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Phytochemical and Antimicrobial Screening of *Gnetum Africanum* Stem and Root

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Authors' contributions

This work was carried out in collaboration among all authors. The work is a research project of an undergraduate student (author) EJE under the author BCA with the author IWO. All the authors read and approved the final manuscript.

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ABSTRACT

The menace of antibiotic resistance and the antecedent evolution of innocuous microbes into superbugs is an epidemic of global concern. This study investigated the phytochemical and antimicrobial activity of hexane and methanol extracts of *Gnetum africanum* stem and root, on *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi and Aspergillus flavus.* Stem and roots of *G. africanum* were extracted with hexane and methanol using cold maceration technique; standardized chemical tests were employed for phytochemical screening and the agar-well diffusion method used for antimicrobial analysis. The results of phytochemical screening *G. africanum* showed that all the following tested phytochemicals which include steroid, tannins, and saponins were present in both methanol and hexane extracts of *G. africanum* while flavonoids, alkaloids, glycosides and carbohydrate were absent in both the methanol and hexane extracts. Anthraquinone was present in hexane extract but absent in methanolic extract of *G. africanum*. Both the stem and root of *G. africanum* had antimicrobial effects on all the test organisms but the effect of the stem was higher than that of the root. Findings indicate that both extracts had a dose-dependent inhibitory effect on the growth of *S. aureus*, with maximum inhibition zones of 17.50 mm and 16.00 mm at 500 mg/ml for hexane and methanol extracts,

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respectively. The stem and root extracts of hexane and methanol were inactive against *A. flavus.* The findings of this study further reinforce the importance of *G. africanum* stem and root in traditional healthcare practice and its use in culinary. Further investigation is however needed to isolate and purify the bioactive antimicrobial principles for potential development into generic antimicrobials.

Keywords: Antibiotic resistance; Gnetum africanum; antimicrobial activity; phytochemicals; cold maceration technique.

1. INTRODUCTION

The use of medicinal plants for the treatment of microbial diseases is well known and has been documented since ancient times. Medicinal plants have pharmaceutical and antibacterial properties [1]. Plants synthesize many components, which act as a defensive agent, helping to protect them from microbial infection and other diseases. Those compounds are bioactive and can be medicinal, intoxicating or toxic depending on circumstance. Several plants species have been tested for antimicrobial properties but vast majorities have not yet been adequately evaluated [2]. In an effort to expand the search for the new antimicrobial agents from natural sources *Gnetum africanum* Welw of the family Gnetaceae has been evaluated in this study. *G. africanum* Welw is traditionally a wild vine found mainly in the humid tropical forest region of Nigeria, Central African Republic, Cameroon, Gabon, Democratic Republic of the Congo and Angola [3].

The scarcity of knowledge of the phytochemical constituents and antimicrobial properties of *G. africanum* has resulted in their neglect and underutilization. It is expected that the result of this study will initiate the exploitation of the therapeutic potentials of this plant. The study of this plant will provide detailed information on the phytochemical constituent and antimicrobial properties of the plant for broader application in relevant areas. The phytochemical and antimicrobial properties of this plant would attract international recognition and that can create revenue and employment. The aim of this study is to evaluate the phytochemical composition and antimicrobial properties of the stem and root extract of *G. africanum* on four bacteria and one fungi.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh stem and roots of *G. africanum* were collected from farm lands in Ikot Ekpene L.G.A of Akwa Ibom State, Nigeria. They were authenticated at the herbarium of the Biological Science, University of Abuja.

The test organisms include three gram negative bacteria: *Escherichia coli, Klebsiella pneumoniae*, and *Salmonella typhi*, one gram positive: *Staphylococcus aureus* and one fungus: *Aspergillus flavus.*

2.2 Processing of Sample

The root and stem were air dried and screened to remove undesirable materials such as stone and other impurities, after which was milled into powder using the laboratory blender and the powder was kept in an airtight container until when needed for analysis.

