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Antimicrobial Activities and Phytochemical Properties of Mango Leaves Extract (*Mangiferaindica*)

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ABSTRACT

The increasing need for antimicrobial therapeutics and the increasing rate of antibiotics resistant strains of pathogens has made the discovery of alternatives imperative, the cost of drugs and proliferation of fake drugs have force the need for thorough investigation of natural sources of new bioactive molecules to overcome this resistance problem. Mango (Mangiferaindica) plant has been used by traditional medicine practitioners to treat diseases. M. indicacontains tannin, magnilerin, flavonoid, Saponin, Alkaloids, Sterols, Cardiac glycosides. This study was to determine effective concentration of aqueous and ethanolic leaves extracts effect against S. aureus and E. coli using Kirby-Bauer Diffusion Methods. Amoxicillin was used as positive control. Aqueous extract had better potency against S. aureus at all concentrations and as effective as Amoxycillinat 62.5mg/ml whileethanolic extract was not effective at 62.5mg/ml.Ethanolicextract had a better potency between the two extracts against E. coli, Amoxicillinhad similar effect of 20mm with 250mg/ml of ethanolic extract. This shows that higher concentration of M.indica leaf extract is required to be effective against E coli than S. aureus. M. indica leaf extract can be used to treat infectious diseases such as bacteremia that are caused by these two organisms.

INTRODUCTION

Phytochemicals are the chemicals that present naturally in plants. Nowadays these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as man friendly medicines (Haslerand Blumberg, 2019). This paper mainly deals with collection, extraction, qualitative and quantitative analysis of phytochemicals. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients (Mathai, 2020). 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.

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 (Olasehinde*et al*., 2021). The fruits are eaten and used in the production of juice and wine. Traditionally, the mango plant has medicinal applications. Mango extract has been to reportedhave anti malaria effect by Tsabang*et al*., (2022) and was found to display in vitro activities against Plasmodium falciparum. The leaves of*M. indica* have also been reported to possess antibacterial activity (Doughari and Manzara 2018). Ojewole, (2020) reported the anti-inflammatory, analgesic and hypoglycemic effects of *M. indica* stem-bark aqueous extract. Doughari and Manzara (2018) also affirm that both acetone and methanol extracts inhibited the growth of grampositive bacteria, with acetone extract exerting more activities on all the gram positive bacteriawith zone of inhibition between 15 - 16mm, and a gram negative bacterium *Salmonella typhi* (14mm) at 250 mg/ml. Stem bark of *M. indica* showed significant antibacterial and antifungal activities against *Streptococcus pneurnoniae*, *Enterobacteraerogenes*, *Kiebsiellapneurnoniae* and *Candida albicans* with MIC of 0.08mg/ml (Singh *et al*., 2012). *Mangiferaindica* contains alkaloidsand glycosides which are of great importance pharmacologically. Certain aliphatic constituentssuch as coumarin, mangiferin, sequiterpinenoids, triterpinoids and phenolics have also beenreported from the stem barks of different cultivars of *M. indica*. It is believed that the presence ofthese phytochemicals confers on *Mangiferaindica*, its medicinal ability. Studies have shown that aqueous and ethanolic herbal extracts show less toxicity in animal models than NHaxane, acetone, ethanol and other solvents (Olasehinde*et al*., 2019).

The plants provide the basic nutrients needed for the growth of animals and humans like proteins, carbohydrates, fats, vitamins and oils minerals. The phytochemicals are majorly classified as primary and secondary metabolites. The primary metabolites are responsible for the basic development of the plant which includes the sugars, amino acids, proteins, nucleic acids, chlorophyll (Agarwala and Yadav, 2021).

Secondary metabolites are those which are needed for the survival of the plants in a harsh environment, they forms the smell, colour and taste of the plants and secondary metabolites suchas flavonoids. tannins, saponins, alkaloids, steroids. Phytosterolsare found to have other commercial applications like they can be used as colouring agents, as drugs as flavouring agents, insecticides, pesticides, anti-bacterial and antifungal products (Jing *et al*., 2020). Moreover they can also be used to protect humans from many diseases like cancer, diabetes, cardiovascular diseases, arthritis and aging (Abdulwadood*et al*., 2023) etc.

As per a report by World Health Organization (WHO), over 80% of the people of developing countries are relaying on the traditional medicines that are extracted from the plants for their primary health needs. Use of these traditional medicines for the preparation of modern medical preparations is indispensable and thus Phytomedicines are a link between the traditional and modern medicine (Sahira and Cathrine, 2019).This study aimed at the antimicrobial activity and phytochemical properties of mango (*mangiferindica*) extracted leaf.

