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ANTIBACTERIAL PROPERTIES OF *MANGIFERA INDICA* ON *STAPHYLOCOCCUS AUREUS.*

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ABSTRACT

Antibacterial activity of *Mangifera indica* stem bark extracts was determined using disk diffusion, agar and broth dilution methods. In disk diffusion method, inhibition zone sizes were used to determine the susceptibility of *S. aureus* to the extracts. The results showed that the stem-bark extracts *of M. indica* have antimicrobial activity against *S. aureus.* Methanol extracts showed the highest inhibition zone diameter of 25 mm, followed by ethyl acetate, water and hexane extracts with inhibition zone diameter of 22 mm, 14 mm and 10 mm, respectively. The antibacterial activities of different extracts were found to be concentration dependent, in agar and broth dilution methods. The plant extracts were shown to have a MIC range of 0.62 mg/ml to 4.17 mg/ml, in agar dilution method. Results from the broth dilution method had a MIC range of 0.16 mg/ml to 1.25 mg/ml. The control (ampicillin) was however, more effective than plant extracts since only a concentration of 0.03 mg/ml in agar dilution and 0.001 mg/ml in broth dilution method were effective to inhibit the growth of *S. aureus*. The extracts were shown to be bacteriostatic at low concentrations. Phytochemical screening of the extracts revealed the presence of phyto-compounds such as alkaloids and tannins which are known to inhibit bacterial growth by different mechanisms from those of synthetic drugs. These phyto-constituents may be responsible for the *M. indica* antibacterial activity.

Keywords: *Staphylococcus aureus*, antimicrobial activity, MIC, Phytochemical screening, MBC.

INTRODUCTION

Plant derived products like gums, oils and extracts have been used for therapeutic purpose before the introduction of modern drugs (1; 2) and continues to provide health coverage for over eighty percent of the world's population (3). Serious attention is being given to medicinal plants as evidenced by the recommendation given by the World Health Organization in 1970 (4). WHO gave emphasis on the need to include traditional remedies within national drug policies as these plants serve as the best sources of a variety of drugs. It is important to study plants so that a better understanding of their properties, safety and efficacy is derived for improved benefit. The first plant compound with antimicrobial activity was reported in the 1930s (5) and now a multitude of plant compounds are readily available from herbal suppliers and natural-food stores. In Africa, self-medication with these substances is common and growing in popularity (6). The reasons can be attributed to easy accessibility and affordability of plants compared to commercial drugs. Contrary to the belief that natural medicine has no ill effects (2) several people have been hospitalised by consuming plants of unknown properties. To address such challenges, plants must be investigated to validate and standardise their dosages. An estimated 74% of pharmacologically active plant derived components were discovered after following up on ethno-medicinal use. More than 25% of modern medicines are thought to have descended from plants whilst others are synthetic analogues built on prototype compounds, isolated from plants (7). Thus, medicinal plants can be regarded as the richest bio-resource of drugs of modern medicine, folk medicine and chemical entities for synthetic drugs.

There are many drugs in clinical use today that were discovered from the way plants were used in traditional communities. Such discoveries include quinine which has been used to manage malaria for many years (8), digitoxin an indispensable cardiac drug from Foxglove *(Digitalis purpurea*). Other examples are Strychne from *Strychnox nuxvomica* which was isolated as a central nervous system (CNS) stimulant and ephedrine from *Ephedra sinica* which was discovered for asthma (9) and taxol which is used as modern therapy for ovarian cancer (10;11). There is little or no doubt that ethnographic research can provide important clues leading to new drugs for the modern pharmacies.

Pathogens develop natural resistance to antimicrobial agents. Most gram-negative bacteria are impermeable to the antibiotic penicillin G and platensimycin (12). Development of virulent factors among infectious agents varies. Some bacteria can resist phagocytosis, for instance, *Streptococcus pneumonia* and *Haemophilus influenza* produce a

slippery mucoid capsule that prevents the phagocyte from effectively contacting the bacterium. *Staphylococcus aureus* produce leukocidins that destroys phagocytes before phagocytosis. The bacteria produce coagulase, which coagulates fibrinogen in plasma thus protecting the pathogen from phagocytosis and isolates it from other host defences (13). *Pseudomonas aeruginosa* cleaves laminin associated with basement membranes, *E. coli* lyses erythrocytes and weakened host defences. The emergence of multi-resistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (14). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agent (15). The new compound may actually be more effective than the parent compound. Since resistance is based on structural recognition, the new compound may not be recognised by resistance factors.

