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Extraction, Physicochemical Characteristics and Fatty Acids Profile of Kernel Oil from *Mangifera indica* L. Cultivated in Sudan

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Authors' contributions

All authors contributed equally to this work. Author IYE designed the study, performed the statistical analysis and edited the manuscript. Authors FSM and YMM performed the experimental, analyzed the data and wrote the first draft of the manuscript. Authors OAOI and HMA administered the experiments and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed to investigate the physicochemical properties and fatty acids composition of *Mangifera indica* L. seed kernel oil; in addition to investigating the effect of solvent type and extraction duration on extracts properties.
Study Design: Extraction of *Mangifera indica* L. seed kernel oil in different trials under the same conditions using two different solvents for different time of extraction, and determining their physicochemical properties and fatty acids constituents.
Place and Duration of Study: This study was conducted at the Department of Applied and Industrial chemistry International University of Africa (IUA), Khartoum, Sudan, between July and November 2019.
Methodology: The oil from *Mangifera indica* L. seed kernel was extracted using n-hexane and petroleum ether in a soxhlet apparatus for 4 and 7 h. the physicochemical properties of the

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extracted oils were determined using standard official methods. Fatty acid profile of n-hexane extract was identified by gas chromatography/mass spectrometer (GC/MS) after methylation.
Results: n-Hexane exhibits better extraction efficiency (11.40±0.66% for 7 h) than petroleum ether (10.80±0.44% for 7 h). The density and refractive index of the oil were 0.89±0.01 g/cm^3 and 1.46 ± 0.01 at 28ºC respectively. The physicochemical properties of n-Hexane and petroleum ether extracts were acid value (3.35±0.54 and 2.52±0.13 mg KOH/g oil), peroxide value (4.32±0.65 and 5.11±1.03 meq O_2/kg), saponification value (201.05±0.95 and 198.66±1.04 mg KOH/g oil), ester value (197.59±0.67 and 192.54±0.20 mg KOH/g oil) respectively. The statistical analysis of obtained data revealed no significant difference, at 95% confidence interval, between the standard deviation and the mean of two data sets of physicochemical properties of *Mangifera indica* L. seed kernel oils extracted with the two solvents used. GC/MS analysis revealed a total of 18 fatty acids were identified in which the majors are stearic acid (39.79%), oleic acid (36.77%), palmitic acid (10.34%), linoleic acid (6.02%) and eicosanoic acid (3.83%).
Conclusion: The results suggest that mango seed kernel contains stable oil which can be potentially extracted by n-hexane; however, the solvent type has no significant effect on the physicochemical properties of the extracted oil and has the potential usefulness to be used in soap industry.

Keywords: Mangifera indica L.; kernel oil; physicochemical properties; fatty acid; solvent extraction.

1. INTRODUCTION

Mangifera indica L., commonly called mango, belongs to the family *Anacardiaceae* [1]. The mango trees can reach a height of more than 35 -40 m, with a radius of 10 m. Its leaves are evergreen, flat, 15-35 cm long and 6-16 cm wide. Mango fruits ripen after 3-6 months of flowering. Ripe fruits have different sizes and colors depending on the variety [2]. Mango trees grow in the tropics and subtropics of Asia and Africa. India produces 44.14% of the world's mango production [3,4]. *Mangifera indica* L. extracts of bark, leaves, stems and unripe fruits have been conventionally used as antibiotics and in treatment of typhoid fever, dysentery, diarrhea, sore throat disease and digestive disorder [5,6]. Moreover mango seed oil contains a high level of antioxidants and free of charge radical scavenging chemical substances [7]. Mango kernel oil is rich in unsaturated fatty acids and phenolic compounds, making it used as nutritious oil and in the cosmetics industry [8]. Previous studies on the kernel of *Mangifera indica* varieties revealed high levels of saponification value ranging from 143.6 to 207 mg KOH/g oil [1,2,9]. The major fatty acids detected in *Mangifera indica* are stearic acid, oleic acid, linoleic and arachidonic acid [1,3,8]. Mango handling creates huge quantity of waste, where the peeling process and disposal of seeds bring about 45 % of the weight of the fruit as waste. Kernels take-up about 17 - 22% of the fruit [10]. Discharge of this waste material into environment may cause environmental risks which might further increase when exposed to climatic factors. Recent research has tended to utilize waste in production of useful materials from them [11]. Since the main components of mango seeds are starch, fat and protein [10], starch has been successfully produced from mango seed kernel [4] and biodegradable plastic, polyhydroxyalkanoate, was prepared using mango kernel as an alternative to glucose [12]. However, consumers consider the mango kernel as waste, so it is disposed of. Therefore, this study was aimed at investigating the physicochemical properties and fatty acids composition of *Mangifera indica L*. seed kernel oil; in addition to investigating the effect of solvent type and duration time on extracts properties.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Mangifera indica L. fruits of *Totapuri* mangoes cultivar were harvested from Abu-Jubaiha city, South Kordofan State, Sudan. The pulp was separated mechanically from the seeds. The seeds were manually cracked to obtain the kernels. The kernels were ground using a kitchen blender and passed through 200 microns sieve. The kernel powder was then sealed in a plastic container and stored in desiccators at room temperature for further work.

