



## Physico-Chemical Properties, Chemical Composition and Antimicrobial Activity of *Adonidia merrillii* Kernel Seed Oil

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### ABSTRACT

*Adonidia merrillii* is among the most widespread ornamental palms in the world today. However, the seed oil is not yet utilized like other seed oils, for industrial application. The aim of the study was to evaluate some selected physico-chemical parameters, chemical composition and investigate the antimicrobial potential of *Adonidia Merrillii* seed oil. *A. merrillii* seed oil was obtained by Soxhlet extraction method using n-hexane as solvent. The physicochemical parameters were determined using standard methods and the chemical composition was determined by Gas Chromatograph-Mass Spectrometry. The antibacterial activity of the oil was evaluated against some selected food-borne microorganisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Aspergillus niger* and *Penicillium notatum*) using agar well diffusion method. The percentage yield obtained was 7.67±0.09%. The result for the physicochemical analysis revealed; refractive index (1.47±0.25), free fatty acids (44.09±0.53%), peroxide value (8.01±0.65 meq/kg), acid value (88.16±0.52 mg KOH/g fat), iodine value (136.90±2.43 mg I<sub>2</sub>/100g) and saponification value (376.85±2.50 mg KOH/g fat). The GC-Mass Spectrometry showed that the major components of *A. merrillii* seed oil were fatty acids, particularly linoleic acid (34.62%), palmitic acid (16.99%) and oleic acid (12.62%). From the antibacterial activity result, the minimum inhibitory and bactericidal concentration (MIC and MBC) of the oil obtained was between 350 to 950 mg/mL and 950 mg/mL respectively. Hence, these results suggest that *A. merrillii* seed oil may perhaps be a significant spring of new oil in different industries and should be given more attention to ascertain its specific importance and application.

**Keywords:** *Adonidia merrillii*, Seed oil, GC-Mass spectrometry, Physico-chemical parameters, Antimicrobial activity.

### Introduction

*Adonidia merrillii*, syn. *Normanbya merrillii* (Becc.), or *Veitchia merrillii* (Becc.) H. E. Moore (Arecaceae) is generally identified to be *Adonidia* palm, Manila palm or Christmas palm.<sup>1</sup> Palms represent the third chief important plant family for human use. There is still an inadequate study on the industrial applications of some palm trees.<sup>2</sup> *A. merrillii* is an ornamental palm widely cultivated for its exotic appearance. Numerous wholesome products are produced from palms, which include the popular *Elaeis gunneensis*, *Cocos nucifera*, *Phoenix dactylifera*, and several palm lipids.<sup>3</sup> Fats and oils (lipids) which are naturally occurring in plants or animals are molecules that are soluble in organic nonpolar solvents. The molecules of lipid contain bulky portion of hydrocarbon and less polar functional groups, and this explains their solubility nature.<sup>4</sup> Fats exist as solid or semi-solid triglycerides at room temperature while oils exist as liquid triglycerides at room temperature. It is known that fat and oil make the major three classes of food after carbohydrates and are considered as essential nutrient in our diet.<sup>5</sup>

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Lipid intake is essential for human life. A healthy adult, needs approximately five grams a day of linolenic acid, an unsaturated fatty acid that cannot be synthesized within the body.<sup>6</sup> Among fats and oils from plant source, the ones from fruit or kernel seeds exist as a significant group. These are very good source of carotenoids, flavonoids, tocopherols, fatty acids, phytosterols, tocotrienols and some bioactive compounds which have beneficial effect relative to definite functions of the human body. As a result of their advantageous effects on the human skin, they remain very useful in cosmetics.<sup>7</sup>

The nutritional, fatty acids, tocopherols and carotenoids contents of the pulp and kernels of *A. merrillii* in Brazil have been reported.<sup>5,8</sup> The extracts of *A. merrillii* fruits, clearly revealed the presence of pyrogallol, vanillic acid, naringin, gallic acid, rutin, caffeic acid and syringic acid as the major flavonoid compounds and phenolic acid from the HPLC results.