Modern drugs are associated with several side effects like nausea and headaches. Man has resorted to plants for treatment due to high prices of synthetic drugs. Plants are regarded as cheaper and safe alternative source of drugs. However, cases of overdose or self poisoning through use of medicinal plants have been increasing. An investigation of the antibacterial activity of stem-bark extracts of *Mangifera indica* on *Staphylococcus aureus* was carried out with a view to screen for phyto-chemical compounds and determine susceptibility of the bacterium. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of different plant extracts on *S. Aureus* was also determined so was the bacterial inhibition time course in water extracts.

MATERIALS AND METHODS

Study area

The bark of *Mangifera indica* Linn Variety Zill was collected in January 2012 from Bindura along Shamva road. The area lies in the following 3D GPS location; Northing 0327972, Southing 80842212, altitude 1002 m. The area has savannah grassland with loam soil, the mean annual rainfall of the area is about 750 mm to 1000mm. Study area is shown in figure 2 below.

Materials used in the research were autoclaved. Glass rods and loops were disinfected by dipping in alcohol and then flaming on a Bunsen burner. The working bench was swabbed with 70% alcohol before and after each experiment.

Preparation of plant material

Fresh stem-bark were collected from a *Mangifera indica tree*, variety Zill. The stem-barks were washed thoroughly with water and then air dried at room temperature for five days. After drying, the pieces were ground into powder and then sieved using a sieve. Two kilograms of powdered plant extracts were transferred into airtight containers and stored at room temperature.

Extraction of the crude extracts from stem-bark powder.

Plant active components were extracted using the cold extraction method (10). Four different extraction solvents namely methanol, ethyl acetate, hexane and distilled water were used.

To 500ml each of pure methanol, ethyl acetate, hexane and sterile distilled water were added 50g portions of the stem-bark powder in sterile conical flasks and allowed to soak at room temperature for 48 hours. A Khan shaker set at 120 rpm was used to improve extraction of phyto-chemicals. The filtrate was obtained by means of a vacuum filter pump through a 127c-1 filter funnel aided by a Whatman® filter paper. Filtering was repeated three times with same plant material until the solution was clear. The filtrate was evaporated in a weighed flask, with a water bath set at 40°C. Drying was done to allow the calculation of the yield of the extraction process. The extraction efficiency was quantified by determining the weight of each of the extracts and the percentage yield was calculated as (weight of dry extracts in grams /initial dry plant extracts) × 100. The procedure was done separately for the four solvents used. A small proportion of dry extracts was stored for phyto-chemical analysis. For the preparation of dilutions of dry extracts for antibacterial assay, dry extracts were reconstituted by re-dissolving in their respective extracting solvent. The final filtrates were filter-sterilized by using cellulose nitrate filter with a pore size of 0.45 µm. Sterile extracts obtained were stored separately in labelled, sterile capped bottles, in a refrigerator at 4°C before use during the antibacterial sensitivity tests.

Preparation of culture media

Nutrient agar media

Nutrient agar powder weighing 15.5 g was suspended in 500 ml of cold distilled water, the mixture was stirred and boiled to dissolve.

For plates

The media was sterilized by autoclaving at 121°C (15psi) for 15 minutes. After autoclaving the liquid agar was cooled to a temperature range of 44°C to 47°C and poured aseptically into sterile plates (90mm diameter). The agar was allowed to cool and solidify at room temperature. Labeled plates with nutrient agar were stored at 4°C. A single plate was examined for sterility by incubating at 35°C for 24 hours.

For agar slants
The media was dispensed in 10ml aliquots into universal bottles and then autoclaved. Molten agar was slanted and allowed to cool in the universal bottles in a sloping position.

Mannitol Salt Agar

60g of powder was suspended in 500ml of distilled water. A suspension was dissolved by stirring and boiling, and then sterilised in an autoclave. Labelled plates with Mannitol Salt agar were stored at 4°C. Plates were wrapped in plastic to prevent contamination.

Mueller Hinton Agar
Dehydrated medium weighing 9.5g was suspended in 250ml of distilled water. The mixture was brought to boil, with constant stirring until complete dissolution. Sterilization was done using an autoclave. Cooled Mueller Hinton agar was poured into sterile Petri dishes on a level, horizontal surface to give uniform depth. Prepared media was allowed to cool and solidify at room temperature. The final pH of the prepared Mueller Hinton agar was checked using a pH metre and it was 7.3 at 25°C. Labelled plates with Mueller Hinton agar were stored at 4°C. Sterility was checked by incubating a plate at 35°C for 24 hours.

