Original Research Article

Susceptibility of *Candida albicans, Staphylococcus aureus and Escherichia coli* to extracts from different partsof mango (*Magniferaindica*) plant

Abstract

Aim: This study was carried out to investigate the Susceptibility of *Candida albicans, Staphylococcus aureus and Escherichia coli*to extracts from young and mature mango (*Magniferaindica*) leaves and stem-bark of the same plant..

Study design: The study employed statistical analysis of the data and interpretation

Place and duration of study: Young and mature mango leaves and stem-barks 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 leaves and stem-bark (young and mature of the same plant) were soaked separately in 500 ml of water, ethanol (95% v/v), and acetic acid (99.9% v/v) 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,*Candida albicans, Staphylococcus aureus*and *Escherichia coli* 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 standard control drug (for bacteria) and fluconazole (for *Candida*), with cultures incubated 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). Assays for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also carried out.

Results: Results obtained showed that *Escherichiacoli*was completely susceptible to acetic acid young leaf and young bark extracts at 100 mg/ml concentrations. *Staphylococcusaureus*was susceptible only to Acetic acid young leaf extract at 100 mg/ml. For *Candida albicans*complete susceptibility was with acetic acid young bark at 100 mg/ml. mature leaf extract (100 mg/ml), acetic acid young bark extract (100 to 50 mg/ml), aqueous young bark extract (100 mg/ml) and acetic acid mature *Candidaalbicans*was susceptible to acetic acid young and maturebark extract at 100 mg/ml concentration. Minimum inhibitory concentration (MIC) values of acetic acid young leaf extract for all three organisms were 12.5 mg/ml. MIC of ethanolic young leaf extract for *E. coli was* 12.5 mg/ml whereas that for *C.albicans*was 50 mg/ml. Minimum bacteriocidal concentration values were same as MIC.

Conclusion:*E. coli* and *S.aureus*were found to be most susceptible to acetic acid young leaf and stem-bark mango extracts. For *C. albicans*susceptibility profiles were best with aceti acid young and mature stem-bark extracts. It was also found that mango phytochemicals have broad-spectrum antibacterial activity as well as antifungal properties. The study also reveals that young mango parts contain higher bioactive substances than mature parts. Finally, it was concluded that acetic acid extracts produced the highest antimicrobial effects whereas aqueous extracts produced the least.

Keywords: *Susceptibility Test, Mango young leaf; young stem-bark; mature leaf; mature stem-bark; acetic acid; ethanol; Staphylococusaureus; Escherichia coli ;andCandida albicans.*

Comment [RA1]: Title should read : Susceptibility of .....to extracts from Mango (*Magniferaindica*) . (the parts should be mention in the methodology)

Comment [RA2]: remove

Comment [RA3]: remove

Comment [RA4]: should all come under methodology. Where study site, duration of study, identification of plant, parts of plant and all other methods used are explained.

Comment [RA5]: Temperature too high. Samples should be air dried or dried at about 50^{o}C

Comment [RA6]: Too many, compound words. Suggestion- susceptibility, Mango, *Staphylococcus, Escherichia*, *Candida*

1. INTRODUCTION

Mango (*Mangiferaindica*) is a fruit tree present in the wild or in many countries of the world, including Nigeria. It is grown for its sweet succulent fruits. Other parts of the plants, particularly the leaves and stem-bark, however, have found uses in forkloric medicine, even from ancient times [1]. Today, in most African and Asian countries, up to 80% of the populations rely on traditional medicine for primary health care needs [2]. Reliance on traditional medicine stems from the fact that many germs are developing resistance to commonly used synthetic drugs and antibiotics [3,4]. Medicinal plants offer the best alternative because they possess great efficacy against most clinical isolates [5,6].

Traditional medicine practice is one that is shrouded in secrecy. The practitioners see it as gift from the "*gods*" that must be guarded. Since the practice is income generating, they believe that it must be protected from the public so as to maintain patronage. For these reasons, many plants of medical values in traditional settings are not subjected to scientific tests. In the absence of tests, herbal medicine practitioners claim multiple remedies for their individual preparations. The World Health Organization (WHO) notes, however, that inappropriate use of traditional medicines or practices can have negative or dangerous effects; and that further research is needed to ascertain the efficacy and safety of several of the practices and medicinal plants used in traditional medicine system [2].

