

ANTIBACTERIAL ACTIVITY OF *Mangifera Indica* LEAVES ON MULTIDRUG RESISTANT CLINICAL ISOLATES

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Abstract

Antibacterial activity of methanolic, ethanolic and hot water extracts of *Mangifera indica* leaves was investigated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*. The assay was carried out using the agar well diffusion method with varying concentrations (5, 25, 50, 75, 100, 150 mg/ml of the extracts and the minimum inhibitory concentration was determined by broth dilution method. The results obtained showed that the ethanolic extracts of *M. indica* leaf had higher antibacterial activities than the methanolic and aqueous extracts. All bacteria used in this study were susceptible to ethanolic extract of *M. indica* leaf but none was susceptible to the aqueous extract. The methanolic extract was also effective against the organisms with its greatest activity on *P. aeruginosa* at a concentration of 150 mg/ml. Minimum inhibitory concentration (MIC) of the ethanol extract of *M. indica* leaf was obtained at a concentration of 75 mg/ml for *Salmonella typhi*, 100 mg/ml for *Escherichia coli* and 200 mg/ml for *Staphylococcus aureus*. No cidal effect was observed for the extracts against any of the test organisms. This study has shown that *M. indica* leaves possess phytochemicals with pharmacological properties and justifies its use in Nigerian traditional medicine.

Introduction

Plants have been a valuable source of natural products for maintaining well-being in the last decade, with more increasing studies for natural therapies. Medicinal plants might be the best source to obtain a variety of drugs. According to WHO, a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Junaid et al., 2006). The primary benefits of using plant-derived medicines are that

they are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. Many of the plant materials used in traditional medicines are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine in this part of the world although the use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (Sharif and Banik, 2006, Kubmarawa *et al.*, 2007). Ethnobotanical surveys carried out in Nigeria and elsewhere in the world have reported a number of plants that are used in the treatment of infectious diseases, including *Mangifera indica* (Bukonya *et al.*, 2003; Mustapha *et al.*, 2013). *Mangifera indica* have been used traditionally to treat diseases. The leaves decoction is taken as a remedy for fever, chest complaints, diabetes and other illnesses (Aderibigbe *et al.*, 2001). Investigations on the biological activities of *Mangifera indica* showed that these plants have a potential to be used as antioxidant agents (Khan *et al.*, 2003). This objective of this study is to test for the presence of phytochemicals in extracts of *M. indica* leaves, evaluate the antibacterial activity of leaf extracts against selected clinical pathogens and assess the bacteriostatic/ bactericidal efficacy of the extracts.

Materials and Methods

Collection of Plant Materials

The fresh leaves of *Mangifera indica* were collected in a clean polythene bag at Tanke, Ilorin, Kwara State. It was thereafter taken to the Plant Biology Department of the University of Ilorin for identification and authentication.

Sources and Maintenance of Test Organisms

Bacterial isolates were obtained from Wesley Guild Hospital, Ilesha, Osun State and the Medical Laboratory of the Microbiology and Parasitology unit of the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara State. The test organisms were inoculated on nutrient agar slant and stored in a working refrigerator at 4 °C as a stock culture. Bacteria were subcultured onto fresh media at intervals to maintain the viability of the test organisms. The bacterial isolates collected were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*.

Preparation of Extract

The leaves were properly washed with distilled water and air dried. The dried leaves were then blended using a household electrical blender into fine powder. The leaf powder was stored sealed in five labeled reagent bottles for further use. The total weight of the powdered plant sample was taken and then stored until it is required.

Extraction

One hundred gram (100g) of the fine powder of the grounded *Mangifera indica* leaves was immersed in 500ml of ethanol, methanol and hot water respectively to get a concentration of 200mg/ml. The extraction was done by subjecting it to agitation on a rotator shaker at 200 revolution per minute (rpm) for 3days. The resulting suspension was then filtered using muslin cloth and Whatman No.1 filter paper and the filtrate was allowed to evaporate to dryness at 45°C in a water bath

Phytochemical Screening

The leaves extract of *Mangifera indica* (Edward variety) were analyzed for the presence of alkaloid, saponins, anthraquinone, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides according to standard methods (Odebiyi and Sofowora, 1978; Sofowora, 1982).

Screening for Alkaloids: Three gram of extract was stirred with ethanol containing 3% tartaric acid. The filtrate was shared into 3 beakers and tested for alkaloids as follows: into the first beaker, Hagar's reagent was added, into the second beaker, Mayer's reagent was added and into third beaker, Marquin's reagent was added. Precipitation in any of the 3 test indicates the presence of alkaloids.