Nutrient broth
Nutrient broth powder weighing 6.5g was dispensed in 500ml of distilled water. After mixing the solution, it was heated gently to dissolve. The broth was distributed into capped tubes in 9 ml aliquots and autoclaved. The broth was kept in a sterile dark room at a temperature range of 15-20°C.

Sterility proofing of extracts
After membrane filtration, extracts were tested for sterility, by streaking on freshly prepared sterile nutrient agar which was incubated for 24 hours at 37°C.

***S. aureus* as a test organism**
Using a sterile cotton swab, the bacterial culture was swabbed on the surface of pre-poured nutrient agar plates. Inoculated plates were inverted and incubated for 24 hours at 35°C. After incubation, colonies formed were used for confirmatory tests. Identification of *Staphylococcus aureus* was based on cultural, morphological and biochemical characterization.

Growth on mannitol salt agar
Bacterial cells were streaked on pre-poured MSA media, a selective medium with 7-9 % sodium chloride. After incubation, colonies of *S. aureus* were confirmed by a yellow colour and by turning the medium around the colony yellow due to the drop in pH around the colony.

Colony morphology on nutrient agar media
Nutrient agar was inoculated with a small amount of test culture using sterile loops. Inoculated plates were incubated at 37°C for 48 hours. Spherical, pinhead colonies which are convex with entire margins were observed on nutrient agar, after incubation period.

Gram stain
A few drops of distilled water were added onto a sterile glass slide. A loopfull of the bacterium was transferred and spread in circular motion over a small area of the slide. The smear was allowed to air dry. The microorganism was heat fixed by placing the bottom of the slide to heat for approximately 30 seconds without exposing. Forceps were used to hold the slide above the sink, the surface of the slide was flooded with crystal violet for 1 minute. Rinsing of the slide with distilled water was done for 5 seconds. Gram iodine solution was applied and allowed to act for 1 minute as a mordant. The slide was thoroughly rinsed with distilled water. Excess water was drained from the slide and the slide was blotted so that alcohol used for decolourization is not diluted. A few drops of 95 % ethanol were applied onto the slide for 10 seconds and washed off with tap water. The slide was drained to remove excess water. A few drops of safranin solution were used to counter stain the slide for 30 seconds, rinsed off with tap water, drained blotted out to dry with bibulous paper. The slide was read with oil immersion lens of the slide of the microscope at high power (x 1000).

Catalase test
A small amount of growth from the culture was placed onto a sterile microscope slide. A few drops of hydrogen peroxide were added onto the smear and mixed with a sterile toothpick. A negative result would be no bubbles or a few scattered.

Maintenance of pure cultures
Colonies confirmed to be *S. aureus* were sub-cultured on MSA media, three times to obtain pure cultures. Pure cultures were sub-cultured onto new agar slants, incubated at 37°C for 24 hours and stored in the refrigerator at 4°C. These cultures were frequently sub-cultured on new agar slants to maintain their viability.

Preparation of Turbidity standard for inoculum preparation
0.5 Mcfarland standard was prepared by adding 0.5ml of 0.048M $BaCl_2$ (1.17 % w/v $BaCl_2.2H_2O$) to 99.5 ml of 0.18M sulphuric acid (H_2SO_4) (1 % w/v) with constant stirring. A barium sulphate precipitate was checked for optical density using matched curvettes with 1 cm path and distilled water as a blank standard. A UV-Vis spectrophotometer was used to measure the absorbance at 625 nm. An absorbance of 0.1 was obtained which was in the accepted range of 0.08-

0.13. The suspension was distributed into tubes of the same size as those used for test inoculum's adjustment. Sealed tubes were stored in the dark at room temperature. The approximate cell density corresponding to 0.5 McFarland is 1×10^8 cfu/ml.

Physiological sterile saline preparation
Sodium chloride of 4.25g was dissolved completely in 1 litre of distilled water by heating. It was autoclaved and stored at ambient temperature in sterile containers with caps tightened to prevent evaporation.

Phytochemical analysis of plant extracts
The stembark extracts were evaluated for the presence of phytochemical compounds using standard methods (16). Phytochemical examination was carried out separately for all the extracts, and the procedure was done three times for confirmatory purpose.