**Figure 1:** (a) Mature fruits and (b) seeds of *A. merrillii*. Ali *et al.*<sup>9</sup>

Determination of antioxidant activities using DPPH radical scavenging, ABTS scavenging assay and NO scavenging activity revealed that the methanol extracts possess greater antioxidant activity compared to water and ethyl acetate extracts.<sup>9</sup> Ali *et al.*,<sup>9</sup> also reported that the composition in the extracts of the fruits are not toxic, and could be considered as potential therapeutics in the development of an anticancer drug. A good number of work has been done on seed oils by several researchers, mainly because seed oil is now gaining more demand for industrial applications and even for human consumption.<sup>10</sup> This work was triggered by the fact that reports on the kernel seed oil of *A. merrillii* is not available and has been scarce. Meanwhile, there is a need to search for oils from non-conventional sources because of the rise in the world's population and the increasing demand for oils to complement the ones available and to meet definite uses.

## Materials and Methods

### Reagents

All reagents used in this study were of analytical grade and solutions were prepared from distilled water. These are: n-Hexane, Methanol, Potassium iodide, Glacial acetic acid, Chloroform, Sodium thiosulphate, Diethylether, Ethanol, Phenolphthalein, Sodium hydroxide, Alcoholic potassium hydroxide, Hydrochloric acid, Wij's solution, Starch solution, Chloroform and Sulphuric acid.

### Collection of plant materials and preparation

The mature ripe fruits of *A. merrillii* were collected in the month of April 2021 from Ugbowo Campus, University of Benin, Nigeria. Plant samples were identified and authenticated by an Herbarium expert, Dr. O. Timothy, Department of Plant Biology and Biotechnology; University of Benin, where a voucher specimen number (UBH-A528) was deposited. The fruits pericarps were peeled to expose the seeds. The seeds were air-dried, and the endocarps were removed to obtain the kernels. These kernels were thereafter ground to fine powder.

### Oil extraction and recovery

Oil extraction was carried out according to the method described by Nzikou *et al.*<sup>11</sup> using Soxhlet extraction technique. The powdered seeds were packed into the extraction chamber and normal hexane poured into the round bottom flask of the Soxhlet extractor. The oil in the seeds was leached for some hours in each case until all the powdered seed was extracted. An exhaustive oil extraction was achieved when no oil was obtained anymore. After the extraction, the resulting mixture containing the oil was concentrated and the solvent was recovered by rotary evaporator and residual oil was oven dried at 60°C for one hour and thereafter exposed to air in order for the oil to be totally free from n-hexane. The seed oil was stored in a refrigerator and further utilized for analyses.

### Percentage yield

The oil recovered was weighed and recorded. The percentage yield was expressed as oil content (%) as follow:

$$\text{Oil content} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100 \quad (2.1)^{11}$$

### Physical and chemical properties

The Physicochemical properties of the oil for melting point, specific gravity, refractive index, density, moisture content, iodine value, acid value, free fatty acid, saponification value and peroxide value were determined as described by other researchers<sup>13-15</sup> with slight modifications.

### Identification of the constituents of the oil using Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis of the oil constituent was carried out as reported by Wara.<sup>16</sup> This technique involved subjecting the oil to methylation process to increase the volatility of the oil for auto injection into the Mass Spectrophotometer. The sample was analyzed using Agilent technologies 7890A GC and 5977B MSD with experimental conditions of GC-MS system were as follows: Hp 5-MS capillary standard non-polar column, dimensions: 30M, ID: 0.25 mm, Film

thickness: 0.25 µm. flow rate of mobile phase (carrier gas: He) was set at 1.0 mL/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1 µL. Samples dissolved in methanol were run fully scanned at a range of 40-650 m/z and using Nist mass spectral library search programme. This was carried out in Ahmadu Bello University, Zaria, Nigeria.

### Antimicrobial Activity of *A. merrillii* oil on bacterial and fungi isolates

All tested bacterial strains which are *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the University of Benin Teaching Hospital (UBTH), Benin City, while the fungi strains which are *Aspergillus niger* and *Penicillium notatum* were obtained from the Department of Microbiology Laboratory, University of Benin. The antimicrobial sensitivity assay was performed using agar well diffusion method as described by Cheesbrough<sup>17</sup> with slight modification. The different concentrations that were made from the oil for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are 350, 550, 750 and 950 mg/mL. All positive petri dish from the MIC were transferred for minimum bactericidal concentration (MBC).

