of 37^{o}C. These zones were more pronounced in the plates containing *S. aureus* than in those containing *E. coli.* Although the extract from *A. occidentale* inhibis the growth of the test organisms, *E. coli* and *S. aureus,* the commercial antibiotic, *Ampicillin* was a better growth retardant.

Table 1: Zones of inhibition of plant extract on test organisms (mm.)

Test organisms	Direct participation	Sun dried	Oven dried
Escherichia coli	7.6 ± 1.27	6.6 ± 1.22	5.9 ± 0.29
Staphylococcus aureus	10.3 ± 1.27	9.6 ± 1.22	6.6 ± 0.29

Table 2: A comparison of the zones of inhibition of the plant extract of *A. occidentale* and the commercial antibiotic, *Ampicillin* on the test organisms (mm)

Test organisms	ant extract of *A. occidentale*	Commercial antibiotic, *Ampicillin*
Escherichia coli	7.6 ± 1.27	6.6 ±1.22
Staphylococcus aureus	10.3 ± 1.27	9.6 ± 1.22

The test organisms, *E. coli* and *S. aureus* were all sensitive to the extract obtained from the leaves of the cashew plant, *A. occidentale.* This manifested in the creation of inhibition zones in the various media in the plates used for the inoculation (Fig. 2). These zones of inhibition were greater in the plates inoculated with *S. aureus* than with *E. coli* indicating that *S. aureus* is more sensitive to the extract than *E. coli.* This difference in sensitivity could be attributed to the fact that *S. aureus* is a Gram +ve organism whereas *E. coli* is Gram –ve. Since Gram +ve organisms lack an outer membrane in their cell walls whereas Gram –ve organisms do possess it, this outer membrane may be responsible for the difference in the degree of sensitivity of these organisms to the crude plant extract of *A. occidentale.*. This could be due to the fact that the outer membrane may prevent a substantial amount of the extract having contact with the cell wall.

Table 3: Zones of inhibition at various concentrations (mg/ml) of the commercial antibiotic, *Ampicillin* on the test organisms.

	Various concentrations of *Ampicillin* (mg/ml)			
Test organisms	8.0	12.5	25	125
Escherichia coli	12.6 ± 0.41	13.2 ± 0.49	14.5 ± 0.24	18.7 ± 1.43
Staphylococcus aureus	13.6 ± 0.41	14.4 ± 0.49	15.1 ± 0.24	22.2 ± 1.43

Table 4: Traditional uses of *Anacardium accidentale*

S/No.	Uses	Main action(s)	Countries
1.	Diarrhoea, dysentery, stomach ulcer.	Antibacterial antiseptic.	Ghana, Nigeria, Togo
2.	Eye and ear infections.	Antibacterial antiseptic.	All over Africa, Brazil
3.	Prevents excessive bleeding	Promotes blood coagulation	All over Africa
4.	Healing of wounds	Antibacterial antiseptic.	All over Africa
5.	Malaria		All over Africa
6.	Anti-diabetic	Antibacterial antiseptic.	Ghana, Haiti, Kenya, Mexico, Nigeria
7.	Fever, poisoning, warts, toothache.	Antibacterial antiseptic, anti-inflammatory.	Haiti, Turkey.

All the two test organisms were much more susceptible to the commercial antibiotics (ampicillin) than to the crude plant extract. This may e due to the fact that the active ingredient in ampicillin is a a refined and purified form whereas the active ingredient in the plant extract is in a crude, impure, unrefined form and in a mixture with other unknown compounds. The growth observed in the bacteriocidal/bacteriostatic test is an indication that the active ingredient is bacteriostatic rather than being bacteriocidal. This is also attributable to the impure unrefined state of the active ingredient in the plant extract or that the active ingredient in the extract is not quantified and concentrated to the level that will make it bacteriocidal. It is our suggestion, therefore, that further studies on extracts of *A. occidentale* should involve refining or purifying and concentrating the active ingredient to determine its

real effect on pathogenic microorganisms. The results of this study are in conformity with the results obtained by earlier workers (Himejina and Kubo, 1991; Mustapha and Hafsat, 2007). The antimicrobial properties of *A. occidentale* are derived from the presence of a polyphenol known as Anacardic acid and other compounds such as Tatrols and Tanins. This plant, *A. occidentale* is thought to be effective in the treatment of malaria (in some African countries), asthma, diarrhoea, dysentery, leprosy, warts and sore throat (Table 4).

Conclusions: More research is required to fully establish the usefulness of this plant, *A. occidentale* to humanity as a source of drugs for the alleviation of illnesses caused by micro and macro-organisms.

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