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Biocontrol of Food Spoilage Microorganisms using Leaf extracts from *Magnifera indica* (Mango) and *Psidium guajava* (Guava).

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ABSTRACT
The shelf life of processed food is greatly reduced by spoilage microorganisms, which has resulted in great losses to food processors, wholesalers, retailers and even consumers. This study was aimed at controlling food spoilage microorganisms using leaf extracts from mango and guava. Fried meat (beef, chicken, grass-cutter, and goat meat), fried fish and fresh bread were purchased from Benin City, and left to spoil, after which spoilage organisms were isolated, identified and characterized using standard microbiological techniques. Mango and guava leaf extracts (ethanolic and aqueous), with Potassium sorbate and sodium nitrite was used. Significant highest (<0.05) (15.67±0.88 mg/ml) antibacterial activity of Mango leaf extract was recorded against *Escherichia coli*, and significant highest (<0.05) antifungal activity (19.67±0.33) was recorded against *Penicillium oxalicum*. Ethanolic leaf extracts of mango had better MICs (6.25 mg/ml) against *E. coli* tested than guava (25 mg/ml). In addition, ethanolic leaf extracts from Mango did not have any effect on *Bacillus polymyxa*. This study suggests that extracts from Mango and Guava leaves can be explored for controlling spoilage organisms; however, ethanolic extracts are more potent than aqueous extracts.
Keywords: Food spoilage, Mango, Guava, Minimum inhibitory concentration, *Escherichia coli*

INTRODUCTION
Microorganisms cause spoilage to food by putrefaction, fermentation or rancidity. These processes lead to a decrease in the flavour and quality of the food substances. Although several preservatives have been used for the preservation of food, chemical preservatives have sometimes posed health challenges. Most recently, essential oils and extracts from plants have been employed for food preservation [1]. Essential oils are considered secondary metabolites and are important for plant defense, as they often possess antimicrobial properties [2]. Almost 3,000 different essential oils are known and 300 are used commercially in the flavour and fragrances market [3]. Jaradat and Zaid [4] reported that many plants are being used as herbal preparations because of their inherent antimicrobial, antimalarial, anti-inflammatory, anti-parasitic, anti-diabetic, antihelminthic, anti-obesity, anti-cancer and anti-viral activities. Leaves of *Mangifera indica* (Mango) and *Psidium guajava* (guava) are known to possess antimicrobial properties, which have been used for the treatment of infectious diseases [4-9]. They inhibit the growth of pathogenic microorganisms, thereby preventing diseases [10,11]. The potential toxicity of chemical based food preservatives has propelled the interest of many researchers for alternative food preservatives from plant sources [12]. Guava, for example, is a tropical and sub-tropical tree grown in Africa, Asia and South America, that has been reported to possess large amounts of useful phytochemicals such as tannins, phenols, triterpenes, flavonoids, vitamins, essential oils etc., which have the potential for controlling food spoilage organisms [12]. Mango has been reported to possess a chemical agent known as magniferin in different concentrations in its various parts (fruit, leaf, bark, stem) and this seems to be responsible for some antimicrobial and toxicological effects [8]. This study therefore focuses on the efficacy of mango and guava leaf extracts in the biocontrol of food spoilage microorganisms.

MATERIALS AND METHODS
Five different types of fried meat (beef, chicken, grass-cutter, goat meat), fried fish and five different brands of bread were purchased in

Benin City. The samples were neatly kept at room temperature and allowed to spoil for a period of five days. Spoilage was confirmed by changes in organoleptic properties, before spoilage organisms were isolated.

MICROBIAL ENUMERATION OF FOOD SAMPLES

Nutrient agar and potato dextrose agar were prepared from commercially available powdered forms (following the manufacturer's instruction) for microbial enumeration and pour plate method was employed as described by [12].

CHARACTERIZATION AND IDENTIFICATION OF ORGANISMS

The microbial isolates were characterized and identified based on their morphological, cultural and biochemical reactions as described by Bergey's Manual of Determinative Bacteriology [13].

