

Extraction and Antibacterial Studies of Oil from three Cultivars of Mango Kernel obtained from Makurdi - Nigeria

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ARTICLE INFO

Article history:

Submitted: 2020-01-06

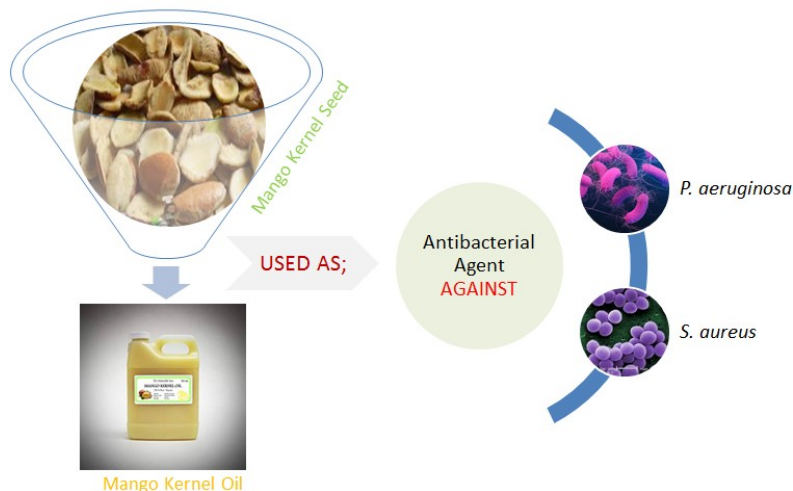
Revised: 2020-02-12

Accepted: 2020-02-28

Available online: 2020-03-11

Manuscript ID: [PCBR-2001-1075](#)

GRAPHICAL ABSTRACT



KEYWORDS

Bacteria
Mango
Kernel
Oils
Phytochemicals

ABSTRACT

The ability of bacteria to develop resistance to many antibiotics cannot be undermined given the multifaceted health challenges in the present times. For this reason a lot attention is on botanicals and their products in search of new antibacterial agents. On the other hand, mango kernel oils (MKO) can be heavily valorized by taking advantage of the myriads bioactive phytochemicals it contains. Herein, we buttressed the use of MKO as bioactive agent against bacteria. The MKOs for the study were extracted by soxhlet means with ethanol and hexane for 4 h from 3 different mango kernels, namely; "local" (sample A), "julie" (sample B) and "john" (sample C). Prior to the extraction, ground fine particles of the kernels were obtained from the seed kernels dried in oven at 100 °C for 8 h. Hexane gave higher yield of the oils than ethanol. It was also qualitatively confirmed that the mango kernel oils contain some phytochemicals such as phenol, quinone, saponin and terpernoid. The results of the antibacterial activities of the MKO against both gram positive (*staphylococcus aureus*) and gram negative (*pseudomonas aeruginosa*) at different concentrations showed that the oils extracted with ethanol gave better antibacterial properties than those of the hexane. More so, the bioactivities were best with the local mango kernel oil. Indeed this work has completely validated the previous claim that MKOs are effective antibacterial agent. Thus, these oils (especially the ethanol-derived ones) can be used as bacteriostatic and antibacterial agent in say food, cosmetics and allied industries.

1. Introduction

Infectious diseases are causing death worldwide partly because of multidrug resistant strains of bacteria and increase in untreatable bacterial infections [1, 2]. This situation has necessitated a search for new antibacterial compounds hence, researchers and pharmaceutical industries are increasingly turning their attention to herbal products in order to develop better drugs against pathogenic microbial strains [2, 3]. Fortunately, most plants produce a large number of secondary metabolites with antimicrobial effects on pathogens, individually or in combination [4-6]. In addition, medicinal plants have various effects on living systems, thus they are used as; sedatives, analgesic, antipyretics, cardio-protective, anti-inflammatory, antioxidants, antispasmodics, immunomodulatory[7], antioxidant[4], and immunomodulatory[7, 8]. Importantly these plants' chemical diversity is thought to protect plants against microbial pathogens[3]. Of course waste or by-products of plants also contain these vital metabolites.

In addition, one of the agro-wastes, currently causing pollution problems and also underutilized is the peels and kernels of the mango fruit[3]. First and foremost, mango (*Mangifera indica* L.) is a member of the family *Anacardiaceae*. Mango is one of the most important and abundant tropical fruits in Nigeria with a global production exceeding 35 million tons[3, 4]. Mango fruits are processed in various products as puree, canned slices, syrup, nectar, leather, pickles, chutney and jam[9], with concurrent generation of significant amount of wastes. In general, there is an abundant supply of mango by-products such as seed kernels and peels which are considered as wastes from mango fruit utilization[2, 3, 9]. Furthermore, it is found that 40 to 60% waste is generated during processing of mangoes; which consists of 12 to 15% peels and 15 to 20% of kernels. To start with, the mango seed represents 10 to 25% of the whole mango fruit weight. The kernel inside the seed represents 45 to 75% of the seed and about 20% of the whole fruit. Similarly, another literature has it that, the seed

