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Antimicrobial Activities of Moringa, Neem and Ginger Plant Extracts against Bacteria Associated with the Spoilage of Fruit Juice

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Authors' contributions

This work was carried out in collaboration among all authors. Author CVN designed the study, wrote the protocol. Author CGU wrote the first draft of the manuscript. Authors CVN, HNO and CEU performed the statistical analysis. Authors UDN, EKA, WNC and KCO helped with the analyses of the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aims to evaluate the antibacterial activity of Moringa, Neem, and Ginger plant extracts on the bacteria species isolated from fruit juice samples.
Place and Duration of Study: Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, between October 2019 and November 2019.
Methods: The fruit juice sample was prepared and cultured on Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB), *Salmonella Shigella* Agar (SSA), and Blood Agar using streak plate techniques. Four (4) bacteria species were isolated and identified from the fruit juice sample. These organisms served as the test isolates. Two (2) solvents (methanol and water) were used to get a comparative result. Disc diffusion method was used to determine the antibacterial effects of the Moringa, Neem, and Ginger on the test organisms.
Results: The methanolic extract of Moringa, Neem and Ginger was found to exhibit high degrees of antibacterial activities against the test isolates. This was shown by the clear zones of inhibition

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produced by the methanolic extract on the test microorganisms. The highest *in-vitro* antibacterial activity is 16 mm, which was exhibited by the methanolic extract of *Moringa* at the highest concentration of 200 mg/ml against *Staphylococcus aureus.* In comparison, the Methanolic extract exhibited no antibacterial activity (0.0 mm) at the lowest concentration of 50 mg/ml against all the test organisms. The minimum bactericidal concentration from this study revealed that methanolic and aqueous extract was active against *Staphylococcus aureus, Shigella species, Bacillus species,* and *Escherichia coli.* However, the *water* extract of *Moringa* demonstrated more significant antibacterial activity on *Shigella species, Bacillus species,* and *Escherichia coli* with the range of 200 mg/ml each. In contrast, methanol extract of neem demonstrated antibacterial activity on *Shigella species* alone, with the range of 200 mg/ml each.

Conclusion: Moringa*,* Neem, and Ginger extract had both a bacteriostatic and bactericidal activity when tested *in vitro* using methanolic and aqueous preparation of Moringa, Neem, and Ginger extract. Therefore, these plants may be used successfully for treating illness caused by *Staphylococcus aureus*.

Keywords: Antimicrobial; Moringa oleifera; neem; ginger; plant extract; fruit juice.

1. INTRODUCTION

Fresh fruits are a regular part of the daily diets of Nigerians and are known for their high nutritional and health values [1]. Some components of fruits (phytochemicals) are potent antioxidants and function to modify the metabolic activation and detoxification/disposition of carcinogens, or even influence processes that alter the course of the tumor cell [2]. Spoilage of fruit may occur by the mechanical fracture of fruits; by autolysis of chemical and mineral contents or by the microbial flora of fruit itself and contaminating microbes, which is the primary concern to study. Foodborne illnesses have been reportedly associated with the consumption of fruit juices in several places [3]. However, the contamination of fresh produce is a primary public concern, as foodborne diseases are increasingly becoming a global public health problem [4], resulting in a substantial amount of worldwide annual morbidity and mortality [5].

Various pathogens are associated with the contamination of fruits, and with different outbreaks of gastroenteritis, associated with the consumption of contaminated fruits that have been recorded at various times [6]. Fruit juice spoilage bacteria include acid-tolerant bacteria such as acetic acid bacteria, lactic acid bacteria, *Clostridium*, *Bacillus*, members of the Enterobacteriaceae family (*Klebsiella sp., Citrobacter* spp., and *Serratia* sp.), and some heat resistant bacteria such as *Alicyclobacillus acidoterrestris* and *Propionibacterium cyclohexinicum*. Some yeasts,like *Pichia candida, Saccharomyces,* and *Rhodotorula* are commonly encountered and generally responsible for spoilage of processed foods [7]. Plant extracts or their active components have been recognized for their antiviral, antimycotic, antiparasitic, insecticidal, antioxygenic, and antibacterial properties [8]. They even have promising activity against several antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Salmonella enterica* [9].

The antimicrobial activity of a compound can be influenced not only by its composition and extraction method but also by the volume of inoculum present. The concentration of the extract, type of pH media used, and the growth phase of the organism can also influence the antimicrobial activity. Also, the use of an emulsifier or solvent to aid in suspension, as well as incubation times and temperatures, have also been reported [8]. However, several studies have shown various plant extracts to be effective in Gram-negative bacteria. *Pseudomonas aeruginosa,* for example, is more resistant to the extracts [10].