EXTRACTION OF LEAF EXTRACTS

Fresh leaves of *Mangifera indica* (mango) and *Psidium guajava* (guava) were harvested by hand and placed in sterile black polythene bags, and transported to the laboratory. The leaves were air dried differently and blended. Fifty grams of each leaf type was weighed and soaked in 250ml of absolute ethanol in a conical flask for 24 hours, and covered to prepare the ethanolic extract. Again, 50 grams of each leaf type was soaked in distilled water for 24 hours to prepare the aqueous extract. The ethanolic extract solutions were then filtered and the filtrates concentrated at 40^{o}C in a water bath to get the crude extracts. These transferred into a sterile conical flask and kept in the refrigerator at 4^{o}C until when needed.

DETERMINATION OF ANTIMICROBIAL PROPERTIES OF THE PLANT EXTRACTS

The antimicrobial properties of the plants extracts were individually tested on the spoilage isolates at different concentrations (mg/ml) using the agar well diffusion technique using a modified method of Boateng and Diunase [15]. These activities were compared with the activities of sodium nitrite against bacterial isolates and potassium sorbate (against fungal) isolates at different concentrations (mg/ml).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION, MINIMUM BACTERICIDAL CONCENTRATION/MINIMUM FUNGICIDAL CONCENTRATION

The minimum inhibitory concentration (MIC) was used to determine the lowest effective concentration of the different types of extracts against the spoilage microorganisms isolated. This test was carried out using a disc impregnated with the leaf extracts as described by Boateng and Diunase [15]. Bacteria MIC and minimum bactericidal concentration (MBC) were incubated at 37^{o}C for 24 hours, while Fungal MIC and minimum fungicidal concentration (MFC) were incubated at 27^{o}C for 72 hours. Growth was observed after incubation and diameter zones of inhibition were measured and recorded.

DATA ANALYSIS

Data collected were analyzed using descriptive statistics on SPSS version 20.0.

RESULTS AND DISCUSSION

Antimicrobial effect of aqueous extracts of mango leaves on some selected spoilage organisms

Table 1 below shows that aqueous leaf extracts of mango had no antifungal effect between 25 and 3.125 mg/ml, whereas, there was still considerable antibacterial effect at 25 mg/ml against *Pseudomonas aeruginosa* and *Escherichia coli*. The highest antibacterial effect of the leaf aqueous extract was recorded against *E. coli* (11.67±1.45) at 100 mg/ml; while the highest antifungal effect was recorded against *Penicillium oxalicum* (13.00±1.52) at 100 mg/ml.

Antimicrobial effect of ethanolic extracts of mango leaves on some selected spoilage organisms

The ethanolic extracts from mango leaf showed a better antimicrobial activity, as seen from the higher zones of inhibition expressed in Table 2. The highest zone of inhibition for the ethanolic extract from mango leaves was highest against *E. coli* (15.67 ± 0.88 mm) followed by *Pseudomonas aeruginosa* (15.00 ± 0.58 mm) at 100 mg/ml. At 12.5 mg/ml and below however, only *E. coli* was sensitive to the mango leaf

ethanolic extract (5.33 ± 2.62 mm), whereas other spoilage organisms tested did not show any response. On the other hand, highest antifungal activity (19.67 ± 0.33 mm) was recorded against *Penicillium oxalicum* at 100 mg/ml, and it was still active even up until 25 mg/ml causing a diameter zone of inhibition of 8.33 ± 1.67 mm. The extract did not show any antifungal activity against *Rhizopus stolonifer*, and to the other fungi at any concentration below 25 mg/ml of the extract.

Table 1: Antimicrobial effect of aqueous extracts of mango leaves on some selected spoilage organisms at different concentrations (mg/ml)

Organisms	Diameter Zones of Inhibition (mm)					
	100mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**
Bacteria						
Staphylococcus aureus	11.00±0.58	7.33±1.20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lactobacillus casei	9.33±0.67	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Bacillus polymyxa	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Listeria monocytogenes	10.00±1.16	7.00±1.16	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Pseudomonas aeruginosa	11.00±1.00	9.33±1.53	5.67±0.67	0.00±0.00	0.00±0.00	0.00±0.00
Micrococcus luteus	4.33±4.33	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Streptococcus mitis	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Escherichia coli	11.67±1.45	9.00±1.00	6.67±0.88	0.00±0.00	0.00±0.00	0.00±0.00
Fungi						
***Trichoderma* sp.**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Penicillium oxalicum	13.00±1.52	9.33±1.20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
***Saccharomyces* sp.**	11.33±2.52	8.33±2.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 2: Antimicrobial effect of ethanolic extracts of mango leaves on some selected spoilage organisms at different concentrations (mg/ml)

