Advances in Medical and Dental Sciences, 3(1): 1-3, 2009
ISSN 1995-0764

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ORIGINAL ARTICLE

Evaluation of Antimicrobial Activity of *Anacardium occidentale* (Linn.)

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Ayepola, O.O and R.O. Ishola,: Evaluation of Antimicrobial Activity of *Anacardium occidentale* (Linn.), *Adv. in Med. Dent. Sci., 3(1): 1-3, 2009*

ABSTRACT

The extracts of the leaves and stem bark of *anacardium occidentale* were screened for phytochemically for the presence of secondary metabolites and for *in vitro* antibacterial activity. The phytochemical screening revealed the presence of alkaloids and tannins. The methanol and aqueous leaf extracts and the methanol extract of the stem bark were tested against *Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Candida albicans* and *Escherichia coli* using the agar dilution method. The leaf methanol extracts presented a higher activity than the aqueous extracts. The antibacterial activity was greatest against *K. pneumoniae, Bacillus anthracis* and *Candida albicans.* Also the leaf extracts showed greater activity than the stem bark extracts. Of the six organisms tested, the stem bark extracts had activity against three. The mean diameter of the zones of inhibition exhibited by the extracts was between 13mm and 22mm. *Bacillus subtilis* showed the highest zone of inhibition (22mm) to the leaf methanol extract. The extracts compared favourably with ampicillin used as a standard control.

Key words: Phytochemical screening, antibacterial activity, *Anacardium occidentale*, agar dilution method.

Introduction

The antimicrobial properties of various medicinal plants have been exploited over the years in the treatment of diseases all over the world [6]. Plant species have been used in the formulation of various proprietary medicines and it is therefore important to determine the active chemical components of such plants in order to develop more effective drugs [9]. *Anacardium occidentale* belongs to the family Anacardiaceae. Various parts of the plant have been used medicinally in many countries of the world. An infusion of the stem bark and leaves of the plant is used as a remedy for tooth ache, and sore gums while the astringent bark is given for severe diarrhea and thrush [2]. It is used in many countries as treatment for diabetes mellitus [10]. In the Southeastern part of Nigeria, the leaf extracts is used to bath malaria patients [5].

In this study, the leaves and the stem bark of *Anacardium occidentale* is being screened for their antimicrobial activities against selected bacteria and a fungal yeast.

Materials and methods

Collection of Plant Materials

The plant materials were collected from Ajegunle area of Lagos State, Nigeria in July 2008. The plant sample was identified and authenticated by a botanist in the Department of Biological Sciences of Covenant University.

Plant Extraction:

The fresh leaves and stem bark of *A. occidentale* were oven-dried and finely ground. A weighed

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quantity of the powder was extracted with methanol by soxhlet extraction. A weighed quantity of the powdered leaves was also extracted with sterile distilled water. The extracts were allowed to evaporate to dryness and stored in airtight bottles until ready for use.

Phytochemical Screening

Qualitative phytochemical analysis of the stem bark of *Anacardium occidentale* was carried out using the methods described by Harbone, [4] and Trease and Evans, [11] to test for the presence of alkaloids, saponins, and tannins.

Sources and Maintenance of Microorganisms

Gram positive organisms (*Staphylococcus aureus, Bacillus subtilis*), Gram negative organisms (*Klebsiella pneumoniae, Escherichia coli, Salmonella typhi)* and a fungal yeast *Candida albicans* were obtained from the research laboratory of the Microbiology Department, Covenant University, Ota, Nigeria. The bacterial strains preserved on nutrient agar at 4^0C were revived in Mueller Hinton broth and incubated overnight at 37^0C. Bacterial suspensions were made to give approximately 10^6cfu/ml.

Determination of Antibacterial Activity

The antibacterial activity of the extracts was determined using the agar well diffusion method of Adeniyi, *et al.,* [1] with modification. Mueller Hinton agar plates were seeded with 0.2mL of an overnight culture of each bacterial isolate. The seeded plates were allowed to set and a standard cork borer of 7mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 100µL of each extract at a concentration of 16mg/ml and 32mg/ml. Ampicillin at 1mg/ml was used as positive control. The plates were incubated at 37^0C for 24h after which the diameter of zones of inhibition were measured.

