Extraction, Utilization, Characterization And Confirmation Of The Structure Of Gorli Oil From The Dry Seeds Of The Traditional Medicinal Plant Caloncoba Echinata In Sierra Leone

Lahai Koroma, T.B.R. Yormah, L.M. Kamara, G.M.T. Robert

Department of Basic and Environmental Sciences, Eastern Polytechnic, Kenema, Sierra Leone
Department of Chemistry, Fourah Bay College, University of Sierra Leone, Sierra Leone
Department of Chemistry, Njala University, Njala, Bo District, Sierra Leone
E-mail: *lahaikoroma2001@gmail.com*

ABSTRACT: A novel source of non-edible vegetable oil (LKO3) for soap making and cosmetic production has been extracted from the dry seeds of Caloncoba echinata plant in Sierra Leone. 31.5% of fat was obtained from the dry weight of seeds of the plant much higher than palm oil obtained from the Palm tree fruits. Unlike other unsaturated fatty acids, LK03 is solid at room temperature having a fairly high melting point (68.5 °C) and boiling point (247.5 °C) with Saponification Value (89.76) and Iodine value (99.00) supporting the use of the oil in soap making and cosmetic production. Antimicrobial sensitivity tests carried out on the Oil (LK03) using bacterial and fungal isolates showed very potent antibacterial activity on Escherichia coli and Staphylococcus aureus known to stimulate wound and skin infections and antifungal activity on Epidermophyton floccosum and Candida albicans known to cause dermatophytes. These results support the use the oil extracted from the seeds of Caloncoba echinata in healing of wounds and skin eruptions in Traditional Medicine. The structure of LK03 was confirmed by both NMR400 Bruker Bio.Spin GmbH (Germany), LCMS/ GCMS analysis (China), by McLafferty Rearrangement and identified as d-13 (2-cyclopentenyl) tridecanoic acid.

KEY WORDS: Antimicrobial activity, Soap production, Cosmetic production, saponification and iodine values, Epidermophyton floccosum, Candida albicans and dermatophytes.

1. Introduction

Caloncoba echinata plant has been identified as one of the plants that are rich in fats/oils **[1, 2].** The plant, apart from being a source of fats/oils (a source of a lot of essential oils) for cosmetic production; it is also widely used in traditional medicine in Sierra Leone. Traditionally the oil extracted from the seed is used in Sierra Leone as medicines for the treatment of leprosy, cutaneous and subcutaneous parasitic infections (dermal infections), as insecticides and arachnicides, hair dressing and skin eruptions due to viral diseases such as smallpox, chickenpox and measles **[1, 2 and 3].** In Liberia, the root bark and seeds are used as local medicine mostly for treating skin-diseases **[4]**. The pounded seeds are used sometimes with success against lice and mange in the Ivory Coast **[5].** Fatty acids have three distinct biological roles. On the one hand, they are energy-rich molecules, and on the other hand, they play an important role as structural components of membranes of bacterial and eukaryotic cells **[6, 7, 8, and 9]** and as raw material for several industries. Fatty acids are also membrane components of prokaryotic and eukaryotic cells, playing an important role in cell structure and integrity. The profit accrued on soap and cosmetic production largely depends of the cost of palm oil, coconut oil and palm kernel oil which are also major food ingredients in Sierra Leone. These vegetable oils are all edible oils and using them as raw materials for soap making and cosmetic purpose further increase their demand. This accounts for the high price of palm oil, coconut oil and palm kernel oil in Sierra Leone thereby reducing the growing need for Industrial Enterprises **[10]** which use vegetable oils as their main raw materials. The present work investigates extraction of non-edible oil from the dry seeds of **Caloncoba echinata**, antimicrobial activity tests, and characterization using both wet chemical and instrumental methods of analysis of the oil extracted.