Organisms	Diameter Zones of Inhibition (mm)					
	100 mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**
Bacteria						
Staphylococcus aureus	14.33±0.67	11.33±0.88	8.00±0.58	0.00±0.00	0.00±0.00	0.00±0.00
Lactobacillus casei	11.33±1.20	7.67±1.20	7.33±0.67	0.00±0.00	0.00±0.00	0.00±0.00
Bacillus polymyxa	4.00±2.08	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Listeria monocytogenes	12.33±0.88	11.67±0.67	7.33±0.58	0.00±0.00	0.00±0.00	0.00±0.00
Pseudomonas aeruginosa	15.00±0.58	13.33±0.67	11.67±0.88	9.33±0.88	0.00±0.00	0.00±0.00
Micrococcus luteus	11.00±1.00	8.67±0.67	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Streptococcus mitis	8.67±1.45	4.00±2.08	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Escherichia coli	15.67±0.88	15.00±0.00	12.00±1.16	10.33±1.20	5.33±2.62	0.00±0.00
FUNGI						

***Trichoderma* sp.**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Penicillium oxalicum	19.67±0.33	15.33±0.67	8.33±1.67	0.00±0.00	0.00±0.00	0.00±0.00
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
***Saccharomyces* sp.**	14.67±0.33	11.67±0.67	9.33±0.88	0.00±0.00	0.00±0.00	0.00±0.00
Rhizopus stolonifer	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Antimicrobial effect of aqueous extracts of guava leaves on some selected spoilage organisms

Highest antibacterial activity were recorded for aqueous extract of guava plant leaf as observed from the measured zones of inhibition (Table 3) against *E. coli* (11.67±0.67 mm), followed by *Pseudomonas aeruginosa* (11.67±0.33 mm). Highest antifungal activity was observed for *Trichoderma* sp. (11.33±1.45 mm) followed by *Penicillium oxalicum* (5.00±2.89 mm). The other fungal isolates were completely resistant to the aqueous extract, while other bacterial and fungal isolates tested were completely resistant to 12.5 mg/ml of the extract and below. Overall, seven of the eight bacterial isolates tested were sensitive to the aqueous extracts, while only two of the fungal isolates tested were sensitive.

Antimicrobial effect of ethanolic extracts of guava leaves on some selected spoilage organisms

Highest antibacterial activity of ethanolic extracts of guava leaves were recorded at 100 mg/ml against *E. coli* (13.67±1.86 mm) as shown in Table 4, followed by *P. aeruginosa* (13.67±0.88 mm), then *S. aureus* (13.00±0.58 mm). In addition, highest antifungal activity of the ethanolic leaf extracts was recorded at 100 mg/ml against *Trichoderma* sp. (13.00±1.15 mm), followed by *Penicillium oxalicum* (10.67±0.88 mm) and then *Rhizopus stolonifer* (9.33±0.67 mm). However, this ethanolic extract shows superior activity to the aqueous extracts, as the zones of inhibition were far higher and more potent. Overall, all the bacteria tested were sensitive at various concentrations to the ethanolic extract while only three fungal isolates were sensitive.

Antibacterial effect of sodium nitrite (preservative) on some selected spoilage bacteria

Sodium nitrite is a common preservative that is used by many food-processing companies to increase the shelf life of processed food. In this study, Sodium nitrite had effect against only six of the spoilage organisms (*S. aureus, L. casei, B. polymyxa, L. monocytogenes, Micrococcus luteus, Streptococcus mitis*) isolated and identified as seen in Table 5.

Sodium nitrite is a common preservative that is used by many food-processing companies to increase the shelf life of processed food.

Then highest antibacterial activity was observed at 100 mg/ml against *Listeria monocytogenes* (12.00 mm), followed by *Bacillus polymyxa* and *Streptococcus mitis* (10.00 mm). *Pseudomonas aeruginosa* and *E. coli* were completely resistant to this preservative. Whereas, mango and guava extracts showed considerable antibacterial activity against these two spoilage organisms.