Leaves of mango plant contain phytochemicals which include glycosides (particularly mangiferin), saponins, tannins and euxanthin acid [7].Barks of the plant have steroids, glycosides, saponins, resins, phenols, flavonoids, and alkaloids, among others [8,9]. Quantitative analysis of the compounds present in mango parts has shown that mangiferin is the predominant component [10]. For this reason, mango is the chief source of this compound [11].

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, moderation in bark, and low in mature leaves and roots [12].Mangiferin has been shown to contribute immensely to antimicrobial actions of mango extracts [13,14]. Other compounds such as alkaloids, flavonoids, saponins and tannins, extractable from mango parts, have also been credited with antimicrobial activities [15].

Extracts of mango leaves and stem-bark 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* [6]. Among these, *Staphylococcus aureus, Escherichia coli* and *Candida albicans* are commensal associated with the human body but may cause serious diseases when present in huge populations or at certain site [16].

Escherichia coli, commonly known as *E. coli*, is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium that is commonly found in the lower intestine (colon) of warm-blooded animals [17]. Most *E.coli* strains are harmless as part of the normal microflora of the gut, and can benefit man by producing vitamin K_2 (that help in blood clotting), and preventing colonization of the gut by pathogenic bacteria [18,19]. A few strains however are pathogenic causing diseases such as gastroenteritis, urinary tract infections, neonatal meningitis, hermorrhagic colitis, Crohn's disease (inflammatory bowel disease) and wound infections [20]. *Escherichia coli* is also a common cause of bronchopneumonia in children and the elderly [21].

Staphylococcus aureus is a Gram-positive, round-shaped, facultative anaerobic bacterium that commonly occurs in the upper respiratory tract (the nares) and skin of humans. Although *S. aureus* normally acts as commensal microflora of the human body, it can become an opportunistic pathogen. Diseases caused by *S. aureus* include abscesses (example, boils and whitlows); respiratory infections (example, sinusitis and pneumonia), wound infections, urinary tract infections, meningitis, sepsis syndromes

(injury to body tissues due to immune response to infections), endocarditis, and food poisoning [22,21].

*Candida albicans*is yeast capable of producing pseudo-mycelium. It can be found in the gastrointestinal tract, mouth, and lower female reproductive tract of healthy adults [23]. This organism is usually a commensal, but can become pathogenic when conditions favour its overgrowth [23]. *Candida albicans* is commonly used as a model organism for fungal pathogens [24]. Disease caused by this organism is called candidiasis. The disease may affect the mouth, skin, lower reproductive tract of females, respiratory tract, digestive tract of infants, and general body organ [16].

It has been established that distribution of mango phytochemicals vary with the plant parts and state of maturity [12]. It has also been seen that extraction of bioactive substances from plant parts is dependent on the solvent used. For these reasons and to address WHO's position on the use of plant parts in the treatment of human diseases, the present study is untaken to investigate young and mature mango leaf and stem-bark extracted with different solvents for efficacy in the inhibition of some clinical microorganisms.

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) [25].

2.2 Collection of Mango Specimens

The most popular mango variety is the one with elongated persistent green fruits popularly called green mango [26]. Mature and young leaves of mango (*Mangiferaindica)* were collected from the same tree in 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. Similarly, mature and young stem-barks were collected from same sample tree using a matchet or knife into polythene bags for transfer to the laboratory.

2.3 Collection of Test Cultures

Cultures of *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* were obtained from the diagnostic laboratories of Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria

Comment [RA7]: Are they type cultures? If not how were the isolates confirmed? Was there no need for subculture ?