2. MATERIALS AND METHODS

Collection and preparation of dried plant materials

The fresh rip fruits of the traditional medicinal plant **Caloncoba echinata**, were harvested from the Gola Forest in Kenema District, Sierra Leone, processed and dried in the sun for several days. Different methods of extracting oil from vegetable plant various sources have been reported **[11, 12, 13, and 14]**. In this research work, the dried seeds from **Caloncoba echinata** plant were crushed in a mortar with a pestle and then transferred into boiling water bath at 100 ^{o}C. The oil floated onto the surface of the boiling water, the crude oil collected labelled as **LK03** and stored in an inert atmosphere in special containers for the following activities;

- Test for unsaturation
- Determination of Saponification value of the oil
- Determination of iodine value
- Antimicrobial activity
- Characterization by Wet Chemical and Instrumental methods of analysis

The percentage of fat/oil was determined using the formula below:

$$\text{Percentage of oil extracted} = \frac{\text{Mass of crude oil}}{\text{Mass of dry seeds}} \times 100$$

3. EXPERIMENTAL

3.1. Test for solubility and unsaturation of Sample LK03

0.5g of the oil which was solid at room temperature was placed in several test tubes and 15 cm^3 of the solvents; diethyl ether, diethyl acetate, dichloromethane, acetone, cyclohexane, petroleum ether, methanol and xylene added to each test tube. To test for unsaturation, 1% of acidified $KMnO_4$ solution was added drop wise to liquid **LK03** in a beaker, stirred and colour change observed

3.2. Determination of Saponification value of the oil and iodine value of LK03

Saponification and iodine values of Sample **LK03** were determined using standard procedures **[15, 16, 17, 18, 19, 20, 21 and 22]** with calculations determined using the equations below;

$$\text{Saponification value} = \frac{(b - a) \times 0.02805 \times 1000}{\text{Weight of oil}}$$

Where a = Volume of HCl required for the test sample in ml
b = Volume of HCl required for the control in ml
(b-a) = Difference in the volume of acid.

$$\text{Iodine value} = \frac{(Vb - Va) \times 0.01269 \times 1000}{\text{Weight (g) of the sample}}$$

Where,
Vb = ml thiosulphate for blank; **Va** = ml thiosulphate for sample
N = normality of thiosulphate solution

Note: Amount of fat/oil taken should be adjusted such that the excess iodine in the added 25mL of **Wij's** Solution has about 60% of excess iodine of the amount added, i.e., if **(Vb – Va)** is greater than **Vb/2**, repeat the smaller amount of sample. The results are reported in **Table 1 and 2**

3.3. Antimicrobial activity of LK03

Test microorganisms
Stock cultures maintained at 4 ^oC of Bacterial and fungal isolates were obtained from the Microbiology Department of the Connaught Hospital, Freetown. A total of six clinically antibiotic-resistant microorganisms, including four bacterial strains and two fungal strains mostly recorded in the hospital infections were used in this study as shown below;

Bacteria isolates

Escherichia coli	Gram negative
Salmonella typhimurium	Gram negative
Staphylococcus aureus	Gram positive
Bacillus anthracis	Gram positive

Fungal isolates

Candida albicans	Fungi
Epidermophyton floccosum	Fungi

Standard Drug
Ciprofloxacin for bacterial isolates
Nystatin for fungal isolates

In vitro antibacterial and antifungal activities were examined using various solvents added to the **LK03** The zones of growth inhibition around the discs were measured after 24 hours of incubation at 37^o C for bacteria using Mueller-Hinton agar and 96 hours for the fungal at 28^o C using Potato dextrose agar media. The **Stokes disc diffusion** technique **[23]** was used to determine sensitivity. According to this technique, the zone if inhibition produced by **LK03** was compared directly with that of the control antibiotic (Ciprofloxacin), antifungal (Nystatin) and the blank solvent on the same Petri dish. The sensitivities of the microorganism species to the solvent extracts were determined by measuring the diameters/sizes of the inhibition zones (including) the diameter of the disc, and the values **<8mm** were considered as not active against the test organisms. The results are shown in **Table 3**

3.4. Wet Chemical methods and Instrumental methods of analysis of LK03

a. Wet Chemical methods of analysis
Elemental analysis was carried out on Sample **LK03** for the presence of carbon, hydrogen, oxygen, nitrogen, sulphur and halogens using standard procedures. The Middleton's Test was used to detect the presence of nitrogen, sulphur and halogens, Acid Test**,** Phenol Test and Test for Aromaticity.

