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

Abstract

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

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 (GC-MS) system was used for the qualitative fatty acid determination. Simple cold method saponification was used in producing the soap.

Results: The powdered seed (50g) yielded 59.21±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 mgKOH/g, 121.6±0.1 $gI_2/100g$, 231.25±0.02 mgKOH/g, 3.02±0.01 meq H_2O_2respectively. The relative density and refractive index of the oil are at 0.5983±0.0001 (g/cm^3) 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(cm^3) respectively.

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

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

1. Introduction

Lannea microcarpa called Wild grape in English is also known as "Fàárúú" in Hausa Language [1]. It is found in derived savannah and drier forest mostly in Sudanian zones of West Africa. The fruits of the plant are edible and traded commercially [2]. Ethnobotanical investigations on local oilseed specie in Burkina Faso revealed that oil from *Lannea microcarpa* tree oil seed is frequently used for food, cosmetics and traditional medicine by local people [3]. Physicochemical properties of biodiesel from African grapes (*L. microcarpa* Engl. & K. Krause) was assessed [4]. Physico-chemical properties of bio-diesel from wild grape seeds oil and petro-diesel blends as chemically stable, environmentally friendly and economically viable for use in compression ignition engine as a blend to partly replace the automotive gasoline oil was reported [5]. Evaluation of proximate composition of seeds and main physicochemical properties and thermal stability of oil extracted from *L. microcarpa* seeds were reported [6] This work appears to be the first report of physico-chemical, Gas Chromatography-Mass Spectrometry (GC-MS) analysis and soap production from wild grapes (Figure 1c.) seed oil.

2. Materials and Methods

2.1. Sample Collection, Identification and Preparation

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

2.2. Oil Extraction Procedure

The hexane extract was obtained by complete extraction using the Soxhlet extractor (GG-17, SHUNIU). The 50 g of each powdered sample was put into a porous thimbleand placed in a Soxhlet extractor, using 150 cm^3 of n-hexane (with boiling point of 40- 60°C) as extracting solvent for 6 hours repeatedly until required quantity was obtained. The oil was obtained after evaporation using Water bath at 70^{o}C to remove the excess solvent from the extracted oil. The oil was then stored in refrigerator prior to GC-MS analysis.

2.3. Percentage Yield

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

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

2.4. Determination of Colour

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

2.5. Determination Density of oil

This was performed according to literature report [8]. The 10ml 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.

$$\text{Density of oil} = \frac{W1 - W\circ}{V\circ}$$

Where W1 = weight of empty measuring cylinder + oil.
Wo = weight of measuring cylinder, Vo = volume of oil used.

2.6. Physico-Chemical Analysis

The physico- chemical analysis of the *L. 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 *L. 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 (GC-MS) 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^{o}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

Saponification Procedure: As reported in literature [13]. 200 grams of sodium hydroxide pellets was dissolved in 1000cm^3 volumetric flask and the volume made to the mark with distilled water. The required quantity of alkaline solution was mixed with *L. microcarpa* seed oil (ratio

