

Physico-chemical, Gas Chromatography-Mass Spectrometry (GC-MS) Analysis and Cold Saponification of Wild grapes (*Lannea microcarpa*) Seed Oil.

Comment [WA1]: The scientific name must be in italics and follow the rules of the International Code of Botanical Nomenclature, the authors name you can find on the "The Plant List" website. I suggest removing the popular name of the species

Comment [WA2]: Never put punctuation in the title.

Abstract

Aims: Physico-chemical, Gas Chromatography-Mass Spectrometry (GC-MS) and Cold saponification was carried out on Wild grape (*Lannea microcarpa*) seed oil with the aim of identifying the quality and quantity of the oil and its suitability in soap production.

Comment [WA3]: Put the scientific name first.

Study Design: Experimental and instrumental study was done to determine the physicochemical characteristics, fatty acids present in the seed oil and its suitability for soap production.

Place and Duration of Study: The study was conducted at the Biochemistry Laboratory, Kebbi State University of Science and Technology, Aliero, Nigeria from May to June, 2014.

Methodology: The hexane extract of the sample was obtained by complete extraction using Soxhlet extractor, physicochemical analysis was carried out. A gas chromatography coupled with mass spectroscopy detector (GCMS) system was used for the qualitative fatty acid determination. Simple cold method saponification was used in producing the soap.

Results: The powdered seed (50 g) yielded $59.21 \pm 0.01\%$ of the oil. Results from the physicochemical analysis showed the seed oil to be dark purple in colour and partially soluble in water with the acid, iodine, saponification and peroxide values at 16 ± 0.01 mg KOH/g, 121.6 ± 0.1 gI₂/100 g, 231.25 ± 0.02 mg KOH/g, 3.02 ± 0.01 meq H₂O₂ respectively. The relative density and refractive index of the oil are at 0.5983 ± 0.0001 (g/cm³) and 1.43 ± 0.01 respectively. Qualitative GC-MS revealed the following fatty acids; Decanoic acid, Palmitic acid, Stearic acid, Margaric acid, 1-octadecanoic acid, Oleic and Erucic acid. The soap produced from the seed oil has pH and Foam height, 10.18 ± 0.01 and 105.1 ± 0.1 (cm³) respectively.

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27 | **Conclusion:** The results showed the potential of the seed oil in soap and other cosmetic
28 | preparations.

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Comment [WA4]: This is not a conclusion, the author just repeated the results. Rewrite.

30 | **Keywords:** *Lannea microcarpa*, seed oil, physico-chemical, GC-MS, Saponification

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Comment [WA6]: Follow the International Code of Botanical Nomenclature.

32 | 1. Introduction

33 | *Lannea microcarpa* called Wild grape in English is also known as “Fààrúú” in Hausa Language
34 | [1]. It is found in derived savannah and drier forest mostly in Sudanian zones of West Africa.

35 | The fruits of the plant are edible and traded commercially [2]. Ethnobotanical investigations on
36 | local oilseed specie in Burkina Faso revealed that oil from *Lannea microcarpa* tree oil seed is
37 | frequently used for food, cosmetics and traditional medicine by local people [3].

Comment [WA7]: After quoting the scientific name the first time, one should abbreviate the genre in the remainder of the text: *L. microcarpa*

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38 | Physicochemical properties of biodiesel from African grapes (*Lannea microcarpa* Engl.&
39 | K.Krause) was assessed [4]. Physico-chemical properties of bio-diesel from wild grape seeds oil
40 | and petro-diesel blends as chemically stable, environmentally friendly and economically viable
41 | for use in compression ignition engine as a blend to partly replace the automotive gasoline oil
42 | was reported [5]. Evaluation of proximate composition of seeds and main physicochemical
43 | properties and thermal stability of oil extracted from *Lannea microcarpa* seeds were reported [6]
44 | This work appears to be the first report of physico-chemical, Gas Chromatography-Mass
45 | Spectrometry (GC-MS) analysis and soap production from wild grapes (Figure 1c.) seed oil.

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46 | The author should increase the introduction because the subject is of
47 | considerable relevance and can be better presented in the introduction.

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48 | 2. Materials and Methods

49 | 2.1. Sample Collection, Identification and Preparation

Comment [WA8]: All plant material used in studies, whether chemical, ethnobotanical or biological activities, must be collected and deposited in a herbarium for identification by a system. You must include herbarium number.

