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Original Research Article

Formulation of Antiseptic Ointments from *Mangifera indica* Kernel, Leaf and *Psidium guajava* Leaf Extracts

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ABSTRACT

The increasing resistance of some priority pathogenic bacteria to some known antibiotics warrant more research into development of new antibacterial agents. The main study objective was to formulate antiseptic ointments from *Mangifera indica* kernel, leaf and *Psidium guajava* leaf extracts and investigate the antibacterial activity. The study also aimed at comparing the efficacy of the plant extracts and ointments as antibacterial agents. The study design was experimental. Study population were laboratory scientists and janitors selected using purposive sampling. Powdered plant samples were extracted with 100% methanol and phytochemical analysis was conducted. The ointments were formulated based on the British Pharmacopoeia method of simple ointment formulation. Microbial analysis was conducted using agar diffusion and dilution methods. Data was analysed using SPSS statistical software. All plant samples tested positive for the presence of alkaloids, saponins, flavonoids and terpenoids. There was significant difference in mean zone of inhibition among the extracts and ointments against all test organisms with mango kernel exhibiting the highest activity. *Staphylococcus aureus* exhibited the highest susceptibility to all extracts and ointments. Minimum inhibitory concentration for mango kernel ointment was 25 mg/mL for all test organisms while bacteria growth was observed at all concentrations for mango leaf and guava leaf ointments. Mango kernel extract and ointment exhibited highest significant antibacterial activity (20.70 ± 1.05 and 18.00 ± 0.89, respectively). This study demonstrated that mango kernel ointment possesses greater efficacy as antibacterial agent compared to mango leaf and guava leaf ointments.

Keywords: Mango kernel, phytochemical, *Psidium guajava*, antimicrobial ointment.

Introduction

Plants have been used for centuries in various cultures of the world for the treatment of diseases and they have become of great interest in modern medicine.[1] *Mangifera indica* belongs to the family Anacardiaceae and genus Mangifera.[2] *Psidium guajava* is an evergreen shrub which belongs to the family Myrteceae and genus Psidium. The genus Psidium comprises approximately one hundred and fifty species of small trees and shrubs in which only twenty species produce edible fruits. *Psidium guajava* is the most commonly cultivated specie.[3] *M. indica* kernels are agro-wastes which are available in large quantities in tropical countries.[4] Agricultural wastes have been reported to contribute a significant proportion to the total waste matter in developing countries of the world.[5] Although used for composting and energy production, management of this waste is limited as the amount of waste reused is far less than that produced hence there is need to explore other beneficial use.[6] The challenges associated with increasing resistance of some pathogenic bacteria such as *Escherichia coli, Salmonella specie* and *Staphylococcus aureus* to conventional antibiotics and the environmental threat posed by some conventional antibacterial/anti-septic agents such as triclosan warrant a need for further research into production of environmentally friendly antibacterial agents from plant parts.[7,8] According to the World Health Organisation, these top priority pathogenic bacteria are chief causes of diseases and death especially in developing countries. The WHO stated that antibiotic resistance is one of the biggest threats to global health, food security and development. Infections, such as pneumonia, salmonellosis, are becoming harder to treat as the antibiotic used to treat them are becoming less effective.[9] Thus, there is increasing need for non-toxic, environmentally friendly antimicrobial products to combat transmission of pathogenic bacteria. A study conducted by Olasehinde *et al*.,[10] investigated the antibacterial property of *M. indica* leaves extract against *S. aureus, E. coli, Micrococcus virians, Micrococcus leteus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The study reported that crude aqueous and ethanol extracts of mango leaves exhibit antibacterial action against both gram-positive and gram-negative bacteria. Alok *et al*.,[11] investigated the antibacterial activity of mango kernel extract against *S. aureus and P. aeruginosa*. The result of the study showed that the extract exhibited antibacterial activity against the test organisms. Nwinyi *et al*.,[12] evaluated the antibacterial activity of ethanol leaf extract of *P. guajava* leaves on clinical isolates of *E. coli* and *S. aureus*. The study found that the extract possesses significant antibacterial activity against the two organisms. Dzotam and Kuete[13] investigated the antibacterial and antibiotic modifying activity of methanol extract of *P. guajava* against selected bacteria isolates and found that methanol extract of guava leaves demonstrated significant inhibitory activities against both sensitive and Multi Drug Resistant (MDR) bacteria confirming previous reports on its antimicrobial activity. The main objective of this study is to formulate antimicrobial ointments from mango kernels, mango leaves and guava leaves and investigate the