2.3 Extraction of the Sample

The stem and root of the plant were properly rinsed in tap water and rinsed again in distilled water. The cleansed stem and root plant materials were air-dried for 3 days. The plant materials were pulverized using an electric blender to obtain a powdered form which was stored in airtight plastic containers until needed for the experiment. The powdered leaf (120 g) was separately macerated in 1 L of methanol in a conical flask for 24 hours, and the mixture agitated mechanically at intervals. The mixture was filtered afterward using Whatmann No. 1 filter paper in a Buchner funnel. The filtrate obtained was concentrated in a water bath at 70°C to obtain a gel-like concentrate. The concentrate obtained was stored in the refrigerator at 4°C until needed for analysis. The hexane was similarly prepared, except that 1 L of distilled water was used for the maceration.

2.4 Sterilization of the Extract

The various extracts (hexane and methanol) was filter-sterilized using a membrane filter (pore size 0.45 um). The sterile filter glass was connected to a suction pump and the sterile filter attached to

the holder, the filter membrane was carefully placed on the filter platform using a sterile pair of forceps and the funnel put in place and locked. The various solvent extract (hexane and methanol) was carefully poured into the funnel and suction applied. Thereafter, the filtrate evaporated to dryness at 50°C in a water bath. Then stored in sterile universal bottles under refrigeration conditions at 4°C until further use.

2.5 Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out using standardized procedures of Trease and Evans [4]. The tests are briefly described below:

2.5.1 Determination of saponins

Exactly 0.5 g of the dried extract was stirred with water in a test tube. Frothing (foam) which persists on warming was taken as evidence for the presence of saponins.

2.5.2 Determination of tannin

The ferric chloride test was employed. A quantity of each extract (200 mg) was heated with 20 mL of water for 5 min in appropriately labelled test-tubes. Each solution was allowed to cool and then filtered. 1 mL of each filtrate was diluted with 5 mL distilled water in a test tube; a few drops of 0.1% ferric chloride solution were added. A characteristic blue, blue-black, green or blue-green colour and precipitate would indicate the presence of tannin.

2.5.3 Determination of alkaloids

Each extract (200 mg) was stirred with 5 mL of 1% aqueous HCl on a water bath and then filtered. Of the filtrate, 1 mL was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; the occurrence of orange-red precipitate was taken as positive. To the second 1 mL, Mayer's reagent was added and the appearance of buff-coloured precipitate will be an indication for the presence of alkaloids.

2.5.4 Determination of flavonoids

Lead acetate test: A quantity of each extract (500 mg) was dissolved in water and filtered. To 5 mL of each of the filtrate, 3 mL of lead acetate solution was then added. The appearance of a buff-coloured precipitate indicates the presence of flavonoids.

2.5.5 Determination of cardiac glycoside

The Salkowski test was employed for this. The extracts (0.5 g) were dissolved in 2 mL of chloroform. Sulphuric acid was then carefully added to form a lower layer. A reddish-brown colour at the interface will indicate the presence of a steroidal ring (i.e. a glycone portion of the cardiac glycoside).

2.5.6 Determination of reducing sugars

To 10 mL of each extract, a few drops of Fehling's solution A and B were added; an orange-red precipitate indicates the presence of reducing sugar.

2.5.7 Determination of terpenoids

5 mL of each extract was mixed in 2 mL of chloroform. 3 mL of H_2SO_4 would then be added to form a layer. A reddish-brown precipitate coloration at the interface formed indicated the presence of terpenoid.

2.6 Determination of Phenols

The extract is treated with 3-4 drops of ferric chloride solution. Formation of bluish-black colour will indicate the presence of phenols.

2.7 Determination of Anthraquinone

0.5 g of extracts place in 10 mL of chloroform in a test tube and shaken for five minutes. The extract was filtered. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinone.

2.8 Determination of Glycoside

Exactly 0.5 g of dried extract was dissolved in 2.0 mL of glacial acetic acid containing one drop of ferric chloride solution. Then under lay with 1.0 mL of concentrated sulphuric acid. A brown ring at the interface indicates the presence of a glycoside.

2.9 Characterization and Identification of Isolates

Isolates obtained from the Teaching hospital was analysed to confirm their purity. Test carried out were Gram staining, spore formation, catalase test, motility test, and sugar fermentation according to the methods of Cheesebrough [5].