### Statistical Analysis

Values obtained were analysed as mean and standard deviation of triplicates determination.

## Results and Discussion

### Physico-chemical Properties of *A. merrillii* Seed Oil.

The quality and probable use of the oil was analyzed by evaluating physicochemical properties such as density, refractive index, specific gravity, moisture content, melting point, peroxide, acid, iodine, and saponification values. The results are presented in Table 1. The percentage yield obtained was 7.67±0.09%, which is relatively low compared to many seed oils. This may be attributed to the choice of extraction method employed, type of solvent used, age of plant, season of harvest and even geographical region.<sup>18</sup> The oil is somewhat red in colour and semi-liquid at room temperature (Figure 2). The ratio of the speed of light at a definite wavelength relative to its speed in the lipid is termed refractive index (RI) of an oil. RI is extensively used in quality control to assess the level of purity of many materials.<sup>19</sup> The refractive index of the oil obtained was 1.47±0.25 which is in close range with the findings of conventional oils such as soybean (1.468)<sup>20</sup> and palm kernel (1.455)<sup>21</sup> and the standard range between 1.4677 - 1.4707 for refined-pomace, refined and virgin oils according to Codex Standards for lipids naturally occurring in vegetable/plant.<sup>22</sup>

**Table 1:** The physicochemical properties of *A. merrillii* seed kernel oil

Parameter	Obtained results
% oil yield (%)	7.67 ± 0.09
Colour	Red
Appearance	Semi-liquid
Refractive index	1.47 ± 0.25
Density (g/mL)	0.87 ± 0.52
Melting point (°C)	39.10 - 41.10
Moisture content (%)	4.71 ± 0.02
Saponification value (mg KOH/g fat)	376.85 ± 2.50
Free fatty acid (%)	44.09 ± 0.53
Iodine value (mg I <sub>2</sub> /100g)	136.90 ± 2.43
Acid value (mg KOH/g fat)	88.16 ± 0.52
Peroxide value (meq/kg fat)	8.01 ± 0.65

Values are means ± standard deviation of triplicate analysis



**Figure 2:** Concentrated *A. merrillii* Kernel Seed Oil

The density obtained was  $0.87 \pm 0.52$  g/mL, an indication that the oil is less dense relative to water and thus in addition to other factors, it would find application in the production of cream which will influence the spread and flow of the oil on the skin. Oils with the density of lower values are greatly appreciable to consumers.<sup>23</sup> Yahaya *et al.*,<sup>24</sup> established that specific gravity is a frequently used parameter with others in the assessment of oil purity.

The specific gravity of an oil is equivalent to its density. The melting point of the oil was found to be between the range of  $39.10$ - $41.10$ °C which is close to that of palm oil. Melting point measures the temperature at which the oil starts melting. Generally, melting point is used in the determination of purity of substances. This value serves as an indicator of the varieties of fatty acid in the triglyceride.<sup>14</sup> The moisture content was found to be  $4.71 \pm 0.02$ %; the greater the moisture content of an oil, the better the quality used for baking, food texturing, frying and as well as industrial manufacturing of detergents, oil paints, cosmetics and soaps.<sup>25</sup> The oil had a moisture value that is higher than the maximal limit (0.2%) for volatile substances at  $105$ °C in lipids<sup>26</sup> nevertheless, even insignificant moisture contents can still be threatening to products of lipids, because the existence of residual water stands as a catalyst of nearly all chemical reactions leading to degradation. As a result of this, moisture content indicates the level of some other quality parameters and can help to forecast consequent changes in the period of storage. The existence of high value of moisture content correspondingly leads to an increase in oxidative degradation.<sup>27</sup> Generally, acid value (AV) in oil indicates the quality of fatty acids that are present. From Table 1, the AV obtained was relatively high ( $88.16 \pm 0.52$  mg KOH/g fat) compared to many seed oils e.g. varieties of melon seed oil reported by Oyeleke *et al.*<sup>23</sup> However, the AV revealed that free fatty acids are present in the oil and as well accounts for the degree of hydrolysis by means of oxidation and lipolytic enzymes.<sup>28</sup> When acid value is low in an oil, it shows that it will be stable upon storage for a longer period of time, preventing peroxidation and rancidity. This might be ascribed to the existence of naturally occurring antioxidants for instance vitamins A and C as well as other likely phytochemicals (e.g. flavonoids). AV also serve as a pointer for an oil edibility and application in soap and paint manufacturing industries.<sup>29</sup> The high AV of the oil indicates that the oil might be unsuitable for cooking purpose (edibility), nevertheless, it can be useful for manufacturing of shampoos, paints, and liquid soaps.<sup>30</sup>

