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Phytochemical Screening and Antimicrobial Efficacy of Aqueous and Methanolic Extract of *Mangifera indica* (Mango Stem Bark)

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ABSTRACT [ENGLISH/ANGLAIS]

Increasing resistance of microorganisms to antibiotics and other orthodox drugs has resulted in the search for more organic molecules from plant source with antimicrobial properties. Therefore investigation of African medicinal plants for their antimicrobial activity rank highest among biological tests carried out on the plants and their isolates. Phytochemical screening of the crude stem bark extracts of *Mangifera indica* revealed the presence of tannins, saponins, alkaloids, flavonoids, cardiac glycosides and phytosterols. The antimicrobial activity of the aqueous and methanolic extract of *Mangifera indica* stem bark were assayed using disc diffusion method on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. At concentration of 50 mg/ml, both the aqueous and methanolic reconstituted extract inhibits the growth of these organisms though with varying degree of susceptibility depending on the bacterium and the extracting solvent. The MIC values ranged from 6.25 - 50 mg/ml, while the MBC values ranged from 12.5 - 50 mg/ml. The antimicrobial activity obtained in this study indicate the presence of bioactive compounds and support the claim by the local communities for use of *Mangifera indica* stem bark decoction for treatment of infections such as diarrhea. The stem bark extract of *M. indica* tree can be used as a source of broad spectrum antibiotic because it inhibits the growth of both Gram positive and Gram negative organisms.

Keywords: Phytochemical screening, antimicrobial, *Mangifera indica*, microorganisms

RÉSUMÉ [FRANÇAIS/FRENCH]

Augmentation de la résistance des microorganismes aux antibiotiques et autres médicaments orthodoxes a abouti à la recherche de plus de molécules organiques à partir des sources végétales aux propriétés antimicrobiennes. Par conséquent le plus élevé enquête sur les plantes médicinales africaines pour leur grade activité antimicrobienne parmi les tests biologiques effectués sur les plantes et leurs isolats. Criblage phytochimique des extraits bruts d'écorce de Mangifera indica révélé la présence de tanins, des saponines, des alcaloïdes, des flavonoïdes, des glycosides cardiaques et des phytostérols. L'activité antimicrobienne de l'extrait aqueux et méthanolique d'écorce de la tige de Mangifera indica ont été analysés en utilisant la méthode de diffusion du disque sur Pseudomonas aeruginosa, Staphylococcus aureus et Escherichia coli. A une concentration de 50 mg / ml, à la fois l'extrait aqueux et méthanolique reconstitué inhibe la croissance de ces organismes mais avec un degré variable de la sensibilité en fonction de la bactérie et le solvant d'extraction. Les valeurs de CMI variait de 6,25 à 50 mg / ml, tandis que les valeurs variaient de MBC 12,5 à 50 mg / ml. L'activité antimicrobienne obtenus dans cette étude indiquent la présence de composés bioactifs et soutenir la revendication par les collectivités locales pour l'utilisation de décoction d'écorce Mangifera indica souches pour le traitement des infections telles que la diarrhée. L'extrait d'écorce de la tige de M. indica arbre peut être utilisé comme une source d'antibiotique à large spectre, car elle inhibe la croissance des deux organismes Gram positifs et Gram négatifs.

Mots-clés: Criblage phytochimique, antimicrobien, Mangifera indica, micro-organismes

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INTRODUCTION

Investigation of African medicinal plants for their antimicrobial activity rank highest among biological tests carried out on the plants and their isolates [1]. The increasing resistance for antibiotics has also resulted in the search for more organic molecules from plants with antimicrobial properties [2]. *Mangifera indica* belongs to the family Anacardiaceae which consists of about sixty genera and six hundred species, which are mainly tropical trees shrubs. It is widely used as a source of food, medicines and timber. In Nigeria, different parts of *Mangifera indica* (mango tree) are commonly used as herbal preparations in the treatment of tooth ache, gastrointestinal disorders, dysentery, diarrhea

gastrointestinal tract infections, respiratory and urinary tract infections [3]. The leaves are used for floral decoration at Hindu marriages and religious ceremonies [4, 5].

MATERIALS AND METHODS

Collection of plant Materials

Samples of *Mangifera indica* (stem bark) were obtained from Damba Village Gussau, Zamfara State and were identified at Biological Sciences Department, Ahmadu Bello University Zaria, Nigeria.

