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Assessment of Biologically Active Components and Nutritional Contents of Seed Kernels of Opioro and Julie Mango Varieties: A Right Step for Drug Discovery

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Authors' contributions

This work was carried out in collaboration among all authors. Author KCN performed the experiment, conduct statistical analysis and wrote the first draft. Author OCU harmonized and proofread the manuscript. Author NCN proofread and supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at investigating the biologically active compounds and nutritional compositions in the seed kernels of Opioro and Julie varieties with medicinal qualities. The seed kernels were milled, dissolved in methanol and concentrated with rotary evaporator. The chemical compounds in these extracts were analyzed with Gas Chromatography and Mass Spectrometry while the mineral and proximate compounds were assessed with the methods of the Association of Official Analytical Chemists (AOAC). Both Opioro and Julie did not contain similar chemical compounds. A total of 22 chemical compounds were obtained from Opioro while only 13 compounds were discovered in Julie. Five bioactive compounds from Opioro namely cholest-5-en-3-ol, (3.alpha.) (31.63%), 2-Pyridinamine, N-(4,5-dihydro-5-methyl-2-thiazolyl)-3-methyl- (14.74%), 2-Methyl-7-phenylindole (6.96%), benzo[h]quinoline, 2,4-dimethyl (6.61%) and 7-Methyl -2-phenyl-1H-indole (4.27%) were abundant. In Julie, only Octadecanoic acid, 6-Octadecenoic acid, 1, 2, 3-Benzenetriol and n-Hexadecanoic acid were plentiful with the highest peak percentage of 39.76%, 16.05% and 5.41% respectively. The protein, fibre, moisture and ash contents from Opioro and Julie were not significantly different but, the carbohydrates varied (P<0.05) between 71.36±0.37%

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and 70.22±0.44% and fats varied (P<0.05) between 7.20±0.48% and 9.21±0.31% respectively. Phosphorus (1610.00±14.00 ppm), calcium (4595.00±7.07 ppm), and magnesium (1984.50±21.92 ppm) were high in Julie while zinc (18.197±0.25), copper (14.960±0.09), iron (319.615±2.28) and manganese (337.940±5.80) were more in Opioro than Julie. The seed kernels of Opioro and Julie have abundant bioactive compounds with medicinal properties. The essential mineral elements and rich proximate compounds in both extracts are potentially required and can be capable of improving the health and nutritional conditions of both humans and animals.

Keywords: Proximate; GC-MS; medicinal properties; seed kernels.

1. INTRODUCTION

Today, due to the need to improve the nutritional quality of our diets, medicinal plants and their products have long been sought for. Due to the availability of these plants, most traditional herbalist used different parts to treat recalcitrant diseases. The efficacy of these extracts depends on the phytoconstituents inherent in the plants [1]. Since the introduction of commercial drugs by pharmaceutical companies years ago, the desire to use plants as therapeutic agent dropped. The antibiotic resistance often experienced when taking these commercial drugs reinvigorated the interests of the users on medicinal plants [1]. However, medicinal plants are not entirely safe for consumption as previously believed as they are harmful and could cause several health-related defects [2,3]. The phytoconstituents in some medicinal plants are known to be lethal [4], therefore, investigation of the chemical constituents of each medicinal plant is necessary to avoid experiencing any harmful effects while consuming them or their products.

Mango (Mangifera indica L.) is one of the few plants with several economic benefits. It belongs to the family of Anacardiaceae majorly grown in humid regions [5]. The different parts of several varieties of mango have been studied for their nutritional compositions, mineral contents. medicinal properties and sources of biofuel [6]. The consumption of Julie for instance, reduces stultification caused by improper breakdown of foods while peels of Opioro prevent ageing and other skin diseases [7]. Since the need to develop novel therapeutic agents that can reduce antibiotic resistance [8] and also improve the quality of our diets, mango seed kernels are better alternative [9].