Saponification value (SV) measures oxidation upon storage, it also shows deterioration levels in oils. This was found to be  $376.85 \pm 2.50$  mg KOH/g fat which is significantly high; it is known that high saponification value in oils lead to an increase in the rate of volatility.<sup>29</sup> It also boost the value of the oil since it reveals that there are low molecular weight constituents in one gram of the oil which can produce greater combustion energy.<sup>31</sup> The high SV clearly revealed that the oil may find application in making oil-based ice-cream, shampoos, soaps, lather shaving creams and some other cosmetic products.<sup>32</sup> High saponification value is as a result of high percentage of lower fatty acids. Therefore, the high saponification value of the oil shows that high percentage of lower fatty acids are present, then, there would be greater molecules of glycerides in one gram of fat compared to when the acids have high molecular weight

(long-chain acids). Consequently, since three molecules of potassium hydroxide are needed by each molecule of glyceride for the process of saponification, fats having low molecular weight glycerides will similarly have greater saponification values.<sup>33</sup>

Free fatty acid (FFA) is the measure of the percentage weight of a certain fatty acid (e.g., percentage oleic acid).<sup>34</sup> Higher amounts of FFA are not desirable in crude vegetable oils since they cause great losses of the neutral oil in the course of refining. In crude lipid, free fatty acids evaluate the quantity of oil that would be lost all through the refining steps aimed to get rid of fatty acids.<sup>35</sup> High concentrations of FFAs particularly linoleic acids are unwanted in refined oils since they cause off-flavours and reduce oil shelf life.<sup>36</sup> The amount of free fatty acid in oil is a pointer to its overall importance. An extreme level of free fatty acids reduces oil smoke point and then will influence 'popping' of the oil when used in cooking. From the results of the oil obtained, the percentage of free fatty acid is  $44.09 \pm 0.53$ %; this is relatively high compared to other seed oils like castor and cotton seed oils as reported by Warra *et al.*<sup>12</sup> The high free fatty acid content shows that the oil would easily go rancid when not appropriately stored.<sup>38</sup>