Screening for saponin: About 0.5g of the plant extract was shaken with water in a test tube. Frothing, which persist on warming was taking as a preliminary evidence for the presence of saponin. Few drops of olive oils was added to 0.5g of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicates the presence of saponin (Odebiyi and Sofowora, 1978).

Screening for tannin: Water extract of the sample was treated with 15% ferric chloride test solution. The resultant color was noted. A blue color indicated the presence of hydrolysable tannin. Or into 10ml of freshly prepared potassium hydroxide (KOH) in a beaker, 0.5g of the extract was added and shaken to dissolve. A dirty precipitate observed indicates the presence of tannin (Odebiyi and Sofowora, 1978; Sofowora, 1982).

Screening for steroids (Salkowski's test): About 100mg of *Mangifera indica* extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interface is indicative of the presence of steroidal ring (Sofowora, 1982).

Screening for flavonoid: About 2g of the powered leaves was completely detained with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered while, still hot. The filtrate was cooled and used. Five millimeters of 20% NaOH was added to equal volume of the detained water extract. A yellow solution indicates the presence of flavonoid.

Screening for anthraquinone (Borntrager's test): About 0.5g of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5min. the extract was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone.

Screening for cardiac glycosides (Keller Killiani's test): About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayer with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of cardenolides.

Screening for reducing sugar (in glycosides): The residue was re-dissolved in water in the water bath. Two millimeters of the solution in a test tube was added 1ml of Fehling's solution A and B. the mixture was shaken and heated in a water bath for 10min. A brick-red precipitate indicates a reducing sugar.

Antimicrobial Assay of *Mangifera indica* Leaf Using Agar Well Diffusion

The antimicrobial screening was carried out using the agar well diffusion method as described by Lino and Deogracious (2006) with slight modifications. The test bacteria were first inoculated into tubes of nutrient broth separately and incubated at 37°C for 18 hours. Each of the cultures was then adjusted to 0.5 McFarland turbidity standards and inoculated (0.2 ml each) onto Muller Hinton agar (MHA, Oxoid) plates . A sterile cork borer was then used to make seven wells (6 mm diameter) for different concentrations of the extract on each of the plates containing cultures of the different test organisms. The dried aqueous and organic extracts were separately redissolved in sterile glycerol at concentrations of 50,100, 150, 200 and 250mg/ml and 0.5 ml of each of the extracts was then introduced into the wells using sterile Pasteur pipettes. The culture plates were allowed to stand on the working bench for 30 minutes for pre diffusion (for the extract to diffuse into the agar) and were then incubated at 37°C for 24 hours. After 24 hours, antimicrobial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around each of the extracts (Lino and Deogracious, 2006).

Antibiotic Susceptibility Testing

Each of the organisms was inoculated into sterile nutrient broth and the media were incubated for 24 hours. Pour plate method was adopted for the inoculation of organisms. Positive antibiotic disc was pressed down on the media containing *Staphylococcus aureus* and the negative discs for *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*. The plates were incubated 37 °C for 24 hours. Zones of inhibition around each disc were measured. The antibiotics used and their corresponding concentrations are as follows:

For Gram negative: Ceftazidime (Caz) 30µg, Cefuroxime (Crx) 30µg, Gentamicin (Gen) 10µg, Ceftriaxone (Ctr) 30µg, Erythromycin (Ery) 5µg, Cloxacilin (Cxc) 5µg, Ofloxacin (Ofi) 5µg, Augmentin (Aug) 30µg.

For Gram positive: Amoxicillin (Amx) 25µg, Cotrimoxazole (Cot) 25µg, Nitrofurantoin (Nit) 300µg, Gentamicin (Gen) 10µg, Nalidix acid (Nal) 30µg, Ofloxacin (Ofi) 30µg,

Augmentin (Aug) 30µg, Tetracycline (Tet) 30µg.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Only the organisms that were found susceptible to the extracts of *Mangifera indica* were inoculated into sterile nutrient broth and incubated at 32 °C for about 16 hours. 1ml of each of the varying concentrations of extracts of *M. indica* that were found potent (150mg/ml, 100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 5mg/ml) were thereafter inoculated with 0.3ml of the overnight broth culture of the test organisms. A negative control was setup to contain only nutrient broth and the extract. The inoculated and control tubes were then incubated at their proper growth temperatures for 16 hours for bacterial cultures. The tubes were observed for turbidity after incubation. The lowest concentration that shows no turbidity (indicating no growth of the test organism) was taken as the MIC (Adetunji *et al.*, 2013). The minimum bactericidal concentration (MBC) of the plant extract on the clinical bacterial isolates was carried out according to Ajaiyeoba *et al.*, (2003). Briefly, 1 ml bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and subcultured on to MHA and incubated at 37°C for 24 hours. After incubation the concentration at which there was no single colony of bacteria was taken as MBC (Ajaiyeoba *et al.*, 2003).

