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Antibacterial Properties of Young and Mature Mango Leaves (*Mangifera indica*) Extract on Some Clinical Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study was carried out to investigate the antibacterial properties and efficacy of mango (*Mangifera indica*) leaf extracts on some clinical isolates as test organisms.
Study Design: The study employed statistical analysis of the data and interpretation
Place and Duration of Study: Young and mature mango leaves were collected from the Botanical Garden, Kenule Beeson Saro-Wiwa Polytechnic, Bori, Nigeria, and taken to the laboratory for analyses.
Methodology: The samples were dried in an oven at 80^{o}C for 3 days. Thereafter, 50 g of each ground mango leaf (young and mature leaves) were soaked separately in 500 ml of water, ethanol (95% v/v), and acetic acid (99.9% v/v) respectively for another 3 days. The soaked materials were filtered through Whatman No. 1 filter paper into sterile beakers and evaporated to dryness in a water bath at 80^{o}C. The dried extracts obtained were reconstituted with water at concentrations of 100, 75, 50, and 25 mg/ml. Test organisms, *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Proteus mirabilis, Bacillus cereus,* and *Pseudomonas aeruginosa* were obtained after proper laboratory screening of isolates from the diagnostic laboratory of the Rivers State University Teaching Hospital, Port Harcourt, Nigeria, for confirmation of identity and storage in universal bottles in a refrigerator. Sensitivity tests were carried out with the agar well diffusion method against the test organisms, using tetracycline as the standard control drug, with cultures incubated

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accordingly. The measured zones of inhibition were compared with the controls and interpreted as resistant, intermediate, or susceptible to mango extracts in accordance with the interpretive guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS). Assay for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was also carried out.

Results: Results obtained showed that acetic acid young leaf extract at 100mg/ml produced 50 % susceptibility and 50 % intermediate response of test bacterial species. Generally, at 100 mg/ml, acetic acid young leaf extracts yielded 50% susceptibility and 50% intermediate response among both Gram-positive and Gram-negative bacteria. Ethanolic extracts gave 100% intermediate sensitivity of Gram-negative species and 50% each of resistant and intermediate response in Gram-positive forms. Aqueous extracts also produced no susceptibility among the test organisms as there was 100% resistance. Extracts of mature mango leaves of all solvents and at all concentrations used yielded no susceptibility response among the test bacterial species on the NCCLS scale. Minimum inhibitory and bactericidal concentrations were found to range from 25 mg/ml to 50 mg/ml. Additionally, it was observed that the sensitivity of organisms to mango extracts increased with concentration.

Conclusion: In conclusion, acetic acid has a better extracting potential than ethanol and water as a solvent for the extraction of mango parts. More so, young mango leaves extracted with acetic acid possess higher broad-spectrum antibacterial properties than the mature mango leaves extracted from the same plant. It is therefore recommended that young mango leaves, extracted with acetic acid, be used for the treatment of microbial infections at concentrations not below 50 mg/ml.

Keywords: Mango young leaf; mature leaf; acetic acid; ethanol; Staphylococcus aureus; Escherichia coli; Pseudomonas aeruginosa; Proteus mirabilis; Salmonella typhi; Bacillus cereus.

1. INTRODUCTION

Plant organs – leaves, flowers, fruits, stems, roots, and barks contain numerous chemical substances. These phytochemicals have been extracted by smoking, squeezing, or use of appropriate solvents for use in the treatment of human diseases [1]. Diseases controlled with mango extracts include bone fractures, ear infections, diarrhea, dysentery, malaria, ulcer, typhoid fever, sore throat, and urinary tract infections [2]. The list of conditions treatable with this plant is inexhaustible.