Seed kernels of mango varieties are generally discarded as waste; however, recent studies have shown that their seed kernel extracts have rich medicinal and nutritional benefits due to their phytoconstituents [10]. The therapeutic or nutritional benefits of the seed kernels of Opioro and Julie varieties to the best of our knowledge have not been reported by any scientific literature. Thus, there is need to determine their bioactive components in order to ascertain their efficacy. Therefore, this study being novel, was conducted determine the nutritional to composition, mineral contents as well as the bioactive compounds present in Opioro and Julie varieties

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The Opioro and Julie varieties of mango fruits were collected from Umuagbaghi village in Aba South Local Government of Abia State, Nigeria. The fruits were authenticated by a taxonomist, Mrs Chikodiri from the Department of Plant Sciences and Biotechnology, Abia State, University, Uturu. The varieties were assigned voucher numbers (ABSU/PSB/250) for Opioro and (ABSU/PSB/251) for Julie and deposited at the herbarium. The seed kernels were haul out from the thick endocarp after peeling off the fruit. The seed kernels were cut into pieces and air dried under shade for two weeks. The seed dried seed kernels were around into fine powder with MX-AC210 model, 170 mm x 236 mm x 268 mm Panasonic, Japan and stored in a refrigerator at 4°C.

2.2 Preparation of Extract

Briefly, the extracts of Opioro and Julie were prepared by dissolving 50 g of each milled sample in 500 ml of methanol using hydrodistillation method. After 12 hours of continuous stirring, the extracts were filtered through Whatman No. 1 filter paper and concentrated with rotary evaporator under reduced pressure at 45°C and kept in air tight containers at 4°C for further analysis.

2.3 Determination of Bioactive Compound by GC-MS

The phytoconstituents present in each Mangifera indica L. seed kernels were analyzed in the University of Lagos Research Laboratory with the method described by Kazeem et al. [11]. Clarus-500 Perkin-Elmer Gas Chromatograph coupled with a mass detector was used for the analysis. Ten grams of each milled sample was soaked in 30 mL methanol and then filtered out with 2 g sodium sulphate. The resultant extract was concentrated to 1 mL by passing nitrogen into the solution, separates polar compounds and non-polar compounds in the extract. Clarus 500 GC used in the analysis utilized a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm x 0.25 nm ID x 1 µm df) and Helium gas partitioned the different compounds in the extract at a constant flow of 1ml/min. The Turbor gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software was used to detect 2 µl of each extract injected into it. The heater was allowed to operate at 110°C for 2 min during 36th min GC extraction process. The temperature of the injector required by the mass analyzer was then maintained at 250°C. In the operation of the Clarus 500 MS, the different parameters such as the Inlet line temperature: 200°C; Source temperature: 200°C were also standardized. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 min.

2.4 Proximate and Mineral Elements Analysis

Mineral elements (macro and micro), moisture, ash, crude fiber, protein, carbohydrate and crude lipid of the two varieties were determined according to the method described by Association of the Official Analytical Chemists [12].

3. RESULTS

3.1 Bioactive Compounds Obtained through GC-MS

A total of 22 bioactive compounds were obtained in Opioro, out of which 5 compounds were abundant, namely, cholest-5-en-3-ol, (3.alpha.) (31.63%), 2-Pyridinamine, N-(4,5-dihydro-5methyl-2-thiazolyl)-3-methyl- (14.74%), 2-Methyl-7-phenylindole (6.96%), benzo[h]quinoline, 2,4dimethyl (6.61%) and 7-Methyl -2-phenyl-1H- indole (4.27%). Thirteen bioactive components were identified from Julie. Out of the thirteen compounds, Octadecanoic acid (39.76%), 6-Octadecenoic acid (31.15%), 1, 2, 3-Benzenetriol (16.05%) and n-Hexadecanoic acid (5.41%) were majorly present (Table 1).