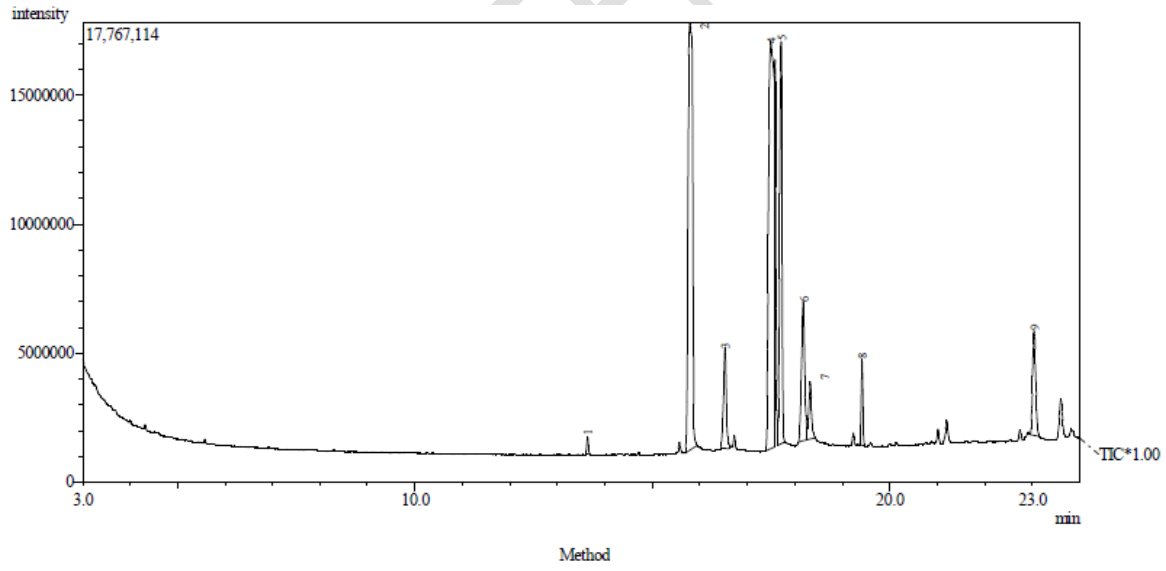
105 1:1 v/v). The oil was warmed gently and poured into the beaker followed by the alkali solution to
106 form an intimate mix and then stirred frequently for 7 minutes using stirring rod until reaction
107 reached equilibrium. The saponification mixture was then poured into mould and allowed to dry
108 (cure) for 24hours.
109
110 *2.8. pH Determination*
111 The pH was determined using pH meter (350 JENWAY Model). A 5g of the soap shavings were
112 weighed and dissolved with distilled water in a 100ml volumetric flask. The electrode of the pH
113 meter was inserted into the solution of the soap and the pH reading was recorded.
114
115 *2.9. Foam Ability Test*
116 A 2g of the soap was added to a 500 cm^3 measuring cylinder containing 100 cm^3 of distilled
117 water. The mixture was shaken vigorously so as to generate foams. After shaking for some time,
118 the cylinder was allowed to stand for 10 minutes. The height of the foam in the solution was
119 measured and recorded.


120
121 (a) (b)

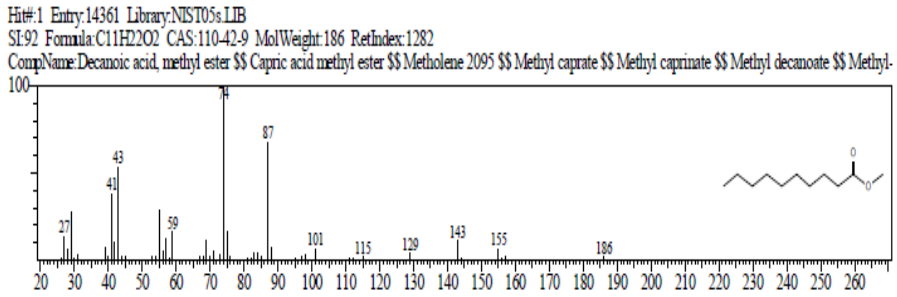
(c) (d)

FIGURES 1.
(a) *Lannea microcarpa* fruits**(b)** *Lannea microcarpa* deshelled seeds
(c) *Lannea microcarpa*seed oil (d)*Lannea microcarpa*oil fresh soap

Results and discussions



(a)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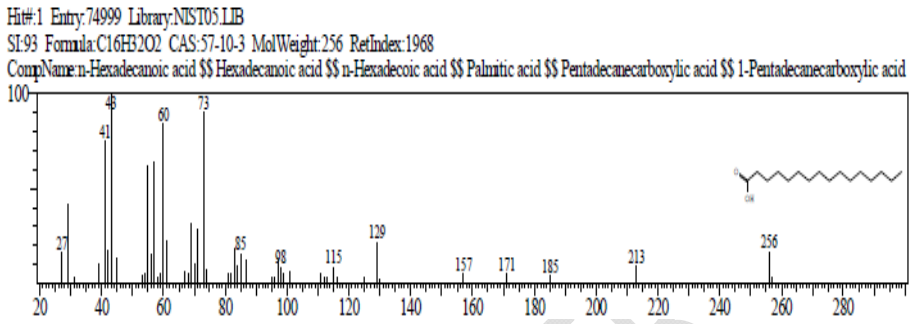
100
74
87
43
41
27
59
101
115
129
143
155
186
20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260

