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Phytochemical Analysis and Antibacterial Activity of *Khaya grandifoliola* Stem Bark

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Abstract: The powdered crude sample of *Khaya grandifoliola* was subjected to phytochemical analysis using standard experimental procedures. The phytochemical evaluation revealed the presence of alkaloids, tannins, saponins and flavonoids. The methanolic extract of *Khaya grandifoliola* stem bark was screened for antimicrobial activity against bacterial isolates MRSA, *Bacillus subtilis*, *Klebsiella pneumonia* and *Proteus mirabilis* at different concentrations. The isolates showed a minimum inhibitory concentrations (MIC) of 0.4 mg mL⁻¹ except *B. subtilis* of 0.002 g mL⁻¹.

Key words: Plant extract, minimum inhibitory concentration, secondary metabolites, microbiological assay

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and the society. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga and Gomina, 2000).

Plants as sources of remedy for many diseases date back to the early century (Karou *et al.*, 2005). In Africa, particularly West Africa, new drugs are often beyond the reach of the poor. Hence, upto 80% of the population use medicinal plants as remedy against infections and diseases (Kirby, 1996; Hostettmann and Maston, 2002).

Treatment offered by traditional healers is primary health care that has sustained the Nigerian community before and after colonization and the medicinal plants used by African traditional healers are selected not on the basis of their chemical constituent, but on their perceived ability to restore patients disease condition to normal.

The use of plants as antibacterial agents is gradually attracting attention probably due to the high cost, unavailability and resistance of the drugs.

Khaya grandifoliola is a medicinal plant endemic to Nigeria. It is a tall, woody tree belonging to the family Meliaceae and commonly called African Mahogany (Hutchinson and Dalziel, 1978). It is widely distributed

across West Africa from the Guinea coast to Cameroon and extending eastward through Congo Basin to Uganda and some parts of Sudan. It grows up to 40 m high and 5 m girth. The bark is grey in colour and yield a bitter gum when wounded.

Khaya species are valuable indigenous traditional medicine in West Africa. Its bitter bark is mostly the part that is used to make concoctions to treat some illness like fever, lumbago, cough, rheumatism, stomach ache, gastric pains and as remedy against worm infestation. The anti malarial activity of the stem bark was also reported (Agbedahunsi *et al.*, 1998; Makinde *et al.*, 2006). The stem bark was also found to possess anti ulcer property (Njifutie and Njikam, 2006), anti anaemic (Adeyemi and Gbilade, 2006), hypoglycaemic, hypoproteinaemic and hypocholesterolaemic effects (Bumah *et al.*, 2005). Some of the chemical constituents reported include limonoids (Zhang *et al.*, 2008).

The aqueous extract of *K. grandifoliola* is used in traditional certain in Nigeria as remedy against cough, mycobacterium tuberculosis and bacterial infections.

This present study was necessitated in order to justify the folkloric usage of the plant. Herein, we report the effect of the aqueous extract of the plant on methicillin resistant *Staphylococcus aureus* (MRSA) implicated in many bacterial infections which are drug resistant.

MATERIALS AND METHODS

Collection and identification of plant material: The stem bark of the tree plant *Khaya grandifoliola* was collected from a local government area in Benue State, Nigeria around April, 2007. The plant was identified by Mr. Sunny A of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, where a voucher specimen (218) was deposited.

Preparation and extraction of plant sample: The fresh stem bark of *K. grandifoliola* was chopped into pieces and sun dried for a period of two weeks, reduced to fine powder with the aid of a mechanical grinder. The milled powder sample was collected and stored in glass jars, tightly covered and kept for further studies.

Extraction of the plant material (800 g) with methanol (3 L) by maceration for 48 h and filtration of the extract was carried out at room temperature 25°C. The reddish brown extract was concentrated to dryness using a rotary evaporator at 30°C at reduced pressure. The dried extract was stored in a refrigerator at -4°C until use.

Phytochemical screening: Phytochemical tests were carried out on the powdered sample using standard procedures, to identify the constituents as described by Sofowara (2008), Trease and Evans (2002) and Harborne (1973).

Carbohydrate: 0.1 g of the powdered sample (*Khaya grandifoliola* powdered stem bark) was measured into a beaker and 20 mL of distilled water was added. The beaker was heated in a water bath for over 5 min. The mixture was filtered using a filter paper into another beaker to obtain a filtrate, which was used to test for

Molisch's test for carbohydrates: Two milliliter of the filtrate from above was measured into a test tube and 2 drops of alcoholic solution of α -naphthol added, then the test tube was slanted and concentrated sulphuric acid added down the side into the test tube without mixing.