Mango belongs to the plant family *Anacardiaceae*. It is found in the wild or may be cultivated. The mango tree is medium to large, reaching up to 40m depending on the type and variety. The canopy is rounded and dense with evergreen leaves. Flowers of mango are terminal and borne in large groups. The fruits vary in size, shape, color, fiber content, flavor, and aroma according to species [3]. The leaves of the mango plant contain phytochemicals which include glycosides (particularly mangiferin), saponins, tannins, and euxanthin acid [4]. Barks of the plant have steroids, glycosides, saponins, resins, phenols, flavonoids, and alkaloids, among others [5,6]. Mango roots are also rich in phytochemicals. Qualitatively, the roots contain similar compounds as the stem-bark [7]. Quantitative analysis of the compounds present in mango parts has shown that mangiferin is the predominant component [8]. For this reason, mango is the chief source of this compound [9].

Although mango parts contain similar phytochemicals, their quantitative distribution varies from one part to another. Mangiferin (a glycoside and polyphenol), for instance, is high in young leaves, moderate in bark, and low in mature leaves and roots [10]. Mangiferin has been shown to contribute immensely to the antimicrobial actions of mango extracts [11,12]. Other compounds such as alkaloids, flavonoids, saponins, and tannins, extractable from mango parts, have also been credited with antimicrobial activities [13]. Alkaloids are organic water-soluble nitrogenous bases that may contain sugar as part of the molecule. They are known to possess antimicrobial properties. Flavonoids are polyphenolic compounds produced by plants to curb infection by pathogens. Saponins are soap-like compounds that can interfere with cell membrane function. Tannins are also polyphenolic compounds with many hydroxyl groups and capable of bioactivity.

Aqueous and ethanolic extracts of mango leaves and stem have been found to have activity against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia,*

Pseudomonas aeruginosa, Enterococcus faecalis, Salmonella typhi, Listeria monocytogenes, Escherichia coli and *Candida albicans* [14]. With methanol, ethanol, and benzene as extracting solvents, antibacterial activities of mango leaves were found against *Proteus Vulgaris, Pseudomonas fluorescens, Shigella flexneri, Klebsiella pneumonia,* and *Salmonella typhi* [15].

The relevance of the use of plant extracts in healthcare delivery systems stems from the fact that many organisms are developing resistance to commonly used antibiotics. This necessitates the search for novel substances with therapeutic values from plants. At present, a number of solvents, particularly organic solvents, are used for the extraction of bioactive substances from plant parts for use in medicine. Solvents commonly used for the extraction of phytochemicals are water, ethanol, methanol, acetone, chloroform, benzene, ethyl acetate, and hexane [5,16]. Organic solvents have better extractive properties than water [16]. It has also been seen that the activity of extracts against microorganisms increases with an increase in the concentration of phytochemicals present [17]. Though a lot of work has been done to show the therapeutic values of plant parts, especially those of mango, studies that reveal the effect of the state of maturity of plant organs, on bioactivity are scarce. Also, there is a rarity of studies on the efficacy of organic acids, particularly acetic acid, in the extraction of phytochemicals. Hence, the present study investigates the antibacterial properties of young and mature mango leaves extracted with different solvents which include acetic acid.

2. MATERIALS AND METHODS

2.1 Study Area

The present study was carried out in Kenule Benson Saro-Wiwa Polytechnic, Bori. Bori is the host of this Polytechnic and is the capital city of Khana Local Government Area, Rivers State, Nigeria. Bori is located in the south-south region of Nigeria with coordinates $4^{o}40'22''$ $N7^{o}22'$ $13''$ E. Bori is an agricultural hub in Rivers State and involves in the production of yam, cassava, oil palm, corn, cocoyam, vegetables, and fruits (including the mango).

2.2 Collection of Mango Specimens

The most popular mango variety is the one with elongated persistent green fruits popularly called green mango [18]. Mature and young leaves of mango (*Mangifera Indica)* were collected from the Botanical Garden, Kenule Beeson Saro-Wiwa Polytechnic, Bori. The leaves were collected from the tree canopy by means of a matchet into clean polythene bags and taken to the laboratory.

