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PHYTOCHEMICAL AND ANTIBACTERIAL INVESTIGATIONS OF CRUDE EXTRACTS OF LEAVES AND STEM BARKS OF *ANACARDIUM OCCIDENTALE*

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ABSTRACT
Phytochemical analysis and antimicrobial investigation of aqueous and ethanol extracts of leaves and stem barks of *Anacardium occidentale* was carried out using the disc diffusion method. Results revealed the presence of tannins, glycosides, saponins, resins, flavonoides and alkaloids. The extracts exhibited antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus feacalis*, *Streptococcus albus* and *Streptococcus pneumonia.* The plant contains substances that can be used in the formulation of very potent antimicrobial agents that can be used for the treatment of bacterial infections.

KEYWORDS: microbial organisms, bioactive substance, plant parts, phytochemcial screening.

INTRODUCTION

Herbal medicine has been shown to be effective in the treatment of different kinds of ailments such as jaundice, malaria fever, cough, snake bite, goiter, diabetes, miscarriages, dysentery, gonorrhea (Gerbremariam *et al.*, 2001); Omojasola and Awe, 2004).

The cashew plant, *Anacardium occidentale* (Fig. 1) is originated from ACAJU a Portuguese word. It is a member of genus *Anacardium* of the family ANACARDIACEAE. It contains about 400 species made up of shrubs and trees which are now grown in many parts of the world. Over 50% of annual cashew production of 400,000 tonnes come from South Asia (especially India) and East Africa (Tanzania) (Opeke, 2005). Studies have been carried out on the antimicrobial potentials of crude extracts of different leaves, barks, bulbs, stems and roots (Atata and Sani (2003); Olafimihan (2004); Hassan *et al.*, 2004); Prescott *et al.*, (2002); Adeleye and Ogunwale (2004), but no detailed information on the phytochemical and antimicrobial potentials of *Anacardium occidentale* to the best of our knowledge. The paper therefore reports on the phytochemical and antimicrobial potentials of leaves and stem bark extracts of *Anacardium occidentale*.

Fig 1: *Anacadium occidentale* used for the analysis

MATERIALS AND METHODS

Collection and preparation of plant samples

The fresh leaves and stem barks of *Anacadium occidentale* used for this analysis were collected behind the Reed Hall, West Campus of Federal College of Agriculture, Akure, Ondo State in May 2008. The samples were authenticated at the Department of Horticulture of the College. The samples were washed in clean water, rinsed in

distilled water, air dried, finely ground, sieved and stored in air tight, containers at ambient temperature prior to analyses. 25 g of each of the powdered samples was soaked in 400 ml of solvents (water and ethanol) for 3 h after which they were filtered using filter paper, and the filtrates stored and labeled separately prior to analyses.

Source of microorganisms
Standard strains of *Staphylococcus aureus*, NCTC 6571, *Bacillus subtilis* ATCC 11779, *Corynebacterium pyogenes*, ATCC 10242, *Escherica coli* NCTC 10418 and *Salmonella typhi* NCTC 52311 were obtained from Department of Microbiology, Lagos State University Teaching Hospital (LUTH), Lagos. All inocula were standardized using the methods described by Bauer *et al.*, (1966)

Phytochemical Analysis
The extracts were evaluated for tannins, saponins carbohydrates, glycosides, resins, phlobatannins, alkaloids, sterols and flavonoids (Cannell, 1998).

(a) Test for Carbohydrates: Molisch Test.
To 2ml of each extract in test tubes, a few drops of Molisch reagent was added, followed by 1ml of conc H_2SO_4 slowly down the size of the tube so that the acid forms an immiscible reddish brown layer with the extract solution (light brown layer).

(b) Test for Alkaloids
To 3 ml of each extract mixture in a test tube, 1ml of 1% HCl was added and to 2 ml of extract mixture 2 drops of Mayer's, Wagner's and Dragendroff reagents were added separately. A creamy white (Mayer), a reddish brown (Wagner) and an orange brown (Dragendroff) precipitates in the ethanol and water extracts was taken as evidence of the presence of alkaloids.

(c) Test for Tannins
Two (2) drops of 5% $FeCl_3$ was added to 1ml of each extract in separate test tubes and the appearance of a dirty-green precipitate was considered as indication for the presence of tannins.