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antibacterial property of their respective formulations and extracts. It is also aimed at exploring other beneficial use of mango kernel wastes possibly as an antibacterial agent.

Materials and Methods

Experimental Design

The study adopted experimental study design entailing laboratory analysis. Study population were laboratory scientists and janitors working in the microbiology laboratories at the University of Ibadan and University College Hospital (UCH) Ibadan, Oyo, Nigeria. Purposive sampling technique was adopted and used to recruit all twenty eligible participants. Ethical approval was requested and approved by the UI/UCH Research Ethics Review Office. Sterile cotton swabs moistened with 0.85% sterile normal saline were used to collect swabs from the palms and fingers of volunteers.

Collection, identification and preparation of plant samples

Healthy leaves and fruits of *Mangifera indica* and *Psidium guajava* leaves were obtained from a private farm in Arepo, Ogun state. The plants were identified and authenticated at the Herbarium, Department of Botany, University of Ibadan and given voucher numbers UIH2282 (*Mangifera indica*) and UIH22823 (*Psidium guajava*). The leaves were washed with distilled water, air dried for 14 days and grounded into powder. The mango fruits were peeled and the flesh separated from seed. The kernels were manually removed from the seed and ground into powder. The powdered plant samples were stored in sealed polythene bags and refrigerated at 4ºC until utilized.

Extraction of plant samples

The maceration method of extraction was used according to the procedure described by Ayoola *et al*.[15] 1000 g of each powdered plant sample was soaked in 100% methanol at room temperature for 48 hours in an enclosed glass jar with periodic shaking. Multiple extractions were conducted on each sample to obtain maximum yield of extracts. The extracts were filtered after 48 hours using Whatmann filter paper No.42. The extracts were concentrated using a rotary evaporator with the water bath set at 40ºC. All extracts were kept at 4ºC until further use.

Phytochemical analysis of plant samples

Qualitative phytochemical analysis was conducted according to the methods used by Ejikeme *et al*.,[16] and Ayoola *et al*.[15] The qualitative phytochemical parameters tested include the presence of tannins, alkaloids, saponins, flavonoids, phenols, plant steroids, terpenoids and cardiac glycosides.

Test for tannins: Zero-point five grams (0.5 g) of extract was boiled in 10 mL water in a test tube placed in a water bath for 10 minutes and then filtered using Whatmann filter paper No. 42 (125 mm). Three drops of 0.1% ferric chloride was added to the filtrate and observed for brownish green or a blue black colouration.

Test for alkaloids: Zero-point five grams (0.5 g) of extract was placed in a conical flask and 10 mL of 5% tetraoxosulphate (VI) acid (H_2SO_4) in 50% ethanol was added. The mixture was boiled for 2 minutes and filtered through Whatmann filter paper No. 42. 2 mL of dilute ammonia solution was added to 5 mL of the filtrate. Five millilitre (5 mL) of chloroform was added to the solution and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. Dragendorff's reagent (Bismuth potassium iodide solution) was added to the acid extract and the formation of reddish-brown precipitate was regarded as positive for the presence of alkaloids.

Test for flavonoids: Five millilitre (5 mL) of distilled water was added to 0.5 g of extract weighed into a beaker, the mixture allowed to stand for 2 hours and filtered using a Whatmann paper No. 42. 5 mL of 1M dilute ammonia solution was added to the filtrate followed by addition of 1 mL of concentrated tetraoxosulphate (VI) acid. Observation of yellow colouration which disappeared on standing indicated the presence of flavonoids.