Saponins: Two milliliter of the filtrate from above was measured into another test tube and 10 mL of distilled water was added it was shaken vigorously for over a minutes.

Tannins: Powdered *Khaya grandifoliola* stem bark (2.5 g) was weighed into a conical flask and mixed with 50 mL of water, boiled in a water bath for 5 min. The mixture was filtered hot using a filter paper and the filtrate

collected in a beaker. Two milliliter of the filtrate was mixed with 10 mL of distilled water and then a drop of iron chloride was added.

Flavonoids: Five milliliter of dilute ammonia solution was added to a portion of the aqueous filtrate followed by the addition of concentrated sulphuric acid, (1 mL) to 2 mL of potassium hydroxide solution and allowed to mix. Then into the acid base mixture, a small quantity of aqueous filtrate of the sample was added and observed for colour change.

Anthraquinones: Powdered sample (2.5 g) was shaken with 5 mL of benzene and then 2.5 mL of 10% ammonia solution was added and shaken.

Alkaloids: Extraction of 5 g of the powdered sample was carried out by boiling in 50 mL of distilled water in a water bath for 30 min. It was then filtered into a test tube and the filtrate collected. The filtrate was tested with alkaloidal reagents (Dragendorfs, Wagner's and Mayer's reagent) and results compared to blanks.

Tropane alkaloids: Vitali- Morin test; powdered sample (0.5 g) was mixed with chloroform, boiled and allowed to extract. The mixture was filtered using a filter paper into a beaker. Two milliliter of the filtrate was measured into a small crucible and evaporated to dryness. The residue was moistened with a few drops of concentrated nitric acid and evaporated to dryness on a water bath. A few drops of 10% of potassium hydroxide solution in alcohol was added and mixed.

Isoquinoline alkaloids: Emetine was used as standard. Powdered sample (1.0 g) was added to 2.5 mL of water and 10 mL of HCl. The mixture was allowed to stand for 5 min then filtered. Two milliliter of the filtrate was taken and a few crystals of potassium chlorate were added.

Antimicrobial evaluation

Susceptibility testing: The agar-well diffusion method was used to determine the antimicrobial activity of the extract (Barry and Thornsberry, 1995). The bacteria used for this study were obtained from the department of microbiology, University of Benin Teaching Hospital, Nigeria

The susceptibility of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates using the E test strip was first carried out by the disk diffusion method using mannitol salt agar. Each isolates was grown in a nutrient broth for 18 h. One milliliter of the cultures of

these organisms were sub-cultured into 9 mL nutrient broth and shaken in a water bath for 4 h at 80 throws min^{-1} and the dilutions made to obtain 10^5 cells mL^{-1} . One milliliter of this final dilution was used to flood an already prepared Mueller-Hinton agar and the excess drained into disinfectant jar.

The extract was tested at 20 mg mL^{-1} concentration. This was prepared by dissolving 2 g of the crude extract in 5 mL of sterile distilled water. 0.005 mL (50 μL) was then delivered into wells (5 mm in diameter) bored into the surface of the already seeded Mueller-Hinton agar plates. Standard antibiotics made from ciprofloxacin (0.010 g mL^{-1}) and gentamycin (0.010 g mL^{-1}) were assayed along using the agar-well diffusion technique. The plates were allowed to stand on the bench for 30-40 min and then incubated at 37°C for 24 h.

Determination of Minimum Inhibitory Concentration (MIC):

The modified agar-well diffusion technique (Okeke *et al.*, 2001) was used to determine the MIC of the extract. A two fold serial dilution was prepared by first reconstituting in sterile distilled water, then diluting to achieve a decreasing concentration of 20.0, 16.0, 12.0, 8.0, 4.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.2 and 0.1 mg mL^{-1} , respectively. Each dilution was introduced into Mueller-Hinton agar plates already seeded with standardized inoculums (approximately 10^5 cfu mL^{-1}) of the test bacterial isolates. All test plates were incubated at 37°C for 24 h. The least concentration of extract showing a clear zone of inhibition was regarded as the MIC.