2.2 Extraction of Oil

Oil was extracted from the kernel using two different solvents (n-hexane and petroleum ether) for different times of extraction (4 and 7 h) in a Soxhlet apparatus. The extraction procedure

was conducted in triplicate for each solvent. 140 g of kernel powder was encapsulated in gauze of canvas and inserted into the soxhlet extractor each time and the oil was extracted using the mentioned solvents for duration of 4 h and 7 h. At the end of the period, the solvent was recovered by rotary evaporator and residual oil was oven dried at 75ºC for one hour. The extracted oil was then allowed to cool to room temperature in a desiccator before analysis. The percentage extraction yield of oil was calculated using equation (2.1).

$$\text{Percentage extraction yield of oil} = \frac{\text{mass of oil}}{\text{mass of sample}} \times 100\% \quad (2.1)$$

2.3 Physicochemical Characteristics of the Oil

The density and refractive index were determined according to the procedures described by (ASTM International) [13,14].

Peroxide value (PV) was measured by titration according to the American Oil Chemist' Society AOCS official method [15]. The sample was dissolved in acetic acid/isooctane solution and excess amount of potassium iodide was added, the liberated iodine was titrated against standard sodium thiosulphate solution. The PV was expressed in meq O_2/kg.

Saponification value was determined according to AOCS official method [16], two grams of the oil sample was treated with a known excess amount of alcoholic KOH, and the mixture was heated on a water bath for two minutes then the unreacted KOH was titrated with standardized hydrochloric acid using phenolphthalein as indicator. The SV was expressed in mg KOH/ g of oil using equation (2.2).

$$\text{Saponification value} = \frac{(X - Y) \times N \times 56.1}{W} \quad (2.2)$$

Where: X = blank titre value (ml); Y = Sample titrate value (ml); N = normality of HCl; 56.1 = the molecular weight of KOH; W = weight of sample (g).

Acid value determined using the procedures described by (AOAC) [17]. In a typical procedure, 2.0 g of sample was dissolved in aqueous ethanol solution (1:1) and the mixture was titrated against standard KOH solution using phenolphthalein as indicator. The acid value was calculated mathematically using equation (2.3).

$$\text{Acid value (mg KOH g}^{-1}) = \frac{V \times N \times 56.1}{W} \quad (2.3)$$

Where: V is the volume (ml) of standard KOH; N = normality of KOH; W = weight of oil used (g); the number 56.1 is the molecular weight of KOH.

Ester value was obtained by subtracting the acid value from the saponification value [17]. Ester value represents the number of milligrams of potassium hydroxide required to saponify the esters present in one gram of the oil.