51 | The seeds of *Lannea microcarpa* were obtained directly from fruit of the plant in the month of
52 | May, 2014 at Yauri town, Kebbi state, Nigeria. They were dried and crushed into powder using
53 | mortar and pestle and stored in a plastic container prior to oil extraction.

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55 | 2.2. Oil Extraction Procedure

56 | The hexane extract was obtained by complete extraction using the Soxhlet extractor (GG-17,
57 | SHUNIU). The 50 g of each powdered sample was put into a porous thimble and placed in a
58 | Soxhlet extractor, using 150 cm³ of n-hexane (with boiling point of 40- 60 °C) as extracting
59 | solvent for 6 hours repeatedly until required quantity was obtained. The oil was obtained after
60 | evaporation using Water bath at 70°C to remove the excess solvent from the extracted oil. The
61 | oil was then stored in refrigerator prior to GC-MS analysis.

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63 | 2.3. Percentage Yield

64 | The oil which was recovered by complete distilling of most of the solvent on a heating mantle
65 | was transferred to a beaker. The beaker was then placed over water bath for complete
66 | evaporation of solvent for about 2 hours and volume of the oil was recorded and expressed as oil
67 | content (%) in line with literature report.

69 |
70 | Oil content (%) = $\frac{\text{Weight of the oil}}{\text{Weight of sample}} \times 100$

72 | 2.4. Determination of Colour

73 | The colour of the oil sample was determined by observation using several independent
74 | competent individuals. Oil colour was correlated using colour charts [7].

76 |

2.5. Determination of Relative Density

This was performed according to literature report [8]. The 10 mL of the oil was measured in a pre-weighed measuring cylinder. The weight of the cylinder and oil was measured; the weight of the oil was then obtained by subtracting the weight of the cylinder from the weight of the oil and cylinder. The density of the oil was obtained using equation below.

$$\text{Density of oil} = \frac{W_1 - W_0}{V_o}$$

Where W₁ = weight of empty measuring cylinder + oil.

W₀ = weight of measuring cylinder, V_o = volume of oil used.

2.6. Physico-Chemical Analysis

The physico- chemical analysis of the *Lannea microcarpa* L. Seed oil was carried out using the methods reported [9;10;11]

2.3. GC-MS Analysis

The analysis of the fatty acids in the *Lannea microcarpa* oil sample was done at National Institute of Chemical Technology (NARICT), Zaria, Nigeria, a Shimadzu QP2010 plus series gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (GCMS) system was used. The temperature programmed was set up from 70 °C to 280 °C. Helium gas was used as carrier gas. The injection volume was 2 µL with injection temperature of 250 °C and a column flow of 1.80 mL/min for the GC. For the mass spectroscopy ACQ mode scanner with scan range of 30-700 amu at the speed of 1478 was used. The mass spectra were compared with the NIST05 mass spectral library [12]. .

2.8. Preparation and Analysis *Lannea microcarpa* seed oil Soap

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103 | *Saponification Procedure:* As reported in literature [13], 200 grams of sodium hydroxide pellets
104 | was dissolved in 1000 cm³ volumetric flask and the volume made to the mark with distilled
105 | water. The required quantity of alkaline solution was mixed with *Lannea microcarpa* seed oil
106 | (ratio 1:1 v/v). The oil was warmed gently and poured into the beaker followed by the alkali
107 | solution to form an intimate mix and then stirred frequently for 7 minutes using stirring rod until
108 | reaction reached equilibrium. The saponification mixture was then poured into mould and
109 | allowed to dry (cure) for 24 hours.

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111 | 2.8. pH Determination

112 | The pH was determined using pH meter (350 JENWAY Model). A 5 g of the soap shavings were
113 | weighed and dissolved with distilled water in a 100 mL volumetric flask. The electrode of the
114 | pH meter was inserted into the solution of the soap and the pH reading was recorded.

116 | 2.9. Foam Ability Test

117 | A 2 g of the soap was added to a 500 cm³ measuring cylinder containing 100 cm³ of distilled
118 | water. The mixture was shaken vigorously so as to generate foams. After shaking for some time,
119 | the cylinder was allowed to stand for 10 minutes. The height of the foam in the solution was
120 | measured and recorded.



