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Phytochemical and Antimicrobial Properties of *Mangifera indica* Leaf Extracts

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Abstract: There have been reports of increasing development of drug resistance among human pathogens as well as undesirable side effects of certain antimicrobial agents.It is therefore necessary to search for new agents that are better, cheaper and without side effects for treating infectious diseases especially in developing countries. In this study, phytochemical composition and antimicrobial activities of aqueous and ethanolic extracts of leaves of *Mangifera indica* were investigated. Standard methods were employed to screen for the phytochemicals. Agar well diffusion method was used to determine the antimicrobial effects of aqueous and ethanolic extracts of *M. indica* leaves against seven different clinical isolates namely:*Stapylococcus aureus*, *Micrococcus virians*, *M. leteus*, *Escherichia coli*, *Klebsellia pneumoniae*, *Pseudomonas aeruginosa* and a fungus, *Candida albicans*. Phytochemical screening showed the presence of active pharmacological components such as tannins, saponins, cardiac glycoside, flavonoid and alkaloids. Aqueous extract demonstrated a higher activity than the ethanolic extract. *S. aureus* showed highest sensitivity to the aqueous extracts with MIC 31.25mg/mL. Least sensitivity was observed in *K. pneumoniae* and *Candida albicans* with MIC 125mg/mL each in the two extracts.*M. indica* exhibited significant antimicrobial activity comparable to gentamicin which is used as control in this study.

Keywords: Resistance, Antimicrobial, Phytochemicals, Sensitivity

Introduction

Continuous spread of infectious diseases is a major apprehension for health institutions, pharmaceutical companies and government think-tanks all over the world. Failure of treatment, particularly with the current escalating trends of multi-drug resistance (MDR) to the available modern drugs or antibiotics among emerging and re-emerging bacterial pathogens leads to serious risks [1].

Prior to this century, medical practitioners whether allopath (medical doctors), homeopaths, naturopaths, herbalists or shamans had to know the plants in their areas and how to use them since many of their drugs were derived from plants [2–5]. Around 1900, 80% of the drugs were derived from plants, however, in the decades that followed, the development of synthetic drugs from petroleum products caused a sharp decline in the pre-eminence of drugs from live plant sources [6–8]. However, with the recent trend of high percentage resistance of microorganisms to the present day antibiotics, efforts have been intensified by researchers towards a search for more sources of antimicrobial agents [1, 9].

Mangifera indica is commonly called mango (English), manako (Hawai‘i), manggo’am (Fiji), tharyetthi (Myanmar), mangot, mangue or manguier (French), aam, am or amb (Hindi), bobbiemanja, kanjannamanja, magg, manggaboomormanja (Dutch), mamung (Thailand), manga or mango (Spanish), manga (Portuguese), manga, mempelamorampelam (Malaysia), manggaor mempelamn (Indonesia), mangobaum (German), paho (Philippines) and xoài (Vietnam), mongoro (Yoruba, Nigeria), mangolo (Igbo, Nigeria) and mangoro (Hausa, Nigeria). The fruits are eaten and used in the production of juice and wine. Traditionally, the mango plant has medicinal applications.Mango extract has been reported to have anti malaria effect by Tsabang *et al* [10] and was found to display *in vitro* activity against *Plasmodium falciparum* [11].

The leaves of *M. indica*have also been reported to possess antibacterial activity [12]. Ojewole [13] reported the anti-inflammatory, analgesic and hypoglycemic effects of *M. indica* stem-bark aqueous extract. Doughari and Manzara [12] also affirm that both 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 *Salmonella typhi* (14 mm) at 250 mg/ml. Stem bark of *M. indica* showed significant antibacterial and antifungal activities against *Streptococcus pneumoniae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Candida albicans* with MIC of 0.08 mg/ml [14]. *Mangifera indica* contains alkaloids and glycosides which are of great importance pharmacologically. Certain aliphatic constituents such as coumarin, mangiferin, sequiterpinenoids, triterpinoids and phenolics have also been reported from the stem barks of different cultivars of *M. indica* [15]. It is believed that the presence of these phytochemicals confers on *Mangifera indica*, its medicinal ability.