2.4 Determination of Fatty acids by GC-MS Analysis

For analysis of the fatty acid composition of *Mangifera indica* L. seed kernel oil, the oil was converted into fatty acid methyl esters (FAMEs) then identified by gas chromatography/mass spectrometer. For conversion of mango oil into FAMEs, about two grams of the sample were treated with 7 ml of 0.5 M of 0.50 N methanolic NaOH solution, the mixture was heated for 3 min at 60ºC and left to stand overnight at room temperature, then extracted with 10 ml n-hexane. 5 µl from the n-hexane extract was diluted with 5 ml of diethyl ether. The solution was filtered through a syringe filter 0.45 µm and dried with 1g of anhydrous sodium sulphate. 1µl of the diluted sample was injected in the GC/MS instrument. GC/MS analysis was performed with GC-QP2010-Ultra Shimadzu, coupled with Shimadzu TQ8040 plus mass spectroscopy detector. Capillary column (Rtx-5ms - 30 m × 0.25 mm × 0.25 µm). The sample was injected by using a split mode, the split ratio was 1:50. Helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60ºC to 300ºC with a rate of 10ºC/min, the injection port temperature was 300ºC, the ion source temperature was 200ºC and the interface temperature was 250ºC. The sample was analyzed by using scan mode in the range of m/z 40-500 charge to ratio and the total run time was 27 minutes. Identification of components was achieved by comparing the spectral data obtained with those available in the National Institute of Standards and Technology (NIST) libraries.

2.5 Statistical Analysis

Oil extractions and all analyses were performed in triplicates using dry sample and the results were expressed as means ± standard deviation. The standard deviations and the means of the two data sets of the physicochemical properties

are compared using F test, equation (2.4), and Student's *t* test, equation (2.5), respectively [18]. Multiple comparisons of means were done by the LSD (least significance difference) test. 95% confidence interval was considered significant. Statistical analysis of the data was carried out using MS Excel (2007) - version 12.0.4518.1014.

$$F_{calculated} = \frac{s_1^2}{s_2^2} \quad (2.4)$$

$$t_{calculatd} = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 - n_2}} \quad (2.5)$$

3. RESULTS AND DISCUSSION

3.1 Optimization of Solvent Used for Extraction

Petroleum ether and n-Hexane were used to extract oil from the mango seed kernel in different trials under the same conditions. The extraction yield, as shown in Table 1, increased as the time of extraction increases from 4 to 7 hours; for n-hexane the yield percentage was 5.46±0.49% and 11.40±0.66% respectively and for petroleum ether it was 4.61±0.75% and 10.80 ±0.44% respectively. Similar reports of Nwaokobia, et al. [2] and Kemal, et al. [19], declared that the yield has been shown to be time and particle size dependent. n-Hexane solvent gives the best yield with duration time of extraction 7 h this result is in agreement with that presented by Sikdar, et al. [3], the ether extract is less than 25.57% reported in a previous study [20].

3.2 Physicochemical Properties

The obtained results presented in Table 2 showed that there is no significant difference in density and refractive index of *Mangifera indica* L. kernel oil extracted by n-hexane and petroleum ether. The density of mango kernel oil was between 0.89±0.01 g/cm^3; this value is within the range reported in previous studies [9,21]. The refractive index was found to be 1.46 ±0.01 at 28ºC for both n-hexane and petroleum ether extracts. This value is agreed with that obtained by Kemal, et al. [1], Nzikuo, et al. [21] and Nwaokobia, et al. [2] which lies within the range of some butter and edible oils like cocoa butter (1.455 to 1.458), cotton seed oil (1.458 to 1.466) and shea butter (1.463 to 1.468) [1].

Peroxide value is one of the most widely used test for oxidative rancidity in oils; it is a very useful parameter for appreciating the first stages of oxidative deterioration. The results showed that the peroxide values of *Mangifera indica* L. kernel oil (4.32±0.65 to 5.11±1.03 meq O_2/kg oil) are lower than the allowed value for crude vegetable oils.

Basically, the acid value is used to quantify the amount of acid (free fatty acids, acid phosphates or amino acids) present in a sample. For oils, it is a measure of the free fatty acid content. From Table 2 it is shown that both n-hexane and petroleum ether extract have low acid values, 3.35±0.54 and 2.52±0.13 mg KOH/g oil respectively. These values are less than the Codex standard value for virgin vegetable oils (4.0 mg KOH g^{-1} Oil) [22]. The acid value of both extracts agreed with that obtained by Kemal, et al. for Ethiopian *Mangifera indica* seed kernels (2.39 mg KOH/g) [19].