(a)

(b)

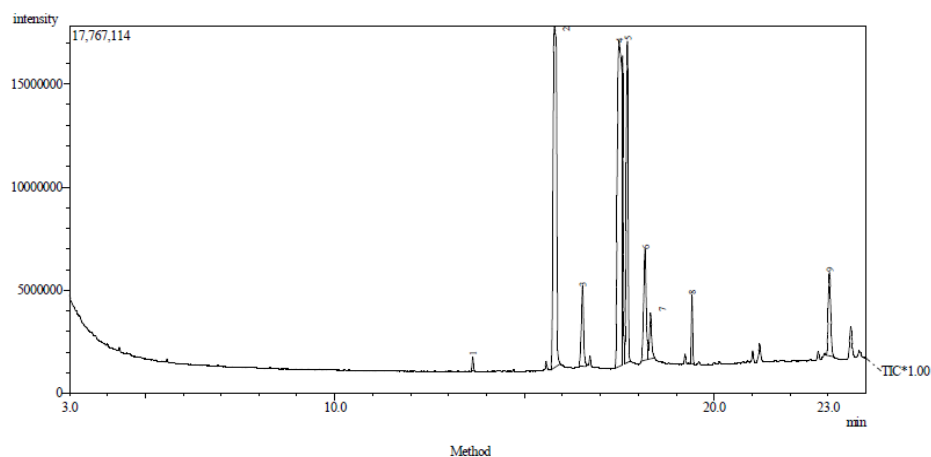


(c)

(d)

FIGURES 1.

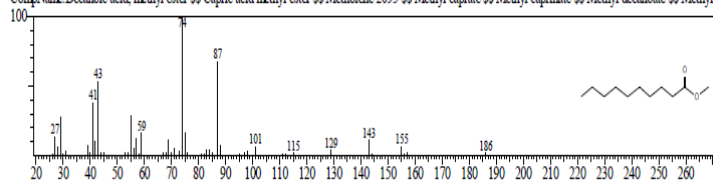
(a) *Lannea microcarpa* fruits (b) *Lannea microcarpa* deshelled seeds
(c) *Lannea microcarpa* seed oil (d) *Lannea microcarpa* oil fresh soap



132 (a)

133

Hit#1 Entry:14361 Library:NIST05a.LIB
 SI:92 Formula:C11H22O2 CAS:110-42-9 MolWeight:186 RefIndex:1.282
 CompName:Decanoic acid, methyl ester \$ Capric acid methyl ester \$ Metholene 2095 \$ Methyl caprate \$ Methyl caprinate \$ Methyl decanoate \$ Methyl-

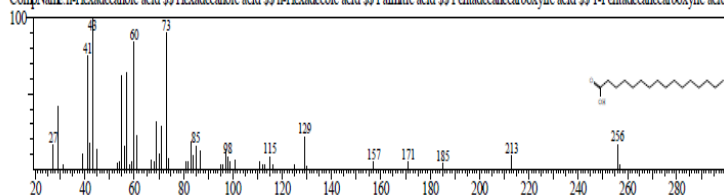


134

135 (b) i

136

Hit#1 Entry:74999 Library:NIST05.LIB
 SI:93 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RefIndex:1.968
 CompName:n-Hexadecanoic acid \$ Hexadecanoic acid \$ n-Hexadecanoic acid \$ Palmitic acid \$ Pentadecanecarboxylic acid \$ 1-Pentadecanecarboxylic acid

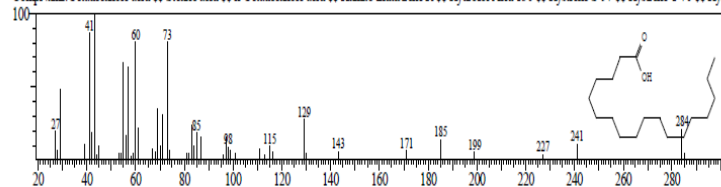


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138 (b) ii

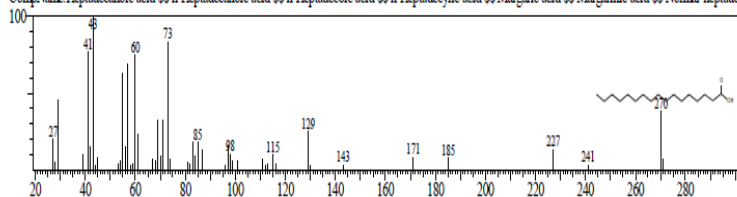
139

Hit# 3 Entry: 22977 Library: NIST05s.LIB
 SI: 90 Formula: C18H36O2 CAS: 57-11-4 MolWeight: 284 RetIndex: 2167
 CompName: Octadecanoic acid \$\$ Stearic acid \$\$ n-Octadecanoic acid \$\$ Hunko Industriene R. \$\$ Hydrofol Acid 150 \$\$ Hystrene S-97 \$\$ Hystrene T-70 \$\$ Hyr