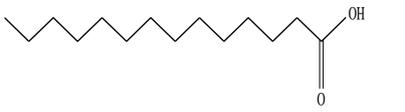
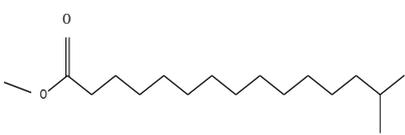
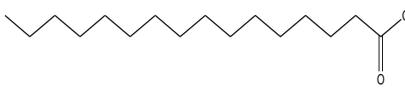
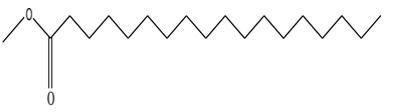
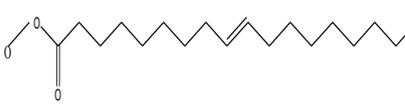
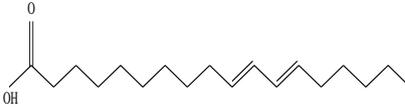
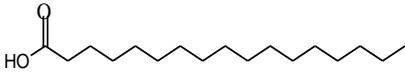
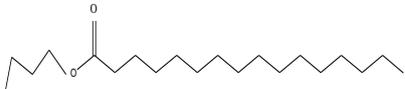
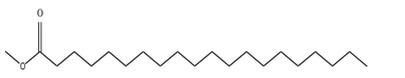
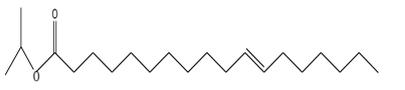
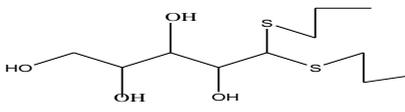
The most popular pointer of oxidation in lipids is peroxide value (PV). There is greater PV in unrefined oils, relative to oils refined. The peroxide value obtained is  $8.01 \pm 0.65$  meq/kg fat. Higher PV are suggestive of high levels of oxidative rancidity in the oil and likewise suggest low or zero antioxidant levels, however, some specific antioxidants may be used to decrease rancidity for instance propyl gallate and butyl hydroxyl anisole.<sup>10</sup> The World Health Organisation in 1994, stipulated an acceptable limit of peroxide concentration of not greater than 10 milliequivalent of oxygen/kg of oils, hence, the value obtained in this study is within the WHO tolerable range for PV and may be considered and further investigated for its suitability for consumption. The level of unsaturation in seed oils is relative to their iodine value (IV). Due to high level of unsaturation in seed oils, it makes them an important raw material in cosmetic industries.<sup>37</sup> For the oil, the IV obtained is  $136.90 \pm 2.43$  mg I<sub>2</sub>/100g. The IV of this oil is a reflection of its susceptibility to oxidation. It should be noted that oils classified as 'non-drying' have an IV which is less than 100 mg I<sub>2</sub>/100g. Similarly, Aremu *et al.*,<sup>39</sup> also reported that lower IV in oils correspondingly lead to lesser number of unsaturated chemical bonds, hence, lower vulnerability to oxidative rancidity. Consequently, 'non-drying oils' also known as liquid oils are unsuitable in the manufacturing of paint and ink as a result of their characteristic non-drying effects but then could be valuable in soaps manufacturing.<sup>40</sup> Oils having an IV that is equal to or greater than 130 mg I<sub>2</sub>/100g are good 'drying oils'. Therefore, the oil obtained can be classified as a drying oil and may be used as alkyl resins for the formulation of paint and as well as varnishes. Higher IV clearly indicates high proportion of unsaturated fatty acids in seed oils, and so, the quantity of iodine which will be absorbed by the unsaturated fatty acids would equally be higher.<sup>41</sup> Oils possessing such distinctive character may therefore be found suitable as raw materials in vegetable oil-based ice cream production.<sup>42</sup>

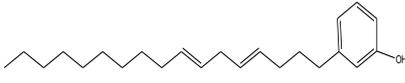
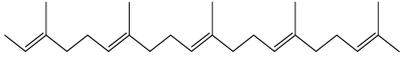
#### *Compounds identified in GC-MS Study of A. merrillii kernel seed oil*

The chemical compounds identified in the oil, with their trivial names, molecular formula, molecular mass, chemical structure, retention time and percentage composition are given in Table 2.

Nineteen (19) compounds were identified for *A. merrillii* seed kernel oil representing a total of exactly 100% of the oil (Table 2); among which are fatty acids, esters, hydrocarbons, aromatic compounds, and other useful organic compounds. Fatty acids constitute over 65% of the total constituents of the oil; linoleic acid had the highest percentage composition (34.62%), then, palmitic acid (16.99%), but least amount of Myristic acid (0.30%). Linoleic acid (34.62%), palmitic acid (16.99%), and stearic acid (3.09%) were found in the oil. The presence of palmitic acid and stearic acid made this oil fit for the manufacturing of detergents, soaps, cosmetics, shaving creams, shampoos, and pharmaceuticals. From research, linoleic acid possesses acne reductive, skin-lightening, moisture retention and anti-inflammatory effects when topically applied on the skin and for these reasons, it is becoming more popular in cosmetic industry.<sup>43-45</sup>

