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In vitro antibacterial activity of crude leaf extracts of *Mangifera indica* Linn

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The active components of leaves of *Mangifera indica* L. were extracted using cold water and organic solvents (acetone and methanol) and were tested against *Staphylococcus aureus, Streptococcus pyogenase, Streptococcus pneumoniae, Bacillus cereus, Escherichia coli, Pseudomonas aerugenosa, Proteus mirabilis, Salmonella typhi* and *Shigella flexnerri* using the agar well (cup plate) diffusion method. Both the acetone and methanol extracts inhibited the growth of gram positive bacteria, with acetone extract exerting more activities on all the gram positive bacteria with zone of inhibition between 15 - 16 mm, and a gram negative bacterium S. typhi (14 mm) at 250 mg/ml. Whereas, water extract was not active on any of the bacterial pathogens tested at any of the concentration of the extract used. The activities of the plant extracts on the inhibited pathogens using the zone of inhibition were not as effective as the standard commercial antibacterial disks of gentamicin and erythromycin ($t = 2.23$, $p < 0.05$). Increased temperature (60 and 100°C for 1 h) had a multiplier effect on the activity of the extracts, but alkaline pH decreased the activity. Preliminary phytochemical analysis revealed the presence of tannins, glycosides, saponins and phenols. The MIC and MBC of the extracts was in the range of 12.5 - 75 and 25 - 175 mg/ml respectively. There is a basis for the traditional use of the plant as a local health remedy.

Key words: Antibacterial activity, MIC, MBC, gram-positive bacteria, gram-negative bacteria, *Mangifera indica,* extract.

INTRODUCTION

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). The advent of science into the search for antibiotics largely depends on some of these medicinal plants as raw materials. For many years, medicine had depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants. 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). At present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly form plants and in homeopathic or ayurvedic medicines, medicinal plants, their parts and extracts dominate the scenes. The commercial value of various innumerable drugs and pharmaceuticals derived from tropical forest systems on worldwide basis is projected at 20 billion dollars a year (Sharif and Banik, 2006). Infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and governments all over the world (accounting for over 50, 000 deaths every day), especially with the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics (Franklin and Snow, 1989; Prescott et al., 2002). It is therefore very necessary that the search for newer antibiotic sources be a continued process. Plants are the cheapest and safer alternative sources of antimicrobials (Pretorious and Watt, 2001; Sharif and Banik, 2006; Doughari et al., 2007). *Mangifera indica* L. (Family: Anacardiaceae) is commonly called *mango* (English), *manako* (Hawai'i), *manggo'am* (Fiji), *tharyetthi* (Myanmar), *mangot*, *mangue or manguier*

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(French), *aam*, *am* or *amb* (Hindi), *bobbie manja*, *kanjanna manja*, *magg*, *manggaboom* or *manja* (Dutch), *mamung* (Thailand), *manga* or *mango* (Spanish), *manga* (Portuguese), *manga*, *mempelam* or *ampelam* (Malaysia), *mangga* or *mempelamn* (Indonesia), *Mango-baum* (German), *paho* (Philippines) and *xoài*(Vietnam), *mongoro* (Yoruba, Nigeria), mangolo (Igbo, Nigeria) and mangoro (Hausa, Nigeria) (Emeruwa, 1991). The genus *Mangifera* originates from the Asia, and is found greatly cultivated in Sumatra, Java, Borneo, Peninsula, Malay, India and Myanmar and practically in every tropical and sub tropical country with India having the largest area for its cultivation (Emeruwa, 1991). Other common species of this genus include *Mangifera gedebe, Mangifera minor*, and *Mangifera mucronulata*. The tree is large and tall (up to 40 m) with a rounded canopy or foliage with leathery leaves and big fleshy edible drupes as fruit (Neon, 1984). The fruits are eaten, and used for juice and wine production. Traditionally the mango plant has medicinal applications. In Côte d'Ivoire the leaf-decoction is used as a febrifuge. The bark infusion has been used as gargle to treat mouth infections in children (Tonga). In India and Nigeria, the infusion of the leaves singly or combined with leaves of *Citrus sinensis* is used in treating diarrhea, dysentery, gastrointestinal tract dis-orders, typhoid fever, sore throat and scurvy (Fowler, 2006; Ledesma et al., 2002; Owuor et al., 2002). The infusion is also drunk as tea for treating stroke and as a relief from pains and exhaustion (India). The fruits have also been eaten with salt and honey as source of vitamin A and for the treatment of blood disorders. Infusion of the ground seeds have been used as remedy for diabetes. Sap from the leaves and unripe fruits have been eaten as colic and to treat irritations scorpion and bee stings. In view of the importance of *M. indica* in ethnobothany as health remedy we set out to investigate the antibacterial activity of its leaf extracts against some pathogenic bacteria and also to determine the phytoconstituents that may be present.