b. Instrumental analysis of LK03

LC/MS method: HPLC parameters (China)

1. HPLC Parameters: X-bridge C18 Column, 50mm x 4.6 mm I.D., 5 □m with Mobile Phase A using 0.02%NH_4A_C solution and Mobile Phase B using Acetonitrile (CAN), Column Temperature: 25□C □□2□C; Flow rate 1.0mL/min, UV(254nm) detection unit, 6.5min Typical Run Time injection volume of 5□L

2. MS Parameters:
Mass range (100 ~ 1000, 100 ~ 2000): Fragmentor 80/70; Gain 1/1; threshold 50/100 with Step size 0.1 and Ionization APCI/ESI

3. Reagents and Solutions
Water, Watsons Distilled or equivalent, Acetonitrile, HPLC Grade, Ammonium Acetate, HPLC Grade, Dichloromethane, AR Grade, Ethyl acetate, HPLC Grade, Filters: For all analytical samples: 0.45-□m Nylon syringe filter

Procedure
10.0 mg of Sample **LK03** was accurately weighed and quantitatively transferred into a 20 mL volumetric flask. Dissolved and diluted to volume (**20 mL**) with DCM. The first 0.5 mL was discarded and the filtrate was then collected into a HPLC vial. 5.0 □L of the sample solution was injected into an LC-MS system and analyzed under UV 254 nm.

4. GC/MS method
GC-MS Parameters: **Column:** Agilent HP-5 MS, 30 m x 0.25mm I.D., 0.25 □m; Inlet Temperature: 250℃ : Split Rate: 20:1, Gas Flow Rate: Nitrogen, 25 mL/minute; Detector: Flame ionization (FID); FID Detection Temperature: 300℃; Typical Run Time: 12.333 minutes; Injection Volume: 0.2 □L; MS source: 230℃ ; MS Quad: 150

5. Reagents and Solutions
Dichloromethane, AR Grade

6. Procedure
10.0 mg of sample **LK03** was accurately weighed and quantitatively transferred into a 20 mL volumetric flask. It was dissolved and diluted to volume (**20 mL**) with DCM. Mixed well, and then filtered an aliquot of the sample solution through a 0.45 □m Nylon syringe filter. The first 0.5 mL was discarded and the filtrate was then collected into an HPLC vial. 3.2 □L and 0.2 □L of the sample solutions were injected into a GC-MS system.

7. ^{1}H and ^{13}C NMR Spectra (Germany) by Bayer, Peter. Prof
Elemental analysis was performed on a Carlo Erba 1106 elemental analyzer. ^{1}H and ^{13}C NMR Spectra were measured in $CDCL_3$ solutions using a Bruker AM400 model. The ^{1}H NMR spectra was recorded at 75.035 Hz/cm and J-values are given in Hz. ^{13}C spectra was recorded at 377.3Hz/cm. Melting points were uncorrected and determined on an Electrothermal 9100 apparatus.

4 RESULTS AND DISCUSSIONS

Results of the percentage of oil extracted from the Seeds of Caloncoba echinata plant

1. Dry seeds

Mass of dry seeds = 16.85kg

Mass of oil obtained = 5.31 kg

$$\text{Percentage of oil extracted} = \frac{\text{Mass of crude oil}}{\text{Mass of dry seeds}} \times 100$$

$$= \frac{5.31\,kg}{16.85kg} \times 100$$

$$= 31.5\%$$

Hence 16.85 kg of the dry seeds of **Caloncoba echinata** plant gave 5.31 kg **(31.5%)** of fat and Labelled as **LK03. Results and discussions on solubility and unsaturation tests carried the oil extracted from the seeds of Caloncoba echinata** The results of solubility tests carried out on sample **LK03** indicates that the Oil is soluble in diethyl ether, ethyl acetate, dichloromethane, cyclohexane, petroleum ether and xylene but insoluble in acetone, methanol and water. **LK03** tested positive for unsaturation as it absorbed 1% of acidified $KMnO_4$ solution in theoretical amounts to give a colourless solution. Unlike other unsaturated acids, **LK03** is a solid at room temperature having a fairly high melting point. It behaves more like saturated acids regard to its physical characteristics. **Results of the determination of Saponification and iodine value of LK03** The results of the determination of Saponification and iodine value of **LK03** are as shown below;