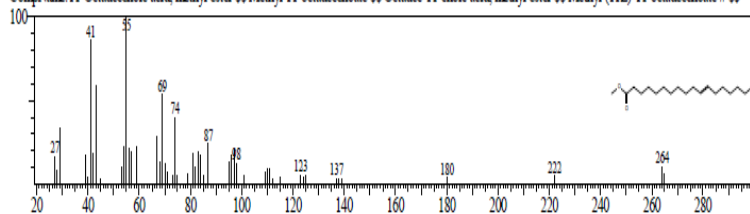
(b) iii

Hit# 5 Entry: 22212 Library: NIST05s.LIB
 SI: 89 Formula: C17H34O2 CAS: 506-12-7 MolWeight: 270 RetIndex: 2067
 CompName: Heptadecanoic acid \$\$ n-Heptadecanoic acid \$\$ n-Heptadecylic acid \$\$ Margaric acid \$\$ Margaric acid \$\$ Normal-heptade



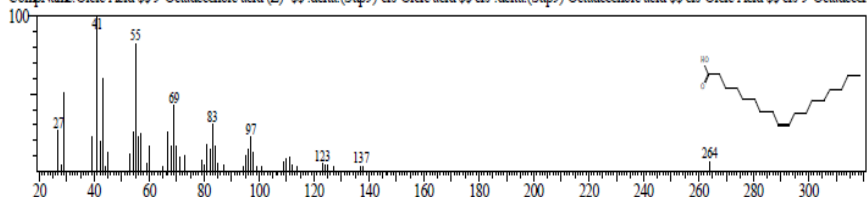
(b) iv

Hit# 1 Entry: 98778 Library: NIST05s.LIB
 SI: 93 Formula: C19H36O2 CAS: 52380-33-3 MolWeight: 296 RetIndex: 2085
 CompName: 11-Octadecenoic acid, methyl ester \$\$ Methyl 11-octadecenoate \$\$ Octadec-11-enoic acid, methyl ester \$\$ Methyl (11E)-11-octadecenoate # \$\$



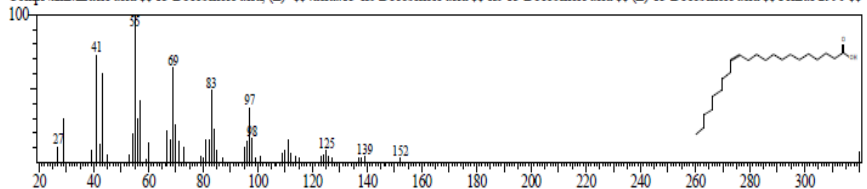
(b) v

Hit#1 Entry:22869 Library:NIST05s.LIB
 SE94 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:2175
 CompName Oleic Acid \$9-Octadecenoic acid (Z)- \$delta (Sup9)-cis-Oleic acid \$cis- delta (Sup9)-Octadecenoic acid \$cis-Oleic Acid \$cis-9-Octadec



(b)vi

Hit#2 Entry:121691 Library:NIST05s.LIB
 SE91 Formula:C22H42O2 CAS:112-86-7 MolWeight:338 RetIndex:2572
 CompName Erucic acid \$13-Docosenoic acid (Z)- \$delta 13-cis-Docosenoic acid \$cis-13-Docosenoic acid \$Prifrac 2990 \$



(b)vii

FIGURE 1.1 (a) Typical GC-MS total ionic chromatogram (TIC) of hexane extract of *Lannea microcarpa* L. seed oil. **(b) i- vii** GC-MS fragments of hexane extract of *Lannea microcarpa* L. seed oil.

Comment [WA9]: Dear author, please put images at a better resolution so the reader can observe.