2.10 Gram Staining of the Isolates

The isolates were gram stained using the following procedure: Smear of culture was prepared on clean slides by emulsifying a little quantity of the growth on a drop of normal saline. The smear was then allowed to air-dry. After which, heat-fixing was done. Crystal violet was then added as a primary stain for 30s and then drained off with distilled water. Lugol's iodine was then added and allowed to react for 30s. After which, it was washed off with distilled water. Acetone alcohol was added, and immediately, it was washed off with distilled water. After which, the smear was counter-stained with safranin for 1min and washed with distilled water. The smear was then allowed to dry. A drop of oil immersion was placed on the stained smear and viewed with a high objective lens of the microscope.

2.11 Test for Spore Formation

Smear of the organism was made on a clean microscope slide. After which, the slide was air-dried. The smear was then heated-fixed. Small piece of blotting paper (absorbent paper) was placed on the slide (smear side up), then it was placed on a wire gauze on a ring stand. The smear was flooded and kept with malachite green. The slide was then heated gently till it started to evaporate via Bunsen burner. The heat was removed. After which the slide was reheated to heat needed to keep the slide steaming for about 3-5 minutes. As the paper began to dry, two drops of malachite green was added to keep it moist. After 5 mins, the slide was carefully removed from the rack. The blotting paper was removed and the slide was allowed to cool to room temperature for 2 minutes. The slide was thoroughly rinsed with tap water (to wash the malachite green from both sides of the microscope slide). The smear was stained with safranin for 2 minutes. Both sides of the slide were then rinsed to remove the secondary stain. After which, the slide was blotted and air-dried. The organism is then observed under 100x (oil immersion) total magnification.

2.12 Catalase Test

A colony of culture was picked using a sterile wire loop and then emulsified in a few drops of hydrogen peroxide on a clean microscope slide. Absence of effervescence indicates catalase negative while the presence of effervescence indicates catalase positive.

2.13 Motility Test

A discrete colony of the overnight culture was placed on a microscope slide containing a drop of peptone water, and covered with a cover slid after a minute. Then, viewing microscopically with 40x objective was done.

2.14 Sugar Fermentation

Exactly 10 mL of peptone water was introduced into different sterile test tubes. One gram of the respective carbohydrates, i.e. D-glucose, lactose, sucrose, galactose, mannose, fructose, and maltose was added into each of the test tubes that contained the peptone water and labeled accordingly. They were stirred to dissolve completely by warming over a Bunsen burner.

Bromocresol purple broth base was used as basal medium. One percent filter-sterilised sugar solution using 0.2 um millipore filter (corning) was added asceptically into sterilised bromocresol purple broth base. The tubes were plugged with cotton wool and sealed with foil before sterilisation in autoclave at 115°C for 15 minutes. After the sterilisation of the medium, the cultural organisms were inoculated into each of the tubes, respectively and Durham's tubes were inserted in inverted positions into each of the tubes. The results were assessed with reference to an uninoculated control after anaerobic incubation at 30°C for 5 days. Tubes in which bromocresol purple color changed to yellow indicated utilization of sugar or acid production. Gas production was shown by the presence of bubbles on the surface of the medium and on upward movement of the inverted Durham's tubes.

2.15 Preparation of Stock Solution of Extract

The method of Cheesebrough [5] was adopted for the stock solution preparation. The stock solution of the four extracts was prepared by dissolving 0.5 g of each extract in 2 ml of dimethyl sulphoxide (DMSO) to give concentration of 500 gm/mL. 0.25 g for 250 mg/mL 0.125 g for 125 mg/mL and 0.06 g for 62.5 mg/mL, respectively. Then well labelled and stored until when required.

2.16 Determination of Antibacterial Activity of Extracts

The antibacterial activities of the extracts were determined using agar well diffusion [6]. Mueller Hilton agar plates were seeded with 0.05 mL of 24 hours culture of each bacterial isolates (equivalent to 10^{7}-10^{8} CFU/mL). The seeded plates will be allowed to set and then dry. A sterile cork borer of 6 mm diameter was used to bore 4 uniform wells on the surface of the agar and the bottom of the hole sealed with a drop of molten agar. Exactly 0.05 mL of each concentration of the extracts (500, 250, 125, and 62.5 mg/mL) was placed in the wells, respectively.