content of different varieties of mangoes range from 9% to 23% of the fruit weight and the kernel content of the seed ranges from 45.7% to 72.8%. More so, Pitchaon [9] reported that mango kernel contributes about 17-22% of the fruit. Hence, the mango kernels contain ± 15 wt% of oils[5]. Mango seed kernel, hitherto considered as waste, contain substantial levels of health-enhancing compounds and natural antioxidants[4]. Therefore, more than one million tons of mango seeds are being treated as waste, subsequently leading to environmental pollution. However, with appropriate treatment and study, the kernel (seeds) might be possibly used as a food ingredient, medicine and many other purposes[2].

Mango seed kernel oil has been shown to have high antibacterial activity, supports the development of the placenta and fetus, and helps in the metabolic activities of teeth, the retina and skin, while preventing anemia. In addition, it also helps to tighten the capillary vessels and can be used as nutrient rich food oil or as ingredients for functional or enriched foods[5]. *Mangifera indica* seed kernel oil has high unsaponifiable matter content (4.58%) which makes it suitable for use in cosmetics industry[10]. Mango chemicals is hoped to promote research into its potential as a novel antibacterial agent against pathogenic micro-organisms and the allied applications[2]. Using the mango kernel oil will not only give room for alternative products that is renewable and sustainable, but will also help to ameliorates some of the challenges associated with total dependence on petrochemical thereby cushioning the adverse effects of synthetic chemicals on health, environment and economy[11]. There is no doubt that the mango kernel is grossly underutilized and unpopular vis-à-vis its potentials and applications; in spite of the readily availability of this resource in many communities across the globe. Many researches on mango kernel are hitherto for academic exercises and solely consignment of the book shelves. This trend is unhealthy for sustainability, socio-economic development and blatant waste of resource. Hence, this research is aimed at comparative analysis of the antibacterial activities of 3 different mango kernel oils.

2. Materials and methods

2.1. Materials and Reagents

Ripe mangoes, absolute ethanol, hexane, lead acetate, ice, sodium hydroxide (NaOH), test organism (*staphylococcus aureus* and *pseudomonas aeruginosa*), Mueller- Hinton Agar (MHA), barium chloride, distilled water, concentrated sulphuric acid (H₂SO₄), ferric chloride solution, chloroform, mythelated spirit. Chloramphenicol (positive control); hexane and ethanol (negative control) were used. These chemicals and reagents were of Analytical Grade.

2.2. Sample Collection and Pretreatment

The 3 different mango fruits (*“Local”*, *“Julie”* and *“John mango”* cultivars –see **Fig. 1**) were purchased at Wurukum market in Makurdi – Nigeria. The samples were prepared before the extraction by the following processes: removal of pulp, drying of kernels and grinding of dried kernel. The seed kernels were dried in an oven at 100 °C for 8 h. The seeds were then cracked and the kernel was removed. All the kernels were ground using a mortar pestle. Then the ground kernels were further ground using a kitchen blender to make the particle size of the kernel powder less than 2000 microns. The kernel powder was then stored in a plastic container in a cool and dry place as previously carried out[9].

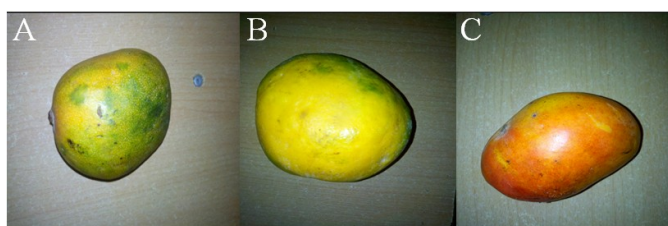


Fig. 1- Varieties of Mango samples, A = Local Mango, B = Julie Mango, C = John Mango

2.3. Extraction of the Mango Kernel Oils

The oils were extracted from the powdered kernel (100 g) using a soxhlet extractor with ethanol (polar protic and a greener solvent) and hexane (non-polar solvent) for 4 h in each case. The oils of mango kernel sample A, sample B and sample C,

respectively were subsequently recovered from the solvents with rotary evaporator.

2.4. Phytochemical Screening

The oils were then qualitatively screened for phenol, quinine, saponin tannins, flavonoids, steroids, compounds using the standard procedures[10].

2.5. Antibacterial Analysis

2.5.1. Bacteria strains used

The test bacteria strains used were *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 29953.