Heat resistant fungi and other spore-forming bacteria such as *Clostridium pasteurianum* and *Bacillus coagulants* have been used as targets for industrial processes such as fruit juice pasteurization [11]. Convention thermal pasteurization in tropical fruit juices is thought to reduce the overall product quality since tropical fruits are heat sensitive [12]. Alternative technologies to reduce microbial loads include irradiation, high pressure, biocontrol, pulsed electric field, pulsed light, oscillating magnetic fields, ultrasound, and U.V. treatment [13].

A variety of plant and spice based anti-microbials are used for reducing or eliminating pathogenic microorganisms and increasing the shelf life of food. Natural herbs and spices are used for several purposes including food or medicine, maintaining proper sanitation, health, and personal hygiene and to boost longevity [14]. Plant extracts have also shown great potential in the food industry and approved by various regulatory agencies such as the U.S. Food and Drug Act (USFDA), the European Union standards, and Codex Alimentarius and Food Standard Safety of India (FSSAI) [15]. There have been many studies published on the activities of plant extracts and essential oils against different microbes, including foodborne pathogens [16]. Hence, the study of the antimicrobial activities of selected plant extracts against bacteria associated with the spoilage of fruit juice.

2. MATERIALS AND METHODS

2.1 Sample Preparation and Isolation of Microorganisms

2.1.1 Sample inoculation

At the laboratory, 50 ml of juice samples were measured and then transferred into various conical flasks. Ten-fold dilutions were prepared under aseptic conditions from each sample using 10 ml of distilled water as diluents. This result in a dilution of 10^{-1} decimal dilutions of 10^{-2}, 10^{-3} and 10^{-4} was then prepared by serial dilution. Diluted suspensions of 0.1 ml samples were plated over Nutrient agar Medium, Eosin Methylene Blue agar, Mannitol Salt Agar, and *Salmonella Shigella* agar using a pour plate method. The plates were incubated at $37^{o}C$ for 24 to 48 hours. Colonies that appeared on the plates were further purified for identification using the techniques of Srinivasan et al [17].

2.1.2 Identification of bacterial isolates

Isolates were analysed based on morphological features, Gram staining [18], and biochemical characterization, which includes; catalase, oxidase, coagulase, citrate, motility, indole, and urease tests of the isolates were carried out to verify the identity of the organisms [19]. The bacterial isolates were identified, and confirmatory identities of bacteria were made [20].

2.1.3 Preparation of Moringa, Neem and Ginger extracts

The freshly collected Moringa, Neem, and Ginger in bulbs and leaf foams were purchased from a local market in Ahiaeke, and 500 g of that was cleared of dirt, washed in running tap water three (3) times, to remove dirt. It was rewashed with sterile distilled water. Then it was blended and crushed using mortar and pestle into a paste form. Two (2) solvents were used to get comparative results. Five (5) grams of moringa, neem, and ginger paste were measured into a conical flask, mixed with 100 ml each of cold distilled water and methanol respectively, stirred for 10 minutes and filtered using a filter paper. The extracts generated are classified as: Methanol/Moringa (M/M), Aqueous/Moringa (A/M), Methanol/Neem (M/N), Aqueous/Neem (A/N), Methanol/Ginger (M/G), Aqueous/Ginger (A/G).

2.1.4 Preparation of different concentration of the extracts

The aqueous extract was reconstituted by weighing 0.8 g quantity of each extract into a sterile test tube, and made up to 2 ml using distilled water to give a concentration of 400 mg/ml. Also, the methanolic extract was also reconstituted by the dissolution of 0.8 g of each crude methanolic extract weighed into a sterile test tube made up to 2 ml with dimethyl sulphoxide (DMSO) 50% respectively to get a concentrate of 400 mg/ml [21]. This 400 mg/ml concentration of the extracts was then, doubly diluted in sterile water to obtain concentration 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml.

2.2 Media Preparation

Four different types of media were used for the isolation of each organism from the fresh fruit juice, respectively. The media include Mannitol Salt Agar (MSA) and Blood Agar (B.A.), for isolation of *Staphylococcus aureus*, Eosin Methylene Blue (EMB), for isolation of *Escherichia coli* and *Salmonella Shigella* Agar (SSA), for isolation of *Salmonella* and *Shigella* spp., Nutrient Agar for general purpose. The media used for sensitivity test and MIC was Nutrient Broth and Mueller-Hinton Agar, as previously done by Srinivasan et al [17].