The saponification values (201.05±0.95 mg KOH/g for n-hexane extract and 198.66±1.04 95 mg KOH/g for petroleum ether extract) are significantly same. Hence, the saponification value of mango oil is not dependent on the extraction solvent used. A high saponification value may suggest use of the oil in the soap industry. Therefore, mango oil has a very high chance of being used for the manufacturing of soap. Both saponification values of the mango oil falls within the literature range [2,21,23].

The ester value is the number of mg of KOH required to saponify the esters present in 1 g of the sample, and is possible identify and differentiate the fats with this value. Ester value was high in hexane extract 197.59±0.67 mg KOH/g oil than petroleum ether extracts 192.54± 0.20 mg KOH/g oil. Both ester values fall within the literature range of ester values [2,21].

Table 1. Effect of solvent and duration time on extraction of *Mangifera indica* L. kernel oil

	n-Hexane		Petroleum ether	
Solvent volume (ml)	250	250	250	250
Sample used (g)	140	140	140	140
Duration time (h)	4	7	4	7
Extraction yield (%)*	5.46±0.49	11.40±0.66	4.61±0.75	10.80±0.44

**Values are means of triplicate ± standard deviations*

Table 2. Physicochemical properties of *Mangifera indica* seed kernels oil

Property	Hexane extract	Petroleum ether extract
Density (g/cm^3)	0.89 ± 0.01	0.89 ± 0.01
Refractive index	1.46 ± 0.01	1.46 ± 0.01
Peroxide value (meq O_2/kg)	4.32 ± 0.65	5.11 ± 1.03
Acid value (mg KOH/g)	3.35 ± 0.54	2.52 ± 0.13
Saponification value (mg KOH/g)	201.05 ± 0.95	198.66 ± 1.04
Ester value (mg KOH/g)	197.59 ± 0.67	192.54 ± 0.20

Values are means of triplicate ± standard deviations. (n = 3 and P = .05)

Table 3. Calculated F and student's t values

	Yield% (4h)	Yield% (7h)	D	RI	AV	PV	SP
$F_{calculated}$	2.35	2.26	1.47	2.05	18.47	1.53	1.20
$t_{calculated}$	1.65	0.95	0.23	0.28	2.51	1.40	2.43

Abbreviations: D = density, RI = refractive index, AV = acid value, PV = peroxide value and SP = Saponification value. Confidence interval 95%, n_1 = 3 and n_2 = 3

Table 4. Main fatty acids content of *Mangifera indica* L. kernel oil

Lipid numbers	Common (IUPAC) name	Formula	Ret. Time	Area %
Saturated fatty acids				
C16:0	Palmitic acid (hexadecanoic acid)	$C_{16}H_{32}O_2$	15.284	10.34
C17:0	Margaric acid (Heptadecanoic acid)	$C_{17}H_{34}O_2$	16.258	0.21
C18:0	Stearic acid (Octadecanoic Acid)	$C_{18}H_{36}O_2$	17.234	39.77
C20:0	Arachidic acid (Eicosanoic acid)	$C_{20}H_{40}O_2$	18.943	3.83
C22:0	Behenic acid (Docosanoic acid)	$C_{22}H_{44}O_2$	20.560	0.81
C24:0	Lignoceric acid (Tetracosanoic acid)	$C_{24}H_{48}O_2$	22.061	1.02
Monounsaturated fatty acids				
C18:1n-9	Oleic acid ((*Z*)-octadec-9-enoic acid)	$C_{18}H_{34}O_2$	17.011	36.77
C20:1n-11	Eicosenoic acid ((*Z*)-icos-11-enoic acid)	$C_{20}H_{38}O_2$	18.741	0.41
Polyunsaturated fatty acid				
C18:2n-9,12	Linoelaidic acid ((9*E*,12*E*)-octadeca-9,12-dienoic acid)	$C_{18}H_{32}O_2$	16.934	6.02