Detection of steroids (Salwoski's test)
100mg of dry extracts were dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were added to form a lower layer. A reddish brown colour at the interface was indicative of the presence of steroidal ring.

Detection of Cardiac glycosides (Keller Killian' test)

100mg of dry extracts were dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric. A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of cardenolides.

Detection of Saponins (Froth Test)
Extracts were diluted with distilled water to 20ml which was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.

Detection of Resins
100mg of dry extracts were dissolved in ethanol and 5 ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5 ml of concentrated sulphuric acid was added. Bright purple colour produced indicated the presence of resins.

Detection of Phenols (Ferric chloride test)
100mg of dry extracts were dissolved in ethanol and 2 ml of distilled water followed by a few drops of 10 % aqueous ferric chloride solution were added. Ten percent aqueous ferric chloride solution was prepared by mixing 5ml of ferric chloride to 45 ml of distilled water. Formation of a blue-green colour indicated the presence of phenols.

Detection of Tannins (Ferric chloride test)
50mg of dry extracts were dissolved in 2ml of distilled water and mixed with 2 ml of ferric chloride. A blue-black precipitate indicated the presence of tannins.

Detection of Terpenoid (Liebermann-Burchard test)
2ml of chloroform and 1 ml of concentrated sulphuric acid were added to 1mg of the dry extract. A reddish-brown colour indicated the presence of terpenoid.

Detection of Glycosides
50 mg of dry extract dissolved in 1ml ethanol was mixed with 1 ml of water and then aqueous sodium hydroxide was added. A yellow colour observed indicated the presence of glycosides.

Detection of Flavonoids
200mg of dry extracts were dissolved in 10ml of ethanol and filtered. A few drops of HCl and magnesium ribbon were added to 2 ml of the filtrate. Pink tomato red colour indicated the presence of flavonoids.

Detection of Phlobatannins
100mg of dry extract dissolved in 2ml distilled water was boiled with 1 % aqueous HCl (1ml HCl mixed with 9ml of distilled water). Deposition of a red precipitation was taken as evidence for the presence of phlobatannins.

Acidic compound
50mg of dry extract was dissolved in 2ml of ethanol and Sodium bicarbonate solution added. A positive result is indicated by the production of effervescence. For a negative result, there was no effervescence.

Anthraquinone (Borntrager's Test)
About 200mg of the extract was placed in a dry test tube and 2 ml of chloroform added for 5 minutes. The extract was filtered and the filtrate was shaken with 2 ml of 10% ammonia solution. A pink violet or red colour shows the presence of anthraquinone.

Determination of antibacterial activity
Antimicrobial activity was performed by standard methods like the disk diffusion method on Mueller Hinton agar and MIC was calculated using dilution methods. Cells used for antibacterial assays are harvested at log phase while they are most active.

Preparation of inoculums

Direct colony suspension method was used to make a suspension of *S. aureus,* approximately corresponding to 1×10^8 cfu/ml for *S. aureus*. Three to four colonies from overnight grown (18 hours) were suspended in saline using a sterile loop. The turbidity standard was shaken vigorously before use, and used to make a visual comparison with the

density of the suspension against a white background with black lines. Density of the suspension was adjusted to 0.5 Mcfarland either by adding sterile saline. The standardised culture was used within 15 minutes of preparation for sensitivity tests.

Disk diffusion test

Antibacterial activities of the extracts were tested on Mueller-Hinton agar by disk diffusion method. Six Mueller Hinton agar plates were warmed to room temperature while inverted. Moisture was drained from the plates by inverting. To maintain sterile conditions this procedure was done in a laminar flow cabinet. A sterile cotton swab was added to the inoculum adjusted to the standard opacity, and excess fluid drained by pressing the swab on the walls of the tube. The inoculum was spread evenly over the entire surface by swabbing in three directions (15). Inoculated plates were allowed to dry for ten minutes before depositing the disks (17).

Sterile paper disc having a diameter of 10 mm, were impregnated with 1ml of each extract at a concentration of 200mg/ml and were allowed to dry for 15 minutes. After drying, paper disks were placed on the agar plate using sterile forceps. Five filter paper disks were placed on each plate and were placed at the same distance from each other and the edge, to prevent overlapping of inhibition zones. Sensitivity discs were pressed with forceps to make complete contacts with the surface of the medium. Plates were kept at room temperature for 30 minutes (pre-diffusion time), inverted and incubated at 37°C for 24 hours, in an aerobic atmosphere. A pair of divider was used to mark the diameter of the zone and a transparent ruler was used to measure the distance in mm. The experiment was repeated three times for each extract and the mean diameter was taken. The mean diameter zones produced were compared to the expected reference chart (18).