Furthermore, two holes were bored on a plate at an equidistant position and 0.2 mL of streptomycin was introduced into one of the holes to serve as positive control and 0.2 mL DMSO into the other hole to serve as negative control for each test organism. The plates were allowed for pre-diffusion for 40-45 minutes followed by 24 hours incubation at 37°C for bacterial isolates. The degree of antibacterial activity of each extract will be measured as the inhibition zone diameter in millimetres. The sensitivity test was done in duplicates. The mean of the two reading was taken to be the zone of inhibition of the bacterial concentration.

2.17 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined by dilution to various concentrations according to the macro broth dilution technique [6]. Standardized inoculum of the test organisms was added to series of sterile tubes of 5 mL nutrient broth containing 500, 250, 125, and 62.5 mg/mL of the extracts 37°C for 24 hours. The MIC was read as the least concentration that inhibited the growth of the test organism.

2.18 Determination of Minimum Bacterial Concentration (MBC)

The MBC was determined by collecting 1 ml of broth culture from the tubes used for the MIC determination and sub-culturing onto fresh extract. The plates were incubated at 37°C for 24 hours. The least concentration that did not show any growth after incubation was regarded as the MBC [7].

3. RESULTS

The results obtained from the phytochemical and antimicrobial screening of *Gnetum africanum* were carried out are presented in the tables.

3.1 Phytochemical Screening of Extracts of *G. africanum*

The results of phytochemical screening showed in Table 1 indicates the presence of phyto-chemical constituents.

Table 1. Phytochemical screening of methanol extract and hexane extract of *G. africanum*

Phytochemicals	Methanol extract	Hexane extract
Tannins	+	+
Alkaloid	-	-
Steroid	+	+
Phenols	+	+
Flavonoid	-	-
Carbohydrate	-	-
Glycolsides	-	-
Saponins	+	+
Anthraquinone	-	+

Keys: + Present, - absent

3.2 Antimicrobial Activity of Hexane Extracts of Stem and Root of *G. africanum*

The antimicrobial activities of hexane extracts of stem and root of *G. africanum* against *Aspergillus flavus*, *Staphylococcus aureus, Escherichia coli, Salmonella typhi*, and *Klebsiella pneumoniae*, respectively are shown in Table 2.

3.3 Antimicrobial Activity of Methanol Extracts of Stem and Root of *G. africanum*

The antimicrobial activities of methanol extracts of stem and root of *G. africanum* against *Aspergillus flavus*, *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Klebsiella pneumoniae*, respectively are shown in Table 3.

Table 2. Antimicrobial activity of hexane extracts of *G. africanum* showing mean zones of inhibition in millimeter

Test organisms	Concentration in mg/mL			
	500	**250**	**125**	**62.50**
Stem				
A. flavus	11.0±1.00	9.5±0.00	NA	NA
S. aureus	19.0±1.00	18.5±0.50	15.0±0.60	13.0±1.00
E. coli	13.0±0.70	11.5±1.50	10.0±1.00	8.5±0.50
S. typhi	13.5±0.60	12.0±1.00	11.0±0.70	9.5±0.50
K. pneumoniae	15.0±0.4	14.0±0.50	12.5±0.60	10.0±0.10
Root				
A. flavus	10.0±0.00	NA	NA	NA
S. aureus	17.5±1.00	15.0±0.40	12.5±1.50	10.0±0.60
E. coli	11.0±0.10	9.5±0.50	8.0±1.50	6.0±0.40
S. typhi	10.0±0.00	9.0±0.50	7.0±0.50	5.0±0.00
K. pneumoniae	10.5±0.50	9.5±0.50	7.5±0.00	6.0±0.00
Controls				
A. flavus (KE)	24.0±2.00	22.0±1.00	18.5±2.00	16.0±1.00
S. aureus (CH)	30.0±2.00	28.0±2.00	23.0±1.00	19.0±2.00
E. coli (CH)	26.0±1.00	25.0±1.50	21.0±2.00	18.5±1.50
S. typhi(CH)	24.5±1.50	23.5±0.50	20.0±1.00	18.0±1.00
K. pneumoniae (CH)	25.0±1.00	24.0±1.00	20.5±1.50	18.5±050