(b) i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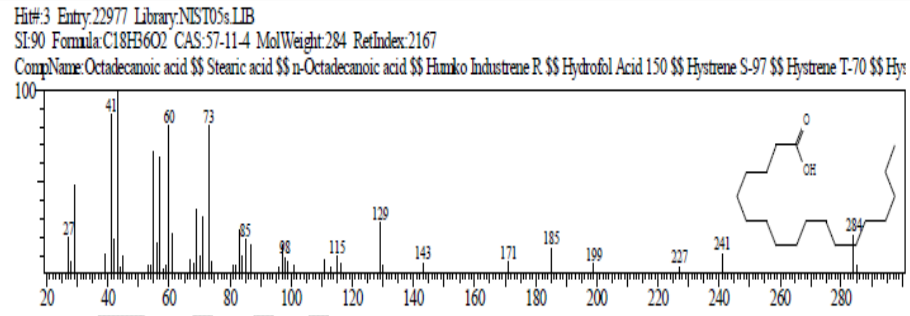
100
43
41
60
73
27
85
98
115
129
157
171
185
213
256
20 40 60 80 100 120 140 160 180 200 220 240 260 280

(b) ii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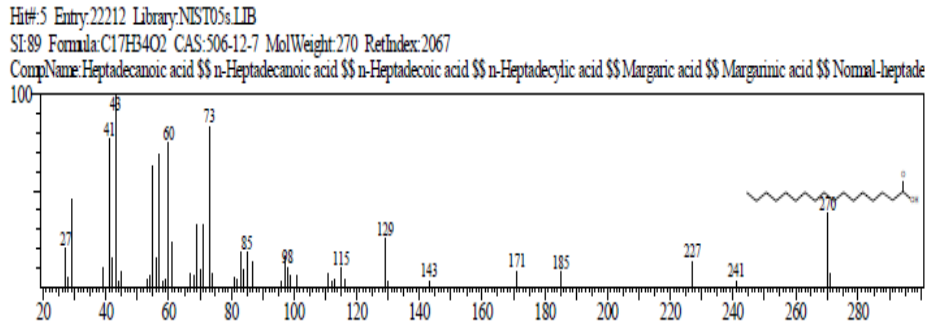
100
41
60
73
27
85
98
115
129
143
171
185
199
227
241
284
OH
20 40 60 80 100 120 140 160 180 200 220 240 260 280