MATERIALS AND METHOD

Collection and Preparation of Plant Materials

Samples of *Mangifraindica* (leaves) were obtained from back of SANS complex in the Federal Polytechnic Bida, Niger State. 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 albicons* were collected from microbiological laboratory the Federal Polytechnic Bida, Niger State.

Preparation of Aqueous Extracts:

Samples (100g) of the dried powdered of the plant leaves were soaked in 1000ml of distilled water contained in a 2000ml flask. The flask was plugged with cotton wrapped with foil and then allowed to stand for 48hours. The suspension was shaken vigorously and filtered using a muslin cloth. The filtrates were concentrated using a rotary evaporator. The concentrated extract wasstored in airtight sample bottle until required. For the preparations of crude extracts forantimicrobial screening, the extract was reconstituted in Dimethyl Sulphoxide (DMSO) to500mg, 250mg, l25rng and 62.5mg/ml by dissolving 05g in 1ml. 0.5g in 2ml, 05g in 4m1 and 0.5g in 8ml DMSO respectively.

Preparation of Ethanolic Extracts:

Samples (100g) of the dried powdered of the plant leaves were soaked in 1000 ml of ethanol contained in a 2000ml flask. The flask was plugged with cotton wrapped with foil and then allowed to stand for 72hours. The suspension was shaken vigorously and filtered using a muslin cloth. The filtrates were concentrated 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 05g in lrnl, 0.5g in 2 ml, 05g in 4ml and 0.5g in 8mlrespectively.

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 by Singh *et al*., (2022)

Qualitative Analysis of Phytochemical Constituents

Tannins

The powdered leaf sample (0.5g) was boiled in 20ml 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 blackcoloration which shows the presence of tannins.

Saponins

The powdered leaf sample (2.0g) was boiled in 20m1 of distilled water in a water bath and filtered off; the filtrate was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3drops ofolive oil and for theformation 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_2S0_4 (1ml) was prepared in a test tube, 5ml of aqueous extract from the powdered leaf sample was mixed with 2m1 of glacial CH_3COOH containing 1 drop of $FeCI_3$. The above mixture was carefully added to 1ml of concentrated H_2SO_4so that the concentrated H_2S0_4 settled beneath the mixture. The presence of cardiac glycoside constituent was indicated by appearance of a brown ring.

Alkaloids

The plant sample (5.0g) was prepared in a beaker and 700ml of 10% CH_3COOH 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*. (2019) 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.Duplicate well of 7mm diameter were bored on each plate using sterile cork borer and filled withequal 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°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. aeruginosaand C. albicans*,) due to its sensitivity against the growth of the isolates. Nutrient broth (5ml) was dispensed into each of the 56 testtubes and sterilized at 121^{o}C for 15 minutes and allowed to cool to 40-45^{o}C. 0.5m1 of 0.5Mcfarland standard of each test isolates were introduced into 8 different tubes while 5m1 of each extract concentrations (500, 250, 125, and 62.5mg/ml of aqueous and ethanolic extract) were introduced into 7 different tubes containing each isolates, labeled accordingly and incubated at 37^{o}C for 24 hours.

RESULTS AND DISCUSSION

Results

Table 4.1.1: Phytochemical properties of leaf extracts of *Magniferaindica*

Phytochemicals	**Aqueous Leave Extract**	**Enthanolic Leave Extract**
Tannin	+	+
Flavonoids	-	+
Saponin	+	+
Alkaloids	+	+
Sterols	-	-
Cardiac glycoside	+	+

The Keyword: + = present, - = absent

Mango's leaves extract, bacterernia, inhibition zone, antibacterial effect aqueous and ethanol je extracts were subjected to phytochernical screening and the results as shown on table 4.1.1 showed that tannin,saponins, alkaloids and cardiac glycosides but sterols and flavonoids wereabsent in aqueous extract while saponin, tannin, flavonoids, alkaloids and cardiac glycosides were all present but sterols was absent in the ethanolic extract.