RESULTS AND DISCUSSION

The phytochemical components of *K. grandifoliola* is presented in Table 1. Results obtained from the qualitative phytochemical tests carried out on the powdered sample revealed that the stem bark contained a wide array of phytochemicals these include carbohydrate, saponins, tannins, flavonoids, anthraquinones, alkaloids and specific alkaloids such as emetine (isoquinolic alkaloid) and strychnine (indole alkaloids). The absence of tropane alkaloids and brucine (indole alkaloid) was also observed. Knowing the phytochemical constituent can help one to speculate on the medicinal value of the stem bark. Flavonoids have been reported to have antibacterial and antimicrobial properties (Tsuchiya *et al.*, 1996). Tannins have antimicrobial (Ya *et al.*, 1988) and antioxidant properties. Crude saponin extract from *Sorghum bicolor* has antimicrobial activity (Seotan *et al.*, 2006). Alkaloids have pronounced physiological effect particularly on the nervous system (Sofowora, 2008; Levetin and McMahon, 2003). The presence of these

Table 1: Phytochemical analysis of *Khaya grandifoliola* stem bark

Phytochemicals	Stem bark
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Anthraquinone	+
Carbohydrate	+++

Table 2: Zone of inhibition (mm) produced by extract and standard antimicrobial agents

Organisms	Crude extract (0.020 g mL^{-1})	Cip.(standard) (0.010 g mL^{-1})	Gen.(standard) (0.010 g mL^{-1})
MRSA 1	20.7±1.5	32.3±1.5	23.7±1.5
MRSA 2	18.3±1.5	29.7±1.5	17.7±1.5
MRSA 3	19.7±1.5	26.7±1.5	22.7±1.5
MRSA 4	21.3±3.1	30.3±3.1	19.3±1.5
MRSA 5	21.0±1.7	32.7±1.5	24.7±1.5
<i>B. subtilis</i>	15.0±1.7	24.7±0.6	23.3±0.6
<i>K. pneumoniae</i>	12.3±0.6	34.3±0.6	23.3±1.5
<i>P. mirabilis</i>	15.7±2.5	31.3±3.1	21.7±2.5

Cip: Ciprofloxacin, Gen: Gentamycin

phytochemicals in the stem bark suggests that the plant is pharmacologically active, supporting the claim by traditional healers.

The result contradicted the reported phytochemical components indicating absence of flavonoids in the stem bark of this same plant (Ibrahim *et al.*, 2006).

The results of the antimicrobial activity of the crude extract at 0.020 g mL^{-1} concentration, ciprofloxacin 10 mg L^{-1} and gentamycin 0.010 g L^{-1} tested against the isolates is given in Table 2.

The crude extract showed activity against all the test isolates having a greater activity against the gram positives especially the MRSA isolates. The mean zone diameter measured for the MRSA isolates was the highest, ranging from 18.3- 21.0 mm (Fig. 1). This is of great interest, because, bacterial resistance to antibiotics is a difficult problem facing the world of medicine today. Substances that are able to significantly control the activity of resistant strains of bacteria are highly esteemed; the stem bark of *Khaya grandifoliola* is proving to be one of such.

Similar concentrations were not used for the crude extract and the standards, but the results are comparable because the crude extract is a mixture of pharmacologically and non-pharmacologically active components while the standards are purified antibiotics. The mean zone diameter measured for the crude extract is within a reasonable range of that measured for the standards, indicating that when further isolation and purification of the extract is carried out, increased activity most assuredly would be obtained.

The Minimum Inhibitory Concentration (MIC) for all test isolates was 0.4 mg mL^{-1} except for *B. subtilis* which was 0.2 mg mL^{-1} (Table 3). This concentration is