Each value represents mean± standard deviation from three replicate values
Keys: CH= Chloramphenicol, KE= Ketoconazole, NA=Not active

Table 3. Antimicrobial activity of methanol extracts of *G. africanum* showing mean zone of Inhibition in millimetre

Test organisms	Concentration in mg/mL			
	500	**250**	**125**	**62.50**
Stem				
A. flavus	10.0±0.20	NA	NA	NA
S. aureus	17.0±1.50	15.0±0.30	13.5±0.50	11.0±1.00
E. coli	11.0±1.00	10.0±0.00	9.0±1.00	6.5±0.50
S. typhi	10.0±0.00	9.0±1.00	7.5±1.50	5.5±1.50
K. pneumoniae	10.5±0.50	9.5±1.50	8.5±0.50	6.0±0.00
Root				
A. flavus	NA	NA	NA	NA
S. aureus	16.0±2.00	14.0±1.00	12.0±2.00	10.0±2.0
E. coli	10.0±1.00	8.5±0.50	7.0±1.00	5.5±0.50
S. typhi	9.5±1.50	7.0±1.00	6.0±0.00	5.0±1.00
K. pneumoniae	10.0±0.00	7.5±0.50	6.5±0.50	5.0±0.00
Controls				
A. flavus (KE)	24.0±2.00	22.0±1.00	18.5±2.00	16.0±1.00
S. aureus (CH)	30.0±2.00	28.0±2.00	23.0±1.00	19.0±2.00
E. coli (CH)	26.0±1.00	25.0±1.50	21.0±2.00	18.5±1.50
S. typhi(CH)	24.5±1.50	23.5±0.50	20.0±1.00	18.0±1.00
K. pneumoniae (CH)	25.0±1.00	24.0±1.00	20.5±1.50	18.5±050

Each value represents mean± standard deviation from three replicate values
Keys: CH= Chloramphenicol, KE= Ketoconazole, NA=Not Active

3.4 Minimum Inhibitory Concentration (MIC) of *Gnetum africanum* Stem and Root

The MIC of *Gnetum africanum* against *Aspergillus flavus, Staphylococcus aureus,* and *Escherichia coli*, respectively are shown in Table 4 and 5.

3.5 Minimum Bactericidal Concentration (MBC) of *Gnetum africanum* Stem and Root

The MBC of *Gnetum africanum* against *Aspergillus flavus, Staphylococcus aureus, Escherichia coli, Salmonella typhi*, and *Klebsiella pneumoniae*, respectively are shown in Table 6 and 7.

4. DISCUSSION

The methanol and hexane extracts of *Gnetum africanum* against *Aspergillus flavus, Staphylococcus aureus*, and *Escherichia coli*, respectively showed a dose-dependent activity. However, the following tested phytochemicals which include steroid, tannins and saponins were present in both methanol and hexane extracts of *G. africanum* while flavonoids, alkaloids, glycosides, and carbohydrate were absent in both the methanol and hexane extracts. Anthraquinone was present in hexane extract but absent in methanolic extract of *G. africanum*. Tannins possess astringent taste and help in healing of wounds and inflamed mucous membrane [8]. Tannins are also potent metal ion chelator, proton precipitating agents and biological antioxidant [9]. Flavonoids are mostly known for their antioxidant activities and act as detoxifiers with the ability to modify a cell's reaction to carcinogens, viruses and allergens. They possess antimicrobial, anticancer, anti-inflammatory, and anti-allergy activities [10]. This is in contrast with the findings of Ilodibia et al. [11] who worked on the aqueous and ethanolic leaf and stem extracts of *G. africanum* revealing that alkaloid, tannin, saponin, sterol, flavonoid, and terpenoid were present in both extracts while glycoside was absent. This may be due to the polarity of the solvents employed, which determines their dissolving activity.