**Table 2:** Compounds Identified in GC-MS Study of *A. merrillii* Kernel Seed Oil

S/N	Compounds	Trivial/Other names	MF	MM (g/mol)	Chemical Structure	t <sub>R</sub> (min)	% Composition
1.	Tetradecanoic acid	Myristic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228.37		26.623	0.30
2.	Pentadecanoic acid, 14-methyl ester	Methyl, 14-methyl pentadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45		29.539	13.60
3.	n-Hexadecanoic acid	Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.40		30.128	16.99
4.	8,11-Octadecadienoic acid, methyl ester.	Methyl Octadeca 8,11-dienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.50		31.120	3.51
5.	9-Octadecenoic acid, methyl ester, (E)-	Oleic acid methyl ester (E)-	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.50		31.178	12.67
6.	Methyl Stearate	Methyl Octadecanoate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.50		31.349	2.77
7.	9,12-Octadecadienoic acid (Z,Z)	Linoleic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.45		31.600	34.62
8.	6-Octadecenoic acid	Petroselinic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.47		31.624	8.44
9.	Octadecanoic acid	Stearic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.48		31.711	3.09
10.	Hexadecanoic acid, butyl ester	Butyl Palmitate	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312.50		31.753	0.34
11.	Eicosanoic acid, methyl ester	Arachidic acid methyl ester	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	326.60		32.584	0.33
12.	n-Propyl, 11-Octadecenoate	—	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324.54		32.770	0.34
13.	1-Hexacosene	—	C <sub>26</sub> H <sub>52</sub>	364.70		32.807	0.18
14.	D-Arabinose, dipropyl mercaptal	—	C <sub>11</sub> H <sub>24</sub> O <sub>4</sub> S <sub>2</sub>	284.07		33.466	0.16

15.	Androstane 3,17-dione,12-hydroxyl-(5 $\alpha$ , 12 $\alpha$ )	–	C <sub>19</sub> H <sub>29</sub> O <sub>3</sub>	305.43	–	35.707	0.98	
16.	Behenyl Chloride Chlorodocosane	1-	C <sub>22</sub> H <sub>45</sub> Cl	345.04		34.926	0.20	
17.	(Z)-3-(Heptadec-10-en-1-yl) phenol	–	C <sub>23</sub> H <sub>38</sub> O	330.55		35.086	0.82	
18.	3-([4Z,7Z]-Heptadeca-4,7-diene-1-yl) phenol	–	C <sub>23</sub> H <sub>36</sub> O	328.53		35.495	0.39	
19	2,6,10,14,18-Peantamethyl-2,6,10,14,18-eicosapentaene	–	C <sub>25</sub> H <sub>42</sub>	342.60		36.230	0.27	
Total							100	

Molecular formula (MF), Molecular mass (MM), Retention time (tR)

Linoleic acid reduces the incidence of tumor and inhibits carcinogenesis.<sup>46</sup> Hence, this oil may be useful in food, cosmetic and pharmaceutical industry. Oleic acid (12.67%) in the oil is widely used to prepare lotions and oleates, as well as solvents used in pharmaceuticals. It is an excellent moisturizer and it is part of soaps and lotions formulation in several cosmetic industries due to its skin nourishing ability.<sup>47,16</sup> Myristic acid (0.30%) in the oil is a popular saturated fatty acid and in pair with Lauric acid has been described as saturated fatty acids in assessing the average serum cholesterol levels in humans.<sup>48</sup> Arachidic acid (0.33%), is also a saturated fatty acid and the extracts remain useful in manufacturing lubricants, detergents and photographic materials. Due to its surfactant characteristic, it also finds application in cosmetology.<sup>49</sup> Petroselinic acid (8.44%) found in the oil is a rare acid which contains double bonds at positions six and seven. It is a positional isomer of Oleic acid. It is already used in many formulations of cosmetics and serves as a moisturizer, anti-aging agent, and as a skin-irritation reducing agent in alpha-hydroxy ( $\alpha$ -OH) acid containing compositions.<sup>50-52</sup> In the GC-MS spectra of the FFAs in *A. merrillii* seed oil, few of them appeared in their corresponding methyl ester forms due to methylation of the oil before commencement of analysis. Methylation influenced the volatility rate

of the oil so that it will correspond to the Programme Temperature Volume (PTV) of the GC-MS injector. The methyl derivatives relate to the parent compounds only that the weight of the molecular ions are 14 units higher but have the same patterns of fragmentation.<sup>53</sup> Hence, the methyl esters of the free fatty acids relates to the free fatty acids except that the molecular weight of the methyl esters are higher by 14.<sup>49</sup> The esters found are: Butyl palmitate (0.3%), n-propyl, 11-Octadecanoate (0.34%), Methyl stearate (2.77%), Methyl, 14-methyl pentadecanoate (13.60%) and Methyl octadeca-8,11-dienoate (3.51%). Generally, esters have been known for their characteristic tastes and odours.<sup>54</sup> thus boosting the quality of this oil in soap and cosmetic industry. Hydrocarbons and other compounds found in the oil constitute smaller fraction. The oil can be considered as potential oil that may be used as lubricant.