2.3 Determination of Antimicrobial Activity

The different bacteria (*Escherichia coli, Staphylococcus aureus, Salmonella* species, and *Bacillus* species) isolates were inoculated respectively onto the solid, sterile Muller Hinton Agar. This was done using the spread plate method [22]. After the inoculation of the isolates, the prepared disc was dipped into the concentrated extract and allowed to absorb it. Carefully, with the aid of a flame pair of forceps, the disc bearing extract was transferred to the inoculated plate. Four extract discs were used for each plate, and they were placed about the same distance from one another and not less than 1cm from the edge of the Petri dish. The plates were incubated at 37^0C for 24 hours. After incubation, the plates were examined for the zone of inhibition [23]. The presence of a clear zone around any disc gave a positive result. The diameters of the zone of inhibition were measured with a transparent rule and recorded in millimeter (mm). The test was carried out in duplicates, and the mean values were calculated and recorded. Ciprofloxacin and Rifampicin served as the control [24].

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

Using sterile forceps, the paper disc of different concentration from (200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml) were placed at different portions of the inoculated labelled plates. The plates were incubated at 37^0C for 24 hours [25]. After incubation, the plates were examined for the presence of inhibition zones and the concentration that caused inhibition was recorded, the lowest diluents which caused inhibition was recorded as the MIC [26]**.** The Minimum Bactericidal Concentration (MBC) was determined by streaking the content of the tubes used for minimum inhibitory concentration determination, which were showing reduced turbidity on freshly prepared nutrient agar plates. The Minimum Bactericidal Concentration was then identified as the concentration that completely inhibited the growth of the bacteria [26].

3. RESULTS

Table 1 shows the bacterial isolates from the fruit juice samples, which were identified by their cultural morphology, Gram's reaction, and biochemical reaction. The bacteria identified are *Staphylococcus aureus*, *Bacillus* spp, *Shigella* spp, and *Escherichia coli.*

Table 2 shows the antibacterial activity of the methanolic and aqueous extract of Moringa, Neem, and Ginger. The methanolic extract of Moringa, Neem, and Ginger was found to exhibit high degrees of antibacterial activities against the test isolates. This was shown by the clear zones of inhibition produced by the methanolic extract on the test microorganisms. The highest in-vitro antibacterial activity is 16 mm, which was exhibited by the methanolic extract of Moringa at the highest concentration of 200 mg/ml against *Staphylococcus aureus,* followed by *Shigella* species 15 mm (200 mg/ml). In comparison, the methanolic extract exhibited no antibacterial activity (0.0 mm) at the lowest concentration of 50 mg/ml against all the test organisms. The aqueous extract of neem exhibited the highest antibacterial activity, 14 mm at a concentration of 200 mg/ml against *Escherichia coli*, followed by aqueous ginger extract at 12 mm against *Staphylococcus aureus*.

Table 3 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the Moringa, Neem, and Ginger extracts against the test organisms. Methanolic and aqueous soluble extracts of the three plants were active against *Staphylococcus aureus*, *Shigella* spp, *Bacillus* spp, and *Escherichia coli*. However, the aqueous extract of Moringa demonstrated more significant antibacterial activity on *Shigella* spp, *Bacillus* spp, and *Escherichia coli* with the range of 200 mg/ml each. In contrast, methanol extract of neem demonstrated antibacterial activity on Shigella species alone, with the range of 200 mg/ml each.

4. DISCUSSION

The antibacterial activity of Moringa, neem, and Ginger on some pathogens was investigated with different solvents. Plant extracts or their active components have been recognized for their antiviral, antimycotic, antiparasitic, insecticidal, antioxygenic, and antibacterial properties. In this work, it was observed that the methanolic extract had a more significant inhibitory effect than aqueous extracts. *Shigella spp.* and *Staphylococcus aureus* exhibited the highest sensitivity (15 mm, and 16 mm), respectively, against the methanol extract of Moringa, followed by *Escherichia coli* (14 mm) against aqueous extract of neem. The need for this study is to compare the differences in antimicrobial effects as a result of the extraction solvent. The solvent used in the preparation of the spice (Moringa, Neem, and Ginger) extract plays a major role in the inhibitory effect of the spice (Moringa, Neem, and Ginger) as described by Ekwenye et al. [24].