Table 1: *Showing the results if determination of Saponification Value*

Experiment	Burette Reading (Blank: Va)		Titre (cm^3)
	Initial Reading	Final Reading	
First readings	1.00	26.80	25.80
Second readings	2.50	28.20	25.70
Third readings	3.00	28.90	25.90
			25.80 cm^3
Experiment	**Burette Reading (Blank: Vb)**		**Titre (cm^3)**
	Initial Reading	**Initial Reading**	
First readings	1.00	30.00	29.00
Second readings	2.50	31.50	28.80
Third readings	3.00	32.20	29.20
			29.00cm^3

Results and calculation

$$\text{Saponification Value} = \frac{(29.00 - 25.80) \times 0.02805 \times 1000}{1.0g}$$

$$= \frac{3.2 \times 0.02805 \times 1000}{1.0g}$$

$$= 89.76$$

Hence Saponification Value of Sample **LK03** is 89.76.

The importance of Saponification Value/Number

- Saponification Value/Number is an indication of the amount of fatty saponifiable material in a compounded oil
- It gives information concerning the character of fatty acids and solubility of their soaps
- The higher the Saponification Value/number of a fat free from moisture and unsaponifiable matter, the more soluble the soap that can be made from it.
- It is of special importance to soap makers.

Table 2: *Showing the results if determination of Iodine Value*

Experiment	Burette Reading (Blank: Va)		Titre (cm^3)
	Initial Reading	Final Reading	
First readings	1.00	30.00	22.20
Second readings	2.50	31.50	22.30
Third readings	3.00	32.20	22.10
			22.20 cm^3
Experiment	**Burette Reading (Blank: Vb)**		**Titre (cm^3)**
	Initial Reading	**Initial Reading**	
First readings	1.00	31.00	30.00
Second readings	2.50	32.60	30.10
Third readings	3.00	32.90	29.90
			30.00 cm^3

Results and calculation

The Iodine value of **Sample LK03** was determined using the relation below;

$$\text{Iodine value} = \frac{(Vb - Va) \times 0.01269 \times 1000}{\text{weight (in gm) of sample}}$$

$V_b = 30.00\ cm^3$ $V_a = 22.20\ cm^3$

$$\text{Iodine value} = \frac{(30.00\ cm^3 - 22.20\ cm^3) \times 0.01269 \times 1000}{1.0gm}$$

$$= 98.982$$

Hence Iodine Value of Sample LK03 is 99.00.

The iodine value is a measure of the degree of unsaturation in oils. It is constant for any particular type oil or fat. Iodine value is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid. It is important for the Industrial user to know the amount of free fatty acid present in a given fat/oil since it determines the measure of refining loss. Oils contain both saturated and unsaturated fatty acids. Iodine gets incorporated into the fatty acid chain wherever the double bond exist. Hence, the measure of the iodine absorbed by the oil, gives the degree of unsaturation. Iodine value/number is defined as the 'g' of iodine absorbed by 100g of the oil. The higher the iodine value, the more unsaturated fatty acid (C=C) bonds are present in the fat. Sample **LK03** has been kept for two years without going rancid. This indicates that the fat/oil is suitable for body cream production and other cosmetic products. It is a very stable fat/oil.

The results of antimicrobial sensitivity testing on LK03

The sensitivities of the microorganism species to the solvent extracts were determined by measuring the diameters/sizes of the inhibition zones (including) the diameter of the disk, and the values <8mm were considered as not active against the test organisms. The results of antimicrobial sensitivity testing of the Fat/oil extracted from the seeds of **C. echinata** are shown in **Tables 3** below.