Test for saponins: Zero point five grams (0.5 g) of the plant extract was added to 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for terpenoids: Two millilitres (2 mL) of chloroform was added to 0.5 g each of the extract. 3 mL of concentrated H_2SO_4 was added carefully to form a layer. A reddish brown colouration formed at the interface indicates the presence of terpenoids.

Test for cardiac glycosides: Two millilitres (2 mL) of distilled water was added to 0.2 g of extract. 2 mL of glacial acetic acid containing one drop of ferric chloride solution was added. 1 mL of concentrated H_2SO_4 was carefully added to form an under layer. Formation of brown colouration at the interface indicated the presence cardiac glycosides.

Test for phenols: A few drops of ferric chloride solution was added to 2 mL of the extract in a watch glass. The appearance of a bluish green colour indicated the presence of phenol.

Quantitative analysis of the phytochemicals was conducted according to the method described by Ajuru *et al*.,[17] and Hussain *et al*.[18] The quantitative parameters determined were percentage content of saponins, alkaloids and flavonoids.

Determination of alkaloid content: Two point five grams (2.5 g) of powdered plant sample was weighed into a beaker and 100mL of 10% acetic acid in ethanol was added and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole mixture was allowed to settle for 3 hours, the supernatant was discarded and the precipitate washed with 20mL of 0.1M NH_4OH solution and then filtered. The residue was dried in an oven and weighed using electric weighing balance and the percentage alkaloid content calculated.

Determination of flavonoid content: Two point five grams (2.5 g) of powdered the plant samples were extracted repeatedly using 50mL of 80% aqueous methanol at room temperature for 24 hours. The solution was filtered and the filtrate transferred into an already weighed crucible. The filtrate was evaporated to dryness over a water bath. The crucible and its content was cooled in a desiccator and weighed until constant weight was obtained. The weight of the flavonod is obtained from the difference in the weight of the empty crucible and the crucible containing dried extract.

Determination of saponin: Twenty grams (20 g) of powdered plant sample was dispersed in 200 mL of 20% ethanol in a conical flask. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The extract was then reduced to 40 mL over a water bath at about 90ºC. The concentrate was transferred to a separating funnel and 2mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice. 60 mL of n-butanol was added and the butanol extract was washed twice with 10 mL of 5% sodium chloride solution. The sodium chloride layer was discarded and the remaining solution heated in a water bath for 30 minutes after which the solution was transferred into a crucible and dried in an oven to a constant weight.

Microbiological analysis

Susceptibility test for plant extracts

This was carried out using the agar well diffusion method described by Mohammed *et al*.[19] Mueller-Hinton agar (MHA) was prepared according to manufacturer's instructions. It was autoclaved, cooled and poured into sterile petri dishes. Overnight broth cultures of the bacteria of interest, *S. aureus*, *E. coli* and *Salmonella sp.* were seeded into the petri dishes uniformly and allowed to dry. Holes were bored with the aid of 8 mm cork borer. The holes were filled with 1 mL of different concentrations (100 mg/mL to 6.25 mg/mL) of each extract. Gentamicin was used as the positive control. The agar plates were incubated at 37ºC

for 24 hours. The diameter of zones of inhibition were measured and recorded.

Determination of minimum inhibitory concentration of plant extracts
This was determined using the agar dilution method.[20] Muller-Hinton agar (MHA) was prepared according to manufacturer's specification, dispensed and sterilised. Two grams of extract was dissolved in 10mL of methanol to give a concentration of 200mg per mL (double strength). The extracts were serially diluted into different bottles at different concentrations respectively. 2mL of each dilution was introduced into the agar bottles, mixed, poured into sterile petri dishes and allowed to solidify. Overnight broth culture of the test organisms was seeded unto the petri dishes and incubated at 37ºC for 24 hours. The lowest concentration of the extract that inhibited the test organism was considered the minimum inhibitory concentration (MIC).