2.4 Preparation of Mango Extracts

Collected mango parts were washed of debris, chopped (bark), and dried in an oven at $80^{o}c$ for 3 days. The dried materials were ground in a surface-sterilized electric blender to fine particles. Fifty gramme (50 g) amount of each ground plant part was transferred into a sterile one-liter conical flask and 500 ml 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 ($28 \pm 2\ ^{0}C$) for 72 hours as described by Doughari and Manzara [27].Using funnel, soaked mango samples were separately filtered through sterile muslin filter and again through Whatman No. 1 filter paper into sterile beakers. From each filtrate, the solvent was evaporated via water bath at $80^{o}C$ until dryness. The dried substances obtained were stored aseptically in specimen bottles until needed.

Comment [RA8]: Ethanol or acetic acid? Mention the exact solvent used.

2.5 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 [8]. To prepare 100 mg/ml extract, 1g (1000mg) of dried extract was transferred into a sterile measuring cylinder and homogenized with sterile distilled water to a final volume of 10 ml (i.e. 1000 mg/10 ml or 100 mg/ml). To prepare 75 mg/ml, 1.5 g (1500 mg) of extract was homogenized with sterile distilled water

to a final volume of 20 ml. For 50 mg/ml, 1 g (1000 mg) of extract was homogenized in a final volume of 20 ml, and for 25 mg/ml, 0.5 g (500 mg) of extract was homogenized in a final volume of 20 ml with sterile distilled water.

2.6 Preparation of Control Antimicrobial Discs

Control antimicrobial discs were prepared as described by [28]. 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 15minutes. 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 [28]. To prepare 30 μg/disc of each agent, 250 mg each of tetracycline and fluconazole 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 each control in separate Petri dishes and allowed to air-dry (i.e., 250 mg / 167 ml or 1.5 mg/ml or 30 μg / 0.02 ml).

2.7 Sensitivity Test of Organism

Mueller-Hinton agar and Sabouraud dextrose agar were prepared according to manufacturer's direction. Each bacterial suspension was prepared to match 0.5 McFarland standard and transferred by means of inoculating loop, in one loopful amount, onto Mueller-Hinton agar. Similarly, test fungal suspension prepared to match 0.5 McFarland standard was transferred in one loopful amount onto Sabouraud dextrose agar. Inoculum in each case was spread evenly on agar surface using a sterile swab stick [28]. Seeded plates, in duplicates for each organism, were allowed to air-dry on 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 [28]. A set of four concentrations – 100, 75, 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 laboratory bench. For controls, each bacterium was challenged with prepared tetracycline discs and *Candida albicans*with fluconazole 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 agents were allowed to diffuse into their media on laboratory bench for 30 minutes. Thereafter, the bacterial cultures were incubated at 37 ^{0}C for 24 hours, whereas plates of *Candida albicans*were kept at room temperatures for up to 48 hours.

2.8 Measurement and Interpretation of Inhibition Zones

Following incubation, the diameter of zone of inhibition was measured across each well by means of a transparent ruler, in millimetres (mm).Using the controls and the interpretive guidelines published by National Committee for Clinical Laboratory Standard (NCCLS) [29], inhibition zone of ≤ 14 mm was read as resistance of test organism to antimicrobial agent, inhibition zones of 15 – 18 mm as intermediate response; and ≥ 19 mm as susceptibility of organism [28].

2.9 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC)

These tests were carried out using the steps described by Ochei and Kolhatkar [28] and Mustapha *et al*., [7]. 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 MIC test onto Mueller-Hinton agar and Sabuoraud dextrose agar for bacteria and *Candida*, respectively. After incubation of bacterial cultures at 37 ^{0}C and fungal cultures at 28 ^{0}C for 24 and 48 hours, respectively, the culture plates were observed for sterility.

2.10 Statistical Analysis

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

Comment [RA9]: Reconstitution of the extracts for susceptibility testing should have been done with the solvent used for extraction for proper interpretation of result.

Comment [RA12]: reframe

Comment [RA10]: Is this the recommended concentration by the NCCLS for both drugs? Please check.

Comment [RA13]: zones of inhibition as well as drug concentration for susceptibility testing is not generalized but depend on the drug used. Please consult the standard again and specify for tetracycline and fluconazole which were used as your control.