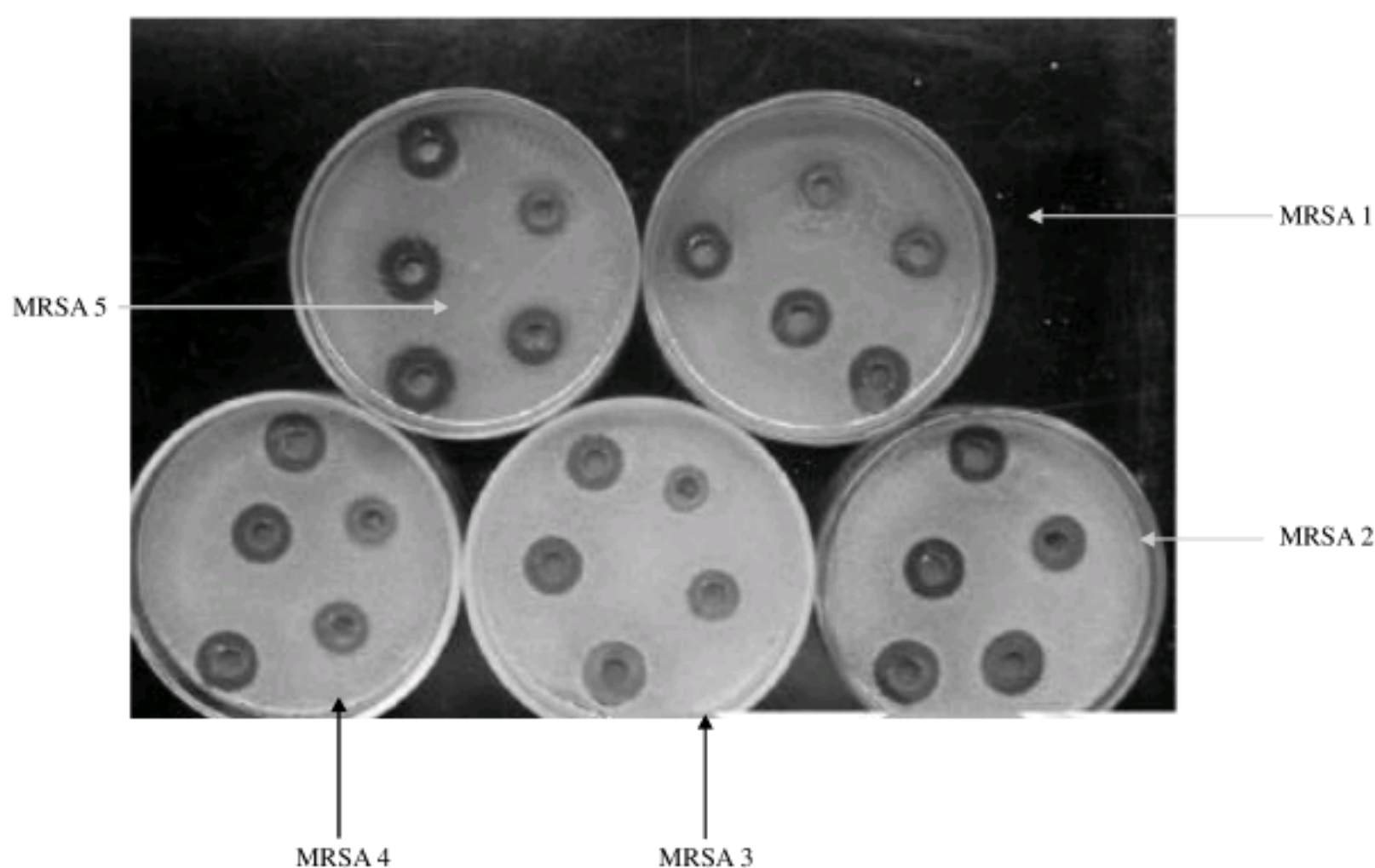


Fig. 1: Zones of inhibition produced by extracts at the tested concentration

Table 3: Minimum Inhibitory Concentration (MIC) of *K. grandifoliola* stem bark extract

Microorganism	MIC (mg mL ⁻¹)
MRSA 1	0.4
MRSA 2	0.4
MRSA 3	0.4
MRSA 4	0.4
MRSA 5	0.4
<i>B. subtilis</i>	0.2
<i>K. pneumonia</i>	0.4

MRSA; Methicillin Resistant *Staphylococcus aureus*

remarkable for a crude extract and further work should be carried out to isolate, characterize and purify the active constituents of this indigenous plant with view to determining its spectrum of activity as well as adding it to already established antimicrobial agents especially those that are active against resistant strains of bacteria.

The antimicrobial activity exhibited by this plant could be attributed to the phytochemicals that it contains. Of particular interest is its activity against the Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates. This is probably due to the presence of flavonoids (Tsuchiya *et al.*, 1996). This is an indication that the stem bark if processed could be a good first line drug for gram positive bacteria (especially the *Staphylococcus* specie). This result proves that the use of the stem bark of *Khaya grandifoliola* to cure several illnesses, especially those caused by microbes, is valid. It is expected that the results from this study would serve as background knowledge for further studies on this plant, which would result to discovering other medicinally useful properties.

CONCLUSION

An aqueous solution of the methanolic extract of the powdered stem bark of *Khaya grandifoliola* possesses antimicrobial potentials against both gram positive and gram negative bacteria, especially on some resistant strains of *Staphylococcus*. It is therefore confirmed as a useful antimicrobial agent. The powdered stem bark is rich in phytochemicals and secondary metabolites such as tannins, alkaloids, flavonoids, anthraquinones and saponins, which are probably responsible for its medicinal properties.

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