2.3 Collection of Test Cultures

Cultures of isolates – *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Salmonella typhi*– were obtained from the diagnostic laboratories of Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria. After preparation into pure cultures, using the streak plate technique, the organisms were screened using cultural characteristics, cell morphology, Gram reaction, and biochemical tests before use.

2.4 Preparation of Pure Cultures

Collected test organisms were streaked on appropriate media to obtain isolated colonies. *Escherichia coli* was streaked on MacConkey agar. *Salmonella* and *Proteus* were streaked on *Salmonella-Shigella* agar, and the remaining organisms were streaked on nutrient agar. An isolated colony of each bacterium was transferred to a nutrient agar slant in a universal bottle. Slant cultures were incubated at $37^{o}C$ for 24 hours. Thereafter, storage of cultures was carried out in the refrigerator.

2.5 Preparation of Mango Extracts

Collected mango parts were washed of debris and dried in an oven at $80^{o}C$ for 3 days. The dried materials were ground in a surface-sterilized electric blender into fine particles. Fifty gram (50g) amount of each ground plant part was transferred into a sterile one-liter conical flask and 500ml of solvent (water, ethanol (95 % v/v), or acetic acid (99.9% v/v)) was added and mixed properly. The soaked plant substances were allowed to stand at ambient temperatures for 72 hours as described by Doughari and Manzara [17]. Using a funnel, soaked mango samples were separately filtered through a sterile muslin filter and again through Whatman No. 1 filter paper into sterile beakers. From each filtrate, the solvent was evaporated via a water bath at $80^{o}C$ until dryness. The dried substances obtained were stored aseptically in specimen bottles until needed.

2.6 Preparation of Plant Extract Solutions

Each plant extract was reconstituted with sterile distilled water to give concentrations of 100, 75, 50, and 25 mg/ml [5]. To prepare 100 mg/ml extract, 1g (1000mg) of the dried extract was transferred into a sterile measuring cylinder and homogenized with sterile distilled water to a final volume of 10ml (i.e. 1000 mg/10 ml or 100 mg/ml). To prepare 75 mg/ml, 1.5 g (1500 mg) of the extract was homogenized with sterile distilled water to a final volume of 20 ml. For 50 mg/ml, 1 g (1000 mg) of the extract was homogenized in a final volume of 20 ml, and for 25 mg/ml, 0.5 g (500 mg) of the extract was homogenized in a final volume of 20 ml with sterile distilled water.

2.7 Preparation of Control Antimicrobial Discs

Control antimicrobial discs were prepared as described by Ochei and Kolhatkar [19]. Using a paper punch, 6-mm discs were cut from Whatman's No.1 filter paper and sterilized in an autoclave at 121° C for 15 minutes. Thereafter, the discs were dried in an oven at 100° C for 30 minutes. The capacity of a 6-mm disc cut from Whatman's No.1 filter paper is 0.02 ml [19]. To prepare 30 μg/disc of tetracycline, 250 mg of the antibiotic was homogenized aseptically with sterile distilled water in a sterile measuring cylinder to a final volume of 167 ml. Thereafter, punched discs were impregnated aseptically with 0.02 ml of the control in a Petri dish and allowed to air-dry (i.e., 250 mg / 167 ml or 1.5 mg/ml or 30 μg / 0.02 ml).

