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# Characterization of selected clinical isolates and antimicrobial properties of ethanol root extract of *Anacardium occidentale* L.

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**ABSTRACT:** Ethanolic root extract of *Anacardium occidentale* L. was evaluated for antimicrobial activities against selected clinical isolates. Bacteria and fungi pathogens obtained from the laboratory unit of a functional health facility for this study included *Escherichia coli*, *Kblesiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus* sp., *Candida albicans* and *Aspergillus niger*. The identities of the bacteria spp. were re-established following standard physical and biochemical routines. Susceptibility screenings were performed to determine the zone of inhibition as well as minimum inhibitory concentration of the plant extract using well diffusion and broth dilution methods respectively. Significant, $P< 0.05$, dose-dependent antibacterial activities were recorded against all the bacterial isolates. However, no detectable activity was observed against either of the fungi species. Although the highest inhibition diameter, 12.2±0.2 mm, was obtained against *Staphylococcus aureus*, it was observed that *E. coli* and *Streptococcus* sp. were the most susceptible organisms based on lower minimum inhibitory and bactericidal concentrations of 62.50 mg/ml respectively. The extract demonstrated broad-spectrum, inhibitory and bactericidal potentials.

**Keywords**: *Anacardium occidentale*; Ethanol; Root; Extract; Bacteria; Fungi; Pathogens

## Introduction

Infectious diseases are a leading cause of death world-wide and growing antibiotic resistance has been a global concern (Bandow *et al.*, 2003; Westh *et al.*, 2004; Ventola, 2015). However, many infectious diseases have been known to be treated with herbal remedies (Anyawu and Okoye, 2017). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Rojas *et al.*, 2003; Jeyachandran and Mahesh, 2007).
*Anacardium occidentale* L. commonly called cashew, is a tropical nut crop that is native to Brazil (da Silva *et al*., 2016). The tree has great economic and medicinal values (Maia *et al.*, 2000). Their high biological activities have been attributed to a high content of tannins (Kudi *et al*., 1999; Gaffar *et al*., 2008). Different parts and bi-products of cashew plant, such as the apple, stem bark, leaf, gum and nut have been reported to be effective in inhibiting the

growth of medically important microorganisms (Marques *et al*., 1992; Akinpelu, 2001; Abulude *et al*., 2009; Goncalves and Gobbo, 2012). However, there is paucity of report on the antibacterial potential of the root of this plant species. This study therefore seeks to evaluate the antimicrobial activities of ethanol extract of *A. occidentale* root against selected clinical isolates.