3.2 Proximate and Mineral Element Contents

The carbohydrates and fats between the two varieties were significantly different (P<0.05). The carbohydrate varied between 71.36±0.37% for Opioro and 70.22±0.44% for Julie. The fat contents in Opioro and Julie were 7.20±0.48% and 9.21±0.31% respectively. The moisture content (10.90±0.15 to 9.72±0.10%), protein content (5.60±0.085% and 6.03±0.12%), ash content (2.71±0.04 to 2.95±0.08%) and crude fibre content (2.18±0.02% and 1.89±0.01%) of Opioro and Julie respectively were not significant (p>0.05). A total of 5 macro-elements and 4 micro-elements were identified and quantified in Opioro and Julie extracts. The values of phosphorus (P), calcium (Ca) and magnesium (Mg) were significantly higher (P<0.05) in Julie than Opioro except potassium (K) and sodium (Na) while the microelements, manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) were appreciably higher (P<0.05) in Opioro than Julie. The result showed that Opioro and Julie contain P (1510.00±14.14 and 1610.00±14.00 ppm), Ca (3740.00±56.57and 4595.00±7.07 , mag Ma (1529.00±41.01and1984.50±21.92 ppm), Κ (7045.00±77.78 and 1535.00±21.21 ppm), Na (36.740±0.38 and 25.860±0.65 ppm), Mn (337.940±5.80 and 57.90±0.74 Fe ppm), 75.00±0.14 Cu (319.615±2.28 and ppm), (14.960±0.09 and 4.285±0.09 ppm), and Zn (18.197±0.25 and 7.665±0.49 ppm) respectively. The proximate and mineral elements were presented in Tables 3 and 4.

4. DISCUSSION

Gas Chromatography and Mass Spectrometry (GC-MS) is an analytical method used in the detection of different components of phytochemicals, fatty acids, esters and other biomolecules. The identification and classification of these compounds were based on the peak area, retention time and molecular weight [22]. The bioactive components, cholest-5-en-3-ol, (3.alpha.), 2-Pyridinamine, N-(4,5-dihydro-5methyl-2-thiazolyl)-3-methyl-. 2-Methvl-7phenylindole and benzo[h]quinoline,2,4-dimethyl were predominantly abundant in Opioro.

Benzo[h]quinoline, 2,4-dimethyl is a derivative of benzo[h]quinoline [15]. This derivative is responsible for inhibiting the carcinogenic and mutagenic effects of superoxides that develop from metabolic activity in a living system through tyrosine kinases topoisomerase 15 inhibition [15].

One of the derivatives of indole compounds, 2-Methyl-7-phenylindole, is responsible for analgesic, anti-inflammatory, antimicrobial and antioxidant activities [16]. Cholest-5-en-3-ol, (3.alpha.) is a group of steroids derived from most plant extracts useful against bacterial

Table 1. The bioactive compounds obtained from the GC-MS analysis of Opioro and Julie
varieties