2.5.2. Evaluation of Antibacterial Activity

The antibacterial activity was carried out as previously reported by Jahangirian *et al.* [12] with some slight modifications. Antibacterial activities of these oils were evaluated using well diffusion method on Mueller-Hinton agar (MHA). The inhibition zones were reported in millimeter (mm). *Pseudomonas aeruginosa* (ATCC 29953) and *Staphylococcus aureus* (ATCC 25923) were used as references for the antibacterial assay. In a nutshell, MHA agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter=6mm) were filled with 100 mg, 50 mg, 25 mg and 12.5 mg of the oil, respectively and the plate were incubated at 37 °C for 18-24 h. After the incubation period, the diameter of the growth inhibition zones was measured. About 0.1 mL overnight culture of each test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) were transferred into 5 mL of nutrient broth and incubated for 3 h at 37 °C and then compared with 0.5 McFarland standard to give the population of test organisms as 10⁶ colony forming unit (cfu mL). Turbidity of the bacterial suspension was measured at 600 nm. Hexane and ethanol were used as negative standard while chloramphenicol was used as positive standard. All tests were performed in triplicate[3].

3. Results and Discussions

3.1. Extraction and Phytochemical Screening of the kernel oil

The yield of oil determined were 13.24%, 9.03% and 8.44% for sample A, B and C, respectively for ethanol; and 18.03%, 17.67% and 17.21% for sample A, B and C, respectively for hexane. Hexane gave higher yield of oil than ethanol after the Soxhlet Extraction. The results of the yield of these oils were in agreement with the report of Kittiphoom and Sutasinee[5]. Furthermore, the various phytochemical screening performed shows tannin, quinone, flavonoid, terpenoid and phenol present and whereas saponin and steroid were absent in our hands and at the given condition. Falusi *et al.* [3]. rather found the phytochemical composition of both

ripe and unripe mango peels extracts as tannins, saponins, flavonoids, terpenoids, alkaloids and phenolic compounds present while glycosides was not detected in the extracts. Once again, the different phytochemicals are known to perform different biological activities in humans and animals. These compounds can be harnessed for industrial and pharmaceutical utilization[1, 13, 14].

3.2. Antibacterial Results

The various inhibition zones of the antibacterial activity of all the samples at different concentration against the test organisms are as presented in Table 1.

Table 1- Inhibition zone of the antibacterial activity of control, A (hexane-oil), A (ethanol-oil), B (hexane-oil), B (ethanol-oil), C (hexane-oil) and C (ethanol-oil) at different concentrations against the test organisms

Sample	Test Organisms	Growth inhibition (mm) of the test organisms at different concentration of the Oils			
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL
Control (Positive)	<i>S. aureus</i>	15.33±0.58	11.33±0.58	9.67±0.58	5.67±0.58
	<i>P. aeruginosa</i>	16.33±0.58	12.33±0.58	10.67±0.58	7.33±0.58
A (Hexane-oil)	<i>S. aureus</i>	-	-	-	-
	<i>P. aeruginosa</i>	-	-	-	-
A (Ethanol-oil)	<i>S. aureus</i>	12.67±0.58	10.00±0.58	7.33±0.58	5.67±0.58
	<i>P. aeruginosa</i>	11.67±1.53	10.00±1.00	7.67±0.58	6.33±0.58
B (Hexane- oil)	<i>S. aureus</i>	-	-	-	-
	<i>P. aeruginosa</i>	6.67±0.58	5.33±0.58	4.33±0.58	4.00±1.00
B (Ethanol- oil)	<i>S. aureus</i>	7.33±1.53	6.67±0.58	6.33±2.31	4.33±0.58
	<i>P. aeruginosa</i>	8.00±1.00	6.67±0.58	5.67±0.58	4.67±0.58
C (Hexane- oil)	<i>S. aureus</i>	-	-	-	-
	<i>P. aeruginosa</i>	7.67±1.00	5.00±1.00	3.67±0.58	0.00±0.00
C (Ethanol-oil)	<i>S. aureus</i>	9.67±1.00	8.33±0.58	6.67±0.58	5.67±0.58
	<i>P. aeruginosa</i>	9.00±1.00	7.00±1.00	5.67±1.53	4.67±1.52

S. aureus – *Staphylococcus aureus* ATTC-25923 and *P. aeruginosa*- *Pseudomonas aeruginosa* ATTC- 29925
 - = No Zone of Inhibition, Values expressed as mean ± SD, n = 3