Comment [RA14]: Wrong procedure. Please revisit this. Inconsistency in spelling of media used, ^{O}C for unit of temperature

Comment [RA11]: How many solvents were used for extraction? If is all the 3 mentioned then it should be 'and' not 'or'

3. RESULTS AND DISCUSSION

3.1 Sensitivity of Test Isolates to Mango Extracts

Tables 1.shows the susceptibility pattern of individual organisms to the various mango extracts used in the present study. According to NCCLS guidelines for determination of susceptibility profiles of micro-organisms to antimicrobial substances, with tetracycline as control, *E. coli* was susceptible to acetic acid young leaf and acetic acid young bark extracts at 100 mg/ml. This organismsshowed intermediate reactions to acetic acid young leaf extract at 75 and 50 mg/ml, ethanolic young leaf extract at 100 to 50 mg/ml, acetic acid young bark extract at 75 and 50 mg/ml, and aqueous young bark extract at 100mg/ml. All mature leaf and mature stem-bark extracts produced resistance of *E. coli* at all concentrations tested.

Staphylococcus aureus demonstrated complete susceptibility only to acetic acid young leaf extract at 100 mg/ml. There was intermediate responses to acetic acid young leaf extract at 75 mg/ml, acetic acid mature leaf extract at 100 mg/ml and acetic acid young bark extract at 100 to 50 mg/ml. Aqueous young stem-bark and acetic acid mature stem-bark extracts produced intermediate reactions in *S. aureus* at 100 mg/ml. Complete resistance of *S. aureus* was observed with ethanolic and aqueous young and mature leaf extracts, as well as ethanolic young and mature stem-bark extracts. There was also total resistance of *S. aureus*to aqueous mature stem-bark extract.

With *Candida albicans* as test organism, it was found that acetic acid young and mature stem-bark extracts, at 100 mg/ml, gave complete susceptibility reactions (Table 1). Intermediate sensitivity of this fungus occurred with acetic acid young leaf, ethanolic young leaf, acetic acid mature leaf and ethanolic mature leaf extracts at 100 mg/ml. Also, at 75 mg/ml concentration, ethanolic young leaf, acetic acid young and mature stem-bark extracts produced intermediate sensitivity reactions. Total resistance of this organism was observed with aqueous young and mature leaf extracts. Also, ethanolic and aqueous young stem-bark extracts gave absolute resistance of *C. albicans.* Similarly, ethanolic and aqueous mature bark extracts produced complete resistance of this organism.

All test bacteria and *C. albicans* were susceptible, at 30 µ/disc,to tetracycline and fluconazole, respectively, used as positive controls in the present study.

Percentage resistance of test organisms to young leaf and mature leaf as well as young and mature stem-bark extracts of the three solvent types taken together is provided in figure 1. *E. coli* showed the least resistance to young leaf extracts whereas *S. aureus*exhibited the highest resistance. To mature leaf extract, *E. coli* showed the highest resistance (100 %) as *S. aureus*and *C. albicans* produced the same lower level resistance. Response of test bacteria to young stem-bark extracts was higher than seen in *C. albicans.* With mature stem-bark extracts, *E. coli* showed the highest resistance whereas *C. albicans* was most affected. Generally young mango parts produced higher effects on test microorganisms than mature parts, except young and mature stem-bark extracts that gave the same results in *C. albicans* Figure 2 shows the percentage resistance of test organisms to extract of solvent types used in the present study. Extracts of acetic acid produced the highest effects on test microorganisms. Those of water have the least effects, except on *S. aureus*where the organism was found to be most resistant to ethanonic extracts.

Phytochemicals are believed to protect plants from invading microorganisms as they possess antimicrobial properties [8]. Among mango phytochemicals, mangiferin is considered the most important, and the plant is the chief source of this compound [11]. In addition to mangiferin, other compounds such as alkaloids, flavonoids, tannins and saponnins have been credited with bioactivity against microorganisms [30]. These phytochemicals, particularly mangiferin, have been found to be more abundant in young mango leaves and stem-bark than in mature mango leaves [12]. This finding suggests that young mango leaves

Comment [RA17]: Correct the unit, indicate the zone of inhibition measured for both drugs which are interpreted as susceptible.