2.8 Antimicrobial Sensitivity Profile

Mueller-Hinton agar was prepared according to the manufacturer's direction. Each bacterial suspension was prepared to match 0.5 McFarland standard and transferred by means of the inoculating loop, in one loopful amount, onto Mueller-Hinton agar. Inoculum in each case was spread evenly on the agar surface using a sterile swab stick [19]. Seeded plates, in duplicates for each organism, were allowed to air-dry on the surface-sterilized laboratory bench. Thereafter, a sterile 6-mm cork borer was used to create wells in the seeded plates, such that wells were at least 22 mm from each other and at least 14 mm from the edge of the plate [19]. A set of four concentrations – 100, 75, and 50,25 mg/ml of each plant part extracted with each test solvent (water, ethanol, or acetic acid) were transferred into labeled wells by means of sterile pipettes. Extracts were allowed to diffuse from the wells into the medium for 30 minutes on the laboratory bench. For controls, each bacterium was challenged with prepared tetracycline discs. The antimicrobial discs were placed on seeded plates and pressed lightly onto the medium for stability using a pair of sterile forceps. The antimicrobial agent was allowed to diffuse into the medium on a laboratory bench for 30 minutes. Thereafter, the bacterial cultures were incubated at 37^0C for 24 hours.

2.9 Measurement and Interpretation of Inhibition Zones

Following incubation, the diameter of the zone of inhibition was measured across each disc or well by means of a transparent ruler, in millimeters (mm). Using the control and the interpretive guidelines published by National Committee for Clinical Laboratory Standard (NCCLS), inhibition zones of $\leq$ 14 mm were read as the resistance of the test organism to the antimicrobial agent, and inhibition zones of 15 – 18 mm as an intermediate response; and $\geq$ 19 mm as the susceptibility of organism [19].

2.10 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

This test was carried out as described by Ochei and Kolhatkar [19] and Mustapha et al. [4]. Minimum bactericidal concentration (MBC) is the lowest concentration of an antimicrobial substance required to kill at least 99.9% of microbial cells present [19]. MBC was determined by subculturing 0.1 ml of the highest concentration of mango extract that showed visible growth as well as all tubes that showed no visible growth in the MIC test onto Mueller-Hinton agar. After incubation at 37 ^{o}C for 24 hours, the culture plates were observed for sterility.

2.11 Statistical Analysis

Data obtained in the present study were subjected to statistical analysis using the analysis of variance (ANOVA) test to establish significant differences among variables.

3. RESULTS AND DISCUSSION

3.1 Identification of Test Bacteria

Cultural, morphological and biochemical characteristics of bacteria were used for the identification of isolates. *Escherichia coli* on MacConkey agar produced small, pink, moist, convex, and entire colonies. Colonies of *Staphylococcus aureus* were small, golden orange, moist, convex and entire on nutrient agar. Colonies of *Salmonella typhi* and *Proteus mirabilis* on *Salmonella–Shigella* Agar (SSA) were milky, convex, and smooth with dark centers due to the production of hydrogen sulfide (H_2S). These species were separated by urase test in which *Salmonella* was negative and *Proteus* positive. *Bacillus cereus* colonies on nutrient agar were large milky, dry, flat, and irregular-edged. *Pseudomonas aeruginosa* on nutrient agar showed moist, smooth, convex colonies that produced blue-green pigmentation.

3.2 Sensitivity of Test Bacteria to Mango Extracts

Table 1 shows the susceptibility profile of bacteria to mango leaf extracts. Acetic acid young leaf extract exhibited 50% susceptibility, 50% intermediate, 0% resistance of test bacterial species at 100 mg/ml. Lower concentrations of 75 and 50 mg/ml gave resistant and intermediate responses only. There was no susceptibility at lower concentrations of acetic acid in young mango leaf extracts. Ethanolic young leaf extract at 100 mg/ml concentration produced 83.33% intermediate reaction and 16.67% resistance of test bacteria. This shows that all concentrations of ethanolic young leaf extract used in the present study gave no susceptibility reaction. Test bacteria were completely resistant to aqueous young leaf extract at all test concentrations.

Acetic acid and ethanolic mature leaf extracts at 100 mg/ml each produced 83.33% resistance and 16.67% intermediate response among the organisms tested. All lower concentrations exhibited complete resistance of bacterial species. Again, there was no response of test organisms to aqueous mature mango leaf extract.