3.3. Antimicrobial results

The results obtained demonstrated inhibitory action against the bacteria by the MKOs at different concentrations. For hexane-oil of sample A (local mango), there were no inhibition activity against both *S. aureus* and *P. aeruginosa*. For hexane-oil of sample B (Julie mango), there were no inhibition against *S. aureus* also; but for *P. aeruginosa*, it

showed zone of inhibition with 100 mg/mL providing the highest value of 6.67±0.58 mm. Similarly, the inhibition against *P. aeruginosa* for 100 mg/mL of the hexane-oil of sample C (John mango) was observed to be 7.67±01.00 mm. Therefore, no bioactivity was observed against *S. aureus* at all the concentration for the three different mango kernel hexane-oils. On the other hand the ethanol-oil of

sample A, B and C all showed zone of inhibition for both *S. aureus* and *P. aeruginosa* at 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL. It can be seen that being polar, the ethanol was able to extract right composition/ requisite amount of phytochemicals than the hexane; hence the ethanol oil extracts were more bioactive than those of hexane. Thus, for both test organisms (*S. aureus* and *P. aeruginosa*), the highest zone of inhibitions were obtained with sample A (ethanol - oil) at 100 mg/mL as 12.67 ± 0.58 mm and 11.67 ± 1.53 mm, respectively. Again Julie mango kernel oil (of sample B) produced least zone of inhibitions at the highest concentration, 100 mg/mL of 7.33 ± 1.53 mm and 8.00 ± 1.00 mm for *S. aureus* and *P. aeruginosa*, respectively. The results showed that the zone of inhibitions formed with these oils were indeed comparative as far as *S. aureus* and *P. aeruginosa* are concerned. The antibacterial activity of these MKOs is attributed to the presence of phytochemicals (such as phenols) in the MKOs which are known to cause damage to cell membrane, causing leakage of cellular materials and ultimately the death of the microorganism[15]. In general, the zone of inhibition is obviously seen to increase with increase in the concentration of the MKOs in the wells. Other works have also reported that the specific bioactive component in mango kernel oil is phenol, 2,4-bis (1,1-dimethylethyl) with concentration of 0.92% [2, 16].

Though chloramphenicol (positive control) inhibition zones are marginally higher than those of the MKOs (especially the ethanol-oils), at least these MKOs have potential here as antibacterial agent. Contrary to our results, Kaur *et al.*, [1] reported antibacterial activity of crude methanolic extract of single and mixed mango seed kernel as having higher activity against methicillin resistant *S. aureus* and *E. coli* than the chloramphenicol at 100 mg/mL. Well and importantly, our findings agree with the following previous research in terms of our ethanol-oil samples producing slightly lesser inhibition as the positive control. The results of zone of inhibition for the ethanol extract of Malaysian mango kernel against bacterial strains showed highest inhibition zone for *Escherichia coli* with Waterlily extract as 18

mm, followed by Shakran extract (17 mm) and Lemak extract (12 mm). Tetracycline (positive control) gave an inhibition zone of 24 mm with respect to *E. coli*. Similar pattern was shown by *Staphylococcus aureus* with Waterlily extract giving the highest inhibition zone (21 mm), followed by Shakran extract with 19 mm and Lemak extract with 17 mm. Tetracycline (positive control) inhibits up to 25 mm in diameter with respect to *S. aureus*. Inhibition zone for *Bacillus subtilis* was high for Waterlily extract with 18 mm and 15 mm each for Lemak extract and Shakran extract. Tetracycline gave an exceptional inhibition zone for *B. subtilis* with 28 mm. Inhibition zone of *Pseudomonas aeruginosa* was high for both Waterlily extract and Shakran extract with 14 mm each but slightly low for Lemak extract (13 mm). Tetracycline has zone of inhibition of 21 mm (higher too) [1]. Similarly Sen and Batra reported that alcoholic extract of *M. azedarach* showed maximum zone of inhibition and minimum inhibitory concentration against all the microorganisms better than petroleum ether and aqueous extracts [17]. Joshua and Takudzwa also showed that the stem-bark extracts of *M. indica* have antimicrobial activity against *S. aureus*. Methanol extracts showed the highest inhibition zone, followed by ethyl acetate, water and hexane extracts. The antibacterial activities of different extracts were found to be concentration dependent [3, 18].

4. Conclusion

Although substantially underutilized, mango kernels can no longer be regarded as waste. Hence this work has buttressed again antibacterial characterization of mango kernel oils extracted by soxhlet means using hexane and ethanol. Firstly, the yield of the MKOs determined were 13.24%, 9.03% and 8.44% for sample A, B and C, respectively for ethanol; and 18.03%, 17.67% and 17.21% for sample A, B and C, respectively for hexane. Hexane gave higher yield of oil than ethanol. Useful phytochemicals such as phenol, quinone, saponin and terpenoid were detected. The MKO extracted with ethanol showed better antibacterial activities against both Gram positive and Gram negative

bacteria than those of hexane. The local MKO showed best antibacterial activity. Therefore, these MKOs can be used as bacteriostatic and antibacterial agent as the case may be. Thus, these plants can be used for further studies to find more about their pharmacological benefits and their potential against fighting various ailments and diseases.

5. Acknowledgements

We thank the Department of Chemistry and Biology, Benue State University - Nigeria for admitting us into their laboratories for the purpose of this research.

6. Conflict of Interest

The authors declare no conflict of interest

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