TABLE 3: *Antibacterial and antifungal activity of oil extracted from the seed in various selected solvents*

No.	Sample LK03	Bacteria [a]				Fungal isolates [a]	
		Ec (%)	*St (%)*	*Sa (%)*	*B (%)*	*Ca (%)*	*Ef (%)*
1	001-DEZ	-	-	-	-	-	-
2	002-A	15(51)	-	-	-	14(44)	-
3	003-DCM	-	-	-	-	-	-
4	004-PZ	-	-	-	-	-	-
5	005-X	14(48)	-	-	-	11(44)	9(39)
6	000 Oil blank	16(55)	-	11(37)	-	18(55)	15(65)
7	Ciprofloxacin	29(100)	34(100)	30(100)	28(100)	20(100)	23(100)

KEY: [a] = Values are zones of inhibition diameter (mm) **Ec** =Escherichia coli, **St** = Salmonella typhimurium, **Sa** = Staphylococcus aureus, **B** = Bacillus anthracis (Bacillus sp.); - = Concentration not sensitive to Test organism, **Oil blank,** % = Percentage efficacy relative to the standard drug Ciprofloxacin, **DEZ** – Diethyl ether, **A** – Acetone, **DCM** – Dichloromethane, **PZ** – Petroleum ether, **X** = Xylene. Ca = Candida albicans, Ef = Epidermophyton floccosum and **Oil blank,** The results in **Table 3** indicate that the oil extracted from the seeds and dissolved in acetone (51%) xylene (48%) and the Oil blank (55%) gave minimum inhibitory activity against **Escherichia coli** and 36% against **Staphylococcus aureus**. It gave minimum inhibitory activity against **Candida albicans** with acetone (44%), xylene (44%) and the blank oil (55%) whilst xylene (39%) and blank oil (65%) for the **Epidermophyton floccosum**. The above results support the use of Sample **LK03** for the healing of wounds and skin eruptions in Traditional Medicine.

Results of Wet Chemical and Instrumental analysis of Sample LK03

a. Elemental analysis

The results of elemental analysis are shown in the **Table 4** below;

Table 4: *Elemental analysis carried out on Sample LK03*

Property tested on Sample LK03		Results
Elemental Analysis	Carbon	X
	Hydrogen	X
	Oxygen	X
	Sulphur	-.-
	Chlorine	-.-
Acid Test		X
Phenol Test		-.-
Aromaticity		-.-
Unsaturation		x

Table 4 indicates that the elements Carbon, Hydrogen and Oxygen are present in Sample **LK03**. **Sample LK03** also tested positive for Acid test and Unsaturation.

a. Instrumental analysis carried out on sample LK03

The Structure of Sample **LK03** has been confirmed using ^{1}**H,** ^{13}**C** and by **LG/MS** and **GC/MS** and by the use of **McLafferty Rule** as shown below. **Report on Instrumental Analysis of sample LK03 extracted from the seeds of Caloncoba echinata (Analytical results from China and Germany)**

Physical Properties of Sample LK03

Gorli Oil : Light yellow semi-solid fat

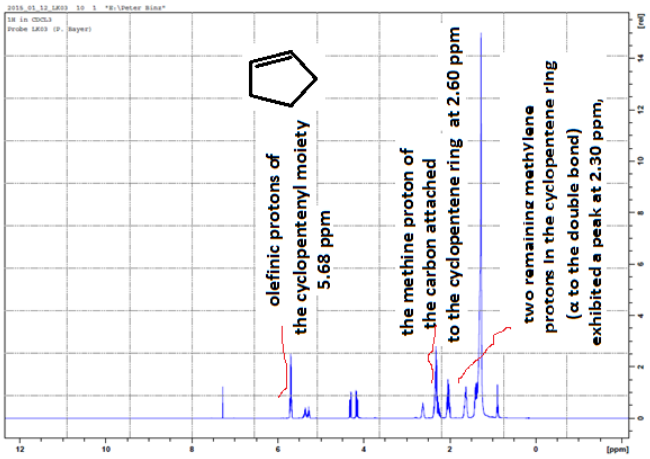
Appearance: Pale yellow semi-solid.
Specific Rotation $[\alpha]_D$ +62
Refractive Index: 1.47990 @ 20.00 °C
Melting Point: 68.5 °C. @ 760.00 mm Hg
Boiling Point: 247.5 °C. @ 760.00 mm Hg
Specific gravity: 0.9549
Solubility: It is very soluble in diethyl ether, ethyl acetate, dichloromethane, cyclohexane, petroleum ether and xylene but insoluble in methanol, water and acetone. The oil produced white suspension in acetone.
Saponification Value: 89.76
Iodine Number: 99.00
Organoleptic Properties:

Odor Strength: None
Odor Description: Faint fatty at 100.00 %.
It has been reported that the oil extracted from seeds of **Caloncoba echinata** plant of the genus Hydnocarpus sp. (Flacourtiaceae) contains cyclopentyl fatty acid in its structure **[24]**. This was confirmed from the analysis of NMR Spectrum **[25, 26]** of **Sample LK03** and LC/MS and GS/MS shown from the reports of Instrumental analysis sent to China and Germany as shown below;

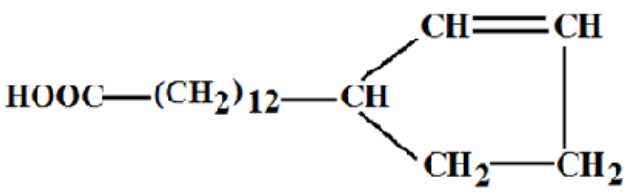
^{1}H in $CDCL_3$

The signal of the olefinic protons of the cyclopentenyl moiety of sample **LK03** was identified at 5.68 ppm **(with the** Peak1D F1="**5.6830**" intensity="**7.477342e-001**" type="**0**"). Methylene proton of the carbon attached to the cyclopentenyl ring and the fatty acid chain at 2.60 (Peak1D F1="**2.6079**" intensity="**3.818820e-001**" type="**0**"). The signal of the methylene protons in the fatty acid chain α to the cyclopentene ring was split, giving two signals at 1.25 (Peak1D F1="**1.2701**" intensity="**1.500000e+001**" type="**0**") and 1.35 ppm (Peak1D F1="**1.3577**" intensity="**1.412711e+000**" type="**0**") as was the signal of the methylene protons α to the carbon in the cyclopentene ring at 2.02 (<Peak1D F1="**2.0225**" intensity="**1.329700e+000**" type="**0**") and 1.27 ppm (Peak1D F1="**1.2701**" intensity="**1.500000e+001**" type="**0**") The two remaining methylene protons in the cyclopentene ring (α to the double bond) exhibited a peak at 2.30 ppm (Peak1D F1="**2.3025**" intensity="**2.276501e+000**" type="**0**") overlapping that of C2 in the fatty acid chain. The spectra were obtained with deuterochloroform ($CDCl_3$) at 400 MHz**. [24]** The proton and carbon nuclear magnetic resonance analysis of **Sample LK03** showed some characteristic signals of the cyclopentenyl ring. The presence of these signals in the proton and/or carbon nuclear magnetic resonance spectrum of oil thus confirmed the presence of cyclopentenyl fatty acids in lipids.

The above spectrum is interpreted as shown below;



The expected structure of LK03 is as shown below;



d-13 (2-cyclopentenyl) tridecanoic acid

Confirmation of the above structure from Mass Spectrometry is as shown below;

Molecular Fragmentation Patterns for LK03 using LCMS analysis;

MOLECULAR ION	MOLECULAR MASS	PERCENTAGE ABUNDANCES
[M']	280.1	2.5
[M']	280.3	10.0
[M' + CH_3COOH]	339.0	0.5
[M' + $(CH_2)_2COOH$]	374.3	2.0
[M' + $(CH_2)_3COOH$]	380.3	10.0
[M' + $(CH_2)_5COOH$]	394.5	2.0
[M' + $2(C_2H_7)$]	414.0	2.0
[M' + $(CH_3COOH) + 2(COOH)$]	429.0	1.0
[M' + $(CH_2)_{11}$]	448.9	0.5