TABLE 1: ZONE SIZE INTERPRETIVE CHART.

		Zone size produced (mm)		
Test organism	**Disk content**	**Resistance**	**Intermediate**	**susceptible**
S. aureus	**1ml**	**≤ 20**	**21-28**	**>29**

Controls

Negative controls were set as follows; different absorbent paper discs were saturated each with a solvent only and another disk with 1 ml sterile saline. They were allowed to dry for 15 minutes at room temperature. The paper disks were later placed on the surface of an inoculated plate with standard density of bacteria and incubated at 37°C for 24 hours.

A positive control was set as follows; absorbent paper discs were saturated with an antibiotic (Ampicillin) at 500µg/ml. The paper disks were placed on the surface of inoculated petri plates. Plates were incubated at 37°C for 24 hours.

Dilution methods

Dilution susceptibility testing methods were used to determine the minimum concentration of plant extracts to inhibit growth of *S. aureus*. This was achieved by two fold dilution of plant extracts in either agar or broth media.

Determination of MIC using agar dilution

The reconstituted sterile plant extracts were serially diluted to obtain a concentration of 10^{-6} (3,25 mg/ml) to (200 mg/ml). Then 1ml of the extract was incorporated in 19 ml molten nutrient agar to give a final concentration range of 10 mg/ml to 0,16 mg/ml. Solidification was allowed in a laminar airflow. A standard loop was used to transfer 100µl (10^8 cfu/ml) of the inoculum. The test organisms were streaked in radial patterns on the agar plate and incubated at 37 °C for 48 hrs. A negative control was set by incorporating 1ml of solvent in molten media, and inoculated with the test organism. Plates with medium only were set as controls for sterility of the medium. A positive control was set with antibiotic (Ampicillin). The antibiotic was serially diluted from a concentration of (0.05mg/ml). Plates were evaluated for the presence or absence of bacterial growth, after incubation period. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of the test organism in the agar plate (7). The presence of one or two colonies was disregarded. All tests were carried out in triplicates. The procedure was repeated three times for each extract. Ampicillin was set as the positive control.

Determination of MIC using broth dilution method

The MIC values were determined by broth dilution assay. Sterile plant extracts were serially diluted (two-fold) to obtain a concentration range of 200mg/ml to 1.625mg/ml. Then, 0.1 ml of each concentration was added to 9ml of nutrient broth containing 0.1ml of standardized test organism of bacterial cells. Negative controls were equally set

up by using solvents and test organisms without extracts. Tubes with medium only were set as controls for sterility of the medium. Test tubes were evaluated for the presence or absence of visible turbidity in the broth after the incubation period. The lowest concentration (highest dilution) of the extract preventing appearance of turbidity (growth) was considered and recorded as the MIC (19). Ampicillin was used as a positive control.

Determination of MBC

From the tubes showing no visible sign of growth/turbidity in MIC, 0.1 ml of the sample was inoculated onto sterile nutrient agar using the streak plate method. The plates were then incubated at 37°C for 48 hours. The least concentration that did not show growth of the test organism was considered as the MBC (20). A plate with media only was set as a negative control to check the sterility of the media. The MBC for ampicillin were also determined.

Determination of the death rate of the bacterial isolate in the extract

Assay for the rate of killing of bacterial isolates by water extracts was determined using the Kelsey and Maurer method (21). This was done by mixing 0,5ml of (10^4 cfu/ml) test isolates to 4.5 ml of MBC (5 mg/ml) of the water extracts. These were held at in a water bath at 37 °C and the killing rate was determined over a period of three hours. Exactly, 0.1ml volume was withdrawn at 30 minutes intervals using a micropipette, and plated out on the surface of solidified nutrient agar containing 3 % tween 80, for viable count. The number of colonies formed on each plate at the time intervals, were counted using a digital colony counter. Incubation was done at 37°C for 48 hours. A control was set containing nutrient media and the test organism but without the extract. The test results were compared with that of the control. Emergent colonies were counted and compared with the count of a culture control. All the counts obtained, were the mean of triplicate tests.