Results

The pH value results of methanolic, hot water and ethanolic extracts in this study as shown in Figure 1 are 3.9, 2.9 and 3.8 respectively indicating all extracts to be acidic. Table 1 shows the phytochemical screening of leaf extract of *Mangifera indica* indicating the presence of saponin, tannin, glycosides, alkaloids, anthraquinone and flavonoids in the ethanolic extract. The methanolic extract shows the presence of tannin, glycosides, alkaloids, anthraquinone and flavonoid except saponin while in hot water extract all the phytochemical constituents are present as in ethanolic extract except alkaloids. Table 2 shows the results of preliminary screening of methanolic, hot water and ethanolic extracts of *Mangifera indica* leaves. The result indicates that none of the extracts shows effect on *E. coli* and *Klebsiella pneumoniae*. Hot water and methanolic extracts of the leaves have no effect on *S. aureus* and *Pseudomonas aeruginosa* except the ethanolic extract. *Salmonella typhi* shows zone of inhibition of 11mm and 10mm on hot water and ethanolic extracts respectively except methanolic

extract with no inhibition. In Figure 2, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* showed sensitivity to varying concentrations of the extract. *Staphylococcus aureus* was the most susceptible organism with sensitivity to all concentrations of the Ethanolic extract, while *Klebsiella pneumoniae* is resistant.

According to Figure 3, *Pseudomonas aeruginosa* showed sensitivity to varying concentrations of the extract and it's the most susceptible organism with sensitivity to all concentrations of the methanolic extract, while *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* were resistant.

Table 3 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of the different leaf extract (ethanol, methanol and hot water) of *M. indica* on the clinical isolates. There is no Minimum Bactericidal Concentration (MBC) because there was bacterial growth in the entire surface of the agar medium. Generally the ethanolic extract showed greater antibacterial activity compared to the methanolic extract while Hot water extract displayed no antibacterial activity as it was unable to inhibit the clinical isolates at all concentrations. The ethanolic extract concentration that were used and found to inhibit *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* were 200, 75, 100mg/ml.

Table 4 shows the Antimicrobial activity of standard Gram positive antibiotic disk and Gram negative antibiotic disk on the test organisms.

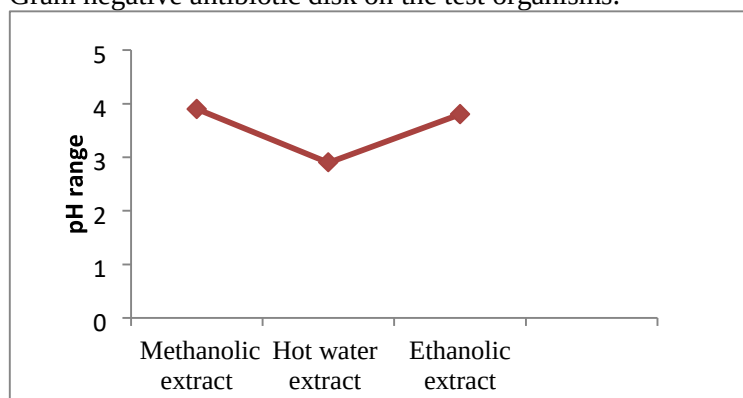


Figure 1: pH value of extracts of *Mangifera indica*

Table 1: Phytochemical Screening of The Leaf Extracts of *M. Indica*

Phytochemical parameters	Methanolic extract	Ethanolic Extract	Hot water extract
Saponin	-	+	+
Tannin	+	+	+
Glycosides	+	+	+
Alkaloids	+	+	-
Anthraquinone	+	+	+
Flavonoids	+	+	+
(-):Absent (+):Present			

Table 2: Preliminary Screening of Leaf Extracts of *Mangifera indica*

S/N	Organisms	Hot water extract	Ethanolic extract	Methanolic extract
1	<i>Staphylococcus aureus</i>	0.0	15	0.0
2	<i>Salmonella typhi</i>	11	10	0.0