Preparation of Plant Materials

Freshly collected stem barks of *M. indica* were cleaned and dried under the shade at normal room temperature for 10 days. Upon drying, the plant material was pounded using mortar and pestle into smaller particles and then blended to powder using an electric blender. 100 grams of the powdered sample was then stored in airtight containers and kept under normal room temperature until required.

Collection of Test Organisms

The test organisms:- (*E.coli, P.aeruginosa* and *S.aureus)* used, were clinical isolates obtained from Microbiology Department, Ahmadu Bello University Zaria. All the test organisms were maintained in a refrigerator at 4 ^{0}C in nutrient agar slants until required.

Preparation of Aqueous Extracts

Ten grams of the dried powdered sample was soaked in 100 ml of distilled water contained in a 500 ml flask. The flask was covered with cotton plug and then allowed to stand for 24 hours. After, 24 hours the suspension was shaken vigorously and filtered using a muslin cloth and filter paper. Then filtrate was then concentrated on a water bath set at 50 ^{0}C. (The percentage yield extract was 12.3%) The concentrated extract was stored in airtight sample bottle until required. For the preparations of dilutions of crude extracts for antimicrobial screening, a reconstituted aqueous extract was prepared by dissolving 50 mg, 100 mg and 200 mg of the extract in 1ml of distilled water to obtain a concentration of 50 mg/ml, 100 mg/ml and 200 mg/ml respectively.

Preparation of Methanolic Extracts

Ten grams of the dried powdered sample was soaked in 100 ml of methanol contained in a 500 ml flask. The flask was covered with cotton plug and then allowed to stand for 24 hours. After 24 hours, the suspension was shaken vigorously and filtered using a muslin cloth and filter paper. The filtrate was then concentrated on a water bath set at 50 ^{0}C. The concentrated extract was then stored in airtight sample bottle in a refrigerator until required. (The percentage extract yield was 15.2%). For the preparations of dilutions of crude extracts for antimicrobial screening, a reconstituted methanolic extract was prepared by dissolving 50 mg, 100 mg and 200 mg of the extract in 1ml of methanol to obtain a concentration of 50 mg/ml, 100 mg/ml and 200 mg/ml respectively.

Phytochemical Screening

Phytochemical screening was carried out to determine the presence of tannins, saponins, sterols, cardiac glycosides, flavanoids and alkaloids, [6, 7, 8, 9, and 10].

Preparation of Microbial Media

Nutrient agar was used for antimicrobial assay. This was prepared according to the manufacturer's specification. The nutrient agar was prepared by dissolving 7 g of the agar in 250 ml of distilled water contained in a 500 ml sterile conical flask. The media was then autoclaved at 121 ^{0}C for 15 minutes. The sterilized media were allowed to cool to a temperature of 45 ^{0}C and then approximately 20 ml was poured into sterile Petri-dish and allowed to gel.

Antimicrobial Screening of Methanolic Extract Using Disc Diffusion Method

Six Petri dishes (plates) were used, three served as test plates for each organism and the other three served as control. Twenty (20) ml sterile of molten agar media was poured into the Petri dishes, rocked several times and allowed to gel at room temperature. Three paper discs with concentration of 50 mg/ml, 100 mg/ml and 200 mg/ml of methanol extracts of *Mangifera indica* stem bark were prepared respectively and then placed on the nutrient agar plates after being inoculated with the test organisms. However the concentration of streptomycin (control) was 40 mg/ml. The plates were incubated at 37 ^{0}C for 24 hours and zones of inhibition are then measured to the nearest millimeter using a ruler [11].

Determination of Minimum Inhibitory Concentration (MIC)

The method described by [12] using the Nutrient broth dilution methods was used. The MIC was determined on

those organisms that were sensitive to the methanol and aqueous extract.

Four clean test tubes were prepared and labelled for each of the organism. Five (5) ml nutrient broth was delivered into each test tube with the aid of a pipette. The tubes were covered with aluminum foil and sterilized by autoclaving at 121 ^{0}C for 15 minutes. After autoclaving the tubes were allowed cooled and 5 ml of the extract containing 50 mg/ml, 20 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively were poured into the tubes for each organism.

Determination of Minimum Bactericidal Concentration (MBC)

The content of the MIC tubes and the content of the preceding tubes in the serial dilution were subcultured into appropriately labeled quadrant of the nutrient agar. All bacterial plates were inoculated at 37 ^{0}C for 24 hours after which they were observed.

RESULTS AND DISCUSSION

There was clear indication that the solvent system plays a significant role in the solubility of the bioactive components and influences the antibacterial activity [13]. From the extraction analysis using different extracrion solvents, methanol was more efficient solvent of extraction than water with a yield of 15.2% from ten grams of the sample while Water made a yield of 12.3%.