Table 4.1.2: Antimicrobial activity of aqueous and ethanolic leaf extract*M.indica*

Test isolate	Aqueous extract in mg/ml and zone of inhibition in mm				Controls	Ethanolic extract in mg/ml and zone of inhibition in mm			
	500	250	125	62.5		500	250	125	62.5
S. aureus	25	20	18	15	20	20	15	15	10
E. coli	25	20	15	12	20	22	18	16	12

Table 4.1.3: Minimum Inhibitory concentration of aqueous and ethanolic leaf extract*M.indica* against test isolates

Test isolate	Aqueous extract in mg/ml and MIC				Ethanolic extract in mg/ml and MIC				control
	500	250	125	62.5	500	250	125	62.5	
S. aureus	-	-	-	+	-	-	-	-	-
E. coli	-	-	-	+	-	-	+	+	-

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

Discussion

Phytochemical screening of the extracts of *M. Indica* showed presence of active pharmacologicalcomponents such as tannins, saponins, cardiac glycoside flavonoid and alkaloids. This observation agrees with the findings of Madunagu*et al*., (2020). These are biologically active components 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 Scalbert (2021).

The antibacterial activity of aqueous and ethanolic extracts of *M. Indica* leaf assayed against two human pathogenic bacteria using Amoxycilin as positive control showed great potency at variable concentration (Table 4.1.2). Poongothai and Rajan (2023) reported that the leaves and flowers of *M.indica* possess antibacterial activity against *E. coli* and other bacteria in the family Enterobacteriaceae, this explains the traditional use of decoction of *M.indica* in the treatment of fever. The bioactive component mangiferin present in *M.indica* was reported to possess remarkable anti-influenza activity (Poongothai and Rajan, 2023). The presence of phyto-constituents in the leaf extracts may be responsible for the antibacterial activity of the plant is medicinally important for the treatment of pneumonia, asthma and inflamed tissues (Olasehinde*et al*., 2017). The active phytochemicals are known to be effective against dysentery (Leven *et al*, 2019).

The antibacterial assay performed using the Agar well diffusion method showed the clear zones of inhibition in diameters. Table 4.1.2 showed the different Sensitivities of' thetest organism tothe different concentration of the extracts. The susceptibility exhibited is dependent on the microorganism and exacting solvents. This agrees with earlier findings that diameter of zones of inhibition from different studies vary from one organism to another, plants, solvent and concentration difference (Mann *et al*., 2018). The patterns shown by 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. This study showed that aqueous extract demonstrated a slightly higher activity against *S. aureus* and *E. coli* at some concentrations than the ethanolic extract.

Aqueous extract had better potency against *S. aureus* at all concentrations as shown on table 3, while amoxycillin gave an inhibition zone of 20mm similar to that of ethanolic extract at 250 mg/ml and aqueous extract at 500 mg/ml. From the same table 3, it is obvious that ethanolic extract had a better potency between the two extracts against *E. coil* while Arnoxycillinhad similar effect of 20mm with 250 mg/ml of ethanolic extract. Ethanolic extract of *M. indica* had minimum inhibitory concentration (MIC) of 62.5mg/ml against *S. aureus* and *E. coli* only while the MIC of 125mg/ml was observed for *E. coli* for aqueous extract had turbidity, This showed that ethanolicextract is more potent against *E. coli* than the aqueous extract, this does not agree with the findings of Olasehinde*et al*., (2019).

Generally, the findings of this work compares with earlier assertions by Ojewole (2020) where zones of inhibition ranging between 12 mm and 16 mm were recorded for extracts of *M. indica*stem bark and leaves against some Gram negative and Gram positive bacteria, it was reported that stem bark of had been found to show significant *M. indica*antibacterial and antifungal activities against *Streptococcuspneumoniae, Enterobacteraerogenes. Klebsiella pneumonia*and *Candida albicans* with MIC of 0.08mg/ml (Singh *et al*., 2022). This study has established that crude aqueous and ethanolic extracts of *M. indica* leaves have good activity against Gram negative bacteria at relatively low concentrations.

CONCLUSION AND RECOMMENDATION

Conclusion

The use of herbs for treatment of' ailments that have shown proven resistance to antibiotics have been boosted by the findings of this study. Both aqueous and ethanolic extracts of *Magniferaindica*have shown potency against *Staphylococcus aureus* which is notorious for causing abscesses (boils), furuncles, cellulitis, pneumonia, bone and joint infections and bacteremia many of which require treatment with intravenous antibiotics. The extract is also effective against*E. coli* which has been implicated in many diseases such as urinary tract infection (UTI), pelvic infection. Bacteremia, meningitis and abdominal infections. Extracts of *M. indica* can be used to prevent untimely death from these pathogens, it is cheaper, handier and readily available in Nigeria.

Recommendations

It is recommended that:

i. Further research should be carried out to reduce cytotoxicity
ii. Determination of the appropriate dosage for different age range or body weight should be carried out.
iii. More fund should he provided to improve the packagingof herbal product to reduce adulteration and quarkry.

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