MATERIALS AND METHODS

Fresh leaves of *M. indica* were collected from the wild in Jimeta, Yola North Local Government Area of Adamawa State, and were identified and authenticated in the Department of Biological Sciences; School of Pure and Applied Sciences, Federal University of Technology, Yola; Adamawa State, Nigeria where a voucher specimen was kept.

Test organisms

The bacterial isolates: *S. aureus, S. pyogenase, S. pneumoniae, B. cereus, E. coli, P. aerugenosa, P. mirabilis, S. typhi* and *S. flexnerri,* were all clinical isolates obtained from the, Department of Microbiology, Federal University of Technology, Yola, Adamawa State, Nigeria. The antibiotic agents (powder); gentamicin and erythromycin were purchased from Jimex Pharmaceutical Stores Jimeta, Adamawa State, Nigeria.

Preparation of extracts

The fresh leaves were washed with distilled water to remove dirt and air-dried to constant weight for 5 days. The dried leaves were then blended using a household electrical blender. The leaf powder was stored sealed in five labeled reagent bottles for further use. The bioactive components were extracted using the methods of Akerele et al. (2008) with slight modification. One hundred milliliters (100 ml) each of methanol, acetone, and water -ethanol (1:1) were added unto 10 g portions of the leaf powder in separate sterile conical flasks and allowed to soak at ambient temperature for 72 h. The extracts were then filtered using Whatman no. 1 filter paper and the filtrates concentrated in vacuo at 40ºC using a rotary evaporator (Akerele et al., 2008).

Determination of phytochemical constituents

The freshly prepared extracts were subjected to standard phyto-chemical analyses for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins and phenols as described by Jigna et al. (2006).

Assay for antibacterial activity

The antibacterial screening was carried out using the agar 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^{O}C for 18 h. Each of the cultures was then adjusted to 0.5 McFarland turbidity standard and inoculated (0.2 ml each) onto Mueller Hinton agar (MHA, Oxoid) plates (diameter: 15 cm). A sterile coke 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 250 mg/ml and 0.5 ml of each of the extracts was then introduced into the wells using sterile Pasteur pipettes. Zero point five milliliters (0.5 ml) of sterile glycerol only was introduced in another well to serve as negative control. Wells containing the standard antimicrobials gentamycin and erythro-mycin (0.5 mg/ml) were included as positive control. The culture plates were allowed to stand on the working bench for 30 min for pre diffusion and were then incubated at 37°C for 24 h. After 24 h, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around each of the extracts and the antibiotics (Lino and Deogracious, 2006).

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

The minimum inhibitory concentration (MIC) of the methanol extracts was determined for each of the test organisms in triplicates at varying concentrations of 175, 150, 125, 100.0, 75, 50.0, 25.0 and 12.5 mg/ml). To obtain these concentrations, varying concen-trations (1 ml) of the extracts containing double strength of the concentrations (350, 300, 250, 200, 150, 100, 50 and 25 mg/ml) in a test tube, 1 ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibacterials gentamicin and erythromycin. A tube containing nutrient broth only was seeded with the test organism to serve as control. All the tubes were then incubated at 37^{O}C for 24 h and then examined for growth by observing for turbidity. 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 bac-

Table 1. Phytochemical constituents of root extracts of *Deuterium microcarpum.*

Extract	Saponins	Tannins	Alkaloids	Glycosides	Balsams	Anthraquinones
Water	-	-	-	+	-	-
Acetone	+	+	-	+	-	-
Methanol	-	+	-	+	-	-

Key: - = absent; + = present

Table 2. Antibacterial activity of leaf extracts of *Mangifera indica* against the test bacteria.