Statistical analysis

Results were expressed as mean ± standard errors and the comparison of the antibacterial activity of the samples with standard antibiotics was evaluated by applying one way analysis of variants.

RESULTS

The extraction efficient ranged from 14.5 % to 0.92 % for the most efficient solvent, water and the least efficient extract, hexane. The colour and state of the final extracts were also observed, refer to fig 1. Results are recorded in table 1.

TABLE 2: MEAN PERCENTAGES OBTAINED AFTER USING DIFFERENT SOLVENTS IN EXTRACTION OF COMPOUNDS FROM THE STEM BARK OF *MANGIFERA INDICA* .

Solvent used in	Weight of starting material	Mean percentage of extracts (w/w)	Standard errors
Water	50	13.67	± 0.33
Methanol	50	10.33	± 0.33
Ethylacetate	50	12,33	± 0.33
Hexane	50	0,009	± 0.00

There was a significant different between the mean percentages obtained after using different solvents. The highest mean percentage was obtained using water extracts. The least percentage was obtained in hexane extracts.

FIGURE 1: COLOURS OF DIFFERENT EXTRACTS OBSERVED AFTER 72 HOURS OF SOAKING PLANT MATERIAL IN DIFFERENT SOLVENTS.

Ethyl acetate extracts showed a black colour, after vaporisation of solvent, a black-solid extracts was obtained. Methanol extracts had a brownish- blackish colour, the extract was solid after vaporisation of solvent. Water extracts were black in colour; a solid extract was obtained after vaporisation. Hexane extracts were yellowish in colour and a gummy extracts was obtained.

A range of phytochemicals were confirmed to be present in stembark extracts of *M. indica*. Results from ethyl acetate and methanol extracts confirmed the presence of all the phytochemicals tested. Phytochemical compounds were almost common in stem-bark extracts. However acidic compounds and anthraquinone were not present in water extracts. Results from phytochecimal analysis are shown in table 2.

TABLE 3: PHYTOCHEMICAL ANALYSIS OF *MANGIFERA INDICA* STEM-BARK EXTRACTS.

Phytochemical Constituency	Water extract	Methanol extract	Ethylacetate extract	Heaxane extracts
Steroids	+	+	+	+
Cardiac glycosides	+	+	+	+
Saponins	+	+	+	+
Resins	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Terpenoid	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phlobatannins	+	+	+	+
Acidic compound	-	+	+	+
Anthraquinone	-	+	+	-

Key : + = present - = absent

The result revealed that the extracts of *M. indica* possess good antibacterial activity against *S. aureus*. Stem-bark extracts inhibited the growth of *S. aureus* and the inhibition zones ranged from 10mm to 25mm. The extracts were less potent than the antibiotic. Negative Controls showed that solvents without extracts had no inhibitory effect on bacterial growth. The mean inhibition zone

diameter of the different extracts to *S. aureus* are shown in table 4.

TABLE 4: ACTIVITY OF CRUDE EXTRACTS FROM THE STEM BARK EXTRACTS OF *MANGIFERA INDICA* ON *S. AUREUS* AT A CONCENTRATION OF 200MG/ML.

	Disk Content				
					Positive Control
(Plant extracts)	H_2O	Meth	Ethyl	Hex	Amp
Mean zone of inhibition	14	25	22	10	29
Standard errors	± 1.00	± 1.15	± 1.0	± 1.52	± 0.577

Key :

H_2O = water Meth = methanol Ethyl = ethyl acetate Hex = Hexane Amp = Ampicillin (Amp)

There was a significant difference in mean zone of inhibition between ampicillin (control) and plant extracts ($P < 0.05$). There was no significant difference between methanol and ethyl acetate extracts ($P = 0.82$). Negative controls: There was no zone of inhibition around disks submerged in solvents only. There was no zone of inhibition around disks soaked in sterile saline solution only.To determine the extent of antibacterial of antibacterial activity, the extracts were subjected to MIC assay by serial two-fold dilution method of extracts and then dilution methods for inhibitory concentration assays.

TABLE 5 : RESULTS FROM AGAR DILUTION METHOD ON MINIMUM INHIBITORY CONCENTRATION (MIC) OF STEM-BARK EXTRACTS ON *S.AUREUS*. VALUES GIVEN ARE BASED ON FINAL CONCENTRATION OF EXTRACT IN MEDIA.

