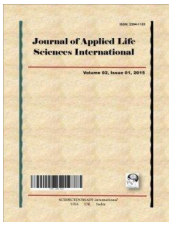

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Phytochemical Screening and Antibacterial Activity of Methanolic Extracts of Ripe and Unripe Peels of Mango (*Mangifera indica* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VOF and IAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AOA and TRE managed the analyses of the study. Author IAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the phytochemical constituents and antibacterial activity of methanolic extracts of ripe and unripe peels of mango (*Mangifera indica* L.) against pathogenic bacterial strains.
Methodology: Both ripe and unripe mango peels were collected separately, air-dried and powdered. Crude methanolic extracts of ripe and unripe mango peels were analyzed for the phytochemical constituents. Different concentrations (100, 200 and 400 mg/ml) of methanolic extracts of the mango peels were prepared using 30% Dimethyl sulfoxide (DMSO). Antibacterial activity was evaluated by agar well diffusion method using seven bacterial species: three reference strains- *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212); and four food-borne pathogens- *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Bacillus cereus*.
Results: Phytochemical analysis revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids and phenolic compounds in both ripe and unripe mango peels extracts while

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glycosides was not detected in the extracts. Both peel extracts exhibited potent antibacterial activities against all the tested pathogenic bacteria and this inhibitory effect increased with the increasing concentrations of the extracts.

Conclusion: The present study has revealed that methanolic extract of ripe and unripe mango peels contains phytochemical constituents and possess antibacterial activity against the tested pathogenic bacterial strains. Hence, mango peel extracts can be utilized as an alternative antibacterial agent in the treatment of diseases caused by pathogenic bacteria.

Keywords: Mangifera indica; peel extract; phytochemical analysis; antibacterial activity.

1. INTRODUCTION

Infectious diseases are leading cause of death worldwide due to multidrug resistant strains of bacteria, reduced susceptibility to antimicrobics and increase in untreatable bacterial infections [1]. In recent years, multiple drug resistance in human pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has necessitated a search for new antimicrobial compounds and for this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against pathogenic microbial strains [2].

Medicinal plants produce a large number of secondary metabolites with antimicrobial effects on pathogens [3]. All parts of plants individually or in combination show antimicrobial properties. A significant part of the chemical diversity produced by plants is thought to protect plants against microbial pathogens [1]. Numerous scientific investigations point at consecutive rich sources of antimicrobics, especially among fruits and vegetables, but only few of them involve waste parts of fruits, i.e. seeds and peels [1]. One of the agro-wastes, currently causing pollution problems and also underutilized is the peels of the mango fruit.

Mango (*Mangifera indica* L.) is a member of the family Anacardiaceae. Mango is one of the most important tropical fruits that are abundant in Nigeria. Thus, there is an abundant supply of mango by-products such as seed kernels and peels which are considered as wastes after consumption or industrial processing of mango fruits [4].

Therefore, the objective of the present study was to evaluate the phytochemical constituents and antibacterial activity of methanolic extracts of ripe and unripe peels of mango (*Mangifera indica* L.) against pathogenic bacterial strains.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Ripe and unripe mango (*Mangifera indica* L.) fruits were collected from Owo Local Government Area, Ondo State, Nigeria. The fruits were washed and the peels were manually removed separately, air-dried for three weeks and powdered using electric blender. The powdered samples were stored in airtight container for analysis.

2.2 Preparation of Extracts

The method of Silva et al. [5] was adopted, fifty (50) grams each of the powdered sample of ripe and unripe mango peels was suspended in 250 ml of 99% absolute methanol separately for a period of about 72 hours with intermittent shaking. The extracts were filtered with double folded muslin cloth and the filtrate evaporated at room temperature. And then reconstituted in 30% dimethylsulphoxide (DMSO) and reserved as stock concentration and then stored.

2.3 Phytochemical Analysis

The methanolic extracts of both ripe and unripe mango peels were screened qualitatively and quantitatively for tannins, saponins, flavoniods, terpenoids, glycosides, alkaloid and phenolic compounds using the standard procedures as described by Sofowora [6], Harborne and Baxter [7], and Trease and Evans [8].

2.4 Microorganisms Used

Seven bacterial pathogenic strains were used: three reference strains- *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Enterococcus faecalis* (ATCC 29212); and four food-borne pathogens- *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis, and Bacillus cereus*.

2.5 Antibacterial Assay

The antibacterial effect of the mango peel extracts was investigated by agar well diffusion method as described by Jafari et al. [9]. The microbial strains tested were initially suspended in sterile Tryptone Soya Broth to a turbidity matching 0.5 Mcfarland standards.