In this study, Sodium nitrite had effect against only six out of all the spoilage organisms (*S. aureus, L. casei, B. polymyxa, L. monocytogenes, Micrococcus luteus, Streptococcus mitis*) isolated and identified as seen in Table 5.

Then highest antibacterial activity was observed at 100 mg/ml against *Listeria monocytogenes* (12.00 mm), followed by *Bacillus polymyxa* and *Streptococcus mitis* (10.00 mm). *Pseudomonas aeruginosa* and *E. coli* were completely resistant to this preservative. Whereas, mango and guava extracts showed moderate antibacterial activity against these two spoilage organisms.

Antifungal Activity of potassium sorbate (control) on some selected fungi responsible for food spoilage

Potassium sorbate was active against three of the fungal spoilage organisms (*Trichoderma* sp., *Penicillium oxalicum* and *Saccharomyces* sp.) at various concentrations between 100 mg/ml and 6.25 mg/ml as seen in Table 6.

Table 3: Antimicrobial effect of aqueous extracts of guava leaves on some selected spoilage organisms at different concentrations (mg/ml)

Organisms	Diameter Zones of Inhibition (mm)					
	100 mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**
Bacteria						
Staphylococcus aureus	9.67±1.20	3.67±1.86	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Lactobacillus casei	5.33±2.91	00.0±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Bacillus polymyxa	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Listeria monocytogenes	7.33±1.20	6.67±0.88	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Pseudomonas aeruginosa	11.67±0.33	6.67±3.38	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Micrococcus luteus	7.00±1.53	2.67±2.67	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Streptococcus mitis	9.00±1.00	5.00±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Escherichia coli	11.67±0.67	6.67±3.53	2.00±2.00	0.00±0.0	0.00±0.0	0.00±0.0
Fungi						
Trichoderma sp.	11.33±1.45	3.33±3.33	2.67±2.67	0.00±0.0	0.00±0.0	0.00±0.0
Penicillium oxalicum	5.00±2.89	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Saccharomyces sp.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
Rhizopus stolonifer	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0

Table 4: Antimicrobial effect of ethanolic extracts of guava leaves on some selected spoilage organisms at different concentrations (mg/ml)

Organisms	Diameter Zones of Inhibition (mm)			
	100 mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**
Bacteria				
Staphylococcus aureus	13.00±0.58	7.33±3.84	6.67±1.67	0.00±0.00
Lactobacillus casei	9.33±0.67	8.67±0.33	6.33±0.33	0.00±0.00
Bacillus polymyxa	6.33±0.88	0.00±0.00	0.00±0.00	0.00±0.00
Listeria monocytogenes	11.67±0.88	9.67±0.67	7.33±1.20	0.00±0.00
Pseudomonas aeruginosa	13.67±0.88	11.67±0.33	8.67±0.88	6.00±1.00
Micrococcus luteus	12.33±1.45	8.33±4.26	0.00±0.00	0.00±0.00
Streptococcus mitis	8.33±2.19	10.67±0.88	0.00±0.00	0.00±0.00
Escherichia coli	13.67±1.86	12.33±1.67	8.33±0.33	0.00±0.00
Fungi				
***Trichoderma* sp.**	13.00±1.15	7.33±1.20	2.67±2.67	0.00±0.00
Penicillium oxalicum	10.67±0.88	7.33±1.45	0.00±0.00	0.00±0.00

Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
***Saccharomyces* sp.**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Rhizopus stolonifer	9.33±0.67	7.33±0.88	0.00±0.00	0.00±0.00

Table 5: Antibacterial effect of sodium nitrite (preservative) at different concentrations (mg/ml) on some selected spoilage bacteria.

Organisms	**Diameter Zones of Inhibition (mm)**					
	100 mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**
Staphylococcus aureus	8.00	6.00	0.00	0.00	0.00	0.00
Lactobacillus casei	5.00	0.00	0.00	0.00	0.00	0.00
Bacillus polymyxa	10.00	5.00	0.00	0.00	0.00	0.00
Listeria monocytogenes	12.00	4.00	0.00	0.00	0.00	0.00
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00
Micrococcus luteus	7.00	0.00	0.00	0.00	0.00	0.00
Streptococcus mitis	10.00	7.00	0.00	0.00	0.00	0.00
Escherichia coli	0.00	0.00	0.00	0.00	0.00	0.00

Highest antifungal properties was observed against *Saccharomyces* sp., and at 12.5 mg/ml, Potassium sorbate was still active against *Saccharomyces* sp. (13.00 mm) and *Trichoderma* sp. (8.30 mm).

