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Antimicrobial Activity of the Leaf and Root Extracts of *Kigelia africana* and *Albizia chevalieri* against *Staphylococcus aureus*

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ABSTRACT

The investigation was carried out to determine the antimicrobial activity of the Leaf and Root extracts of *Kigelia africana* (nonon giwa) and *Albizia chevalieri* (katsari) against *Staphylococcus aureus*. Antimicrobial activity was evaluated by disc diffusion method and MIC and MBC were calculated by double fold dilution method. The antimicrobial activity result shows 20mm zone of inhibition *Kigelia africana* leaf at 100% concentration and 16mm zone of inhibition for *albezia chevalieri* leaf at same concentration while for the roots 14mm and 11mm zone of inhibition at 100% concentration. The maximum inhibition zone is found at 20mm and minimum inhibition zone at 11mm. Also the antimicrobial result shows the MIC of 6.25mg/ml against *Staphylococcus aureus* and MBC of 12.5mg/ml and 10.5mg/ml for both extracts. The study reveals that the extracts possess antimicrobial activity in a dose dependent manner. This antimicrobial activity may be due to the presence of some active compounds, further studies are highly needed for the drug development.

Keywords: Antimicrobial, Leaf, Root extracts, *Kigelia Africana*, *Albizia chevalieri*, *Staphylococcus aureus*.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.*, 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.*, 2000). Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms.

With increase in antibiotic resistance, cost and inaccessibility (especially in rural areas) to some orthodox modern antibiotics, traditional weeds are fast gaining popularity even to urban and civilized dwellers. In addition, considering the wide medicinal application of this plant, this work was set out in order to investigate the antimicrobial activity of leaf extracts of *S. obtusifolia* against some pathogenic bacteria and fungi and to ascertain the chemical constituents that may be present. The use of modern separation techniques in the extraction and isolation of useful bioactive molecules from plant sources had made an otherwise tedious and time consuming process a lot easier (Constantine, 2007; Ankit *et al.*, 2012).

Kigelia africana (Bignoniaceae) is a distinctive tree native to the African continent. The indehiscent, sausage-shaped fruits, and less commonly the pale bark of roots and stems, are used in traditional medicines throughout its pan-continental distribution (Retief and Herman, 1997). The many uses of *K. africana* in therapy of physical and magico-religious or spiritual complaints indicate that this tree is a valuable and popular source of traditional medicine.

Previous investigations of *K. africana* have established antimicrobial activity of the stem bark against *Candida albicans*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* (Akunyili *et al.*, 1991; Kwo and Craker 1996). Binutu *et al.* (1996) reported that extracts of *K. africana* roots and fruits showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and antifungal effects against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Pullularia pullularis*. Anti-cancer potential has been indicated by cytotoxicity of root and bark material in the brine shrimp bioassay against *Artemia salina* (Khan, 1998; Ekeanyawu, 2011). Ankit, *et al* (2012) reported the significant inhibitory activity of stem bark extracts against four melanoma cell lines and a renal carcinoma cell line, and slight activity by fruit extracts. Azuine *et al* (1997) reported inhibitory effects of fruit extracts on induced tumors and inflammation in mice.

The plant *Albizia chevalieri* is a tree that grows up to 12m high or a shrub under harsher conditions of the dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella shaped canopy, bark pale-greyish, twigs pubescent with white lenticels, leaves with 8-12 pairs of pinnate and 20-40 pairs of leaflets each. The bark was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal. It is used in Borno-North eastern Nigeria as purgative, taenicide and also remedy for coughs. A decoction of leaves is used in Northern Nigeria as remedy for dysentery (Burkill, 1995; Ahmed, *et al.*, 2012).

This work was designed to investigate the antimicrobial properties of methanol leave and root extract of *Albizia chevalieri* and *Kigelia africana* with a view to assessing the inhibitory potentials of the plants against the test organism *Staphylococcus aureus*.

MATERIALS AND METHODS

Plant Sample Collection

The leaf and root of *A. chevalieri* (*katsari*) and *K. africana* (*nonon giwa*) was collected at Bauchi Local Government Area, Bauchi State of Nigeria in August, 2014; it was confirmed as *A. Chevalieri* (*katsari*) and *K. africana* in the department of Biological Sciences by a final year botany student. The sample was dried in the laboratory at room temperature and pulverized to powder with a pestle and mortar. The method of treatment followed standard procedures as recommended by Yusuf *et al* (2007); Igidi and Edene, 2014.