Comparing the susceptibility of Gram-positive and Gram-negative bacteria to mango extracts with young and mature parts taken together, it was observed that the organisms were 50 % susceptible, 50 % intermediate and 0 % resistant to acetic acid leaf extracts at 100 mg/ml, when using NCCLS interpretive guidelines. To ethanolic leaf extracts, the response was 100 % intermediate for Gram-negative species and 50 % each for intermediate and resistant responses of Gram-positive forms. Sensitivity of the organisms to aqueous leaf extracts was the same for both forms of bacteria – 100 % resistant (Fig. 1). That is, there was 0 % resistance of organisms to acetic acid young leaf extract whereas ethanolic and aqueous extracts showed 0 % susceptibility of test bacteria on the NCCLS scale.

Plants contain chemotherapeutic components which, from ancient times, have been exploited in herbal medicine for the treatment of disease [5]. These phytochemicals are believed to protect plants from invading microorganisms. Among mango phytochemicals, mangiferin is considered the most important, and the plant is the chief source of this compound [9]. This chemical is obtained at 172 g of mangiferin from 1 kg of young leaves; at 107 g from 1 kg of stem-bark; and 94 g of mangiferin from 1 kg of mature leaves [10]. Since mangiferin is principally responsible for the bioactivity of mango against microorganisms [20, 21], it follows that among the three organs mentioned here (young leaves, stem-bark and mature leaves), the young leaves would show the greatest activity whereas the mature leaves the least. This position is obvious in the results obtained in the present study. Extracts of young leaves demonstrated higher inhibition zones against test organisms than mature mango leaves. Further, it could be easily seen that acetic acid extracts inhibited test microorganisms the most, whereas aqueous extracts showed the least activity. Indeed, statistical analysis using Analysis of variance (ANOVA) tests showed that the sensitivity of organisms to mango extracts in all cases was mango-organ-dependent. It also showed that the different mango parts(at different stages of maturity) produced different levels of susceptibility among test organisms and that the different solvents used for extraction of mango phytochemicals as well as the various concentrations employed were significantly different ($p < 0.05$).

Table 1. Susceptibility profile of test bacteria to mango extracts

Extract	Concentration (mg/ml)	Inhibition zone (mm)and interpretation (R-I-S)						Percentage susceptibility		
		E. coli	*S. aureus*	*P. aeruginosa*	*P. mirabilis*	*S. typhi*	*B. cereus*	*R n(%)*	*I n(%)*	*S n(%)*
Acetic acid young leaf Extract	100	20(S)	20(S)	16(I)	15(I)	21(S)	18(I)	0(0.00)	3(50.00)	3(50.00
	75	18(I)	17(I)	15(I)	13(R)	14(R)	15(I)	2(3.33)	4(66.67)	0(0.00)
	50	16(I)	14(R)	14(R)	11(R)	11(R)	13(R)	5(83.33)	1(16.67)	0(0.00)
	25	12(R)	12(R)	11(R)	9(R)	9(R)	11(R)	6(100.00)	0(0.00)	0(0.00)
Ethanolic young leaf extract	100	18(I)	0(R)	16(I)	15(I)	15(I)	17(I)	1(16.67)	5(83.33)	0(0.00)
	75	16(I)	0(R)	11(R)	10(R)	12(R)	14(R)	5(83.33)	1(16.67)	0(0.00)
	50	15(I)	0(R)	10(R)	8(R)	11(R)	11(R)	5(83.33)	1(16.67)	0(0.00)
	25	10(R)	0(R)	8(R)	0(R)	0(R)	9(R)	0(0.00)	0(0.00)	0(0.00)
Aqueous young leaf extract	100	0(R)	0(R)	13(R)	0(R)	0(R)	11(R)	6(100.00)	0(0.00)	0(0.00)
	75	0(R)	0(R)	11(R)	0(R)	0(R)	8(R)	6(100.00)	0(0.00)	0(0.00)
	50	0(R)	0(R)	8(R)	0(R)	0(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
	25	0(R)	0(R)	0(R)	0(R)	0(R)	9(R)	6(100.00)	0(0.00)	0(0.00)
Acetic acid mature leaf Extract	100	11(R)	15(R)	13(R)	12(R)	12(R)	9(R)	5(83.33)	1(16.67)	0(0.00)
	75	10(R)	11(R)	12(R)	10(R)	10(R)	8(R)	6(100.00)	0(0.00)	0(0.00)
	50	8(R)	10(R)	11(R)	8(R)	8(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
	25	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
Ethanolic mature leaf extract	100	10(R)	0(R)	16(I)	0(R)	12(R)	10(R)	5(83.33)	1(16.67)	0(0.00)
	75	0(R)	0(R)	13(R)	0(R)	10(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
	50	0(R)	0(R)	12(R)	0(R)	8(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
	25	0(R)	0(R)	8(R)	0(R)	0(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
Aqueous leaf Extract	100	0(R)	0(R)	0(R)	0(R)	0(R)	11(R)	6(100.00)	0(0.00)	0(0.00)
	75	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
	50	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	6(100.00)	0(0.00)	0(0.00)
	25	0(R)	u0(R)	0(R)	0(R)	0(R)	0(R)	6(100.00)	0(0.00)	0(0.00)