## Materials and methods

*Collection and preparation of plant material*: Fresh root samples of *A. occidentale* were collected within the University of Benin, Benin City. The roots were washed under running tap water to remove debris, and then they were cut into tiny bits and air-dried at room temperature. The dried roots were pulverized before extraction. Using cold maceration method, 1 kg of the powdered sample was soaked in 5 litres of 99.5% absolute ethanol in an airtight container and kept at room temperature for 4 days and filtered using a clean white cloth to obtain the filtrate. The filtrate obtained was concentrated and evaporated to dryness over a water bath to obtain the residue of ethanol extract of *A. occidentale* root. This was reconstituted into varying concentrations by serial dilutions of a stock preparation for the purpose of further study.
*Preparation of extract diluents*: Three dilutions 20, 40 and 80 mg/ml of the extract were prepared by dissolving in equivalent volumes of distilled water and 20% dimethyl sulfoxide (DMSO).
*Collection of test isolates*: The bacteria and fungi strains used in this study were clinical isolates obtained from the Laboratory Unit of St. Philomena Catholic Hospital, Benin City, Nigeria. They included six bacteria and two fungi species primarily identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Streptococcus* sp., *Candida albicans* and *Aspergillus niger*.
*Characterization of the bacteria isolates*: Using the methods of Chakraborty and Nishith (2008), the isolates were sub-cultured to obtain pure colonies. They were then classified based on morphological characteristics, microscopic features and Gram reaction. Biochemical tests such as oxidase, catalase and coagulase were also performed.
*Gram staining*: Smears of the isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet, then washed out with distilled water. The slides were flooded with dilute Gram's iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with saffranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective.
*Catalase test*: About 3 ml of hydrogen peroxide was placed in a sterile tube then a colony of the test organism was picked with a sterile rod and emulsified in the tube. Evolution of oxygen or effervescence production of gas bubbles indicates a positive result.
*Oxidase test*: A piece of filter paper was placed in a clean Petri dish, then three drops of freshly prepared oxidase reagent was placed on the filter paper, a sterile glass rod was used to pick a colony of the test organism and then smeared on the filter paper with the oxidase reagent and observed for colour change. A dark-purple colouration after a few seconds confirms the organism is oxidase positive.
*Coagulase test*: This was performed by placing a drop of distilled water on one end of two glass slides followed by a colony of the test organism on the slide to make a thick suspension. Then a loopful of plasma was placed on the suspension and mixed gently. Clumping of the organism within 10 seconds will indicate a positive test.
*Antimicrobial susceptibility test*: Antimicrobial activity of the various dilutions of the extract was done by agar well diffusion method (Oliveira *et al*., 2006). Prepared nutrient agar and potato dextrose agar (PDA) plates were inoculated with the selected strains of bacteria and fungi respectively. Four wells were made in the agar surface with sterile 5 mm borer. Three of the wells were filled with varying concentrations of the plant extract, while one of the well was left without plant extract to serve as negative control. The extracts were allowed to diffuse into the medium and the plates were incubated at 37 $^0$C for 24 hours. Antibacterial sensitivity discs (Amoxacilin 30 µg and Ciprofloxacin 10 µg) were used as standard control. The zone of inhibitions of both the extract and the sensitivity disks were measured in millimeters after 24 hours. Three replicates were maintained for each treatment. The PDA plates were observed for 48 hours.
*Minimum inhibitory concentration*: Minimum inhibitory concentration (MIC) of the extract was evaluated by broth dilution method (Truiti *et al*., 2006). Experiments were carried out in 5 ml nutrient broth for bacteria and 5 ml potato dextrose broth for fungi. Serial dilutions of the test extracts were prepared in distilled water and 20% DMSO to yield solutions of 250, 125 and 62.5 mg/ml. Aliquots (20 µl) of each dilution was put into the broth and then the inoculum

was added in an aseptic condition. The tubes were incubated under the same condition as the screening stage. Tubes containing no extract but inoculated with test strains were considered as positive control, while negative control tubes consisted of serial dilution of extracts only. The lowest concentration of each extract showing no visible growth or least turbidity was taken as the MIC.

*Minimum bactericidal concentration*: After determining the MIC, the materials from each tube used in the minimum inhibitory concentration assay that showed no growth after incubation, were streaked onto a solid nutrient agar plate and then incubated at 37 $^0$C for 24 hours. The lowest concentration of the extract that showed no growth after 24 hours was taken as the minimum bactericidal concentration (MBC).

*Statistical analysis*: Quantitative data were analysed using Microsoft Excel 2007 software. Means were separated with SPSS 16.0 by Duncan multiple comparison test at 5 % level of significance.

## Results and discussion

Table 1 reveals that the isolates obtained from the medical facility for this study consisted of rod-shaped, Gram positive and Gram negative bacterial colonies. The physical attributes were consistent with the standard features described for these organisms (Chakraborty and Nishith, 2008). Further biochemical tests were confirmatory for *Staphylococcus auerus, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. However, the species of *Streptococcus* could not be fully determined (Table 2). The most common bacteria causing infections, especially nosocomial infections, are *S. aureus, E. coli* and *P. aeruginosa* (Lowy, 1998; Galoise-Guibal *et al*., 2006; Silva *et al.,* 2007; Cruvinel *et al.,* 2011) and drug resistant strains pose serious health challenges (Edelstein *et al*., 2013; Hussain *et al*., 2014).

**Table 1**: Morphological characteristics and Gram reactions of clinical samples of bacterial isolates

| Organism | Shape | Margin | Elevation | Colour | Gram reaction | Cell type | Cell arrangement |
|---|---|---|---|---|---|---|---|
| *Staphylococcus auerus* | Round | Smooth | Raised | Golden yellow | + | Cocci | Cluster |
| *Streptococcus* sp. | Round | Smooth | Raised | Dark | + | Cocci | Chain |
| *Escherichia coli* | Round | Smooth | Raised | Pink | - | Rod | Chain |
| *Pseudomonas aeruginosa* | Round | Rough | Flat | Green | - | Rod | Singly |
| *Klebsiella pneumoniae* | Round | Smooth | Raised | White | - | Rod | Chain |
| *Proteus vulgaris* | Circular | Even | Raised | Green | - | Rod | Chains |