In this study, *A. flavus,* the ketoconazole and the leaf extract exhibits varied antifungal activity. The susceptibility of the plant extract could be attributed to the absence of a polysaccharide in some of these organisms which is present in the *A. flavus*. The presence of anthraquinone in the hexane extract earlier detected by Timothy et al. [12] which were presumablyabsent in the aqueous extract may be responsible for the

Table 4. Minimum inhibitory concentration of hexane extracts of *G. africanum* showing mean zones of inhibition in millimeter

Test organisms	Concentration in mg/mL			
	500	250	125	62.50
Stem				
A. flavus	+	+	+	+
S. aureus	-	-	-	+
E. coli	-	-	+	+
S. typhi	-	+	+	+
K. pneumoniae	-	-	+	+
Root				
A. flavus	+	+	+	+
S. aureus	-	-	-	+
E. coli	-	+	+	+
S. typhi	+	+	+	+
K. pneumonia	-	+	+	+
Controls				
A. flavus (KE)	-	-	-	+
S. aureus (CH)	-	-	-	-
E. coli (CH)	-	-	-	-
S. typhi(CH)	-	-	-	+
K. pneumoniae (CH)	-	-	-	+

Each value represents mean± standard deviation from three replicate values
Keys: CH= Chloramphenicol, KE= Ketoconazole, NA=Not Active

Table 5. Minimum inhibitory concentration of methanol extracts of *Gnetum africanum* showing mean zones of inhibition in millimetre

Test organisms	Concentration in mg/ml			
	500	250	125	62.50
Stem				
A. flavus	+	+	+	+
S. aureus	-	-	-	+
E. coli	-	+	+	+
S. typhi	+	+	+	+
K. pneumoniae	-	+	+	+
Root				
A. flavus	+	+	+	+
S. aureus	-	-	-	+
E. coli	+	+	+	+
S. typhi	+	+	+	+
K. pneumonia	+	+	+	+
Controls				
A. flavus (KE)	-	-	-	+
S. aureus (CH)	-	-	-	-
E. coli (CH)	-	-	-	+
S. typhi(CH)	-	-	-	+
K. pneumoniae (CH)	-	-	-	+

Each value represents mean± standard deviation from three replicate values
Keys: CH= Chloramphenicol, KE= Ketoconazole, NA=Not active

Table 6. Minimum bactericidal concentration of hexane extracts of *G. africanum* showing mean zones of inhibition in millimetre

Test organisms	Concentration in mg/mL			
	500	250	125	62.50
Stem				
A. flavus	+	+	+	+
S. aureus	-	-	+	+
E. coli	-	-	+	+
S. typhi	-	+	+	+
K. pneumoniae	-	+	+	+
Root				
A. flavus	+	+	+	+
S. aureus	-	-	+	+
E. coli	-	+	+	+
S. typhi	+	+	+	+
K. pneumonia	-	+	+	+
Controls				
A. flavus (KE)	-	-	+	+
S. aureus (CH)	-	-	+	+
E. coli (CH)	-	-	+	+
S. typhi(CH)	-	+	+	+
K. pneumoniae (CH)	-	+	+	+

Each value represents mean± standard deviation from three replicate values
Keys: CH= Chloramphenicol, KE= Ketoconazole, NA=Not active

variation in antimicrobial activity. This study justifies the ethno-medical use of this plant in the management of dermatophytosis and other fungal diseases.

Table 7. Minimum bactericidal concentration of methanol extracts of *Gnetum africanum* showing mean zones of inhibition in millimeter

Test organisms	Concentration in mg/mL			
	500	250	125	62.50
Stem				
A. flavus	+	+	+	+
S. aureus	-	-	+	+
E. coli	-	+	+	+
S. typhi	+	+	+	+
K. pneumoniae	-	+	+	+
Root				
A. flavus	+	+	+	+
S. aureus	-	-	+	+
E. coli	+	+	+	+
S. typhi	+	+	+	+
K. pneumonia	+	+	+	+
Controls				
A. flavus (KE)	-	-	+	+
S. aureus (CH)	-	-	+	+
E. coli (CH)	-	-	+	+
S. typhi(CH)	-	+	+	+
K. pneumoniae (CH)	-	+	+	+