Studies have shown that aqueous and ethanolic herbal extracts show less toxicity in animal models than N-Haxane, acetone, ethanol and other solvents [1].This study therefore investigated and revalidated the phytochemical and *in vitro*antimicrobial properties of aqueous and ethanolic extracts of *Magniferaindica.*

Methods

Sampling: Samples of *Mangifera indica* (leaves) were obtained from Igbesa in Ado Odo/Otta Local Government Area of Ogun State and were identified in the Department

ofBiological Sciences, Covenant University, Ota, Ogun State, Nigeria.

Preparation of plant materials: Freshly collected leaves of *M. indica*were washed with distilled water and dried under the shade at normal room temperature for 10 days. After drying, the plant material was pounded using mortar and pestle into smaller particles and then blended to powder using an electric blender. 200grams of the powdered samples were stored in airtight containers and kept under normal room temperature for further screening.

Collection of test organisms: Clinical isolates of *Escherichia coli*, *Pseudomonas aerugenosa,Micrococcus leteus*,*Staphylococcus aureus*, *Klebsiellapneumoniae*, *Micrococcus virians* and *Candida albicans* were collected from Microbiological Teaching Laboratory of Covenant University Ota in Ado OdoOta Local Government Area of Ogun State, Nigeria. The collected isolates were sub-cultured for 24hours and were adjusted to 0.5McFarland standard.

Preparation of aqueous extracts: Samples **(**100 g) of the dried powdered of the plant leaves were soaked in1000 ml of distilled water contained in a 2000 ml flask. The flask was plugged with cotton wrapped with foil and then allowed to stand for 48 hours. The suspension was shaken vigorously and filtered using a muslin cloth. The filtrates wereconcentrated using a rotary evaporator. The concentrated extract was stored in airtight sample bottle until required. For the preparations of crude extracts for antimicrobial screening, the extract wasreconstituted in Dimethyl Sulphoxide (DMSO) to500mg, 250mg, 125mg and 62.5mg/ml by dissolving 0.5g in 1ml, 0.5g in 2ml, 0.5g in 4ml and 0.5g in 8ml DMSO respectively.

Preparation of ethanolic extracts: Samples **(**100 g) of the dried powdered of the plant leaves were soaked in 1000 ml of ethanol containedina 2000ml flask. The flask was plugged with cotton wrapped with foil and then allowed to stand for 72 hours. The suspension was shaken vigorously and filtered using a muslin cloth. The filtrates wereconcentrated using a rotary evaporator. The concentrated extract was stored in airtight sample bottle until required. For the preparations of crude extracts for antimicrobial screening, the extract was reconstituted in Dimethyl Sulphoxide (DMSO) to 500mg, 250mg, 125mg and 62.5mg/ml by dissolving 0.5g in 1ml, 0.5g in 2 ml, 0.5g in 4ml and 0.5g in 8ml respectively.

Phytochemical screening: Phytochemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out on the extract using the standard procedures as previously described [16].

Qualitative analysis of phytochemical constituents

***Tannins*:** The powdered leaf sample (0.5 g) was boiled in 20 ml of distilled water in a test tube and filtered, 0.1% $FeCl_3$was added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.

***Saponins*:** The powdered leaf sample (2.0 g) was boiled in 20ml of distilled water in a water bath and filtered off; the filtratewas mixed with 5ml of distilled water in a test tube and

shaken vigorously to obtain astable persistent froth. The frothing is then mixed with 3drops of olive oil and for the formation of emulsion which indicates the presence of saponins.

***Flavonoids*:** A few drop of 1% NH_3solution was added to the aqueous extract of each plant sample in a test tube. A yellow coloration is observed if flavonoids compound are present.

Glycosides: Concentrated H_2SO_4 (1 ml) was prepared in a test tube, 5 ml of aqueous extract from the powdered leaf samplewas mixed with 2ml of glacial CH_3COOH containing 1 drop of $FeCl_3$. The above mixture was carefully added to 1ml of concentrated H_2SO_4so that the concentrated H_2SO_4 settled beneath the mixture. The presence of cardiac glycoside constituent was indicated by appearance of a brown ring.

***Alkaloids*:** The plant sample (5.0 g) was prepared in a beaker and 200ml of 10% $CH_3CO\mathbf{O}H$ in C_2H_5OH was added to the plant sample nearly 0.5g.