Key: - = negative, + = positive

**Table 2:** Biochemical characterization of the bacterial isolates

| Organism | Catalase | Coagulase | Oxidase |
|---|---|---|---|
| *Staphylococcus auerus* | + | + | - |
| *Streptococcus* sp. | - | - | - |
| *Escherichia coli* | + | - | - |
| *Pseudomonas aeruginosa* | - | - | + |
| *Klebsiella pneumoniae.* | + | - | - |
| *Proteus vulgaris* | + | - | - |

- = negative, += positive

The ethanol extract of the root of *A. occidentale* demonstrated significant ($P< 0.05$) dose-dependent antibacterial activity against both Gram negative and Gram positive clinical bacterial isolates in the present study (Table 3). The

highest inhibitory diameter (12.2±0.2 mm) was recorded against *Staphylococcus aureus* at an extract concentration of 80 mg/ml. However, the extract had no activity against the fungi isolates.

**Table 3:** Zone of inhibition diameter (mm) of various concentrations of ethanol extract of *A. occidentale* root

| | Control | Extract (mg/ml) | | | Standard antibiotics (µg) | |
|---|---|---|---|---|---|---|
| **Isolates** | **0** | **20** | **40** | **80** | **AM 30** | **CPX 10** |
| *Escherichia coli* | 0.0±0.0 | 6.5±0.3$^{a}$ | 7.7±0.2$^{b}$ | 8.5±0.3$^{b}$ | 10.0±0.0$^{c}$ | – |
| *Klebsiella pneumoniae* | 0.0±0.0 | 7.2±0.9$^{a}$ | 7.3±0.9$^{a}$ | 8.0±1.3$^{a}$ | – | 19.0±0.0$^{b}$ |
| *Staphylococcus aureus* | 0.0±0.0 | 10.2±0.9$^{a}$ | 10.5±0.8$^{a}$ | 12.2±0.2$^{a}$ | – | 16.5±0.5$^{b}$ |
| *Pseudomonas aeruginosa* | 0.0±0.0 | 6.8±0.2$^{a}$ | 9.7±0.7$^{b}$ | 10.3±0.2$^{c}$ | 11.5±0.5$^{c}$ | 18.5±0.5$^{d}$ |
| *Proteus vulgaris* | 0.0±0.0 | 7.7±0.4$^{a}$ | 7.8±0.4$^{a}$ | 11.0±1.0$^{b}$ | – | 25.0±0.0$^{c}$ |
| *Streptococcus* sp. | 0.0±0.0 | 8.3±0.6$^{a}$ | 8.7±0.7$^{a}$ | 9.3±0.6$^{a}$ | 14.0±1.0$^{b}$ | 15.0±0.0$^{b}$ |
| *Candida albicans* | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | NA | NA |
| *Aspergillus niger* | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | NA | NA |

Values are mean ± SEM; n = 3; Means with similar superscripts along a row are not significantly different (P >0.05). NA= Not applicable; AM= Amoxicillin; CPX= Ciprofloxacin.

Figure 1 shows that the MIC ranged from 62.5 mg/ml to 125 mg/ml, while MBC was between 62.5 mg/ml to 250 mg/ml. A minimum inhibitory concentration of 62.5 mg/ml was recorded against *E. coli*, *K. pneumoniae* and *Streptococcus* sp., but a higher concentration (125 mg/ml) was obtained for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*. Again, as in the previous susceptibility screening, MIC could not be established for any of the fungi isolates even at the maximum test concentration used in this study. It was also observed that *E. coli* and *Streptococcus* sp. had the lowest (MBC) at 62.5 mg/ml, while the highest MBC (250 mg/ml) was obtained against *Pseudomonas aeruginosa*.