Bacterial agent	Extract concentration (mg/ml) and diameter zone of inhibition (mm															Control antibiotics	
	50			100			150			200			250			Gen (0.5mg/ml)	Ery (0.5mg/ml)
	WE	AE	ME	WE	AE	ME	WE	AE	ME	WE	AE	ME	WE	AE	ME	dW	dW
S. aureus	0	4	0	0	7	3	0	10	5	0	13	7	0	15	9	17	0
S. pyogenase	0	3	0	0	5	4	0	8	7	0	12	10	0	14	12	21	12
S. pneumoniae	0	5	0	0	9	4	0	11	6	0	13	9	0	15	11	19	12
S. typhi	0	4	0	0	7	0	0	9	2	0	12	4	0	14	6	19	6
P. mirabilis	0	0	0	0	3	0	0	5	0	0	7	3	0	8	5	0	8
E. coli	0	3	0	0	6	0	0	8	3	0	10	5	0	12	7	0	24
S. flexnerri	0	0	0	0	4	0	0	5	3	0	9	5	0	11	7	17	26
E. faecalis	0	6	3	0	9	5	0	12	7	0	14	9	0	16	12	25	27
P. aerugenosa	0	3	0	0	5	4	0	8	6	0	10	8	0	13	10	18	17
B. cereus	0	0	0	0	3	0	0	5	4	0	7	6	0	9	8	18	28

Key: WE = water extract; AE = acetone extract; ME = methanol extract; dW = distilled water; Gen = gentimicin; Ery = erythromycin

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 ^{o}C for 24 h. After incubation the concentration at which there was no single colony of bacteria was taken as MBC (Ajaiyeoba et al., 2003).

Effect of temperature and pH on antimicrobial activity of extracts

Five milliliters (5 ml) of 250 mg/ml concentration of acetone extracts were dispensed in test tubes and treated at 60 and 100 ^{o}C in a water bath for 1 h and tested for antibacterial activity. To determine the effect of pH, the acetone extracts (250 mg/ml) were dispensed in three sets of test tubes and 1 N HCl added dropwise until the pH of the extract is 2.5 or 5 (pH determined using the pH meter) or 1 N NaOH until the pH reaches 8 and the extracts were then allowed to soak for 1 h. After the 1 h period of acid- base treatment, the extracts were again neutralized using 1N HCl and 1N NaOH solutions as the case may be and then tested for antibacterial activity (Doughari et al., 2007).

RESULTS AND DISCUSSION

Preliminary phytochemical analysis revealed that the plant possessed the phytoconstituents tannins, glycolsides, saponins and phenols (Table 1).

Phytoconstituents have been found to inhibit bacteria, fungi, viruses and pests (Marjorie, 1999). The leaves have been reported to possess antibacterial activity against *E. coli* and other bacteria in the family enterobacteriaceae and the bioactive component mangiferin isolated from *M. indica* is reported to possess remarkable anti-influenza activity (Neon, 1984) . The presence of phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant (Marjorie, 1999). Among the extracts tested, the acetone extracts exerted highest activity on bacterial agents tested compared to the aqueous and methanol extracts. The acetone extract at 250 mg/ml for example, 15 mm was recorded as diameter zone of inhibition against *S. aureus* and *S. pneumoniae.* This was followed by 14 mm zone of inhibition each against *S. typhi* and *S. pneumoniae,* and. 13 mm zone of inhibition against *S. pyogenes* (Table 2). Whereas at the same concentration the methanol extracts, exerted highest activity against *E. faecalis* and *S. pyogenes* with diameter zone of inhibition of 12 mm, followed by 10 and 11 mm zone of inhibition against *S. pyogenes* and *P. aerugenosa* respectively. The least activity (5 mm zone of inhibition) was recorded by the water extracts against *S. aureus* at 250 mg/ml. The differrences in the observed activities of the various extracts may be due to varying degrees of solubility of the active constituents in the three solvents used. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents (Marjorie, 1999). The difference in activities among the solvents recorded