	Control				
Plant extracts	H_2O	Meth	Ethyl	Hex	Amp
Mean conc	2.08	0.62	1.04	4.17	0.03
Standard Errors	± 0.417	± 0.00	± 0.20	± 0.833	± 0.00

Key; H_2O - water , Meth – methanol, Ethyl – ethylacetate, Hex – Hexane, Mean conc - mean concentration,

Amp – ampicilin.

There was no significant difference between ampicillin , methanol and ethylacete extracts ($P > 0.05$). There was a significant difference between ampicillin and water, hexane extracts ($P > 0.05$). There was no significant difference between water and ethyl acetate extracts ($P = 0.115$).

TABLE 6: MINIMUM INHIBITORY CONCENTRATION (MIC) OF *M. INDICA* STEM-BARK EXTRACTS USING BROTH DILUTION METHOD.

Control					
Plant extracts	**H_2O**	**Meth**	**Ethyl**	**Hex**	**Amp**
Mean Conce	**1.25**	**0.16**	**0.27**	**1.04**	**0.001**
Standard Errors	**± 0.00**	**± 0.00**	**± 0.54**	**± 0.208**	**± 0.001**

TABLE 7: MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF CRUDE EXTRACTS OF *M. INDICA* ON *S. AUREUS*.

Plant extracts	**H_2O**	**Meth**	**Ethyl**	**Hex**	**Amp**
Concentration (mg/ml)	**5**	**0.3125**	**0.625**	**10**	**0.0125**
Standard errors	**±0.417**	**± 0.000**	**± 0.208**	**± 0.83**	**± 0.00**

Key : H_2O = water Meth = methanol Ethyl = ethyl acetate Hex = hexane

Negative Control : There was no growth in a plate which was set for sterility test.

TABLE 8. RESULTS SHOWING THE NUMBER OF COLONIES OBTAINED ON PLATE COUNT.

Time interval (minutes)		**0**	**30**	**60**	**90**	**120**	**150**	**180**
Average No of colonies	**Test**	**96**	**100**	**17**	**13**	**4**	**2**	**0**
	Control	**98**	**157**	**179**	**182**	**180**	**183**	**185**

Key :

Test = experimental tube with water extracts Control = experimental tube with bacteria only.

DISCUSSION

The basic parameters influencing the quality of an extract are plant parts used as a starting material, the solvent used for extraction, the extraction technology and sterilisation method (7). These findings on extraction potential of the different solvents are consistant with previous investigation, in which the percentage yield of water extract was higher than that of hexane extracts (22). The observed differences in the extract yields of different solvents might be ascribed to the fact that the extract has different solubility or to the polarity of the solvent. Different extractable components were present in different quantities within the extract. The difference in ethyl acetate and methanol solvents compared with hexane extracts may be due to the fact that plant compounds such as phenolics are often extracted in higher amounts in more polar solvents than in non-polar.

Phytochemical analysis conducted on *M. indica* extracts revealed the presence of tannins, flavonoids, steroids, saponins, glycosides and resins among others. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and are thought to be responsible for coagulating the wall proteins of pathogenic organisms. Thus, *M. indica* containing this compound may serve as a potential source of bioactive compounds in the treatment of infectious diseases such as pneumonia. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase (23). They also serve as health promoting compounds as a result of their anion radicals (24). These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds. Alkaloids were also detected and their common biological property is cytotoxicity (25).

The stem-bark extracts of *M. indica* had significant antibacterial potency against the test organism. This result may suggest that all extracts possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs, for therapy of infectious diseases in human. Ethyl acetate and methanol extracts had an inhibition zone diameter of 22 mm and 25 mm respectively, which is close to a standard antibiotic, hence we suggest their effectiveness as antimicrobials.

The active components in the crude extract may be acting synergistically to produce antimicrobial effects (22), the disparity between the activities of the extracts and the standard antimicrobial drug , may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotic (26). Thus a standard drug had the highest zone of inhibition of 29mm. Methanol and ethyl acetate are polar solvents, since they showed the highest antibacterial activities. We could suggest that some of the principal antibacterial components of this plant were polar compounds. Most of the identified components with antimicrobial activity extracted from plants are aromatic or saturated organic compounds which are more soluble in polar solvents such as water and methanol. However water extracts were less potent. This can be attributed to the presence of water-soluble compounds such as polysaccharides and polypeptides, which are commonly more effective as inhibitors of pathogen adsorption and have no real impact as antimicrobial agents (7). The antibacterial activity demonstrated by water extract provides the scientific bases for the use of water extracts in traditional treatment of diseases.