Results and discussion

The results of the phytochemical screening of the plant presented in Table 1 indicated the presence of alkaloids and tannins while it tested negative for saponins.

The results of the antibacterial activity of the extracts of *A. occidentale* are presented in Tables 2and 3. The antibacterial activity of the extracts increased with increase in extract concentration. The diameter of the zones of inhibition ranged between 13mm and 22mm. The methanol extract of the leaves at a concentration of 32mg/ml had higher activity on all the test organisms than the aqueous leaf extract and the methanol stem bark extract. The methanol extract of the stem bark was active on only three of the six test bacteria. *Bacillus subtilis* showed the highest sensitivity to all the extracts with the highest zone of inhibition (22mm). On the other hand *Candida albicans* was inhibited by the methanol extracts of the leaves alone.

Discussion

The present study showed that *A. occidentale* extracts are effective inhibitors of microbial growth. All extracts showed varying levels of activity against both Gram positive and Gram negative bacteria as well as the fungal yeast. The methanol extract of the leaves of *A. occidentale* was highly active against *Bacillus subtilis* and showed a zone of inhibition higher than the control antibiotic (Ampicillin). The extracts also showed considerable activity against the two enteric organisms, *Klebsiella pneumoniae* and *E. coli.* The activity against *E. coli* therefore justifies the traditional use of *A. occidentale* in the treatment of diarrhea and is similar to the work of Kudi *et al.,* [2]. The methanol extract of the leaves of *A. occidentale* was more active against all test bacteria than the aqueous extract. This may be due to the ability of methanol to extract a wider range of antibacterial principles than the aqueous solvent [7]. The leaf methanol extract was also observed to be more active than the stem bark methanol extract. The phytochemical analysis of the stem bark of *A. occidentale* revealed the presence of alkaloids and tannins. Goncalves *et al.,* [3] had earlier reported the presence of tannins, alkaloids, saponins, terpenes and flavonoids in *A. occidentale* leaves. Thus the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components.

This study has demonstrated the potential uses of *A. occidentale* as an antibacterial agent against infections caused by *Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli* and *Candida albicans.* This has also supported the folklore usage of *A. occidentalis* in the treatment of various ailments such as diarrhea, dysentery, pile, toothache, sore gums and oral thrush [2].

Table 1: Phytochemical components of the stem extracts of *A. occidentale*

Phytochemical components	*A. occidentale*
Alkaloids	+
Tannins	+
Saponins	-

Note: + = Present- = Absent

Conclusion

There is need for the development of new antimicrobials to combat the problem of the spread of resistance more importantly from natural sources as this delays the emergence of resistance. It can be concluded that the present results provides a basis for the development of new pharmaceuticals from *A. occidentale.*

Table 2: Antimicrobial Activities of *Anacardium occidentale* Leaves Against Selected Organisms

Organisms	LME 16mg mL^{-1}	LME 32mg mL^{-1}	LAE 16mg mL^{-1}	LAE 32mg mL^{1}	AMP 1mg mL^{-1}
Klebsiella spp	-	20	14	16	25
Salmonella typhi	15	17	-	14	35
Candida albicans	15	20	-	-	30
Escherichia coli	-	16	12	13	35
Staphylococcus aureus	-	16	-	-	20
Bacillus subtilis	17	22	14	16	15

LME= Leaf methanol extract; LAE= Leaf aqueous extract; AMP= Ampicillin (Positive Control)
Resistance= no zone of inhibition

Table 3: Antimicrobial Activities of the Stem Bark of *Anacardium Occidentale* Against Selected Organisms

Organisms	Extract Concentrations		AMP
	16mg mL^{-1}	32mg mL^{1}	1mg mL^{-1}
Klebsiella spp	14	15	25
Salmonella typ	-	-	35
Candida albicans	-	-	30
Escherichia coli	-	-	35
Staphylococcus aureus	-	13	20
Bacillus subtilis	-	17	15

AMP= Ampicillin (Positive Control)
Resistance= no zone of inhibition

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