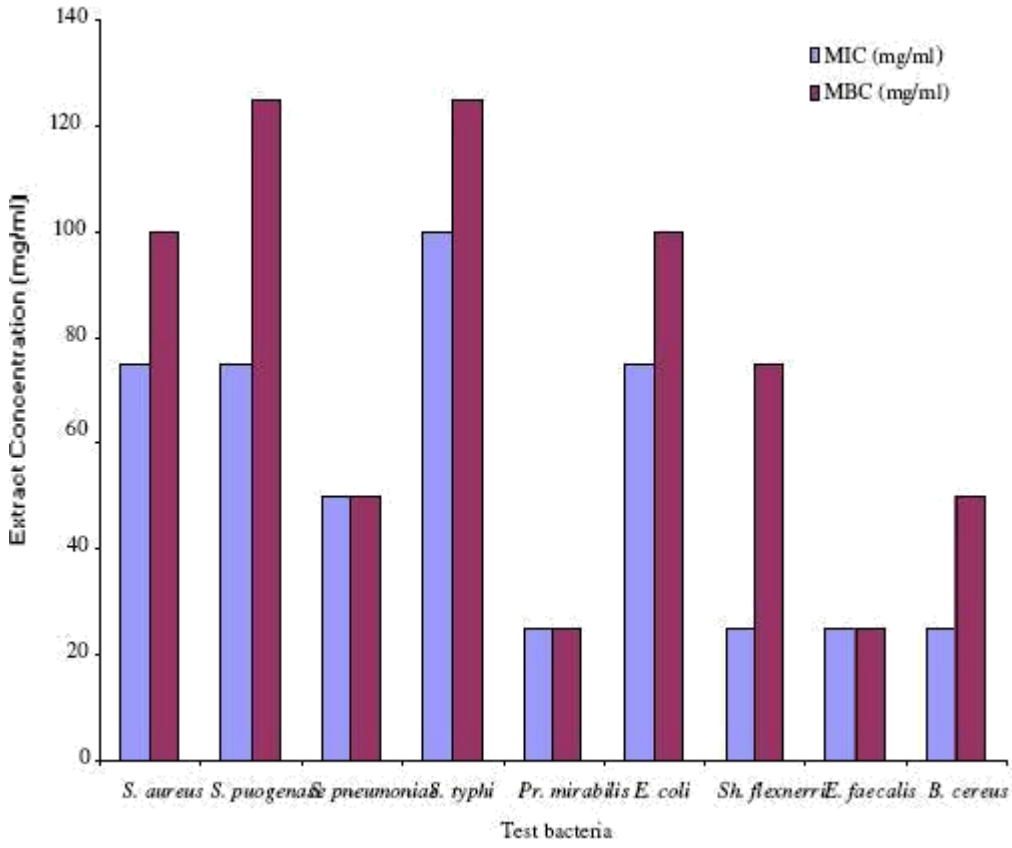

Figure 1. Minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) of leaf extracts of *M. indica* against the test bacteria