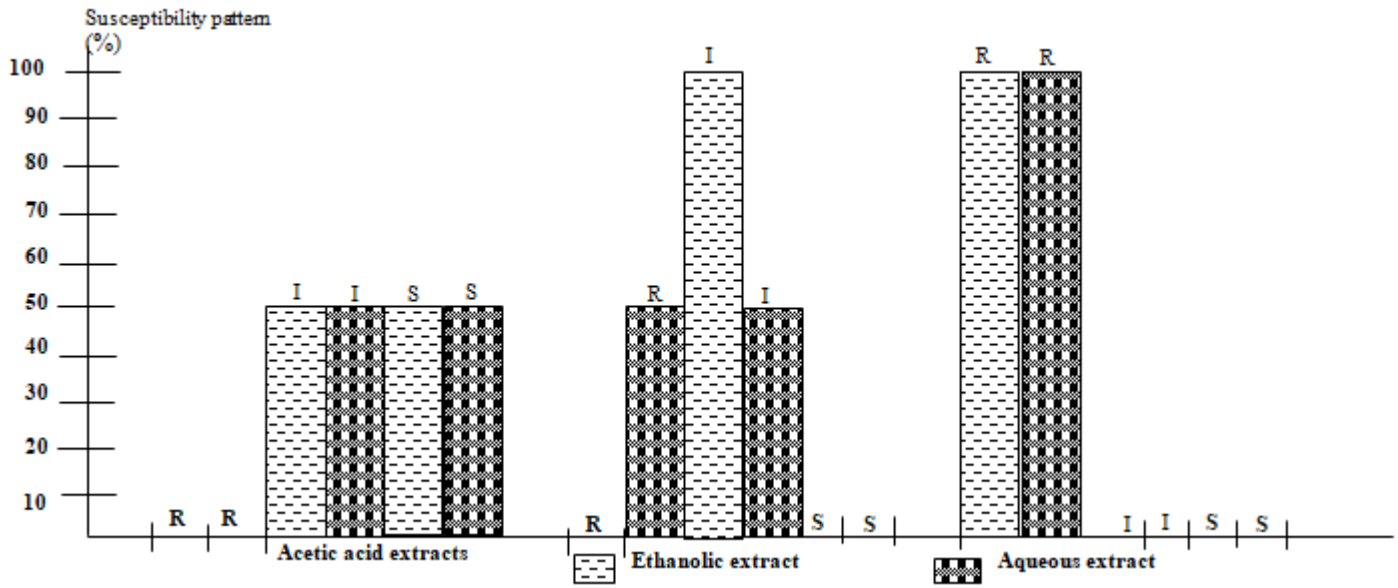

Fig. 1. Percentage susceptibility pattern of Gram-negative, and Gram-positive, bacteria to 100 mg/ml acetic acid, ethanolic and aqueous young and mature mango leaf extracts

