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The Potentials of *Mangifera indica* Extracts as Antimicrobial Agent

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Abstract This work aimed to identify the phytochemical composition of *Mangifera Indica* bark and to test the various extract fractions against some selected microorganisms. Fresh bark of *Mangifera Indica* was collected, air dried and ground to powder, the crude extract was prepared using chloroform, ethanol and distilled water, the dried sample of 50g each of the *Mangifera Indica* bark yielded 9.11g, 5.4g and 1.8g for chloroform, ethanol and distilled water as solvents respectively, the crude extract was subjected to phytochemical screening in which alkaloids, flavonoids and steroids were found to be present and terpenoids were absent in all the three crude extracts. The crude extracts were also tested against some strains of bacteria and found to be active against it, with little resistance. It was further subjected to antifungal test and found to be resistant in all the extracts.

Keywords Mangifera Indica, Phytochemical, Microorganism

Introduction

Mango belongs to the family *Anacardiaceae* and genus *Mangifera*. Mango is native to South and Southeast Asia from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics [1]. Furthermore, it is one of the most popular tropical fruit bearing trees in the world with global production exceeding 30 million tonnes [2]. Similarly, *Mangifera Indica* extracts have been reported to possess antibacterial, antiviral, analgesic, immuno-modulatory and anti-inflammatory activities [3]. In addition, *Mangifera Indica* extracts are said to exhibit antimicrobial activity which are attributed to the presence of various phytochemicals [1]. Mango fruits are edible and used in juice and wine production. Furthermore, it possessed medicinal application, Tsabang et al, [4] reported that its extract possessed antimalarial effect, therefore, it was found to display in vitro activity against *Plasmodium Falciparum* [5]. The *M. Indica* leaves have also been reported to possess antibacterial activity [6]. Ojewole reported the anti-inflammatory, analgesic and hypoglycaemic effects of *M. Indica* stem-bark aqueous extract [7]. Doughari and Manzara also affirmed 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 [6]. In this work, phytochemical constituents contained in the bark of *Mangifera Indica* extracts and their activities against some selected microorganisms are investigated.

Materials and Methods

Sample Collection

The Bark of *Mangifera Indica* was obtained from home garden in Katsina and was identified in the Department of Biology, Umaru Musa Yaradua University, Katsina State, Nigeria.

Preparation of Plant Materials

The already collected Bark of *M. Indica* were washed with distilled water and dried under the shade at room temperature for 30 days. It was then placed inside mortar and pounded with pestle into relatively smaller sizes and then transformed into powder using an electric blender.

Preparation of Chloroform Extract

50g of the powdered *M. Indica* bark was soaked in a flask containing 250 cm^3 of chloroform solvent. The flask was covered with cotton and then allowed to stand for 7 days. It shaken vigorously and then filtered using a filter paper. The filtrates were concentrated using a rotary evaporator at 30°C. The concentrate was stored in airtight sample bottle until required.

Preparation of Ethanolic Extract and Aqueous Extract

The procedure adopted in the preparation of chloroform extract was also used in the preparation of ethanolic and aqueous extracts, using ethanol and distilled water as solvents instead.

Phytochemical Screening

Test for Alkaloids

2g of each extract were placed in test tubes and then 3 drops of Dragendoff's reagent were added. Appearance of an orange red precipitate indicated the presence of alkaloids [8].

Test for Flavonoids

0.27g of magnesium ribbon was added to 7 mg/mL of each of the extracts, concentrated HCl was added drop wisely. Appearance of color change ranging from orange to red indicated the presence of flavones while red to crimson indicated presence of flavonoids [9].

Test for Saponins

0.5g of each extract was dispensed in a test tube. 5 cm^3 of distilled water was added and shaken vigorously. Appearance of persistent froth that lasts for about 21 mins indicated the presence of saponins [9].

Test for Steroids

7mg of each extract was placed into separate test tubes. 2 drops of acetic anhydride and later 3 drops of chloroform were added. This was followed by the 2 drops of concentrated H_2SO_4. Appearance of a brown ring at the interface of the two liquids and a violet color in the supernatant layer indicated the presence of steroids [8].

Test for Tannins

1.0mg of each extract was diluted with 1 cm^3 of distilled water in separate test tube and 2 drops of ferric chloride ($FeCl_3$) solution was added. Appearance of a green – black or blue coloration indicated the presence of tannins [8].

Test for Glycosides

10 cm^3 of sulfuric acid was added to 1g each of the extracts in separate test tubes. The mixtures were heated for 17 min. 5 cm^3 of Fehling's solution was added to tubes and the mixture boiled for 5mins. A brick red precipitate indicated presence of glycosides [9].

Test for Terpenoids

2 cm^3 of chloroform was added to 0.5g of each of the extracts. Then 2 cm^3 of concentrated sulfuric acid was added carefully and shaken gently. Appearance of a reddish-brown coloration of the interphase formed indicated the presence of terpenoids [10].

Test for Phenols

3mg of each extract was treated with 2 drops of ferric chloride solution. Formation of bluish-black color indicated the presence of phenols [9].

Antimicrobial Susceptibility Test

The agar well diffusion method was used for the antimicrobial susceptibility test. Mueller Hilton agar was prepared according to manufacturer's specification. The media were autoclaved and dispensed into sterile petri-dishes and allowed to gel. Standardized inocula of each bacterial isolate were streaked on the agar plate. Four wells of 6mm each were made in each plate with a central well for control using a sterile cork borer.