in this study may be associated with the presence of oils, wax, resins, fatty acids or pigments, which had been reported to be capable of blocking the active ingredients in the plant extract , thus, preventing the plant extract from accessing the bacterial cell wall (Jigna et al. 2006). Of all the bacteria tested the Gram-positive bacteria were slightly more susceptible to the extracts than the Gram-negative bacteria. This finding is in agreement with earlier reports that Gram-positive bacteria are more susceptible to extracts from *Launaea procumbens, Vitis vinifera* and *Cyperus rotundus* than Gram- negative bacteria (Jigna and Sumitra, 2006). Activities of the various extracts were comparable to those of standard antibacterial agents gentamicin and erythromycin ($t = 2.23$, $p < 0.05$). Demonstration of antibacterial activity against the test bacteria is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents. Bacteria used in this study are associated with different types of infections including urinary tract infections, wound infections, otitis media, gastroenteritis, pneumonia and minigitis (Gupte, 2006). The MIC and MBC values were in the range of 12.5 - 175 mg/ml, with the lowest MIC and MBC values of 12.5 mg/ml in each case recorded against *P. mirabilis, E. faecalis* and *B. cereus,* while the lowest MIC value of 12.5 mg/ml was obtained against *S. flexnerri* (MBC 75 mg/ml) (Figure 1). The observed low MIC and MBC values against these bacteria means that the plant has the potential to treat any ailments associated with these bacterial pathogens effectively. At the same time high MIC and MBC values however, is an indication of lack of efficacy of the plant extracts against the test bacteria and/or the possibility that the bacteria may possess the capacity to develop resistance against the plant extracts. In this study, increased temperature enhanced the activity of the extracts, while alkaline pH decreased the activity of the extracts (Tables 3 and 4). The untreated extracts (30°C) for instance gave the activity of 15 mm zone of inhibition against *S. pneumoniae*, but an increased activity of 15 and 17 mm respectively was observed when the temperature was raised to 60 and 100°C respectively. The temperature stability demon-strated in this study could be an indication that the bioactive components present in this plant may be heat stable. Locally, the application of these plant materials requires boiling for prolonged periods which in any way does not affect its efficacy in the treatment of their patients. Temperature stability by plant extracts had

Table 3. Effect of temperature (ºC) on the antibacterial activity of leaf extracts of Mangifera indica against the test bacteria

Bacterial agent	Zone of inhibition (mm)		
	NT(30ºC)	(60ºC)	(100ºC)
S. aureus	15	18	20
S. pyogenase	14	16	19
S. pneumoniae	15	17	22
S. typhi	14	16	19
P. mirabilis	8	10	13
E. coli	12	15	18
S. flexnerri	11	13	15
E. faecalis	16	18	21
P. aerugenosa	13	15	17
B. cereus	9	11	13

Key: NT = non treated.

Table 4. Effect of pH on the antibacterial activity of leaf extracts of *Mangifera indica* against the test bacteria.

Bacterial agent	pH / Zone of inhibition (mm			
	NT pH 4.3	2.5	5	8
S. aureus	15	15	16	11
S. pyogenase	14	15	14	10
S. pneumoniae	15	14	15	9
S. typhi	14	14	15	8
P. mirabilis	8	8	8	5
E. coli	12	12	13	7
S. flexnerri	11	11	11	6
E. faecalis	16	17	16	8
P. aerugenosa	13	12	12	7
B. cereus	9	10	9	4

Key: NT = non treated

earlier been reported (Doughari *et al.* 2007). At acidic pH (pH 2.5) the activity of the extracts against *S. aureus* (15 mm zone of inhibition) decreased to 11 mm. This observation does not agree with earlier reports that acidic pH enhances the activity of plant extracts (Molan, 1992). This implies that the active components of the plant are acid labile and thus may be destroyed or not effective in acidic medium.

Conclusion

M. indica in this study exerted antibacterial activity against both Gram positive and Gram negative bac-teria associated with different type of infections including pneumonia (*S. pneumoniae*), urinary tract infections (*S. aureus*), wound infections (*P. mirabilis*) and typhoid fever (*S. typhi*). The study therefore provides the scientific basis for its traditional application as ethnomedecine. The plant can therefore be used for the treatment of gastro-enteritis, uretritis, pneumonia, otitis media, shigel-losis and typhoid fever. The demonstration of activity against both Gram positive and Gram negative bac-teria is an indication of broad spectrum of activity and thus can be used to source antibiotic substances for drug develop-ment that can be used in the control of these bacterial infections. Further investigations of its activity against a wider range of bacteria and fungi, identification and purification of its chemical constituents, and toxicological investigations of the plant extracts should be carried out with a view to developing novel drugs for human con-sumption.

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