R = percentage resistant, I = Percentage Intermediate, S = Percentage Susceptibility

Table 2. MIC of strongly bioactive leaf extracts on test isolates

Organism	EYE (mg/ml)				EME (mg/ml)				AYE (mg/ml)				AME (mg/ml)			
	50	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**
E. coli	-	-	-	+	ND	ND	ND	ND	-	-	-	+	-	+	+	+
Staph. Aureus	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-	+	-	+	+	+
Pseudo. aeruginosa	-	-	+	+	-	-	+	+	-	-	-	+	-	+	+	+
Bacillus cereus	_	+	+	+	ND	ND	ND	ND	-	-	-	+	ND	ND	ND	ND
Proteus mirabilis	-	+	+	+	ND	ND	ND	ND	-	-	+	+	-	+h	+	+
Salmonella typhi	-	+	+	+	-	+	+	+	-	-	+	+	-	+	+	+
Candida albicans	-	+	+	+	-	+	+	+	-	-	-	+	-	+	+	+

– = No growth; + = growth (turbidity); ND = not done (because extracts produced little or no susceptibility of test organisms); EYE = Ethanol young leaf extract; EME = ethanolic mature leaf extract; AYE = Acetic acid young leaf extract; AME = Acetic acid mature leaf extract

In the present study, all test organisms were resistant to aqueous extracts of a mature mango leaf. This is in agreement with the findings of Doughari and Manzara [17] and Nwankwo and Osaro-Mathew [22]. The poor activity of aqueous extracts could be attributable to the fact that some bioactive phytochemicals have limited solubility in water [5] and therefore may not be available when water is used for extraction. Unlike water which can dissolve only polar substances, ethanol and acetic acid can dissolve both polar and non-polar solutes. The ability of ethanol and acetic acid to dissolve polar and non-polar solutes increases the capacity of these solvents to extract bioactive substances from plant organs [23,24]. For this reason, leaf extracts of ethanol and acetic acid inhibited most of the organisms tested in the present study. Ethanolic leaf extract of mango, however, did not produce inhibition against *S. aureus*. This also agrees with the findings of Mustapha et al. [4] who found that *Staphylococcus aureus* was resistant to ethanolic extracts of mango leaves. Acetic acid, the most successful solvent used in this study, is considered safe because vinegar derived from it is used to season food. The lack of use of acetic acid as a solvent for the extraction of phytochemicals may stem from the fact that it has a high boiling point (118℃) and is therefore difficult to remove from extracts. Its success as a solvent, however, far outweighs this difficulty. Additionally, it was observed that extract yield from young leaves with acetic acid, used in the same volume as other extraction solvents, was the highest. This shows that the extractive power of this organic acid is high.

3.3 Minimum Inhibitory Concentration (MIC) of Mango Extracts of Test Organisms

The minimum inhibitory concentration of ethanolic young leaf extract against *Escherichia coli* was 12.5mg/ml (Table 2). This same concentration was observed for this organism with acetic acid young mango leaf extract. With acetic acid mature leaf extract, the MIC was 50mg/ml.