Comment [RA15]: The organisms

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Comment [RA18]: ethanoic

and stem-bark possess higher antimicrobial properties than mature leaves. This position is obvious in the results obtained in the present study. Extracts of young organs demonstrated higher inhibition zones against test organisms than mature

Table 1: Sensitivity of test organisms to mango extracts

Extract	Conc. (mg/ml)	*Escherichia coli*		*Staphylococcus aureus*		*Candida albicans*	
		Zone of Inhibition (mm)	RIS Category	Zone of Inhibition (mm)	RIS Category	Zone of Inhibition (mm)	RIS Category
Acetic acid	100	20	S	20	S	16	I
young leaf	75	18	I	17	I	13	R
Extract	50	16	I	14	R	12	R
	25	12	R	12	R	10	R
Ethanolic	100	18	I	0	R	17	I
young leaf	75	16	I	0	R	15	I
Extract	50	15	I	0	R	11	R
	25	10	R	0	R	0	R
Aqueous	100	0	R	0	R	0	R
young leaf	75	0	R	0	R	0	R
Extract	50	0	R	0	R	0	R
	25	0	R	0	R	0	R
Acetic acid	100	11	R	15	I	16	I
mature leaf	75	10	R	11	R	14	R
Extract	50	8	R	10	R	13	R
	25	0	R	0	R	0	R
Ethanolic	100	10	R	0	R	16	I
mature leaf	75	0	R	0	R	13	R
Extract	50	0	R	0	R	10	R
	25	0	R	0	R	0	R
Aqueous	100	0	R	0	R	0	R
mature leaf	75	0	R	0	R	0	R
Extract	50	0	R	0	R	0	R
	25	0	R	0	R	0	R
Acetic acid	100	20	S	18	I	19	S
young bark	75	18	I	17	I	15	I
Extract	50	16	I	15	I	10	R
		11	R	10	R	0	R
Ethanolic	100	0	R	0	R	0	R
young bark	75	0	R	0	R	0	R
Extract	50	0	R	0	R	0	R
	25	0	R	0	R	0	R
Aqueous	100	18	I	17	I	11	R
young bark	75	14	R	13	R	10	R
extract	50	10	R	8	R	8	R
	25	0	R	0	R	0	R
Acetic acid	100	12	R	16	I	20	S
mature bark	75	10	R	12	R	16	I
Extract	50	8	R	10	R	14	R
	25	0	R	0	R	0	R
Ethanolic	100	0	R	0	R	0	R
mature bark	75	0	R	0	R	0	R
Extract	50	0	R	0	R	0	R
	25	0	R	0	R	0	R
Aqueous	100	0	R	0	R	0	R
mature bark	75	0	R	0	R	0	R
Extract	50	0	R	0	R	0	R
	25	0	R	0	R	0	R
Control*	30 μg/ml	22	S	25	S	22	S

R = Resistant, inhibition zone ≤ 14mm; I = Intermediate inhibition zone of 15 – 18mm; S = Susceptible, inhibition zone ≥ 19 mm. (Source: NCCLS, 2002). *Control = Tetracycline (bacteria) and Fluconazole (*C. albicans*)

Comment [RA19]: table is too clumsy for good understanding. Could be split into three for the organisms used or split into two to reflect the activity of the extract on the two groups of organism i.e bacteria and fungi. So please reconstruct the table.