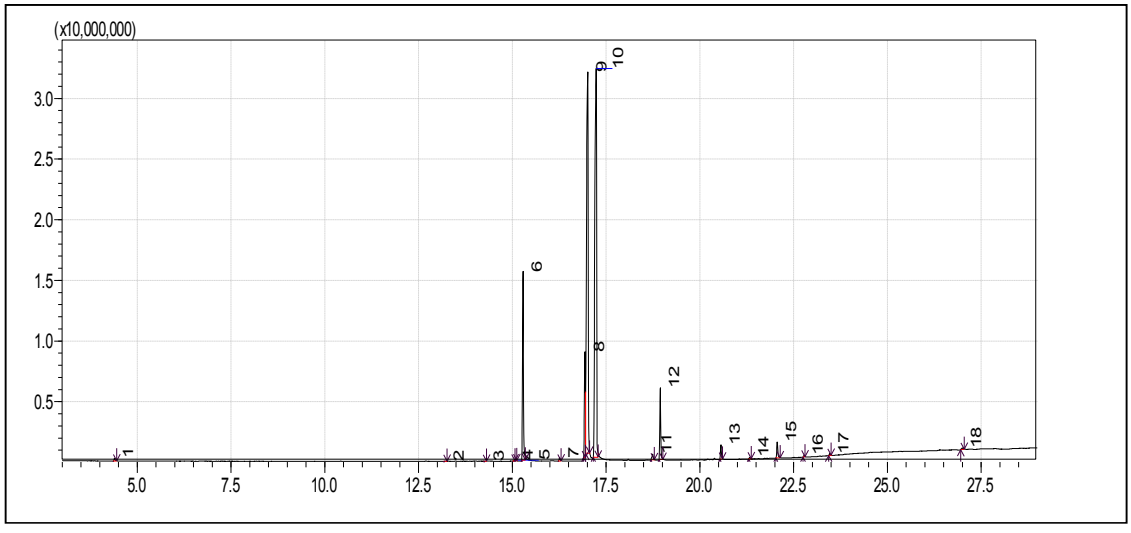

Fig. 1. GC Chromatogram of fatty acids of *Mangifera indica* oil

The results of statistical analysis of data were presented in Table 3. The results revealed that the values of $F_{calculated}$ for seven properties are less than F_{table} (= 19.0) [18], this indicated that the standard deviations of the two data sets are not significantly different from each other at 95 % confident interval. The comparison between the means of the two data sets was performed by student's t-test, equation (2.5) the values of $t_{calculated}$ are obviously less than the critical value for t_{table} (= 2.776) for 95% confidence and 4 degrees of freedom [18]. Therefore, there is more than a 5% chance that the two sets of results lie within experimental error of each other. It was concluded that the results are not significantly different at the chosen confidence level (95%).

3.3 GC/MS Analysis

Fatty acids profile of *Mangifera indica* L. kernel oil was determined using GC/MS the obtained results were shown in Table 4 and the chromatogram of Fig. 1.

The GC-MS data revealed the presence of 18 fatty acids. The major identified fatty acids were stearic acid (39.79%), oleic acid (36.77%), palmitic acid (10.34%), linoelaidic acid (6.02%) and eicosanoic acid (3.83%). These results were compared to the results obtained by Sikdar et al. [3], where it found that their stearic acid and oleic acid (43.32% and 42.25% respectively) were higher than our obtained results for the same acids. About 55.98% of the fatty acid contents of *Mangifera indica* L. kernel oil are saturated and the unsaturated fatty acids represent approximately about 43.2% of the total fatty acids.

4. CONCLUSION

In this study oil was effectively extracted from *Mangifera indica* L. seed kernel (which is generally generated as waste), using n-hexane and petroleum ether as extracting solvents. The extraction yield was found to be time dependent; n-hexane gave a higher yield than petroleum ether. It is necessary to carry out an essential future work to establish through analysis the constituents of the excess yield at 7hrs. However, the solvent type has no significant effect on physicochemical characteristics of the extracted oils. The results showed relatively low acid and peroxide values and high saponification and ester values. This indicates good stability of the oil and gives it potential usefulness in soap industry. The GC-MS analysis showed that *Mangifera indica* L. seed kernel oil has got 18 fatty acids, the predominates of them are stearic acid (39.79%), oleic acid (36.77%), palmitic acid (10.34%), linoelaidic acid (6.02%) and eicosanoic acid (3.83%).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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