Collection of test organism

Clinical isolates was collected from ATBUTH in Bauchi town. The organism was subculture into nutrient agar prepared according to the manufacturer's instructions and sterilized at 121°C for 15minute and incubated at 37°C for 24hrs.

Media for isolation

The media used for the isolation of the organism was nutrient agar, prepared according to the manufacturer's instructions and sterilized at 121°C for 15minutes.

Media for maintenance

The media used for maintenance of the test organism was Mueller Hinton agar, prepared according to manufacturer's instructions and sterilized at 121°C for 15minutes. Suspension cultures of test bacteria were initiated from stock cultures maintained at 4°C on Mueller-Hinton (MH) agar.

Preparation of antibiotic disc

Whatman no.24, 6 mm diameter filter paper was used in the preparation of the paper disc; the filter paper was punctured into a circular disc of 6mm in diameter and were put into a screw capped bottle and sterilized at 160°C for one hour.

Preparation of Extract Using Maceration Method

The leaves were separated and roots washed in clean water, and dried at room temperature (Eloff, 1998). 250 ml of methanol was redistilled using distillation setup. 100 g of the powder was weighed on a balance in a beaker and soaked in 95% 250 ml of methanol in a 500 ml conical flask for 4 days (Prashant *et al.*, 2011). The filtrate was

concentrated and dried in an oven at 45°C and dispensed into a sterile screw capped specimen bottle. This was kept in a refrigerator until required for use.

Characterization and identification of the test organism

Characterization was made by initial examination of the colonies on the plate, physiological examination such as staining reactions and biochemical test such as coagulase test and catalase test, were also carried out to aid identification of the organism.

Evaluation of antimicrobial Activity of plant extracts

The bioassay involves a simple disc diffusion test, for crude extract from each plant, dilution were made of alcoholic extract using methanol respectively. The dilution was made in the range of 10% to 100% and this was done by adding the crude extract to the solvent in the ratio of 10:0, 8:2, 6:4, and 4:6, for 100%, 80%, 60%, and 40% respectively. The various dilutions were put in sterile specimen bottles with screwed caps.

The antimicrobial activity of *S. aureus* was evaluated using the Agar-disc diffusion method which was performed in accordance with the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1990). An 18/24 hour old culture of selected bacteria was mixed with sterile physiological saline (0.85%) and the turbidity was adjusted to 0.5 MacFarland (~106 cfu/mL). Petri dishes containing 20 ml of Mueller-Hinton agar was inoculated using standardized bacterial suspensions. Filter paper discs (Whatman no.24, 6 mm diameter) impregnated with the extracts at various concentrations by soaking discs for 10 Seconds in extract solution prepared, three disc of each were placed on the inoculated petri dishes and incubated for 24 hours after which inhibition zones were recorded in millimeters (Hemendra and Sushil, 2010). A positive control was set up using erythromycin and streptomycin and also a negative control in which the plates were uniformly sealed with the test organism and filter paper disc soaked in the solvent were incubated at 37°C for 24hrs. Each extract was tested in triplicate (3 disc/plates).

Determination of minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC)

The MIC was determined by serial dilution method using serially diluted plant extracts. MIC of the extracts was determined by dilution of the plant extract of various concentrations. Equal volume of each extract and Muller-Hinton Agar were mixed in the test tubes. Specifically 0.1 ml of standardized inoculums was added to each test tube. The tubes were incubated at 37°C for 18-24 hours. Two control tubes were maintained for each test batch. This included antibiotic control (containing extract and growth media without inoculum) and organism control (tube containing the growth medium, saline and the inoculum).

The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control were regarded as MIC. However, the MBC were determined by sub-culturing the test dilution onto a fresh extract free solid medium and incubated further. The highest dilution that yielded no bacterial colony was taken as MBC.

RESULTS

The zone of inhibition of both plant extracts at 100% concentration were; table 1 for leaf of *K. africana* 20 mm, table 2 for leaf of *A. chevalieri* 16mm, table 4 for root of same plant 11mm, and table 3 for root of *K. africana* 14mm, respectively. The antimicrobial test result below shows the susceptibility test against Gram positive bacteria (*S. aureus*).