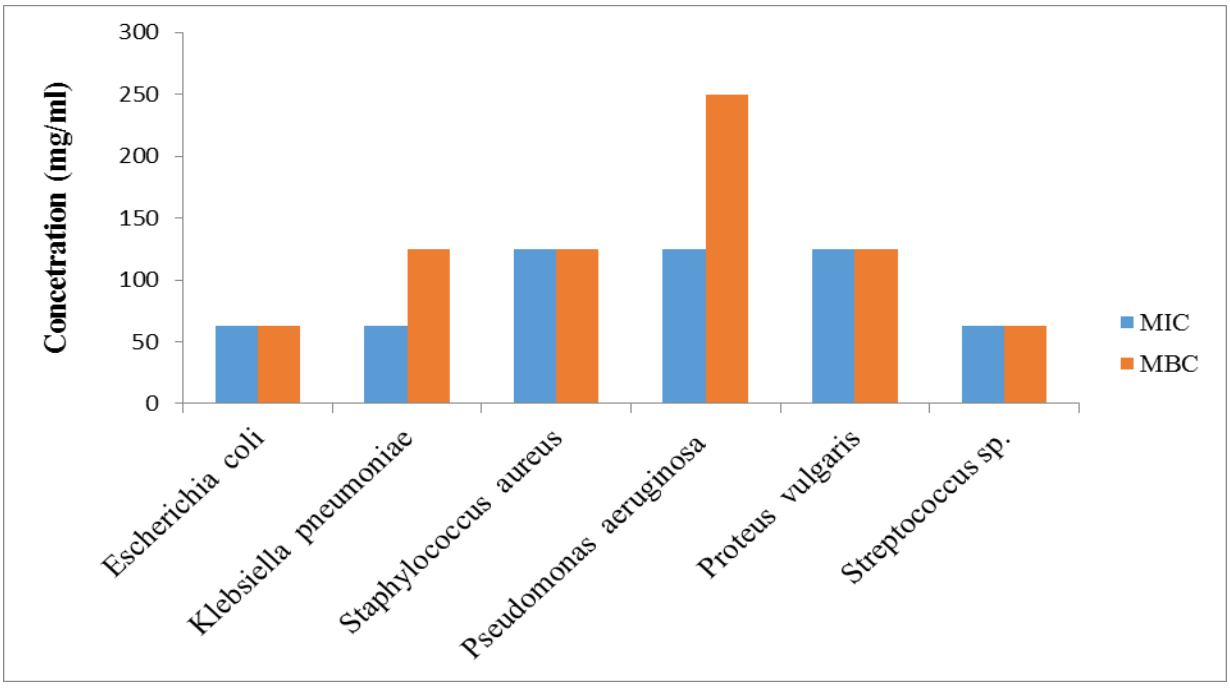


**Figure 1:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol extract of *A. occidentale* root on clinical isolates

In the present study, 20 mg/ml of the extract produced a zone of inhibition diameter of 10.2±0.9 mm against *S. aureus* compared to an earlier report of 9 mm due to 25 mg/ml ethyl acetate leaf extract of *A. occidentale* on a similar organism (Mustapha *et al*., 2015). This may be adduced to differences in the phytoconstituents of both extracts, since bioactivity potential of a plant extract is a function of the active principle present in it. *A. occidentale* has been reported to contain tannins, flavonoids, alkaloids, saponins, steroids, triterpenes and resins, which are active ingredients against microbes (Aderiye, 2001; Sujatha *et al*., 2011). Sometimes, there may be variations in the nature or levels of phytochemicals distributed in different parts of a particular plant species. For example, hydrogen cyanide, was reported in the leaf and stem bark of *A. occidentale*, but lacking in the root and fruit of the same species (Belonwu *et al.*, 2014). The authors however confirmed the presence of alkaloids, saponins, flavonoids, tannins, phenols and anthocyanin in the root extract. Tannins possess antibacterial, antifungal, antiviral, anticarcinogenic and antiparasitic activities (Banso and Adeyemo, 2007; Jaishee and Chakraborty, 2015). Similarly, flavonoids, alkaloids and saponins are sources of antimicrobial activities (Hima *et al*., 2012; Britto *et al*., 2013). Onuh *et al.* (2017) also reported appreciable antimicrobial activities of ethanol extracts of leaf, bark and fruit of *A. occidentale* against some pathogens. The present study however finds reason to suspect that fungi species may generally not be susceptible to *A. occidentale* extracts, hence the obvious lack of information in this regard in most of the literatures examined.

## Conclusion

The ethanol extract from the root of *A. occidentale* plant had significant antibacterial activity on the clinical strains studied, but inactive against the fungi. Definite bactericidal concentrations were also established against the isolates.

## Conflict of interest

The authors wish to state that they have no conflict of interest whatsoever that would influence the results of this study.

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