Compound	Retention	Molecular	Molecular	Peak
	time (min)	formular	weight	area
			(g/mol)	(%)
Opioro variety				
Cyclotetrasiloxane, octamethyl-	3.231	C ₈ H ₂₄ O ₄ Si ₄	296.61	2.71
Tris(tert-butyldimethylsilyloxy)arsane	4.442	$C_{18}H_{45}AsO_3Si_3$	468.7	1.62
2-Hydrazino-4,6-dimethylpyrimidine	4.820	$C_6H_{10}N_4$	138.17	0.88
1,2-Bis(trimethylsilyl)benzene	5.331	$C_{12}H_{22}Si_2$	222.47	0.88
Silicic acid, diethyl bis(trimethylsilyl) ester	6.409	$C_{10}H_{28}O_4Si_3$	296.58	0.66
5H-dibenzo[a,d]cyclohepten-5-amine	9.897	$C_{15}H_{13}N$	207.27	0.82
2-(Acetoxymethyl)-3-	12.575	C ₁₇ H ₁₄ O ₄	282.29	0.69
(methoxycarbonyl)biphenylene				
7-Methyl -2-phenyl-1H-indole	14.785	$C_{11}H_{18}N_5O_{14}P_3$	537.21	4.27
Cyclotrisiloxane, hexamethyl-	16.052	$C_6H_{18}O_3Si_3$	222.46	0.74
Arsenous acid, tris(trimethylsilyl) ester	16.307	C ₉ H ₂₇ AsO ₃ Si ₃	342.49	1.75
Silicic acid, diethyl bis(trimethylsilyl) ester	16.430	$C_{10}H_{28}O_4Si_3$	296.58	3.83
N-Methyl-1-adamantaneacetamide	16.485		207.31	2.41
2-Pvridinamine, N-(4.5-dihvdro-5-methvl-2-	16.641	$C_{10}H_{13}N_{3}S$	207.3	14.74
thiazolvl)-3-methyl-		- 10 13 3 -		
Cvclotrisiloxane, hexamethyl-	17.352	CeH18O5Si4	282.54	0.94
1.1.1.3.5.5.5-Heptamethyltrisiloxane	18.918		221.5	1.30
Indole-2-one 2.3-dihvdro-N-hvdroxy-4-	19.651	$C_{14}H_{12}NO_2$	207.23	0.65
methoxy-3 3-dimethyl-		• 11: 13: 103		0.00
2-Ethylacridine	20 551		207 27	2 47
2-Methyl-7-phenylindole	20 751	CarHaoN	207 27	6.96
Pyridine 1236-tetrahydro-1-methyl-4-[4-	20.918		207.7	1 16
chlorophenyll-	20.010	01211140114	20111	1.10
Benzo[h]quinoline, 2,4-dimethyl-	21.096	$C_{15}H_{13}N$	207.27	6.61
Cholest-5-en-3-ol. (3.alpha.)-	21.529	$C_{27}H_{46}O$	386.7	31.63
Julie variety		21 10		
1.2.3-Benzenetriol	8.397	CeHeO2	126.11	16.05
n-Hexadecanoic acid	14.896	$C_{4a}H_{2a}O_{2a}$	256.42	5.41
9-Octadecenoic acid (Z)- methyl ester	16 052	$C_{10}H_{20}O_2$	296 4879	0.32
6-Octadecenoic acid	16 707	$C_{40}H_{24}O_{2}$	282 46	31 15
Octadecanoic acid	16 974	$C_{18} H_{34} C_2$	284 5	39.76
1 2-Benzisothiazole 3-(hexahydro-1H-	18 163	$C_{18} H_{36} O_2$	264.35	0.53
(12 Denzioutinazole, 3 (nexaligato fit))	10.105	0131116102020	204.00	0.00
Eicosanoic acid	18 /07		312 53	2 1 1
Z-Dontadovno	10.407	C_{20} C_{40}	208 38	∠ 0.22
Nono(2-othylboxyl) phthalato	10.795		200.30	0.22
viono(2-emymexy) primalate	19.700		210.34 2025	0.20
$0.10 \text{ Cycleorgeot } 24(22) \text{ an } 2 \text{ al} \qquad 4.4.4$	19.901		202.J	0.44
9, 19-Cycloergost-24(28)-en-3-01, 4,14-	20.029	$U_{32}\Pi_{52}U_{2}$	400.75	0.31
acetate,				
(3.beta.,4.alpha.,5.alpha.)-	00.007		220 54	0.40
(∠)-3-(Heptadec-10-en-1-yi)phenol	20.807		330.54	0.42
	21.462	$U_{18}H_{36}$	252.5	0.62