(d) Test for Glycosides
Ten milliliters (10 ml) of 50% H_2SO_4 was added to 1ml of each extract in separate test-tubes. The mixture was heated in boiling water for 15 min and 10 ml of Fehling's solution (5 ml each of solutions A and B) was added to the mixture and boiled. The presence of a brick-red precipitate indicated the presence of glycosides.

(e) Fronting Test for saponins
Two milliliters (2 ml) of each extract in a test tube was vigorously shaken for 2 min and the presence of frothing indicated the presence of saponins.

(f) Test for Phlobatannins
This was carried out by boiling 0.5 ml of the extract mixture with 5 ml of water and 1% HCl in a test tube for 2 min. The colour change was regarded as positive for phlobatannins.

(g) Test for Sterols: Lieberman-Burchard Reaction.
The Lieberman-Burchard reaction was used for this purpose. Briefly, 1 ml of conc.H_2SO_4 was added to 1 ml of each extract in a test tube and observed for the appearance of a red colour indicating the presence of sterols.

(h) Shinoda test Flavonoids
In this method, 1g of magnesium power and 1-5 drops of conc. HCl were added to 3 ml of each extract in separate test tubes. The appearance of red colour in the ethanol and water extracts was and indication of the presence of flavanones.

(i) Test for Resin
To 5 ml of each extract in separate test tubes, 5 ml of copper acetate solution was added and the resulting solution shaken vigorously and allowed to separate. The separation of of a green coloured solution was considered positive for resins.

Antibacterial Screening Test Using the Agar- Diffusion Method.
The extracts were screened for antimicrobial activity using the disc diffusion method developed by Bauer *et al*., (1966).

Pure cultures of *Streptococcus faecalis, Staphylococcus albus, Staphylococcus aureus, Streptococcus pneumoniae* and *Klebsiella pnuemoniae* were inoculated in the petri-dishes by streaking in a zig-zag manner until the entire surface was covered. The plates were then incubated for 24 h at room temperature. The crude extract was introduced into the plates at concentrations of 4 mg per / dish and incubated as room temperature for 24 h. The plates were examined and zones of inhibition recorded at the end of this period.

RESULTS AND DISCUSSION
Table 1 showed the results of the preliminary photochemical screening of the leaves and stem bark extracts of *Anacardium occidentale.*. Results revealed either presence or absence of bioactive substances. Results obtained were comparable to results obtained by Faruq *et al*., (2004); Ogukwe *et al*, (2004); Akpan and Udoh (2004) for extract of *Senna italica, Sansevieria trifasciata and Raphia hookeri* leaves respectively.

The higher activity of the ethanol extracts may not be unconnected with the extraction solvent. Ethanol has been shown to be a stronger extractant than water. The presence of the ethanol in addition to achieving better extraction may also enhance the efficacy of the active ingredients (Omojasola and Awe, 2004).

Table 1: Phytochemcial Analysis of leaves and stem bark extracts of *Anacardium occidentale*

	Water		Ethanol	
Parameters	Leaves	Stem	Leaves	Stems
Carbohydrate	+++	+++	-	++
Tannins	+++	++	-	++
Glycosides	++	++	-	++
Saponins	++	-	++	++
Resins	++	++	+++	-
Phlobitanins	-	-	-	-
Flavonoides	++	++	++	++
Alkaloids	++	++	++	++
Sterols	+	-	-	-

+++ - Strongly positive, ++ - positive, + Trace, – Not detected.

Table 2: Antimicrobial activity (mm) of leaves and stem extracts of *Anacardium occidentale* against the test bacteria

Microorganisms	Zone of inhibition (mm)			
	Leaves		Stem	
	Water	Ethanol	Water	Ethanol
Staphylococcus albus	8	9	9	10
Staphylococcus aureus	8	10	9	12
Streptococcus faecalis	7	11	8	10
Streptococcus pnuemoniae	9	11	9	13
Klebsella pnuemoniae	10	12	12	13

The results of antimicrobial screening of the extracts are given in Table 2. Results showed that the extracts were active against all the organisms tested. The average zone for inhibition ranged between 8 and 12 for leaves and 9 to 13 for ethanol meaning variability in the degree of their susceptibility. Although it was confirmed that conventional antibiotics are more active than plant extracts (Emeruwa, 1982) but if extract component of Anacadium are purified, it may enhance the treatment of some ailments.

CONCLUSION

The study has shown that the leaves and stem bark extracts of *Anacadium occidentale* possessed antimicrobial potentials against some bacteria. The inhibitory activities give promise to their potential application in the treatment of microbial - induced ailments.

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