Collection and analysis of swab samples: Analysis of hand swab samples was conducted to ascertain the presence of test organisms on hands of participants after hand hygiene procedure and to assess the antibacterial activity of formulated ointments against organisms of interest.
Total plate count: This was performed according to the method described by De Alwis *et al*.,[21] Sterile cotton swabs dampened in 0.85% saline were used to take samples from the palm, fingers and between fingers of volunteers. The swabs were transported in 5 mL sterile saline and cultured at the laboratory. 0.5mL of each sample were then inoculated in tryptic soy agar (TSA). Plates were incubated at 37ºC for 24 hours. The number of colonies observed were counted after 24 hours and recorded as colony forming units per hand.
Enumeration of organisms of interest: Zero point one millilitre (0.1 mL) of diluted swab samples were spread inoculated unto petri dishes containing eosin methylene blue (EMB for *E. coli)*, mannitol salt agar (MSA for *S. aureus*) and salmonella shigella agar (SSA for *Salmonella sp*.) respectively. The petri dishes were incubated for 24 hours. The number of colonies observed on each plate was counted and the bacterial counts were expressed as the number of colony forming units per hand. The organisms of interest were identified based on their colony morphology, sub-cultured in nutrient agar and incubated at 37ºC.

Formulation of antiseptic ointment
The antiseptic ointment was formulated according to the method used by Nyong *et al.*,[20] based on the British Pharmacopoeia formula for preparation of simple ointment. The plant extract was incorporated into the ointment base by levigation.

The ointment base was measured into evaporating dish and placed on the steam bath. It was allowed to completely melt while stirring with the glass rod. The plant extract was measured into the evaporating dish and melted until consistency was attained (50 mg of extract per gram of ointment base was measured). The extracts were incorporated into respective melted ointment bases to give 5% (w/w) simple ointment BP and transferred into ointment jars using the spatula.

Assessment of antimicrobial property of ointments
The antibacterial susceptibility test was carried out using the agar well diffusion method.[19] Muller Hinton agar was prepared autoclaved, cooled, poured and allowed to solidify. The test organisms *S. aureus, E. coli* and *Salmonella sp.*, were obtained from the sub-cultured hand swab samples collected from volunteers. Overnight broth cultures of respective bacterial species were seeded into the petri dishes and allowed to dry. Holes of 8mm were bored and filled with 1mL of different concentrations of each formulation. Gentamicin was used as the positive control while simple ointment base was used as the negative control. The agar plates were incubated at 37ºC for 24 hours. The diameter of zones of inhibition were measured and recorded. The minimum inhibitory concentration (MIC) of the formulated ointments were determined based on the agar dilution method.[20] MHA was prepared according to manufacturer's specification, dispensed and sterilised. 4 g of ointment was dissolved in 10 mL of dimethylsulfoxide (DMSO) and serially diluted to obtain different concentrations (25 mg/mL to 1.56 mg/mL). 2 mL of each dilution was dispensed into agar bottles, mixed, poured into sterile petri dishes and allowed to solidify. Overnight broth culture of the test bacteria species was seeded unto the petri dishes and incubated at 37^0C for 24 hours. The lowest concentration of the extract that inhibited the test organism was considered the minimum inhibitory concentration for each ointment.