This result is quite instructive because *Penicillium oxalicum* was better controlled by ethanolic extracts of Mango leaf (19.00 mm) as against Potassium sorbate (15.00 mm). However, Potassium sorbate was active against *Trichoderma* sp. (14.00 mm) while Mango leaf extract (ethanolic and aqueous) was not active.

Minimum inhibitory concentrations (mg/ml) and Minimum bactericidal/fungicidal concentrations (mg/ml) of ethanolic extracts of mango and guava leaves on some selected spoilage organisms

Ethanolic extracts of mango leaves had the best MIC (Table 7) against *E. coli* (6.25 mg/ml), followed by *P. aeruginosa* (12.5 mg/ml), and then *S. aureus*, *L. casei* and *Listeria monocytogenes* (25 mg/ml). Ethanolic extracts from Guava leaves had the best MIC value of 12.5 mg/ml against *Pseudomonas aeruginosa*, followed by *S. aureus, L. casei, Listeria monocytogenes* and *E. coli* (all 25 mg/ml). Mango leaf ethanolic extract was more active against fungal spoilage microorganisms, as 25 mg/ml was able to inhibit *Penicillium oxalicum*, as against 50 mg/ml for Guava leaf ethanolic extract. In all, Guava leaf ethanolic extracts was

active against three fungal agents while Mango was active against two fungal agents.

MBC/MFC values for ethanolic extracts were quite on the high side for both Mango and guava leaves (Table 7), ranging between 25 mg/ml and 100 mg/ml. Both Mango and guava ethanolic extracts did not have any cidal effect against *Bacillus polymyxa*, and *Aspergillus niger* at any of the concentrations tested. Mango leaf ethanolic extracts had the lowest MBC against E. coli (25 mg/ml), followed by *S. aureus* (50 mg/ml), *L. casei* (50 mg/ml), *Listeria*

Table 6: Antifungal Activity of potassium sorbate (control) at different concentrations on some selected fungi responsible for food spoilage

Organisms	Diameter Zones of Inhibition (mm)					
	100 mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**
Trichoderma sp.	14.00	12.00	10.00	8.30	0.00	0.00
Penicillium oxalicum	15.00	0.00	0.00	0.00	0.00	0.00
Aspergillus niger	0.00	0.00	0.00	0.00	0.00	0.00
Saccharomyces sp.	16.00	15.00	13.80	13.00	6.00	0.00

Table 7: Minimum inhibitory concentrations (mg/ml) and minimum bactericidal/fungicidal concentrations of ethanolic extracts of mango and guava leaves on some selected spoilage organisms.

Microorganisms	Minimum Inhibitory Concentration (mg/ml)		Minimum Bactericidal/Fungicidal Concentration (mg/ml)	
	Mango	**Guava**	**Mango**	**Guava**
Bacteria				
Staphylococcus aureus	25	25	50	50
Lactobacillus casei	25	25	50	100
Bacillus polymyxa	100	100	-	-
Listeria monocytogenes	25	25	50	50
Pseudomonas aeruginosa	12.5	12.5	50	100
Micrococcus luteus	50	50	100	100
Streptococcus mitis	50	50	100	100
Escherichia coli	6.25	25	25	50
Fungi				
***Trichoderma* sp.**	-	50	-	100
Penicillium oxalicum	25	50	50	100
Aspergillus niger	-	-	-	-
***Saccharomyces* sp.**	25	-	50	-
Rhizopus stolonifer	-	50	-	100

Mango leaf ethanolic extracts also had MFC against *Penicillium oxalicum* (50 mg/ml) and *Aspergillus niger* (50 mg/ml). Guava leaf ethanolic extract had MBC effect against *S. aureus, Listeria monocytogenes* and E. coli at 50

mg/ml, while against *L. casei*, *P. aeruginosa*, *Micrococcus luteus* and *Streptococcus mitis*, it was 100 mg/ml. Against fungal spoilage organisms however, it had a 100 mg/ml MFC against *Trichoderma* sp., *Penicillium oxalicum* and *Rhizopus stolonifer*.