Table 5 and 6 shows the MIC and MBC value of 6.25 and 12.5 mg/mL against *Staphylococcus aureus* for leave extract and 6.25 and 10.5, 6.25 and 12.5 mg/mL against *Staphylococcus aureus* for root extract tested and listed in the table below.

The low MIC value observed for *S. aureus* is a good indication of high efficacy against this bacterium. This outcome is remarkable considering that boil, breast abscess and surgical wound infection etc. (caused by *S. aureus*) is on the rise and also becoming recalcitrant to first-line antibiotics for its treatment in developing countries, including Nigeria. High MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds.

Table 1. Antimicrobial activity measured by zone of inhibition (mm) for methanol leave extract of *K. africana*

Microorganism	100%	80%	60%	40%	Standard/control 1mg/ml 18(Erythromycin)
<i>Staphylococcus aureus</i>	20.00	13.00	10.00	9.00	

Key:-ETM= erythromycin;
R=Resistant Concentration of „a“ control = 1mg/mL

Table 2. Antimicrobial activity measured by zone of inhibition (mm) for methanol leave extract of *A. chevalieri*

Microorganism	100%	80%	60%	40%	Standard/control 1mg/ml 18(Erythromycin)
<i>Staphylococcus aureus</i>	16.00	12.00	11.00	9.00	

Key:-ETM= erythromycin;
R=Resistant Concentration of „a“ control = 1mg/mL

Table 3. Antimicrobial activity measured by zone of inhibition (mm) for methanol root extract of *K. africana*

Microorganism	100%	80%	60%	40%	Standard/control 1mg/ml 18(Erythromycin)
<i>Staphylococcus aureus</i>	14.00	10.00	8.00	6.00	

Key:-ETM= erythromycin;
R=Resistant Concentration of „a“ control = 1mg/mL

Table 4. Antimicrobial activity measured by zone of inhibition (mm) for methanol root extract of *A. chevalieri*

Microorganism	100%	80%	60%	40%	Standard/control 1mg/ml 18(Erthromycin)
<i>Staphylococcus aureus</i>	11.00	10.00	9.00	7.00	

Key:-ETM= erythromycin;
R=Resistant Concentration of „a“ control = 1mg/mL

Table 5. Determination of MIC, MBC, and value for leaf extract *Albizia chevalieri*

Microorganism	MIC (mg/mL)	MBC (mg/mL)
<i>Staphylococcus aureus</i>	6.25	12.5

Determination of MIC, MBC, value for root extract of *Albizia chevalieri*

Microorganism	MIC (mg/mL)	MBC (mg/mL)
<i>Staphylococcus aureus</i>	6.25	10.5

Table 6. Determination of MIC, MBC, value for leaf extract of *Kigeli africana*

Microorganism	MIC (mg/mL)	MBC (mg/mL)
<i>Staphylococcus aureus</i>	6.25	12.5

Determination of MIC, MBC, value for root extract of *Kigeli africana*

Microorganism	MIC (mg/mL)	MBC (mg/mL)
<i>Staphylococcus aureus</i>	6.25	12.5

The table 5 and 6 shows the MIC and MBC value of 6.25 and 12.5 mg/mL against *Staphylococcus aureus* for leave extract and 6.25 and 10.5, 6.25 and 12.5 mg/mL against *Staphylococcus aureus* for root extract tested listed in the Table above.

DISCUSSION

Albizia chevalieri and *Kigelia africana* were extensively used in the management of various infections and allergic diseases. The ethanol extract of both plants were tested against the organism namely *Staphylococcus aureus*. The zone of inhibition of both plant extracts at 100% concentration were; table 1 for leaf of *K. africana* 20 mm, table 2 for leaf of *A. chevalieri* 16mm, table 4 for root of same plant 11mm, and table 3 for root of *K. africana* 14mm, respectively. The below antimicrobial test result shows the susceptibility test against Gram positive bacteria (*S. aureus*).

The ethanol extract exhibited a considerable level of inhibition against the test organism compared to the standard drug at 100% concentration, **table 1 and table 2**, which is suggestive of the presence of some compounds or groups in the extract with similar mechanism of action to that of standard drug used in bacterial activity (Saha and Ahmed, 2009). Various works have already shown that Gram positive bacteria are more susceptible towards plant extracts as compared to the Gram negative bacteria (Hassan and Oyewale, 2004). These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is a multilayered structure. Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. At 100% concentration, **Table 3 and 4** show less antimicrobial activity as compared to the standard drug.