Physicochemical analysis of antiseptic ointment
The physicochemical parameters namely pH, appearance, saponification value, iodine value, acid value and ester value were determined according to the method used by Odoom and Edusei[22] and Olaniyi *et al*.,[23] The ointment was observed for colour/appearance. The pH was determined using a digital pH meter. Zero point five grams (0.5 g) of each ointment formulation was weighed and dispersed in 50mL of distilled water and the pH was measured.
Determination of acid value: Twenty-five millilitres (25 mL) of alcohol (95%), 25 mL of diethyl ether and 1mL of phenolphthalein solution were mixed and neutralized by adding few drops of dilute KOH until a pink colour was obtained. 2g of each ointment was weighed into a conical flask and the prepared solution was added. When the oil is dissolved it was then titrated against 0.1M aqueous KOH until a pink colour wish persist for at least 15 seconds was obtained.
Determination of iodine value: Into a twenty-five millilitre iodine flask was weighed 0.177 g of the ointment. Ten millilitres (10 mL) of carbon tetrachloride and 20 mL of iodine monochloride solution were added. 15mL of 10% KI solution with 100 mL of distilled water was added to the flask. The resulting solution was then titrated against 0.1M sodium thiosulphate ($Na_2S_2O_3$) using starch as indicator till the end point where the blue black colouration becomes colourless was obtained. A blank titration was also carried out at the same time starting with 10 mL of CCl_4. Iodine value was then calculated.
Determination of saponification value: Two grams (2g) of each ointment formulation was weighed into a clean dried round bottom flask and 20 mL of 1M alcoholic potassium hydroxide (KOH) was added. A reflux condenser was attached to the flask and heated for an hour with periodic shaking. Then 1 mL of 1% phenolphthalein indicator was added through the condenser. The content of the flask was cooled and the excess alkali titrated against 0.5M hydrochloric acid until the solution became colourless. The saponification value was then calculated.
Determination of esterification value: Esterification value of the ointments was obtained by subtraction of the iodine value from the saponification value.

Data analysis
The data collected was analysed using the statistical package for social sciences (SPSS) software version 23. Means, standard deviation, tables were used to summarize data. Independent t-test and ANOVA was used to compare means at a significance of $p < 0.05$. GraphPad Prism 7.01 was used to generate trend lines.

Results and Discussion

The phytochemical analysis showed that all the extracts tested positive for the presence of saponins, flavonoids, tannins, phenols, terpenoids and alkaloids and negative for the presence of cardiac glycosides (Table 1). The analysis revealed that mango leaf, guava leaf and mango kernel contain major phytochemicals. Percentage content of alkaloid in the mango kernel sample was found to be slightly higher (21%) compared to mango leaf and guava leaf samples (20.8% and 18.4%, respectively). Alkaloids were reported in an earlier study to confer antibacterial property on some plants.[23]

Microbial analysis of plant extracts
There was significant difference in the mean zone of inhibition (ZOI) for mango kernel extract ($F = 8.239$, $p = 0.002$) and mango leaf extract ($F = 4.143$, $p = 0.027$) among the three bacteria. There was statistically insignificant difference in the mean value of zone of inhibition (ZOI) for *E. coli* between the mango leaf and kernel extract ($t = 1.059$, $p = 0.303$). There was insignificant difference in the mean value of ZOI for

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mango kernel extract and mango leaf extract for *Salmonella sp*. (t = 1.450, p = 0.164) (Table 2 and Figure 2). This study found that *S. aureus* demonstrated significantly high susceptibility to mango kernel extract compared to mango leaf and guava leaf extracts (Figure 1). The study also found that *S. aureus* demonstrated highest susceptibility to mango kernel, mango leaf and guava leaf extracts among the test organisms which was found to be statistically significant (20.70 ± 1.05 for mango kernel extract, 17.00 ± 1.00 for mango leaf extract and 15.40 ± 1.12 for guava leaf extract F = 6.596 p = 0.05). Guava leaf extract did not exhibit any significant activity against *E. coli* and *Salmonella sp*. (Table 4).

There was significant difference in the mean zone of inhibition for mango kernel ointment among the three bacteria (F= 3.401, p = 0.048). The mean value of ZOI for *S. aureus* was significantly highest for mango kernel ointment compared to that of *E.coli* and *Salmonella sp*. Minimum inhibitory concentration analysis (MIC) for mango kernel ointment was 25 mg/mL in 50% dimethylsulfoxide (DMSO) for all the three test organisms while there was bacterial growth at all the concentrations for mango leaf ointment and guava leaf ointment (Table 3).

S. aureus demonstrated highest statistically significant susceptibility to all the formulated ointments among the test bacteria. Susceptibility of *S. aureus, E. coli* and *Salmonella sp*. was found to be highest for mango kernel extract and ointment. However, only the difference in antibacterial activity against *S. aureus* was found to be statistically significant. This demonstrates that mango kernel ointment exhibited greatest antibacterial activity among the three plant samples. Guava leaf ointment did not exhibit any significant activity against *E. coli* and *Salmonella sp.* Mango kernel ointment had the lowest minimum inhibitory concentration against each test organism compared to guava and mango leaf ointments (Table 5).