Determination of MIC of acetic acid young mango leaf extract against *Staphylococcus aureus* showed 12.5mg/ml. Using acetic acid mature leaf extract, the MIC was 50mg/ml. Growth of *Pseudomonas aeruginosa* was inhibited at 25mg/ml of ethanolic young leaf extract. With mature mango leaf extract, the MIC was also found to be 25mg/ml. Using acetic acid young leaf extract the MIC was 12.5mg/ml, and with mature leaf acetic acid extract, it was 50 mg/ml. The lowest concentration of ethanolic young leaf extract that inhibited *Bacillus cereus* in the present study was 25mg/ml. Inhibition by acetic acid young leaf extract was found to be 12.5mg/ml. For Proteus mirabilis, MIC with ethanolic young mango leaf extract was 50 mg/ml. Using acetic acid young leaf extract, the MIC was found to be 25 mg/ml. With mature leaf acetic acid extract, it was 50mg/ml. The lowest concentration of ethanolic young mango leaf extracts against *Salmonella typhi* was 50mg/ml. The same MIC occurred with ethanolic mature leaf extract. When the organism was tested with acetic acid young mango leaf extract, the MIC was 25mg/ml. With mature leaf acetic acid extract, however, MIC was 50mg/ml. In the present study, the lowest concentrations of extracts that inhibited growth were found to be the lowest concentration that killed microbial cells present. This means that the minimum inhibitory concentrations were the same as the minimum bactericidal concentrations.

The minimum inhibitory concentration (MIC) of mango leaf extracts ranged from 12.5 mg/ml to 50 mg/ml, and this had been reported by other workers [25, 5]. The minimum inhibitory concentration observed here is much higher than the inhibitory concentration (30 μg/disc) for the control (tetracycline). This agrees with previous studies where it had been found that the susceptibility of organisms to plant extracts is usually less than that given by standard antimicrobial agents to which test organisms are sensitive [26]. The explanation for this is that plant extracts contain crude substances that do not contribute to bioactivity whereas standard drugs are pure bioactive substances. Minimum bactericidal concentration (MBC) had values similar to minimum inhibitory concentration values. It is, therefore obvious that mango extracts are bactericidal rather than bacteriostatic. This means that the extracts used in the present study killed the organisms tested rather than merely stopping their growth. This position has been reached in many studies [5]. Bactericidal properties of mango extracts would be attributed to mango phytochemicals such as saponins that interfere with cell membrane integrity [27] and mangiferin and tannins that disrupt proteins and protein synthesis [28, 29]. An additional bactericidal mechanism could be the inactivation of adhesion enzymes and cell membrane transport protein by polyphenolic compounds [30].

It was observed that the activities of extracts used in this study were concentration-dependent. That is, the higher the concentration used the higher the activity recorded. Concentrations used here were restricted. When mango organs are used in traditional medicine, doses are usually administered in cups or bottles. These may usually contain enough bioactive substance required for the complete treatment of target ailments.

4. CONCLUSION

Mango parts used in the present study were found to be biologically active, possessing components that show broad-spectrum antibacterial activity. The activity of mango extracts against test organisms was concentration-dependent. It was also found that young mango leaves demonstrated greater bioactivity than mature ones. This finding stands out because most studies used mature mango organs rather than young ones. Solutions for the extraction of mango parts were ethanol, acetic acid, and water. Extracts of acetic acid exhibited the highest antimicrobial activity whereas those of water showed the least activity. Again, the use of acetic acid as a solvent for the extraction of plant materials for use in bioassay is not common among workers studying the antimicrobial properties of plant extracts. The present study is, therefore, quite revealing as it shows the importance of acetic acid in the extraction of bioactive components of plants. Finally, mango young leaf extracts have bactericidal activity with a minimum bactericidal concentration between 12.5 and 50 mg/ml. These values, however, depending on the organism and the extracting solvent used.

5. RECOMMENDATIONS

Based on the findings of the present investigation, it is recommended as follows:

1. Since mango parts may be regarded as safe and the efficacy of extracts is concentration-dependent, high doses of extracts (at least 50 mg/ml) are recommended for oral or topical treatment of ailments.
2. The choice of source material for extraction of biologically active compounds should be young organs as young mango leaves showed higher inhibitory properties against bacteria than mature ones.
3. For extractions of substances from mango parts, acetic acid, which gave the highest susceptibility profile of bacteria, should be employed as a solvent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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