The prepared inocula were swabbed onto the surfaces of Muller Hinton Agar plates with the sterile swab, and left to dry at room temperature. Then wells with 6 mm diameter were created at agar surface; 80 µl of 100, 200 and 400 mg/ml concentrations of the extracts were injected into the wells.

The negative control was 30% DMSO and the antibiotic chloramphenicol was used as positive control. Then the plates were incubated at 37°C for 24 hours and inhibition zone was measured in millimetre.

2.6 Statistical Analysis

Data values were expressed as mean ± standard deviation. Statistical data analysis (t-test and one-way analysis of variance) was performed by using SPSS software version 16.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The results of qualitative phytochemical screening of methanolic extracts of ripe and unripe mango peels revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids and phenolic compounds in both ripe and unripe mango peels extracts while glycosides was not detected in both extracts (Table 1). The presence of these secondary metabolites (phytoconstituents) suggests that both peel extracts might be of medicinal importance. The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases [10].

The results of quantitative phytochemical screening of the ripe and unripe peel extracts of mango are shown in Table 2. It was observed that the ripe mango peels have a higher amount of some of the phytoconstituents (tannins, saponins, and alkaloids) than the unripe mango peels. This finding is similar to the report of Rakholiya et al. [11]. Rakholiya et al. [11] reported that alkaloids and flavonoids were higher in ripe peel than unripe peel of Indian Kesar Mango. The absent of cardiac glycosides in unripe peel extract of mango reported in this study is contrary to the findings of Rakholiya et al. [11].

Table 1. Qualitative phytochemical constituents of methanolic extracts of ripe and unripe mango peels

Constituents	Ripe mango peels	Unripe mango peels
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Glycosides	-	-
Alkaloid	+	+
Phenolic compound	+	+

Key: + = Present; - = Absent

Table 2. Quantitative phytochemical constituents of methanolic extracts of ripe and unripe mango peels

Constituents	Ripe mango peels	Unripe mango peels
Tannins (mg/g)	1.974	1.908
Saponins (%)	21.415	15.950
Flavonoids (%)	3.826	3.826
Alkaloid (%)	4.412	3.744

3.2 Antibacterial Activity

The results of antibacterial activity studies of methanolic extracts of ripe and unripe mango peel are presented in Figs. 1 and 2. Both mango peels extracts exhibited potent antibacterial activities against all the tested pathogenic bacterial strains and this inhibitory effect increased with the increasing concentrations of the methanolic extracts. Methanolic extract of ripe mango peel exhibited higher antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus*. Similarly, when comparing the antibacterial activity of the methanolic extract of unripe mango peel, *S. aureus* (ATCC 25923) and *B. cereus* was highly sensitive than other bacteria. The antibacterial potency of mango peels observed in this study corroborates the findings of Abdullah et al. [12] and Rakholiya et al. [11]. Abdullah et al. [12]

reported that the peel of mango (*Mangifera indica*) showed potent antibacterial activities against both Gram-positive and Gram-negative bacteria.

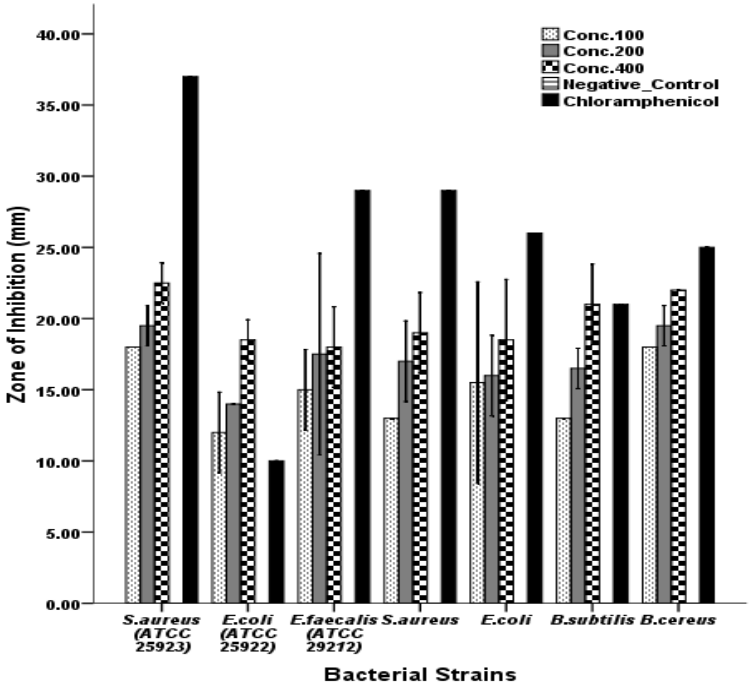

Fig. 1. Antibacterial activity of methanolic extract of ripe mango peel (Concentrations in mg/ml)