Table 1: Physicochemical properties of *Lannea microcarpa* Seed Oil*

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Parameters	Values
Oil yield (%)	59.21±0.01
Colour of oil	Dark purple
Acid value mgKOH/g	016± 0.01
Iodine value gI ₂ /100g	121.6±0.1
Saponification value mg_KOH/g	231.25±0.02
Peroxide value meq H ₂ O ₂ .	3.02±0.01
Relative density (g/cm ³)	0.5983±0.0001
Refractive index	1.43±0.01

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Values are expressed as mean and ± standard deviation of triplicate determinations *

169

170 Table 2. Major fatty acids derived from oil of *Lannea microcarpa* seed.

S/N	Name of fatty acid		MF	MMRI	SI% to T.C.
1.	Decanoic acid.	C ₁₁ H ₂₂ O ₂	186	1282	92
2.	Palmitic acid.	C ₁₆ H ₃₂ O ₂	256	1968	93
3.	Stearic acid .	C ₁₈ H ₃₆ O ₂	284	2167	90
4.	Margaric acid	C ₁₇ H ₃₄ O ₂	270	2067	89
5.	11-octadecanoic acid	C ₁₉ H ₃₆ O ₂	296	2085	93
6.	Oleic	C ₁₈ H ₃₄ O ₂	282	2175	94
7.	Erucic acid	C ₂₂ H ₄₂ O ₂	338	2572	91

171 Note: S/N = Serial number, M.F.= Molecular formula, M.M. = Molecular weight, RI= Retention
172 index SI% = Similarity index, T.C. = Target compound.

173

174

175

176 Table 3:.Physicochemical characteristics of *Lannea microcarpa* seed oil soap*

Parameters	Values/Observation
pH	10.18±0.01
Foam height (cm ³)	105.1±0.1
Solubility in water	Slightly soluble
Color	Very dark purple

177 * Values are expressed as mean ± standard deviation of triplicate determinations

178

179

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181 **Discussion**

182 Physico-chemical, Gas Chromatography-Mass Spectrometry (GC-MS) and Cold saponification
183 | was carried out on Wild grape (*Lannea microcarpa*) Seed Oil and have yielded the following
184 results; oil yield was 59.21±0.01%, higher than 56.50 ± 0.10 (%), reported for thevetia seed oil
185 [14]. and 50.28±0.01 % reported for onion seed oil [15] recommended for cosmetic uses. The

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186 colour of the oil was dark purple. It was reported that many consumers preferred the bright color,
 187 transparent but close to its natural color of oil [16] From the results of the physicochemical
 188 analysis, acid value of 0.16 ± 0.01 mg KOH/g was obtained, higher than 0.35 ± 0.01 reported for
 189 canary melon seed oil [17]. lower than 22.37 ± 1.168 reported for neem seed oil [18]. Lower
 190 acid value makes oil suitable for soap production. Saponification value, 231.25 ± 0.02 mgKOH/g
 191 showed higher value than saponification values (mgKOH/g) 203.00 ± 0.00 and 218.52 ± 0.01
 192 reported for two varieties of sesame seed oils [19] lower than 246.60 mg KOH/g reported for
 193 *Elaeis guineensis* seed oil [20] range of recommended values suitable for soap making. Iodine
 194 value of 121.6 ± 0.1 gI₂/100g obtained is higher than 50.50 ± 8.023 , I₂/100g reported for *Jatropha*
 195 *curcas* L. seed oil [21] lower value than iodine value (mg/100g) of 152.3 , reported for wild
 196 *Corchorusolitorius* seed oil [22] recommended for cosmetics and medicinal purposes. Peroxide
 197 value of 3.02 ± 0.01 meq H₂O₂ was obtained. The peroxide value is used as an indicator of
 198 deterioration of oils. Lower values is an indicator of freshness and purity. Relative density
 199 (g/cm³) value was 0.5983 ± 0.0001 . Refractive index value was 1.43 ± 0.01 lower than 1.4750
 200 reported for Corn oil [23]. Higher than 1.412 reported for Palm Kernel Oil [24] (Olaniyi et al.,
 201 2014. Increase in refractive index values in the triacylglycerols or degree of unsaturation result in
 202 increase in chain length of fatty acids [25]. Qualitative GC-MS revealed the following fatty
 203 acids; Decanoic acid, Palmitic acid, Stearic acid, Margaric acid, 1-octadecanoic acid, Oleic and
 204 Erucic acid. The soap produced from the seed oil has pH and Foam height, 10.18 ± 0.01 and
 205 105.1 ± 0.1 (cm³) respectively, Very dark purple colour and slightly soluble in water. The results
 206 showed the potential of the seed oil in soap and other cosmetic preparations.

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207 The discussion should be improved, enhanced, and using authors
 208 who support their results.

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210 Conclusion

211 From the results of the physico-chemical, GC-MS analysis and the soaps produced from the
 212 hexane extract of *Lannea microcarpa* seed oil indicated its potential for soap and other
 213 cosmetic utilization.

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Comment [WA12]: References are not in accordance with the journal's standards. Refresh them carefully.

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