Analysis of hand swab samples' microbial load: S. aureus, E. coli., and *Salmonella sp.* are among the twelve priority pathogenic bacteria reported by the WHO to pose the greatest threat to human health.[5] The hand swabs in this study were taken after hand washing to assess the possible persistent presence of microbes. *E. coli*, *S. aureus* and *Salmonella sp*. were detected. *E. coli* was detected on the hands of three participants. The microbial load was found to be 60, 90 and 120 cfu/hand for three of the participants. *S. aureus* was detected on the hand of nine participants and the highest count was 10 cfu/hand. *Salmonella sp*. was detected on the hand of one participant and microbial load was 124 cfu/hand. The microbial loads for two participants were too numerous to count (TNTC).

Physicochemical analysis of formulated ointments: Mango leaf ointment has the highest acid value while guava leaf ointment has the highest iodine, saponification and esterification values. The pH of the mango kernel ointment, mango leaf ointment and guava leaf ointment were found to be 4.61, 5.93 and 5.93, respectively.

Table 1: Phytochemical screening of plant samples

Phytochemicals	Mango kernel	Mango leaf	Guava Leaf
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Cardiac glycosides	-	-	-
Terpenoids	+	+	+
Alkaloids	+	+	+
Phenols	+	+	+

Key: + = Present, - = Absent

Table 2: Comparison of ZOI average value across the extract groups for *E. coli*, *S. aureus* and *Salmonella sp.*

Plant extract	Bacteria	ZOI Means ± SEM	F/t test	p-value
Mango kernel	*E. coli*	16.20 ± 0.96	1.059	0.0303*
Mango leaf		14.80 ± 0.90		
Mango kernel	*S.aureus*	20.70 ± 1.05	6.596	0.005*
Mango leaf		17.00 ± 1.00		
Guava leaf		15.40 ± 1.12		
Mango kernel	Salmonella sp.	15.20 ± 1.04	1.450	0.164
Mango leaf		13.20 ± 0.90		

*Statistically significant

Table 3: Comparison of ZOI average value across bacteria for mango kernel and leaf ointments

Bacteria	Ointment Formulation	ZOI Means ± SEM	F-test	p-value
E. coli	Mango kernel	17.00 ± 0.91	3.401	0.048*
	Mango leaf	15.00 ± 0.91	2.668	0.088
S. aureus	Mango kernel	18.00 ± 0.89	3.401	0.048
	Mango leaf	16.40 ± 0.83	2.668	0.088
Salmonella sp.	Mango kernel	14.60 ± 1.03	3.401	0.048
	Mango leaf	13.70 ± 0.73	2.668	0.088

*Statistically significant, ZOI = zone of inhibition

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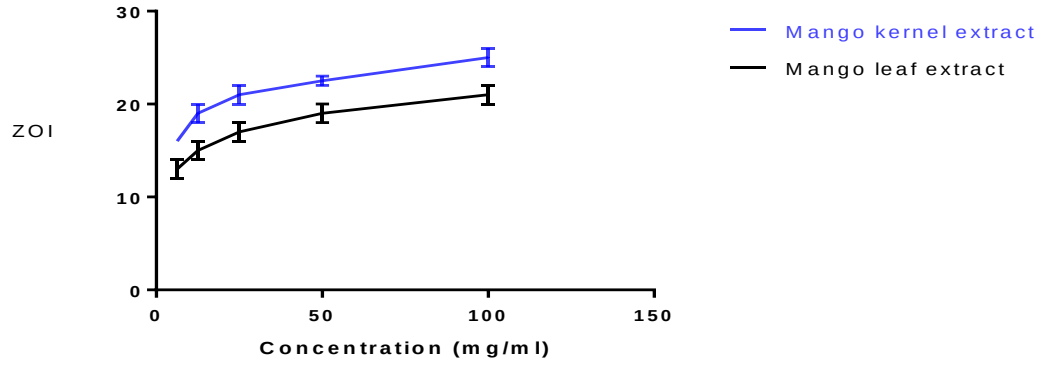