Antimicrobial activity: Agar well diffusion technique as described by Olasehinde*et al*. [1] was adopted for the study. 56 petri-dishes filled with 20ml of Mueller Hinton Agar each (MHA Oxoid) was inoculated with 0.5Mcfarland's standard of each test organisms using sterile swab stick as demonstrated by Cheesbrough[16]. Duplicate well of 7mm diameter were bored on each plate using sterile cork borer and filled with equal volume of plant extracts (0.4ml) with the aid of a sterile micropipette. Control experiment was done using commercially produced Gentamicin.The plates were incubated at 37^{o}C for 18-24hours. Zones of Inhibition were measured in millimeter (mm) and the average values were calculated and recorded.

Determination of minimum inhibitory concentration (MIC): The determination of Minimum Inhibitory Concentration (MIC) was carried out on the extract against the test isolates *(E. coli, K. pneumoniae, M. viridans, M. leteus, S. aureus, P. aeruginosa and C. albicans)* due to its sensitivity against the growth of the isolates. Nutrient broth (5 ml) was dispensed into each of the 56 test-tubes and sterilized at 121^{o}C for 15 minutes and allowed to cool to 40-45^{o}C. 0.5ml of 0.5Mcfarland standard of each test isolates were introduced into 8 different tubes while 5ml of each extract concentrations (500, 250, 125, and 62.5 mg/ml of aqueous and ethanolic extract) were introduced into 7 different tubes containing each isolates, labelled accordingly and incubated at 37^{o}C for 24 hours.

Results and Discussion

The preliminary phytochemical tests carried out on the aqueous leaf extract showed the presence of tannin, saponins, alkaloids and cardiac glycosides but sterols and flavonoids were absent. In the ethanolic extract, saponin, tannin, flavonoids, alkaloids and cardiac glycosides were all present but sterols was absent (Table 1).

Table 1: Phytochemical properties of *M. indica* leaf extracts

Phytochemicals	Leave Extract	
	Aqueous	Ethanolic
Tannin	+	+
Saponin	+	+
Sterols	-	-
Flavonoids	-	+
Alkaloids	+	+
Cardiac glycosides	+	+

Antimicrobial activity of aqueous and ethanolic extracts of M. indica leaf assayed against seven human pathogenic microorganisms, using gentamicin as positive control showed great potency at varying concentration (Table 2). Phytochemical screening of the extracts of M. indicashowed presence of active pharmacological components such as tannins, saponins, cardiac glycoside, flavonoid and alkaloids. This observation agrees with the findings of Madunagu et al. [17]. These components are known to be biologically active because they protect the plant against infections and predations by animals. Phytochemicals generally exert their antimicrobial activities through different mechanisms from that of synthetic drugs [18].

The leaves and flowers of M. indica 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. indicawas reported to possess remarkable anti-influenza activity [19]. The presence of phyto-constituents in the leaf extracts may be responsible for the antibacterial activity of the plant [20–22]. Medicinally, this is important for the treatment of pneumonia, asthma and inflamed tissues.It also plays important roles in herbs for treating dysentery [23]. This justified the use of M. indica in traditional medicine.

The antibacterial assay performed using the Agar well diffusion method showed the clear zones of inhibition in diameters. Table 2 above showed the varied susceptibility of the bacteria and fungi used as test organisms in this study.The susceptibility exhibited are dependent on the microorganisms and extracting solvents. This agrees with earlier findings that length of zones of growth inhibition from different studies vary from one organism to another, plants and concentration difference [24, 25]. The patterns organisms which were sensitive tend to move away from the region around the extract while those that are resistant show no zones of inhibition of growth. In this study, it was observed that the aqueous extract demonstrated a slightly higher activity atsome concentrations than the ethanolic extract. Ethanolic extract was observed to possess more potency against P. aerugenosa and M. virians with zones of inhibition value of 21 mm and 50 mm respectively as shown in Table 2.