(b) iii

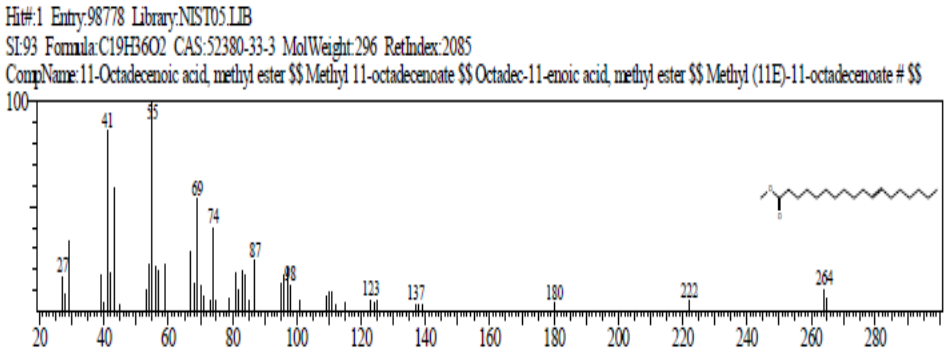


144

145 **(b) iv**

146

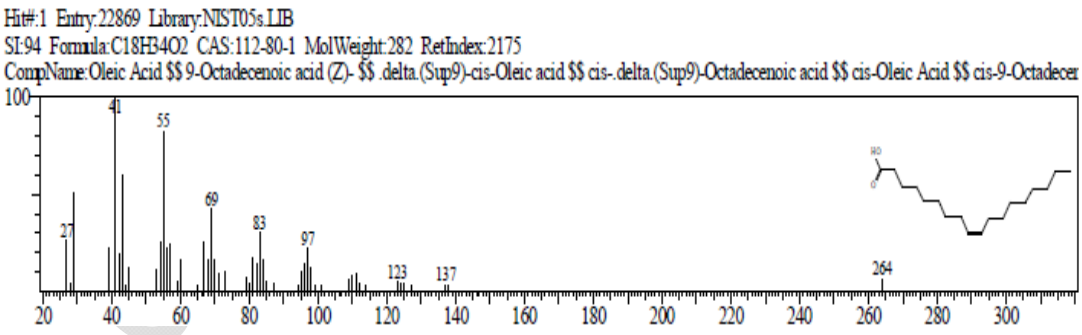
147



148

149 **(b)v**

150



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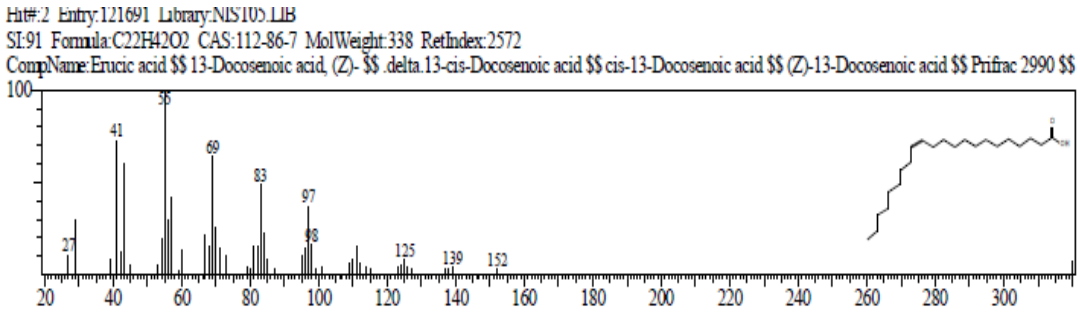
152 **(b)vi**



(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.

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

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 mgKOH/g	231.25±0.02
Peroxide value meq H_2O_2,	3.02±0.01
Relative density (g/cm^3)	0.5983±0.0001
Refractive index	1.43±0.01

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

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

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

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

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

Physico-chemical, Gas Chromatography-Mass Spectrometry (GC-MS) and Cold saponification was carried out on Wild grape (*L. microcarpa*) Seed Oil and have yielded the following results; oil yield was 59.21±0.01%, higher than 56.50 ± 0.10 (%), reported for thevetia seed oil [14]. and 50.28±0.01 % reported for onion seed oil [15] recommended for cosmetic uses. The colour of the oil was dark purple. It was reported that many consumers preferred the bright color, transparent but close to its natural color of oil [16] From the results of the physicochemical analysis, acid value of 016± 0.01 mgKOH/g was obtained, higher than 0.35±0.01 reported for canary melon seed oil [17], lower than 22.37 ± 1.168 reported for *Azadirachta indica* (neem) seed oil [18]. Lower acid value makes oil suitable for soap production. Saponification value, 231.25±0.02 mgKOH/g showed higher value than saponification values (mgKOH/g) 203.00±0.00 and 218.52± 0.01 reported for two varieties of sesame seed oils [19] lower than 246.60 mg KOH/g reported for *Elaeis guineensis* seed oil [20] range of recommended values suitable for soap making. Iodine value of 121.6±0.1gI_2/100g obtained is higher than 50.50 ± 8.023, I_2/100g reported for *Jatropha curcas* L. seed oil [21] lower value than iodine value (mg/100g) of 152.3, reported for wild *Corchorus olitorius* seed oil [22] recommended for cosmetics and medicinal purposes. Peroxide value of 3.02±0.01 meq H_2O_2 was obtained. The peroxide value is used as an indicator of deterioration of oils. Lower values signifies the indicaton of freshness and purity. Relative density (g/cm3) value was 0.5983±0.0001. Refractive index value was 1.43±0.01 lower than 1.4750 reported for Corn oil [23]. Higher value (1.412) was reported for Palm Kernel Oil [24] (Olaniyi et al., 2014. Increase in refractive index values in the triacylglycerols or degree of

unsaturation result in increase in chain length of fatty acids [25]. 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 (cm^3) respectively, Very dark purple colour and slightly soluble in water. The results showed the potential of the seed oil in soap and other cosmetic preparations.

Conclusion

From the results of the physico-chemical, GC-MS analysis and the soaps produced from the hexane extract of *Lannea microcarpa* seed oil indicated its potential for soap and other cosmetic utilization. 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(cm^3) respectively.

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