Figure 1: Comparison curve of ZOI for *S. aureus* at different concentrations

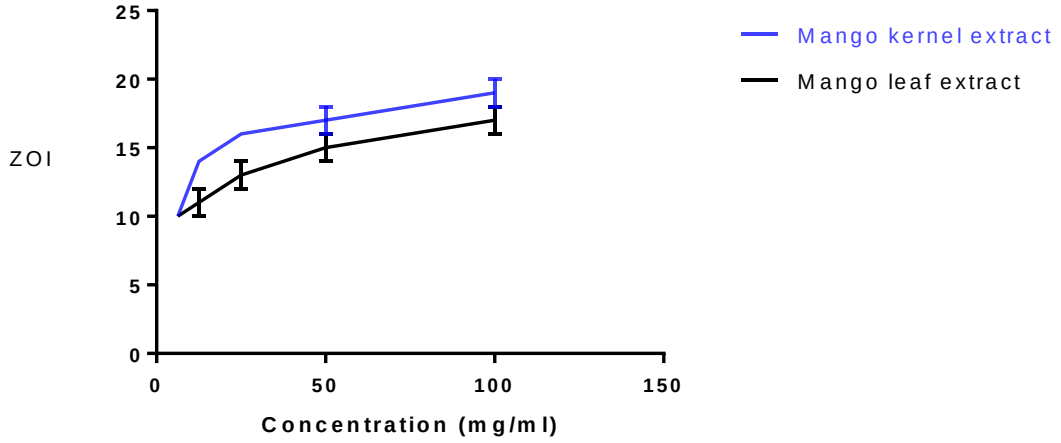

Figure 2: Comparison of ZOI for *Salmonella sp.*at different concentration of extracts

Table 4: Minimum inhibitory concentration analysis for mango kernel, leaf and guava leaf extracts

Conc. in mg/mL	*E. coli*			*S. aureus*			*Salmonella sp.*		
	MKE	MLE	GLE	MKE	MLE	GLE	MKE	MLE	GLE
100	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-
3.125	-	-	-	-	-	-	-	-	-
1.562	-	-	+	-	-	+	-	-	+
0.781	-	-	+	-	-	+	-	-	+
0.390	-	+	+	-	-	+	-	-	+
0.195	+	+	+	-	-	+	-	-	+
0.0975	+	+	+	+	+	+	+	+	+
0.0487	+	+	+	+	+	+	+	+	+
0.0244	+	+	+	+	+	+	+	+	+

Key: Conc. = Concentration of extract in mg/mL, + = Bacterial growth observed, - = No bacterial growth observed
MKE = Mango kernel extract, MLE = Mango leaf extract, GLE = Guava leaf extract, Minimum inhibitory concentration (MIC) in mg/mL

Table 5: Minimum inhibitory concentration analysis for mango kernel, leaf and guava leaf ointments

Conc. in mg/mL	Mango kernel ointment			Mango leaf ointment			Guava leaf ointment		
	E. coli	*S. aureus*	*Salmonella*	*E. coli*	*S. aureus*	*Salmonella*	*E. coli*	*S. aureus*	*Salmonella*
25	-	-	-	+	+	+	+	+	+
12.5	+	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+	+
1.5625	+	+	+	+	+	+	+	+	+

Key: Conc. = Concentration of ointment in 50% DMSO, + = Bacterial growth observed, - = No bacterial growth observed

Conclusion

Mango kernel, leaf and guava leaf extracts and ointments were found to possess antibacterial properties against gram-positive *S. aureus* and gram-negative *E. coli* and *Salmonella sp*. However, mango kernel extract and formulation exhibited highest antibacterial activity against all the test bacteria thus indicating the possible higher efficacy of this ointment as an antibacterial agent compared to mango leaf and guava leaf ointments. This agro-waste has potential use in the pharmaceutical industry and this can be further researched and explored.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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