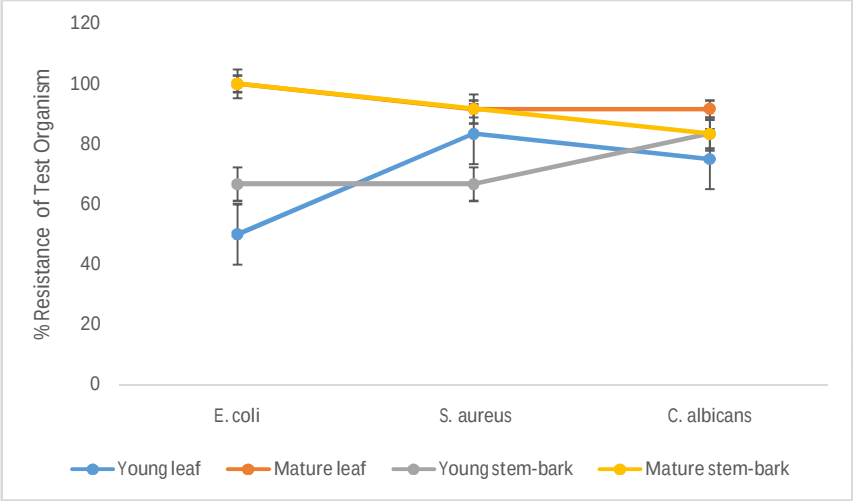

Fig.1 Percentage (%) Resistance of test organisms to young and mature mango parts

Comment [RA20]: Figures are not self-explanatory. Do not give meaning to the result.

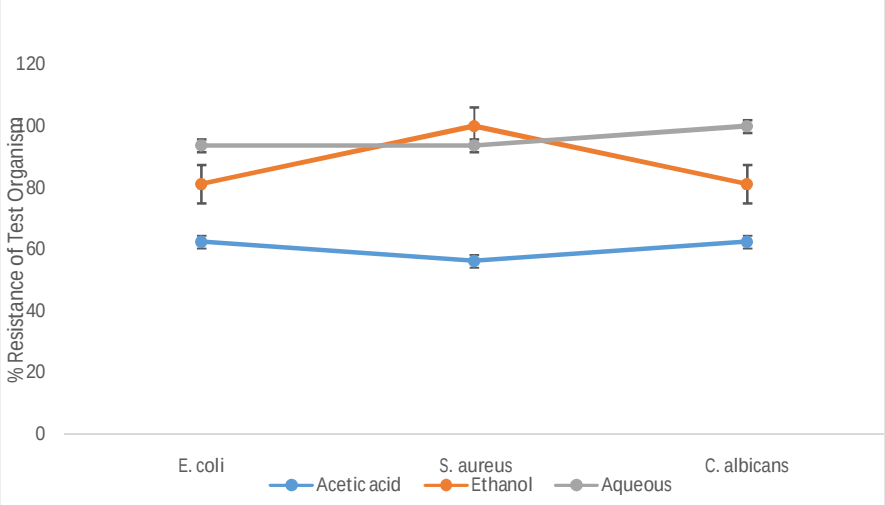

Fig.2 Percentage Resistance of test Organisms to Extracts of the three different Solvents used.

Comment [RA21]: How does this affect the efficacy of the parts of the plant used? Are the results indicating dependence on solvent used?. Are they not exerting their own antimicrobial effects?

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

Comment [RA22]: Strongly bioactive .....????? . table is not clear, methodology not reflected.

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	-	-	-	+	-	+	+	+
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.

mango parts, except young and mature stem-bark extracts that produced the same results in *C. albicans*. Further, it could be easily seen that acetic acid extracts inhibited test microorganisms the most, whereas aqueous extracts showed the least activity. Statistical analysis using Analysis of variance (ANOVA) tests showed that sensitivity of organisms to mango extracts was not mango-organ-dependent. It showed, however, that the different mango organs at different stages of maturity produced different levels of susceptibility in test bacteria species, and that the different solvents used for extraction of mango phytochemicals as well as the various concentrations employed were significantly different ($p < 0.05$).

In the present study, all test organisms were resistant to aqueous extracts of mature mango leaf. This is in agreement with the findings of Doughari and Manzara [27] and Nwankwo and Osaro-Mathew [31]. Poor activity of aqueous extracts could be attributable to the fact that some bioactive phytochemicals have limited solubility in water [8] and therefore may not be available when water is used for extraction. Unlike water that 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 [32,33]. 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. auereus*. This also is in consonance with the findings of Mustapha *et al.* [7] 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. Lack of use of acetic acid as solvent for extraction of phytochemicals may stem from the fact that it has high boiling point (118℃) and therefore difficult to remove from extracts. Its success as a solvent, however, far outweighs this difficulty [33].