3	<i>Pseudomonas aeruginosa</i>	0.0	15	0.0
4	<i>Escherichia coli</i>	0.0	0.0	0.0
5	<i>Klebsiella pneumoniae</i>	0.0	0.0	0.0

Values represent the diameter of zone of inhibition in mm

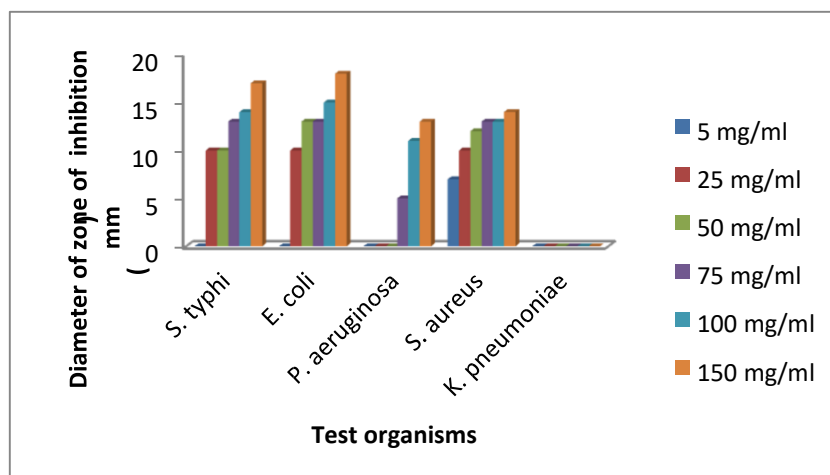


Figure 2: Effect of varying concentrations of the Ethanolic leaf extract of *M. indica*.

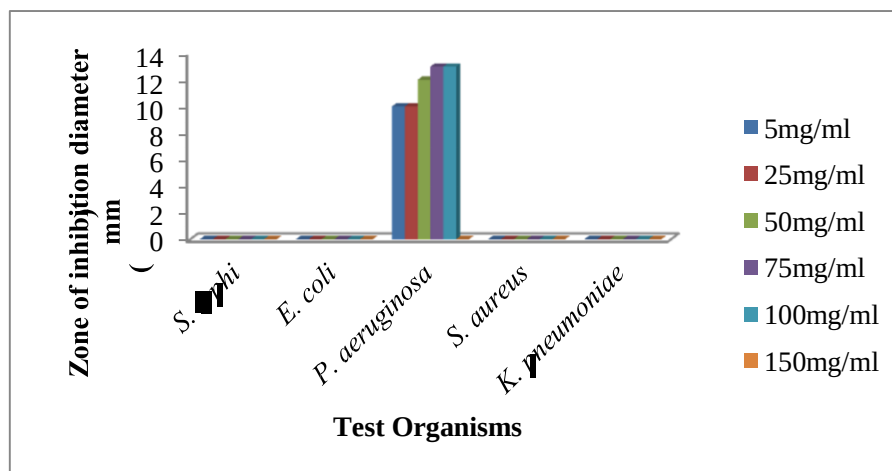


Figure 3: Effect of varying concentrations of the Methanolic leaf extract of *M. indica*.

Table 3: Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal of the leaf extracts of *Mangifera indica*

SN	Organisms	MIC (mg/ml)			MBC (mg/ml)		
		Ethanollic	Methanolic	HWE	Ethanollic	Methanolic	HWE
1	<i>S. aureus</i>	200	0.0	0.0	0.0	0.0	0.0
2	<i>S. typhi</i>	75	0.0	0.0	0.0	0.0	0.0
3	<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0	0.0	0.0
4	<i>K. pneumoniae</i>	0.0	0.0	0.0	0.0	0.0	0.0
5	<i>E. coli</i>	100	0.0	0.0	0.0	0.0	0.0

HWE: Hot water extract.