Table 1: This table shows the result for phytochemical screening of *Mangifera indica* stem bark

Phytoconstituents	Water Extract	Methanol Extract
Tannins	+	+
Saponins	+	+
Sterols	+	+
Cardiac glycosides	+	+
Flavonoids	+	+
Alkaloids	+	+

+: Indicates present; --: Not detected

Phytochemical screening showed the presence of active pharmacological components such as tannins, saponims, sterols, cardiac glycoside, flavonoid and alkaloids. These componenents 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 to that of synthetic drugs [14].

Medicinally, this is important for the treatment of pneumonia, asthma and inflammed tissues.It also plays important roles in herbs for treating dysentery [15]. This justified the use of *M. indica* in traditional medicine.

Table 2: This table shows the antibacterial activity of the stem bark extract of *Mangifera indica*

Organisms	Diameter of zone of Inhibition (mm) Water (mg/ml)			Methanol (mg/ml)			Streptomycin (mg/ml)
	200	100	50	200	100	50	40
P. aeruginosa	16	14	9	17	15	10	39
S. aureus	15	13	10	19	17	14	25
E. coli	16	13	12	19	16	13	28

Table 3: This table shows the Minimum Inhibitory Concentration (MIC) in mg/ml of the stem bark extracts of *Mangifera indica*

Organisms	Water extracts (mg/ml)				Methanolic extracts (mg/ml)			
	50	25	12.5	6.25	50	25	12.5	6.25
P. aeruginosa	-	-	+	+	-	-	-	+
S.aureus	-	-	+	+	-	-	-	+
E.coli	-	-	+	+	-	-	-	+

The antibacterial assay was performed using the disc diffusion method which best showed the clear zones of inhibition in diameters. Table 2, 3 and 4 show the varied susceptibility of the bacterial in the crude extract on the

basis of zones of growth of inhibitions, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). These differences are dependent on the microorganisms and extracting solvents. Lengths of zones of growth of inhibition from different studies vary from one organism to another, plants and concentration difference [13, 16]. The organisms which are sensitive tend to move away from the region around the extract while those that are resistant show no zones of growth of inhibition.

Table 4: This table shows the Minimum Bactericidal Concentration (MBC) in mg/ml of the stem bark extracts of *Mangifera indica.*

Organisms	Water (mg/ml)		Methanolic (mg/ml)		
	50	25	50	25	12.5
P. aeruginosa	-	+	-	-	+
S. aureus	-	-	-	-	-
E. coli	-	-	-	-	+

From the result of the zones of growth of inhibition, it was seen that the methanolic extract demonstrated the higher activity with respect to the different concentrations. For *P.aeruginosa* at 200 mg/ml, 100 mg/ml and 50 mg/ml; zones in diameter were 17mm, 15mm and 10mm. *S.aureus* also at 200 mg/ml, 100 mg/ml and 50 mg/ml; zones in diameters were 19mm, 17mm and 14mm.While in *E.coli* at 200 mg/ml, 100 mg/ml and 50 mg/ml zones in diameters were 19mm, 16mm and 13mm respectively. Activities for water extract are as follows *P.aeruginosa* at 100 mg/ml, 100 mg/ml and 50 mg/ml, zones in diameters were 16mm, 14mm and 9mm. While for *S.aureus* at 200mg/ml, 100mg/ml and 50mg/ml zones in diameters were 15mm, 13mm and 10mm. Then for *E.coli* at 200mg/ml, 100mg/ml and 50mg/ml zones in diameters were 16mm, 14mm and 12mm respectively. Streptomycin at 40mg/ml concentration which servrd as control, showed activity measuring 39 mm for *P.aeruginosa*, 25mm for *S.aureus* and 28mm for *E.coli.*

Both the extracts demonstrated antimicrobial activity but higher in methanolic extract at the varied concentrations. Related reports have been conducted [13, 17]. Since activities were seen in both the methanolic and water extract, it means that the crude extract can further be refined into pure form and use it against pathogens that cause infections in local communities. Streptomycin show highest activity is in its pure form against both gram negative and gram positive bacteria.

CONCLUSION

Based on the results obtained it has been found that stem bark of *Mangifera indica* exhibit antimicrobial activity with methanolic extract being the most potent than aqueous extract which support the claimed by the local commuinties for its potential use as therapeutic agents for the treatment of urinary tract infection, respiratory infection, and stomach pain.

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CONFLICT OF INTEREST

No conflict of interests was declared by the authors