Minimum inhibitory concentration (mg/ml) of the aqueous extracts of mango and guava leaves on some selected spoilage organisms

MIC values against Bacteria and Fungi ranged between 25 mg/ml and 100 mg/ml as shown in Table 8. While *Bacillus polymyxa* and *A. niger* were completely resistant to both Mango and Guava aqueous leaf extracts, *Micrococcus luteus*, *Streptococcus mitis*, *Trichoderma* sp., *Saccharomyces* sp. and *Rhizopus stolonifer* were partly sensitive to the extracts. Best MIC values for Mango extracts were 25 mg/ml for *P. aeruginosa* and *E. coli*, while best MIC value for Guava extract was also 25 mg/ml against *E. coli*.

Table 8: Minimum inhibitory concentration (mg/ml) and minimum bactericidal/fungicidal concentration (mg/ml) of the aqueous extracts of mango and guava leaves on some selected spoilage organisms

Microorganisms	Minimum inhibitory concentration (mg/ml)	
	Mango	**Guava**
Bacteria		
Staphylococcus aureus	50	50
Lactobacillus casei	100	100
Bacillus polymyxa	-	-
Listeria monocytogenes	50	100
Pseudomonas aeruginosa	25	50
Micrococcus luteus	-	100
Streptococcus mitis	-	50
Escherichia coli	25	25
Fungi		
***Trichoderma* sp.**	-	50
Penicillium oxalicum	50	100
Aspergillus niger	-	-
***Saccharomyces* sp.**	50	-
Rhizopus stolonifer	-	100

Minimum bactericidal/fungicidal concentration (mg/ml) of the aqueous extracts of mango and guava leaves on some selected spoilage organisms

All aqueous extracts had very high MBCs and MFCs in this study, as shown in Table 9. Between 50 mg/ml and 100 mg/ml was required for the extracts to elicit any cidal action on the bacterial and fungal isolates. However, *L. casei*, *B. polymyxa*, *Micrococcus*, *A. niger*, *R. stolonifer* could not be killed by any of the concentrations of the aqueous leaf extracts tested. Also, it was observed that the Mango extracts had better cidal effects against fungal isolates as a total of two spoilage fungi (*Penicillium oxalicum* and Saccharomyces sp.) while only one of them was affected by guava leaf aqueous extracts (*Trichoderma* sp.).

Table 9: Minimum bactericidal/fungicidal concentration (mg/ml) of the aqueous extracts of mango and guava leaves on some selected spoilage organisms

Microorganisms	Minimum Bactericidal/Fungicidal Concentration (mg/ml)	
Bacteria	**Mango**	**Guava**
Staphylococcus aureus	100	100
Lactobacillus casei	-	-
Bacillus polymyxa	-	-
Listeria monocytogenes	100	-
Pseudomonas aeruginosa	50	100
Micrococcus luteus	-	-
Streptococcus mitis	-	100
Escherichia coli	50	50
Fungi		
***Trichoderma* sp.**	-	100
Penicillium oxalicum	100	-
Aspergillus niger	-	-
***Saccharomyces* sp.**	100	-
Rhizopus stolonifer	-	-

Chemical control of food spoilage has resulted in many of diseases of serious public health concern in humans [12]. Cases of non-communicable diseases such as diabetes, hypertension, kidney failure, liver poisoning, cancers and genetic diseases have been traced to exposure to chemical agents, which have previously been used in food preservation. Various studies have therefore explored the use of various agents from organic sources in reducing the growth of potential spoilage organisms [20-22]. Ejele *et al*., [1] reported the use of essential oils in food preservation because they are believed to be important in protecting plants from pathogenic attack. They are already being explored commercially in the production of flavourings and fragrances, as over 3,000 essential oils have already been discovered [3].

Herbal preparations from plants are already being used as agents in the treatment of cancer, malaria, diabetes, obesity, helminthic, parasitic, viral, bacterial and fungal diseases among others [4,22]. Leaves of *Mangifera indica* (Mango) and *Psidium guajava* (guava) are known to possess antimicrobial properties, which have been used for the treatment of infectious diseases [4-9]. They inhibit the growth of pathogenic microorganisms thereby preventing diseases [10,11].