Crude extracts were prepared for antimicrobial screening by reconstitution in dimethyl sulphoxide (DMSO) to 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL via dissolving 0.5g in 1 cm^3, 0.5g in 2 cm^3, 0.5g in 4 cm^3 and 0.5g in 8 cm^3 respectively.

The wells were filled with 0.1 cm^3 of different concentrations of the extracts with the use of sterile pipettes. Similarly, 62.5 mg/mL of the standard antibiotic (Erythromycin) was used on separate plates to serve as positive control. The plates were allowed to stand for 15 minutes on a table to allow free diffusion of the extracts. Diameters of zones of inhibition were measured using plastic meter rule in (mm) after 24 hours of incubation at 37 °C [11].

Minimum Bactericidal Concentration (MBC)

Nutrient agar plates were inoculated with sample from each of the tubes that show no turbidity and the plates were incubated at 37°C for 24hr to determine the MBC. MBC was determined by inoculating samples from the minimum inhibitory concentration (MIC) tubes that showed no bacterial growth on Mueller Hilton agar plates separately and then incubated at 37 °C for 24 hr. After the incubation the plates were observed for presence or absence of growth. The least concentration of the extract that showed no bacterial growth was considered as the MBC [12].

Results and Discussion

Table 1: Weight of *Mangifera Indica* Bark Extracts

S/No	Extracts	Actual weight of the extract (g)
1	Chloroform	9.11
2	Ethanol	5.40
3	Aqueous	1.86

The solvent extraction of the dried sample of 50g of the bark of *Mangifera Indica* yielded 9.11g, 5.40g and 1.8g each using chloroform, ethanol and distilled water as a solvent respectively as reported in table 1, the results obtained from the study of *Mangifera Indica* Bark is consistent with findings of Pintu and Arna [13].

Table 2: Result of Antimicrobial test

Isolates	Zone of inhibition against fractions (mm)												
	Chloroform (mg/mL)				Ethanol (mg/mL)				Aqueous (mg/mL)				Erythromycin (mg/mL)
	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5	62.5
Salmonella Typhi	11	9.0	-	-	10	-	-	-	9.0	7.0	7.0	-	27
Pseudomonas Aeruginosa	-	-	-	-	11	-	-	-	-	-	-	-	21
Staphylococcus Aureus	18	13	9.0	7.0	24	18	12	9.0	28	24	11	-	51
Escherichia Coli	11	7.0	-	-	-	-	-	-	-	-	-	-	18

Key: - = Not detected

The antibacterial screening reported in table 2 revealed that the chloroform extract inhibited the growth of *Salmonella* and *E. Coli* at 500mg/mL and 250mg/mL. The extract was also found to be active against *Staph. Aureus*. at all the concentrations, although it was not active against *Pseud. Aeruginosa*. The ethanol extract inhibited the growth of *Salmonella* and *Pseudomonas* at 500mg/mL concentration and inactive on other concentrations. It was

also unable to inhibit the growth of *E. Coli* in all the concentrations, and inhibited the growth of *Staph. Aureu*s. in all the concentrations This result is consistent with the work of Dahiru et al [11].

The aqueous extract inhibited the growth of *Salmonella* and *Staph. Aureus* on 500mg/mL, 250mg/mL and 125mg/mL; and not active against them at 62.5mg/mL, it was unable to inhibit *pseudomonas* and *E. Coli* growth in all the concentrations as previously reported by Ayoola et al [10].

Table 3: Result of Antifungal test

Isolates	Zone of inhibition against fractions (mm)											
	Chloroform (mg/mL)				Ethanol (mg/mL)				Aqueous (mg/mL)			
	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5
Aspergillus Flavus	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus Niger	-	-	-	-	-	-	-	-	-	-	-	-

Key: - = Not detected

Table 3 shows the Antifungal analyses for the extracts under four different concentrations, the various extracts were found to be in active against the tested fungi at all the four different concentrations.

Table 4: Phytochemical Screening Results

S/No	Phytochemical	Chloroform extract	Ethanol extract	Aqueous extract
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Saponins	-	+	+
4	Steroids	+	+	+
5	Tannins	+	-	+
6	Glycosides	+	-	-
7	Terpenoids	-	-	-
8	Phenols	+	-	+

Key: + = Present; - = Not detected

Table 4 presented the phytochemical screening result. The Phytochemicals alkaloids, flavonoids and steroids were found present in chloroform, ethanol and aqueous extracts. While tannins, phenols were found present in both chloroform and aqueous extract, Saponins were found present in both aqueous and ethanolic extracts. While glycoside and terpenoids were found absent in chloroform, ethanol and aqueous extracts. The results obtained on phytochemical study of *Mangifera Indica* (Bark) extracts were similar to what was reported by Pritesh et al [14].

Conclusions

The bark of *Mangifera Indica* was selected on the basis of its uses and subjected to phytochemical screening was found to show the phytoconstituents tested. It was further tested against *Salmonella Typhi, Pseudomonas Aeruginosa, Staphylococcus Aureus and Escherichia Coli* using agar well diffusion method. The chloroform, ethanol and distilled water fractions of the bark of *Mangifera Indica* at a concentration of 500 mg/mL showed a remarkable zone of inhibition of 18mm, 24mm and 28mm against *Staphylococcus Aureus* respectively. The antifungal test did not show any positive result.

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