It was found that *E. coli* was resistant to ethanolic stem-bark extracts and aqueous mature bark extracts of mango. The resistance to aqueous extracts is in agreement with some previous studies [9] where it was found that *E. coli* is resistant to aqueous extracts of mango barks. The organism, however, was sensitive to acetic acid bark extracts and aqueous young bark extracts. This further suggests that bioactive substances are more in young mango organs than in mature ones. Response of *S. aureus*showed that it was not inhibited by ethanolic bark extracts as well as aqueous mature bark extracts. These findings are supported by those of Ashok *et al.*, [9].

3.2 Minimum Inhibitory Concentration of Mango Extracts of Test Organisms

Minimum inhibitory concentration of ethanolic young leaf extract against *Escherichia coli* was 12.5 mg/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 50 mg/ml.Determination of MIC of acetic acid young mango leaf extract against *Staphylococcus aureus* showed 12.5 mg/ml. Using acetic acid mature leaf extract, the MIC was 50mg/ml. Ethanolic extracts of young and mature mango leaves gave MIC of 50 mg/ml in each case. With acetic acid young leaf extract, MIC was 12.5 mg/ml, whereas with mature leaf acetic acid extract, it was 50 mg/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 or mycocidal concentrations.

Minimum inhibitory concentration (MIC) of the strongly bioactive leaf extracts ranged from 12.5 mg/ml to 50 mg/ml, and this had been reported by other worker [34,8]. Minimum inhibitory concentration observed here is much higher than the inhibitory concentration (30 μg/disc) for the controls, tetracycline and fluconazole, used in the present study. This agrees with previous studies where it had been found that

susceptibility of organisms to plant extracts is usually less than that given by standard antimicrobial agents to which test organisms are sensitive [35]. 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 bacteriocidal concentration (MBC) had values similar to minimum inhibitory concentration values. It is, therefore obvious that mango extracts are bacteriocidal rather than bacteriaostatic. This mean that the extracts used in the present study killed the organisms tested rather than mere stopping their growth. This position has been reached in many studies [8]. Bacteriocidal properties of mango extracts would be attributed to mango phytochemicals such as saponins that interfere with cell membrane integrity [36] and mangiferin and tannins that disrupt proteins and protein synthesis [37,38]. An additional bacteriocidal mechanism could be the inactivation of adhesion enzymes and cell membrane transport protein by polyphenolic compounds [39].

It has been found that one polyphenol alone is less effective than many polyphenols used together; which implies that synergism of many mango polyphenols is essential for optimum biological activity [39]. This further shows that mango parts with many polyphenols, coupled with suitable solvents for their extraction, are necessary for demonstration of antimicrobial activity. Gram-positive and Gram-negative bacteria as well as fungi were sensitive to mango extracts. These suggest that mango plant contains bioactive substances that show broad-spectrum antimicrobial activity.

Finally it was observed that 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 complete treatment of target ailments. Aqueous mango stem-bark extract, for instance, administered in high dose (in cups) is used for treatment of malaria and typhoid fever in traditional medicine practices.

4. CONCLUSION

Activity of mango extracts against test organisms was found to be concentration-dependent. It was also found that young mango parts demonstrated greater bioactivity than mature ones, except young and mature stem-bark extracts that produced the same results with *C. albicans*. This finding stands out because most studies used mature mango organs rather than young ones. Solvents for 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, use of acetic acid as solvent for extraction of plant materials for use in bioassay is not common among workers studying 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 parts used in the present study were found to be biologically active, possessing components that show broad-spectrum antibacterial activity.

Comment [RA23]: But there are no indication of phytochemical screening of the plant used in this study.

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 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. For *C. albicans*, however, young or mature mango stem-bark could be the source material.
3. For extraction of substances from mango parts, acetic acid, which gave the highest susceptibility profile of test organisms, should be employed as solvent.

COMPETING INTERESTS

Authors have declared that no competing interest exist.

Comment [RA24]: exists

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