There are also reports in literature that methanol is a better solvent for consistent extraction of antimicrobial substances for medicinal plants (22). This may be attributed to two reason, firstly, the nature and potentiality of biologically active components (alkaloids, flavonoids, essential oils biterpenoids), which could be enhanced in the presence of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number or amount of active constituents responsible for antibacterial activity (27). This is also proved in our study in which methanol extracts exhibited the highest antibacterial activity against *S. aureus* compared to other extracts.

In another study, (27) found out that methanolic extracts had the least antibacterial activity against *S. aureus* using *N. oleander* plant materials. Thus besides choice of a good solvent for extraction of active compounds, antibacterial activity also depends on phyto-constituents present in the plant. The contents of active ingredients in plant materials have been shown to fluctuate constantly with the genetic heterogeneity of a

plant species, differences in soil condition, variation in seasonal cycle, climatic influences, age of plant, alteration in weather, sun and shade fluctuations. Hexane is a non-polar solvent and since the n-hexane extracts showed the least antibacterial activity, this could suggest that a small quantity of the antibacterial component of this plant was lipid soluble. Hexane is the most widely used solvent to extract edible oils from medicinal plants, however its use is decreasing due to its toxicity.

The MIC results showed that the activities of different extracts were concentration dependent. MIC values obtained from agar dilution were higher than broth values. Major differences between the agar dilution and broth dilution was seen for methanol, ethyl acetate and hexane extracts , where MIC values from broth dilution were almost four times lower than MIC values in agar dilution.

Similar differences were reported (28) when comparing the broth dilution with the standard agar dilution method on *Mycobacterium avium* complex strains. Some studies (29) found that the MIC determined by the broth dilution was four to eight times lower than those found by the agar plate method. This trend has been observed by several researchers using antibiotics (29). The differences may be caused as suggested by factors such as higher absorption and degradation of drug in solid, agar medium. Furthermore on solid medium bacteria grow on the surface and a concentration gradient could develop during incubation and growth of bacteria leading to altered or pseudo resistance. In contrast in liquid, broth medium, there are more cell to antimicrobial compound contact as the bacteria is sub-merged in the plant extracts-containing medium.

MBC was determined using tubes showing no turbidity. Results obtained showed that MIC values were lower than MBC values, suggesting that the extracts were bacteriostatic at lower concentration. At higher concentration the extracts were bactericidal as shown in table 4.6. As shown clearly in table 6 and 7 the MBC results varied considerably from the MIC. Low MIC and MBC values obtained of methanol and ethylacetate extracts, is an indication that *M. indica* stem-bark extracts are effective as antimicrobials. However, a high MIC and MBC values for hexane and water extracts, is an indication that they are less effective against *S. aureus* as antibiotics and the bacteria has the potential of developing resistance against the extracts.

From the study of the kinetics of disinfection, it was clear that the number of colony forming units were reduced to zero within the first 180 minutes of exposure to plant extracts. The reduction in bacterial cell number to zero shown in table 8 confirms that water extracts are bactericidal at this concentration. In the first 30 minutes the extracts cell numbers were almost constant, a significant decrease in cell number occurred during the first hour. The mechanism by which plant extracts inhibit bacterial cells is not well understood. The extracts were shown to have effect on bacterial growth after thirty minutes, thus we suggest that the extracts were being absorbed and possibly inhibit cell growth by acting inside bacterial cells. The findings suggest the possibility of using water extracts at bactericidal concentration as a disinfectant, and the time to disinfect bacteria cells (10^4) was determined to be 180 minutes at a concentration of 5 mg/ml.

CONCLUSIONS

The stem-bark extracts were found to have antibacterial activity against *S. aureus*. The stem bark extracts of *M .indica* contains several phyto-compounds. There is need for lead compounds from the plant extracts to be isolated so that they can serve as templates for the production of new antibiotics.

RECOMMENTATIONS

Further research on the antibacterial activities of stembark extracts of *Mangifera indica* should be done using different strains of bacteria. This is important to establish whether the extracts have a broad spectrum activity. Research should also be done on isolation and identification of the main active compounds in the stem-bark of *M. indica*. Moreover, further studies should be done on the bactericidal effect of the plant extracts.

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