Each value represents mean± standard deviation from three replicate values
Keys: CH= Chloramphenicol, KE= Ketoconazole, NA=Not active

Zone diameter of inhibition of hexane extract of *G. africanum* stem at 500 mg/mL against *A. flavus* was 11.0±1.00 mm, *S. aureus*, 19.0±1.00 mm while *E. coli* was 13.0±0.70 mm, and at concentrations of 125 mg/mL and 62.50 mg/mL, the stem was not active against *A. flavus* but had zones of inhibitions against *S. aureus* and *E. coli*, respectively. Similarly, zone diameter of inhibition of hexane extract of *G. africanum* root at 500 mg/mL against *A. flavus* was 10.0±1.00 mm, *S. aureus*, 17.5±1.00 mm while *E. coli* was 11.0±0.10 mm, and at concentrations of 250 mg/mL, 125 mg/mL and 62.50 mg/mL, the hexane root extract was not active against *A. flavus* but had zones of inhibitions against *S. aureus* and *E. coli*, respectively.

The zone diameter of inhibition of the ketoconazole against *A. flavus* was 24.0± 2.00 mm and chloramphenicol had zone diameter of inhibition of 30.0±2.00 mm against *S. aureus* while that of *E. coli* was 26.0±1.00 mm. This agrees with the findings of Abubacker et al. [13] who reported similar result even though they work on flowers, while Stein et al. [14] evaluated similar result from the stem bark.

The higher statistical significant difference in activity of the extracts at higher doses when compared with Ketoconazole and chloramphenicol on *A. flavus, S. aureus*, and *E. coli* ($p<0.05$) could be attributed to the presence of some bioactive components in the extract. Furthermore, zone diameter of inhibition of methanol extract of *G. africanum* root was not active at all concentrations against *A. flavus* whereas *S. aureus* had 16.0±2.00 mm, *E. coli* also had 10.0±0.00 mm, *S. typhi*, and *K. pneumoniae* had 9.5 ± 1.50 mm and 10.0±0.00 mm, respectively at 500 mg/ml. These findings contrasted with the study of Eneh et al. [15] who found out that there was no detectable activity of the aqueous and ethanolic leaf extracts of *G. africanum* against *E. coli* while 13.30 mm was recorded as the maximum zones of inhibition against *S. aureus* at a concentration of 200 mg/mL.

Meanwhile, at concentrations of 250 mg/mL, 125 mg/mL and 62.5 mg/mL, the methanol root extract had appreciable zones of inhibitions against *S. aureus, E. coli, S. typhi*, and *K. pneumonia*, respectively. The zone diameter of inhibition of the ketoconazole against *A. flavus* was 24.0± 2.00 mm and chloramphenicol had

zone diameter of inhibition of 30.0±2.00 mm against *S. aureus* while that of *E. coli* was 26.0 ±1.00 mm, *S. typhi* was 24.5 ±1.50 mm and *K. pneumoniae* had 25.0± 1.00 mm, respectively. This result agrees with the findings of Enyi-Idoh et al. [16] who studied the antibacterial activity of *G. africanum* on diarrhoegenic bacteria, including *S. aureus*. This results obtained in this study showed that *G. africanum* widely inhibited microbial growth, which suggests that the plant can be exploited for the development of antimicrobial drugs.

5. CONCLUSION

This study has revealed the presence of tannin, phenol, steroid, saponins which are secondary metabolites in the stem and root of *Gnetum africanum* against *A. flavus, S. aureus, S. typhi, K. pneumonia*, and *E. coli*, respectively. It has further confirmed that the extracts could be use for the treatment of various infections caused by the pathogens. The isolates which were tested showed purity after analysis were carried out on them. The extracts of *G. africanum* stem and root inhibited microbial growth and is thus a potential lead in the development of new antibiotic. The stem of *G. africanum* had a higher antimicrobial effect than that of the root. The stem was not effective against *A. flavus*, i.e. it cannot be used in the treatment of infections caused by the organism. Interestingly, its effectiveness against *S. aureus, K. pneumoniae, S. typhi,* and *E. coli* can be exploited in developing safer antimicrobial drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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