Compounds	Nature of	Medicinal properties	Reference
	compound		
<i>Opioro</i> variety	-		
Cyclotetrasiloxane, octamethyl-	Organosilicon	High lipophilicity	Gentry et al. [13].
Pyridine, 1,2,3,6-tetrahydro- 1-methyl-4-[4-chlorophenyl]-	Aromatic hydrocarbon	Antioxidant	Oni et al. [14]
Benzo[h]quinoline, 2,4- dimethyl-	Aromatic hydrocarbon	Anti-cancer, anti-inflammatory, anti-malaria, antibacterial	Yadav et al. [15].
2-Methyl-7-phenylindole	Indole	Anti-inflammatory, analgesic, antioxidant, antimicrobial	Kumar & Ritika, [16].
7-Methyl -2-phenyl-1H-indole	Indole	Anti-inflammatory, analgesic, antibacterial	Arora et al. [17]. Doğan et al. [18].
Cholest-5-en-3-ol, (3.alpha.)-	Alcohol	Antimicrobial	
Julie variety			
n-Hexadecanoic acid	Fatty acid	Anticancer, Antioxidant, antifungal, anti-inflammatory, hypocholesterolemic,	Ravi & Krishnan [19].
1,2,3-Benzenetriol	Alcohol	Antioxidant	Han et al. [20].
9-Octadecenoic acid (Z)-, methyl ester	Fatty acid ester	Anti-hypertensive, antioxidant and anti-depressant, ant- inflammatory, anti-microbial, acid inhibitor.	Rahman et al. [21]. Adegoke et al. [22]. Delbeke et al.[23].
6-Octadecenoic acid	Fatty acid	Anti-inflammatory, Antioxidant, antimicrobial, anticancer.	Adegoke et al. [22].
1-Octadecene	Unsaturated hydrocarbon	Antimicrobial, anticancer, antioxidant	Tonisi et al. [24]
Eicosanoic acid	Fatty acid	Anti-carcinogenic, antimicrobial	Tallima & Rashika [25].
Octadecanoic acid	Fatty acid	Anti-carcinogenic, anti- inflammatory	Valenzuela et al. [26]. Kris-Etherton et al. [27].

Table 2. The medicinal properties of bioactive compounds obtained in the GC-MS analysis of Opioro and Julie varieties

Table 3.The Proximate composition (%) of *Mangifera indica* L. seed kernel of *Opioro* and *Julie* varieties

Proximate composition	Opioro (%)	Julie (%)	
Protein	5.60 ± 0.08^{a}	6.03±0.12 ^a	
Moisture	10.90±0.15 ^ª	9.72±0.10 ^a	
Ash	2.71±0.04 ^a	2.95±0.08 ^a	
Fat	7.20±0.48 ^a	9.21±0.31 ^b	
Fibre	2.18±0.02 ^a	1.89±0.01 ^a	
Carbohydrate	71.36±0.37 ^a	70.22±0.44 ^b	

Data in the figure were analyzed with two-way ANOVA and then expressed as mean \pm SD, n = 3. The different letters in the same row have significant difference (p<0.05)

activity [18]. This cholesterol derivative is active against both Gram-positive and Gram-negative bacteria and are ideal in the production of antibiotics that are effective against pathogenic bacteria [28]. These biological compounds present in Opioro could be good therapeutic agent owing to their rich medicinal properties.

Julie is majorly composed of octadecanoic acid, 6-Octadecenoic acid, 1,2,3-Benzenetriol and n-

Hexadecanoic acid. n-Hexadecanoic acid is a plant derived fatty acids which produces cytotoxic effects against cancerous cells and prevents their further multiplication. This is usually done by the inhibition of DNA topoisomerase-1 [29]. Furthermore, it has shown a considerable activity against rectal cancerous cells [19]. 1,2,3-Benzenetriol is a natural occurring compound that inhibits the oxidative activity in human system thereby preventing the damage of human tissues. Octadecanoic acid also referred as stearic acid, is a straight chain fatty acid predominant in both plants and animals [26]. The consumption of diet with octadecanoic acid promotes increase in HDL and a reduction in LDL-c. Unlike other saturated fatty acids, the intake of these acids does not produce any thrombogenic, hemodynamic and cardiovascular