Table 1. Identification of bacterial species from the fruit juice sample

Colonial features	Gram reaction	Cell arrangement	Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Methyl Red	Voges-P	Suspected bacteria
Pink Pigment	_	Short Rod	+	_		_	+	+	+	_	*Escherichia coli*
Golden Yellow	+	Cocci Group	+	_	+	_	+	_	+	_	*Staphylococcus aureus*
Black Spot	_	Short Rod	+	_	N/A	_	_	_	+	_	*Shigella species*
Creamy Mucoid	+	Short Rod	_	+	_	N/A	N/A	_	N/A	N/A	*Bacillus species*

Key: - = Absent, + = Present, N/A= Not Applicable

Table 2. Antibacterial activity of Moringa, Neem, and Ginger extract against test organisms

Test bacteria	Concentration (mg/ml) zone of inhibition (mm)					Extract
	50mg/ml	100mg/ml	150mg/ml	200mg/ml	Control	
Staphylococcus aureus	_	_	10	16	16	M/M
	_	_	_	8	14	A/M
	_	_	11	13	20	M/N
	_	_	8	10	17	A/N
	_	7	_	_	16	M/G
	_	_	_	12	17	A/G
Escherichia coli	_	8	11	14	20	M/M
	_	_	_	8	9	A/M
	_	_	12	14	20	M/N
	_	6	11	14	20	A/N
	_	_	6	11	18	M/G
	_	7	_	8	17	A/G
Shigella spp	_	8	13	15	18	M/M
	_	_	6	8	12	A/M
	_	_	_	_	11	M/N
	_	8	_	_	11	A/N
	_	_	6		15	M/G
	_	_	8		18	A/G
Bacillus spp	_	6	8	10	11	M/M
	_	_	_	10	12	A/M
	_	6	_	_	20	M/N
	_	6	7	_	21	A/N
	_	_	_	10	16	M/G
	_	_	_	10	15	A/G

Key: Antibiotic positive control on gram-positive isolate = Ciprofloxacin 20mg/ml, Antibiotic positive control on gram Negative isolate = Rifampicin 10mg/ml, Methanol/Moringa (M/M), Aqueous/Moringa (A/M), Methanol/Neem (M/N), Aqueous/Neem (A/M), Methanol/Ginger (M/G), Aqueous/Ginger (A/G)

This study also revealed that the methanolic extract of Moringa was found to exhibit high degrees of antibacterial activities against the test isolates. This was shown by the clear zones of inhibition produced by the methanolic extract, specifically against *Staphylococcus aureus* at 16 mm (200 mg/ml). This could be as a result of some antimicrobial or phytochemical components which were not extracted by the aqueous extract, but were extracted by the methanol extract. This result is in conformation with a study by Ayegoro [27], who reported that the antibacterial effect of methanolic preparation of garlic at room temperature and refrigerated at -10ºC were high against *Staphylococcus aureus.*

In this study, no antibacterial activity (0.0 mm) was exhibited by the various methanolic and aqueous extract at the lowest concentration of 50 mg/ml against all the test organisms. This could be that at the lowest concentrations, the antimicrobial component of the extract is below the MIC. This agrees with the study by Onyeagba et al [28] who reported the crude extracts of garlic and Ginger applied singly and in combination did not exhibit any *in vitro* inhibition on the growth of test organisms including *Staphylococcus* spp at low of concentrations (60 mg/ml) of the extracts.

The aqueous extract of neem exhibited the highest antibacterial activity, 14 mm at a concentration of 200 mg/ml against *Escherichia coli*, followed by aqueous ginger extract at 12 mm against *Staphylococcus aureus*. In a similar study, the results of the antibacterial activity of *Azadirachta indica* (Neem) can be well said to be in accordance with several earlier studies. Bohora et al [22] have observed the significant antibacterial activity of Neem leaf extract against *E. faecalis* and mixed bacterial cultures. [29] reported its anti-*Staphylococcal* activity.