Comparing the efficacy of the root and leaves of *K. africana* and *A. chevalieri*

In addition microorganism show variable sensitivity to chemical substances related to different resistance levels between strains (Meda, 2005); the highest activity was exhibited by crude extracts of both plants with the leaf of *K. africana* showing a considerable zone of inhibition as compared to the leaf of *A. chevalieri* (Table 1 and 2). While the root of *A. chevalieri* having less activity compared to that of *K. africana* (Table 3 and 4). Considering the zone of inhibitions, the leaf and root extract of *K. africana* show high zone of inhibition and exhibit more activity while that of *A. chevalieri* also show antimicrobial activity with a zone of inhibition greater than 10mm.

Comparing their efficacy in terms of antimicrobial activities shown by both plant extracts, one can say that both plant extracts exhibited antimicrobial activity with root of *K. africana* showing more activity as compared to the root of *A. chevalieri* (table 3 and 4).

The pharmacology of *A. chevalieri* showed that it possesses anti-microbial, anti-histamine and mast cell stabilizing properties (Graceline, *et al.*, 2013) and it agrees with this study on the anti-microbial activity showed by the plant extract. Also the leave extract of *A. chevalieri* has been reported for effective anti-bacterial and anti- candida activity from Brazilian flora (Tempone *et al.*, 2008) because of the presence of active compounds (alkaloids, amino-acids, saponins, flavones, Quinone's, tannins, carbohydrates) and this is also in agreement with the anti-bacterial activity exhibited by the plant extract of *A. chevalieri* in this study.

Therefore this suggests the efficacy of both plants in pneumonia, bacteremia, and urinary tract infections. However, it may be suggested that plant extracts exhibiting diameter of zones of inhibition greater than 10mm is considered as active (Usman and Osuji, 2007). Thus it is believed that plant extracts is better antimicrobial agents for various pathogenic bacteria.

K. africana plant extracts has many medicinal properties due to the presence of numerous secondary metabolites e.g. iridoids, naphthaquinoid, flavonoids, coumarone, lignin's, saponins, tannins, alkaloids, phenol etc. Some researchers demonstrated its activity as mild (Sikder, 2011). Previous investigations of *K. africana* have established anti-microbial activity of the leaves and stem bark against *S. aureus*, *E. coli* and *Candida albicans* (Akuyili, *et al.*, 1991; Kwo and Craker, 1996). And this also agrees with this study as ant-microbial activity against *S. aureus* was observed.

Moreover, the methanolic extract from the bark showed significant activity against *Salmonella typhi* and *Proteus vulgaris* but poorly active against *Escherichia coli*, *Enterobacter aerogens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* (Jeyachandran, 2007).

Binutu, *et al* (1996) reported that extracts of *K. africana* roots showed anti-bacterial activity against *S. aureus*, *Bacillus subtilis*, and anti-fungi effect against *candida albicans*. This is in agreement with the reputed potency of *K. africana* root and leaf against common bacterial complaints.

MIC and MBC

The antimicrobial activity showed the MIC of 6.25mg/ml against *S. aureus* and MBC of 10.5 and 12.5mg/ml against test organism (**Table 5 and 6**).

The low MIC value observed for *S. aureus* is a good indication of high efficacy against this bacterium. This outcome is remarkable considering that boil, breast abscess and surgical wound infection etc. (caused by *S. aureus*)

is on the rise and also becoming recalcitrant to first-line antibiotics for its treatment in developing countries, including Nigeria. High MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds.

Conclusively, *Albizia chevalieri* and *Kigalia Africana* should be considered as a promising plant with various therapeutic properties and can be further explored pharmacologically against various ailments and for free-radical mediated diseases; therefore this would open-up a refreshing study about the immense utility of *Albizia* and *Kigalia* and encourages the phytochemists to drive on the rest of the species. Additionally, the failure of alternative drug discovery methods to yield useful drugs in key therapeutic areas makes medicinal plants research a viable option despite inherent limitations (Karuppannan, *et al.*, 2013).

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