Table 2: Antimicrobial activity of aqueous and ethanolic leaf extracts of M. indica

Test isolates	Aqueous extracts in mg/ml and Zone of Inhibition (mm)				Control Gentimicin (ug)	Ethanolic extracts in mg/ml and zones of Inhibition (mm)			
	500	250	125	62.5	20	500	250	125	62.5
S. aureus	*25*	*20*	*18*	*15*	*20*	*20*	*15*	*15*	*10*
M. leteus	*20*	*15*	*10*	*10*	*12*	*20*	*15*	*12*	*10*
K. pneumoniae	*24*	*15*	*10*	-	*21*	*21*	*10*	*10*	-
P. aerugenosa	*20*	*18*	*16*	*15*	*12*	*21*	*20*	*12*	*10*
E. coli	*25*	*20*	*15*	*12*	*20*	*22*	*18*	*16*	*12*
M. virians	*30*	*25*	*16*	*12*	*20*	*50*	*20*	*11*	*10*

C. albicans	*25*	*15*	*12*	*10*	*10*	*20*	*14*	*12*	*10*

- = No zone of inhibition

Table 3: Minimum inhibitory concentration of aqueous and ethanolic leaf extracts of *M. indica* against test isolates

Test isolates	*Aqueous extracts in mg/ml and MIC*					*Ethanolic extracts in mg/ml and MIC*				
	500	*250*	*125*	*62.5*	3	*500*	*250*	*125*	*62.5*	3
S. aureus	-	-	-	-		-	-	-	+	
M. leteus	-	-	+	+		-	-	+	+	
K. pneumoniae	-	-	+	+		-	+	+	+	
P. aerugenosa	-	-	-	-		-	-	+	+	
E. coli	-	-	-	+		-	-	-	+	
M. virians	-	-	-	+		-	-	+	+	
C. albicans	-	-	+	+		-	+	+	+	

Key: - = No growth (no turbidity); + = Growth (turbidity)

Aqueous extract had better potency against S. aureus at all concentrations while gentamicin gave an inhibition zone of 20 mm similar to that of aqueous extract at 250 mg/ml and ethanolic extract at 500 mg/ml. For M. leteus, both extracts had similar effects while gentamicin had zone of inhibition of 12 mm which is similar to that ethanolic extract at 125 mg/ml. Furthermore, aqueous extract showed a greater potency against K. pneumoniae at all concentrations.

Ethanolic extract established a better effect at all concentrations against P. aerugenosa while gentamicin has a zone of inhibition of 12 mm, an effect shown by 125 mg/ml of ethanolic extract. From the same table, it is obvious that aqueous extract had a better potency between the two extracts against E. coli while gentamicin had similar effect of 20 mm with 250 mg/ml of ethanolic extract.

Assessing the zones of inhibition against M. virians, it is obvious that ethanolic extract had a better effect of 50 mm at 500 mg/ml but at other concentrations aqueous extract had the activity against the same organism while Gentamicin had an inhibition zone of 20 mm, a zone size also shown at 250 mg/ml of ethanolic extract. Aqueous extract had better potency against C. albicans at all concentrations while gentamicin had inhibition zone of 10 mm, inhibition zone size shown by 62.5mg/ml of both extracts. Aqueous extract of M. indica had minimum inhibitory concentration (MIC) of 62.5 mg/ml against S. aureus and P. aerugenosa only while an MIC of 31.25 mg/ml was observed for M. leteus, K. pneumoniae, and C. albicans had turbity. The MIC for K. pneumoniae, and C. Albicans was found to be 62.5 mg/ml and 125 mg/ml for S. aureus and E. coli respectively.

The zones of inhibition and MIC of M. indica extracts observed in this study compares with earlier findings where zones of inhibition ranging between 12 mm and 16 mm were recorded for

extracts of M. indica stem bark and leaves for Gram negative and Gram positive bacteria [13]. Doughari and Manzara [12] found that both 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 Salmonella typhi (14 mm) at 250 mg/ml. Stem bark of M. indica had been found to show significant antibacterial and antifungal activities against Streptococcus pneumoniae, Enterobacter aerogenes, Klebsiella pneumonia and Candida albicans with MIC of 0.08 mg/ml [6].

This study has established that crude aqueous and ethanolic extracts of M. indica leaves have good activity against Gram positive and negative bacteria and the fungus, Candida albicans at low concentrations.

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