Table 4: Antimicrobial Activity of standard antibiotic discs on the test organisms

	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella Typhi</i>
Ceftazidime	0.0	0.0	0.0	0.0
Cefuroxime	0.0	0.0	0.0	0.0
Gentamycin	14.0	0.0	0.0	23.0
Ceftriaxone	0.0	0.0	0.0	0.0
Erythromycin	23.5	0.0	0.0	26.5
Cloxacillin	0.0	0.0	0.0	0.0
Ofloxacin	17.5	20.5	0.0	29.5
Augmentin	0.0	0.0	0.0	0.0

Discussion

Mangifera indica has been used in most rural communities for the treatment of a handful of infectious diseases due to its apparent medicinal properties that stands it out from other herbal plants. In this study, we evaluated as a preliminary examination the possible antibacterial activity of *M. indica* leaves as a way to give credence to its wide usage in most rural communities. Figure 1 and 2 presents the results of the effect of varying concentration of the ethanolic and methanolic leaf extract of *M. indica* on clinically important bacterial pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella typhi*. It can be inferred from the results that both the ethanol and methanol leaf extracts of *M. indica* had a broader spectrum of activity against the test isolates. However, better antimicrobial activity was recorded with the ethanolic extracts at different zones of inhibition. It could therefore be deduced from this study that ethanol is a better extractor of the bioactive constituents of the *M. indica* leaves than the methanol solvents used. The hot water extract did not exert antimicrobial effect on the isolates due to failure of the active ingredient to dissolve in it.

Antibiotics	Zones of Inhibition (mm)
	<i>Staphylococcus aureus</i>
Amoxicillin	0.0
Cotrimoxazole	0.0
Nitrofurantoin	0.0
Gentamicin	0.0
Nalidixic acid	0.0
Ofloxacin	33.5
Augmentin	0.0
Tetracycline	0.0

The result of this work showed that Methanol extract of *M. indica* had activity on *Pseudomonas aeruginosa* (16 mm). Ethanolic extracts of *M. indica* also showed varying levels of activity on *S. aureus* (14 mm), *S. typhi* (17mm), *E. coli* (18mm), and *P. aeruginosa* (13mm) while *K. pneumoniae* did not inhibit any growth. The notable antimicrobial activities of *M. indica* on bacterial pathogens have been previously reported and these are also in line with the results obtained in this study (Aderibigbe *et al.*, 1999; Aderibigbe *et al.*, 2001; Garrido *et al.*, 2001; Khan *et al.*, 2003; Amrita *et al.*, 2009). The MIC of the ethanol extracts of *M. indica* on the test isolates was determined at 75mg/ml for *Salmonella typhi*, 100mg/ml for *Escherichia coli* and 200mg/ml for *Staphylococcus aureus*. Extract of *M. indica* has been found in this study to be acidic with a pH value 2.9, 3.8, and 3.9 for hot water, ethanol and methanol respectively. The antimicrobial activity of *M. indica* leaf extracts which obviously tells their bioactivity on the test isolates is encouraging owing to the fact that these organisms are of hospital origin.

In the Antimicrobial Susceptibility Test using the standard antibiotics the Gram negative clinical isolates were more susceptible compared to the susceptibility of the Gram positive organism. *Staphylococcus aureus* was susceptible only to Ofloxacin with a zone of inhibition (33.5 mm) and resistant to the other antibiotics. Antibiotics works in three forms in mode of action, range of activity and its chemical components. Ofloxacin is a broad spectrum antibiotic which is used against both Gram negative and Gram positive organisms. The resistance of *Staphylococcus aureus* could be due to it multiple drug resistance and also the mode of action of the antibiotics against the organism. The Gram negative organisms showed susceptibility to one or more antibiotics except *Pseudomonas aeruginosa* which was resistant to all the antibiotics. The resistance of *Pseudomonas aeruginosa* and the other Gram negative isolate used in this study could be due to the cell wall of Gram negative organisms which is complex having inner and outer membranes. However with the use the plant extracts, *Staphylococcus aureus* was the most susceptible at lower concentration which gives the use of medicinal plants more advantage as antimicrobial agents

The ability of the crude extracts of *M. indica* leaf to inhibit the growth of pathogenic bacteria (as obtainable in this study) is an indication that *M. indica* is a medicinal plant and could serve as a source of lead bioactive compounds for the development of potent antimicrobial agents. This further validates its use in traditional herbal medicine in most rural Nigerian communities to treat a variety of bacterial related infections including those associated with respiratory tract infections.

Conclusion

This study has shown that the ethanolic and methanolic extracts of *M. indica* leaf possess antimicrobial activities. *Mangifera indica* contain bioactive compounds that

are effective against the test organisms, thus the crude extracts could be used directly for chemotherapy or treatment of infections caused by these organisms. Further research and characterization is therefore required to identify the specific phytochemicals responsible for the activity and also the quantitation of the concentration at which cidal effects can be exerted on the test organisms

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