Fig. 1. GC-MS chromatogram of ethanolic extract of seed kernels of Opioro variety

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Fig. 2. GC-MS chromatogram of ethanolic extract of seed kernels of Julie variety

Table 4. The mineral composition (ppm) of Mangifera indica L. seed kernel of Opioro and Juli	e
varieties	

Mineral Elements	<i>Opioro</i> (ppm)	Julie (ppm)
Macro-elements		
Phosphorus	1510.00±14.14 ^b	1610.00±14.00 ^a
Calcium	3740.00±56.57 ^b	4595.00±7.07 ^a
Magnesium	1529.00±41.01 ^b	1984.50±21.92 ^ª
Potassium	7045.00±77.78 ^a	1535.00±21.21 ^b
Sodium	36.740±0.38 ^a	25.860±0.65 ^b
Micro-elements		
Manganese	337.940±5.80 ^a	57.90±0.74 ^b
Iron	319.615±2.28 ^ª	75.00±0.14 ^b
Copper	14.960±0.09 ^a	4.285±0.09 ^b
Zinc	18.197±0.25 ^ª	7.665±0.49 ^b

Values in the figure were analyzed with two-way ANOVA and then expressed as mean \pm SD, n = 3. The different letters in the same row have significant difference (P<0.05)

effect [27.30]. Serum markers of inflammation such as cytokines, selectins and bioproteins are prevented by ctadecanoic acid from exerting their activities. which subsequently prevents cardiovascular diseases [26]. 6-Octadecenoic acid, often called petroselinic acid is a fatty acid and an isomer of oleic acid. It is used in pharmaceutical industry for the manufacture of skin maintaining agent; also, very effective in eliminating microorganisms due to its antimicrobial activity [23]. From the GC-MS analysis, the bioactive components in Opioro extract were appreciably more than those obtained from Julie variety. This is an indication that Opioro variety might be a better option for the treatment of diabetes, malaria, carcinogenic, inflammatory, and diarrheal diseases.

Proximate composition between Opioro and Julie was not significantly different except the carbohydrate and fat contents. The carbohydrate content of mango seed kernels is known for their digestibility attributed to high tannin low composition. Tannins and their derivatives, gallotannins produce unhealthy effect when attached to diets, in order to reduce tannin content, boiling is required [31]. Due to the significant value of Opioro, tannins could be more in it than Julie. The mango seed kernels are rich in both polyunsaturated fatty acids and saturated fatty acids such as stearic and oleic acids. With the exception of few, most polyunsaturated fatty acids are responsible for auto-oxidation of most fatty foods which subsequently leads to spoilage and smelling [32]. The polyunsaturated fats of mango seed kernels are exception as they are quite stable and do not undergo auto-oxidation. From the study, the fat contents of Julie were significantly higher than Opioro. The high saturated fatty acids and stability of Julie variety obtained in this study is an indication that it could be a good preservative agent especially for other oils that easily undergo auto-oxidation and rancidity [33]. The preservative properties of Julie could be attributed to their high phenolic content [32]. The mineral analysis performed on mango seed kernels of both varieties showed that except Na, other macro-elements such as P, Ca, Mg, K were more than micro-elements (Zn, Cu, Fe and Mn). However, P, Ca and Mg, were considerably high in Julie while Zn, Fe, Mn and Cu were more in Opioro than Julie. The values of macro and micro-elements obtained in both varieties might suggest the availability of rich nutrients, capable of improving health through maintenance of

normal body metabolism; their shortages might result in serious health problems [34].

5. CONCLUSION

The seed kernels of Opioro and Julie have abundant bioactive compounds with medicinal properties. The essential mineral elements and rich proximate compounds in both extracts are potentially required and can be capable of improving the health and nutritional conditions of both humans and animals. With the rich chemical compounds discovered, more studies are required to ascertain the safety of the extracts in animal models and human cells.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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