#### Antimicrobial Activity of *A. merrillii* Seed Oil on Bacteria and Fungi isolates.

The determined minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of the oil are shown in Table 3 & 4 respectively.

**Table 3:** Minimum Inhibitory Concentration (MIC) of *A. merrillii* Seed Oil

Bacterial Isolates	Zones of Inhibition in mm			
	350 mg/L	550 mg/L	750 mg/L	950 mg/L
<i>Pseudomonas aeruginosa</i>	0	0	0	11
<i>Proteus vulgaris</i>	0	0	16	22
<i>Escherichia coli</i>	13	18	20	25
<i>Staphylococcus aureus</i>	0	0	0	18
<b>Fungi Isolates</b>				
<i>Penicillium notatum</i>	0	0	0	23
<i>Aspergillus niger</i>	0	0	0	18

**Table 4:** Minimum Bactericidal Concentration (MBC) of *A. merrilli* Seed Oil

Bacterial Isolates	Zones of Inhibition in mm			
	350 mg/L	550 mg/L	750 mg/L	950 mg/L
<i>Pseudomonas aeruginosa</i>	0	0	0	8
<i>Proteus vulgaris</i>	0	0	0	7
<i>Escherichia coli</i>	0	0	0	9
<i>Staphylococcus aureus</i>	0	0	0	8
<b>Fungi Isolates</b>				
<i>Penicillium notatum</i>	0	0	0	10
<i>Aspergillus niger</i>	0	0	0	12

The results of this study showed that the oil extract exhibited antimicrobial activity against the test isolates (both bacteria and fungi) at certain concentrations. Microbicidal activity, according to Bauer *et al.*,<sup>55</sup> can be classified into three: firstly 'resistant' if the zone of inhibition is less than 7 mm, secondly 'intermediate' if the zone of inhibition is within the range of 7-9 mm and lastly 'sensitive' if the zone of inhibition is equal to or greater than 10 mm. From Table 3; the higher the zone of inhibition, the stronger the inhibition effect of the oil extract on the tested microorganism isolate. *Escherichia coli*, *Penicillium notatum* & *Proteus vulgaris* showed highest zones of inhibition at a concentration of 950 mg/mL, hence, the oil extract will have a stronger inhibition effect on these three tested isolates relative to others. Similarly, from Table 4, the higher the zone of inhibition, the stronger the microbicidal effect of the oil extract on the tested microorganism isolate. The oil extract showed stronger microbicidal effect on *Aspergillus niger* relative to other tested isolates at 950 mg/mL concentration. The oil extract contains several fatty acids which are active as antimicrobial agents, therefore, antimicrobial activity of this oil could be linked to its fatty acid composition.<sup>56-59</sup>

### Conclusion

From this research work, there is no doubt that the main constituent of *A. merrillii* kernel seed oil is fatty acids. The relatively high acid value, peroxide value and percentage free fatty acid of this oil may be attributed to poor storage system after extraction and had high susceptibility to oxidative rancidity and deterioration. In addition to the antimicrobial activity against some skin and food-borne microbes, *A. merrillii* kernel seed oil may find its application in the chemical and pharmaceutical industries, rather than just being discarded as waste or allowing them to lay waste on the ground. However, it is therefore recommended that other solvents and methods of extraction should be exploited, the acute toxicity, phytochemical analysis and antioxidant activity of the oil should be thoroughly investigated for further utilization or uses. To the best of our knowledge, this is the first report of the physicochemical, antimicrobial, and GC-MS analysis of *A. merrillii* kernel seed oil.

### 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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