Table 3. Minimum inhibitory concentration and minimum bactericidal concentration value of Moringa, Neem, and Ginger against test bacteria

Test bacteria	MIC (mg/ml)				MBC (mg/ml)		
	50	100	150	200		Control	Extract
Staphylococcus aureus	+	+	+	-	200	200	M/M
	+	+	-	-	150	200	A/M
	+	-	-	-	150	200	M/N
	+	+	+	-	200	200	A/N
	+	+	-	-	200	150	M/G
	+	-	-	-	150	150	A/G
Escherichia coli	+	+	-	-	200	200	M/M
	+	+	+	-	200	100	A/M
	+	+	+	-	150	100	M/N
	+	-	-	-	150	100	A/N
	+	+	+	-	200	200	M/G
	+	+	-	-	200	150	A/G
Shigella specie	+	-	-	-	150	200	M/M
	+	+	+	-	200	150	A/M
	+	+	+	-	200	200	M/N
	+	+	+	-	200	200	A/N
	+	-	-	-	150	150	M/G
	+	+	+	-	150	150	A/G
Bacillus specie	+	-	-	-	150	200	M/M
	+	+	-	-	200	100	A/M
	+	-	-	-	150	100	M/N
	+	-	-	-	200	100	A/N
	+	+	-	-	150	200	M/G
	+	+	-	-	200	150	A/G

Key: Antibiotic positive control on gram positive isolate = ciprofloxacin 20mg/ml and Antibiotic positive control on gram positive isolate = Rifampicin 10mg/ml, MBC = Minimum Bactericidal Concentration, MIC = Minimum Inhibitory Concentration, Methanol/Moringa (M/M), Aqueous/Moringa (A/M), Methanol/Neem (M/N), Aqueous/Neem (A/M), Methanol/Ginger (M/G), Aqueous/Ginger (A/G)

Sinaga et al [30] reported that Gram-positive bacterial strains were more sensitive than the Gram-negative ones. Owolabi et al [31] reported the antibacterial activity of Neem leaf water extract against the same test organisms with a clear zone of inhibition from 10±0 mm to 15.5±0.71 mm.

The minimum bactericidal concentration from this study revealed that methanolic and aqueous soluble extract was active against *Staphylococcus aureus, Shigella species, Bacillus species,* and *Escherichia coli.* However, the aqueous extract of Moringa demonstrated greater antibacterial activity on *Shigella species, Bacillus species,* and *Escherichia coli* with the range of 200 mg/ml each. In contrast, methanol extract of neem demonstrated antibacterial activity on *Shigella species* alone, with the range of 200 mg/ml each. Similarly, Sivan et al [32], in a study to determine the minimum bactericidal activity of Ginger, neem and garlic extract on *Helicobacter pylori*, observed that at the same protocol ginger, neem and garlic extracts had the bactericidal effect at a high concentration of 160 mg/ml.

It was also observed in this study that the Moringa, neem, and Ginger extract had both a bacteriostatic and bactericidal activity when tested *in vitro* using methanolic and aqueous preparation of Moringa, neem and Ginger extract. Therefore, these plants may be used successfully for treating food poisoning causative agents like *Staphylococcus aureus*. It may be useful on other microbes on Gram-positive and gram-negative bacteria, so that further *in vivo* and *in vitro* studies are necessary. The standard antibiotics (Ciprofloxacin and Rifampicin) [33] used as control inhibited the test bacteria; it had higher inhibitory activity compared to the Moringa, neem, and Ginger extracts.

The antimicrobial activity of the extracts tested, which reveal bioactivity on organisms such as *Staphylococcus aureus, Shigella species,* *Bacillus species,* and *Escherichia coli,* is encouraging as these organisms range from pathogenic and toxigenic organisms liable to cause foodborne illnesses to spoilage-causing organisms liable to spoil food products. The control of these organisms by the extracts in foods would reveal the potentials of these extracts as preservatives. The findings add impetus to the clarion call by consumers and authorities in food industries for the replacement of chemically synthesized sanitizers/preservatives with "naturally derived" ones [34]. Medicinal plants having antimicrobial compounds in comparison with antibiotics are usually with no side effects, better patient tolerance, relatively less expensive, and acceptable due to the long history of use, and it is renewable [35].

Therefore, the results of this study support the use of Moringa, Neem, and Ginger in health products and herbal remedies in Nigeria as described by [36] and in good agreement with the reports of earlier investigators.

5. CONCLUSION

The results of this finding indicate that methanolic and aqueous extracts of Moringa, Neem, and Ginger have broad-spectrum antimicrobial activity. Hence, they can serve as a natural therapeutic agent against some enteric pathogens. Based on the findings of this research, aqueous extracts and to a lesser extent, methanolic extract of the Moringa, Neem, and Ginger possess antibacterial activity. Also, methanolic extract was found to be more potent than the aqueous extract against four pathogens tested. It was observed that *Shigella* spp. and *Staphylococcus aureus* had the highest sensitivity against the extracts.

CONSENT AND ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee. Consent and approval was also given by the Head of Department, Microbiology, Michael Okpara University of Agriculture, Umudike

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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