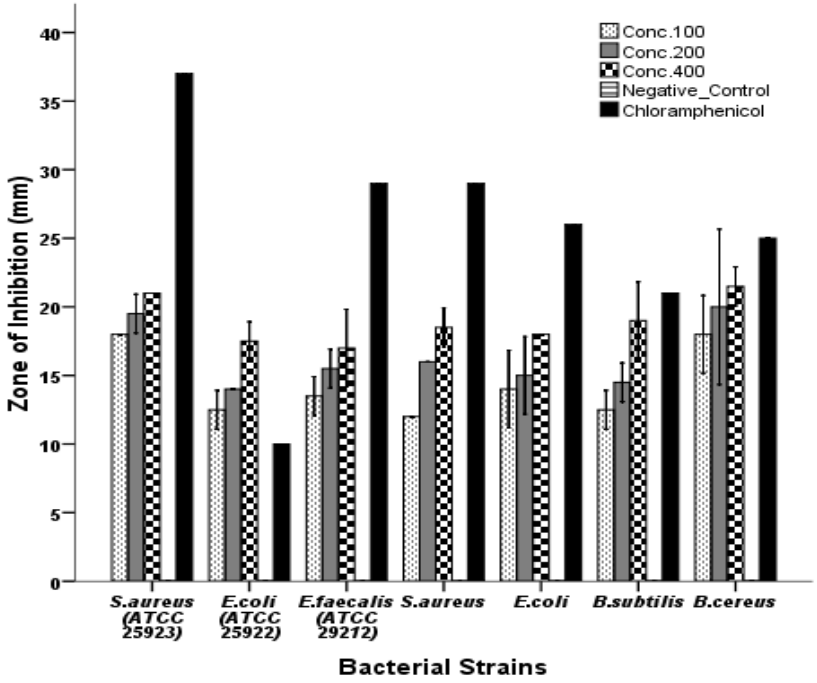

Fig. 2. Antibacterial activity of methanolic extract of unripe mango peel (Concentrations in mg/ml)

Table 3. Comparison of methanolic extracts of ripe and unripe mango peel at 400 mg/ml concentration

Samples	Zone of Inhibition (mm)						
	***S. aureus* (ATCC 25923)**	***E. coli* (ATCC 25922)**	***E. faecalis* (ATCC 29212)**	***S. aureus***	***E. coli***	***B. subtilis***	***B. cereus***
Ripe mango peel extract	22.50^{b}±0.71	18.50^{b}±0.71	18.00^{a}±1.41	19.00^{a}±1.41	18.50^{a}±2.12	21.00^{a}±1.41	22.00^{a}± 0.00
Unripe mango peel extract	21.00^{a}±0.00	17.50^{b}±0.71	17.00^{a}±1.41	18.50^{a}±0.71	18.00^{a}±0.00	19.00^{a}±1.41	21.50^{a}± 0.71
Chloramphenicol (Positive control)	37.00^{c}±0.00	10.00^{a}±0.00	29.00^{b}±0.00	29.00^{b}±0.00	26.00^{b}±0.00	21.00^{a}±0.00	25.00^{b}± 0.00
30% DMSO (Negative control)	-	-	-	-	-	-	-

- = No Zone of Inhibition, Values expressed as mean ± SD, n = 2

Data with different letter in the same column is significantly different at the level P < 0.05

The antibacterial activity of methanolic extracts of ripe and unripe mango peel is significantly lower when compared with the commercial antibiotic chloramphenicol at 400 mg/ml concentration with the exception of the activity against *E. coli* (ATCC 25922). However, there is no significant difference ($P > 0.05$) in the activity of chloramphenicol and the mango peel extracts against *B. subtilis* at 400 mg/ml concentration while activity of chloramphenicol is significantly lower than that of the mango peel extracts against *E. coli* (ATCC 25922) (Table 3).

Comparing the two peel extracts, though the differences for some of the activities are insignificant, there was a slight increase in the antibacterial activity of the methanolic extract of ripe mango peel. This may be attributed to the minor variations in the amount of phytochemical constituents present in both extracts.

The present study has revealed that the methanolic extract of ripe and unripe mango peels contains substantial amount of phytochemical constituents and thus, can be inferred that these phytochemical constituents are responsible for its marked antibacterial activity against the pathogenic bacterial strains. This is consistent with other reports that have shown close relationship between phytochemical constituents and antibacterial activity of fruit peels [13,14].

4. CONCLUSION

Methanolic extracts of ripe and unripe mango peel, which are wastes generated from mango fruits, are a good source of phytochemicals. They also possess antibacterial activity against the tested pathogenic bacterial strains. Hence, mango peel extracts can be utilized as an alternative antibacterial agent in the treatment of diseases caused by pathogenic bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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