Guava, for example, a tropical and sub-tropical tree grown in Africa, Asia and South America, has been reported to possess large amounts of useful phytochemicals such as tannins, phenols, triterpenes, flavonoids, vitamins, essential oils among others which have the potential for controlling food spoilage organisms [12,23,24]. In addition, Mango has been reported to possess a chemical agent known as magniferin in different concentrations in its various parts (fruit, leaf, bark, stem) and this seems to be responsible for some antimicrobial and toxicological effects [8].

This study showed that aqueous extracts from guava were able to control potential spoilage organisms only at very high concentrations. For example, aqueous leaf extracts of mango had no antifungal effect between 25 and 3.125 mg/ml, whereas, there was still considerable antibacterial effect at 25 mg/ml against *Pseudomonas aeruginosa* and *Escherichia coli*. The highest antibacterial effect of the leaf aqueous extract was recorded against *E. coli* (11.67±1.45 mm) at 100 mg/ml; while the highest antifungal effect was recorded against *Penicillium oxalicum* (13.00±1.52 mm) at 100 mg/ml. This weak activity could result from the fact that many spoilage bacteria are Gram negative and Gram-negative bacteria have a thin lipopolysaccharide exterior membrane, which could resist the attack of plant extracts. Earlier reports have mentioned that antimicrobials of plant origin show no effect against Gram-positive bacteria, when compared with Gram-positive bacteria [11]. However, Gram-positive bacteria have a thick peptidoglycan layer, which makes translocation of extracts across the cell membrane easier and more effective

Some studies on guava extracts include the study of Vieira *et al*., [16] that found guava sprout extracts were effective against inhibiting *E. coli*. The study of Hoque *et al*. [17] found no antibacterial activity of ethanolic extracts of guava against *E. coli* and *S. entertidis*. The study of Sanches *et al*. [18] found that the aqueous extract of guava was effective against *Staphylococcus* and *Bacillus*. The study of Nascimento *et al*. [19] found the guava extract was able to have inhibitory effects against *Staphylococcus* and *Bacillus* and no effect on the *Escherichia* and *Salmonella.*

Other studies include that of Amit *et al*. [6,8] on Langra mango extracts, which showed significant activity against five microorganisms (*Escherichia Coli, Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi* and *Staphylococcus aureus*). Olasehinde *et al.* [7] reported that crude aqueous and ethanolic extracts of mango leaves exhibited considerable activity against Gram-positive and Gram-negative bacteria, as well as the fungus, *Candida albicans* even at low concentrations.

In addition, between 50 mg/ml and 100 mg/ml was required for the extracts to elicit any cidal action on the bacterial and fungal isolates. However, *L. casei, B. polymyxa, Micrococcus, A. niger, R. stolonifer* could not be killed by any of the concentrations of the aqueous leaf extracts tested. While *Bacillus polymyxa* and *A. niger* were completely resistant to either the Mango or Guava aqueous leaf extracts, *Micrococcus*

luteus, *Streptococcus mitis*, *Trichoderma* sp., *Saccharomyces* sp. and *Rhizopus stolonifer* were partly sensitive to the extracts. The concentrations at which these extracts showed inhibition and cidal effects are high for a food preservative. A food preservative should only be applied at a concentration that will not interfere with the biochemical and physical characteristics of food, thus preserving its unique taste, texture and structure for the palatability of the consumers [23]. This is a very instructive observation because regular preservatives being used in food processing companies are mainly synthetic, and majority of these preservatives have been known to be carcinogenic and poisonous to the circulatory, hepatic and urinary systems [23,24]. However, with the introduction of natural plant extracts as food preservatives, one can successfully increase the shelf life of processed foods without exposing consumers to the harmful effects caused by synthetic preservatives. The only challenge might just be the extraction procedure, and the concentration of application.

CONCLUSION

Spoilage organisms have greatly affected the shelf life of processed food, and this study has revealed that natural extracts of mango and guava leaf extracts were found to possess antibacterial activity against some spoilage microorganisms, while it was not active against some others. Majority of the microorganisms that were controlled by these extracts were sensitive only at high concentrations of the extracts, which might alter the organoleptic and sensory properties of the processed food. Hence, further research should be carried out to study the changes in the organoleptic and sensory parameters that will result from utilizing these extracts as preservatives. This will further strengthen the final application of such novel improvements in food processing technology.

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