Molecular Fragmentation Patterns for LK03 using GMS analysis;
Major peaks

MOLECULAR ION	MOLECULAR MASS	PERCENTAGE ABUNDANCES
$CH_2CH=C^+$	39.9	1800
$-CH_2H_3COOH$	73.1	4000
[M' - $(CH_2)_3CH=CH(CH_2)_3-6H$']	205.9	1400
[M' - H']	281.1	1900
[M' + CO - 3H']	306.9	700
M' - $C_5H_7(CH_2)_3$ - H']	398.9	400
MOLECULAR ION	**MOLECULAR MASS**	**PERCENTAGE ABUNDANCES**
$CH_2CH=C^+$	39.9	1800
$-CH_2H_3COOH$	73.1	4000
[M' - $(CH_2)_3CH=CH(CH_2)_3-6H$']	205.9	1400
[M' - H']	281.1	1900
[M' + CO - 3H']	306.9	700
M' - $C_5H_7(CH_2)_3$ - H']	398.9	400

Confirmation of the structure using McLafferty rearrangement [27, 28]

McLafferty rearrangement is the predominant fragmentation pathway seen for the $[M - H + 2Li]^+$ ions of monoenoic long-chain fatty acids. The fragmentation process results in a dilithiated product ion of terminally unsaturated fatty acid, which undergoes consecutive McLafferty rearrangement to eliminate a propylene residue, and gives rise to another dilithiated adduct ion of terminally unsaturated fatty acid. In addition to the above-cited fragmentation process, the $[M - H + 2Li]^+$ ions of homoconjugated dienoic long-chain fatty acids also undergo α-cleavage(s) with shift of the allylic hydrogen situated between the homoconjugated double bonds to the unsaturated site. These fragmentation pathways lead to two types of C—C bond cleavages that are allylic (α-cleavage) or vinylic, respectively, to the proximal C—C double bond, resulting in two distinct sets of ion series, in which each ion series is separated by a $—CH_2CH=CH—$ (40 Da) residue. These latter fragmentations are the predominant processes seen for the polyunsaturated long-chain fatty acids. The spectrum feature dependent on the position of unsaturated double bond(s) affords unambiguous assignment

of the position of double bond(s) of long-chain unsaturated fatty acids.

Molecular Fragmentation Patterns for LK03 using McLafferty analysis;

MOLECULAR ION	MOLECULAR MASS	PERCENTAGE ABUNDANCES
-CHCH=C'	38.0	1300
-COOH	45.0	1700
$-CH_2CH=CHCH_3-4H'$	50.0	750
$-CH_2CH=CHCOOH$	85.0	500
$-CH_2CH=CHCOOH-5H'$	80.0	500
$[M' - CH_2CH_2COOH- -COH - H]$	178.0	400
$[M' - 5H+ - 3Li]$	254.0	400
[M' + 2H']	282.0	400
[M' + COOH+Li+3H']	335.0	200
[M' + COOH+Li+8H']	340.0	600
$[M' + COOH+ -C_nH_n- +Li- 2H']$	385.0	200
$[M' - 2(C_2H_7)\ ]$	415.0	400
$[M' - (CH_2)_8COOH - Li - 2H']$	147.0	2100
$[2M' - C_2H_7 + 2Li - 6H']$	503.0	300

The above fragmentation patterns of McLafferty rearrangement are in support of the fragmentations patterns of the LC/MS and GC/MS report sent from China which support the chemical constitution of fatty acid found in the seeds of Flacourtiaceae [29, 30 and 31] and was used in folk medicine for the treatment of leprosy.

5 CONCLUSION

LK03 is a peculiar fatty acid, characterized chemically by the presence of a closed ring of five carbon atoms in its structure. Its empirical formula and structure were first proposed by Power (1905) [29], supported by Shriner and Adams (1925) [30] and later by Perkins (1926) [32]. The structure was also reported by Blaise et al., 1997 [24] and now confirmed in this research work by LC/MS, GC/MS, ^1H and 13C NMR and by McLafferty rearrangement as d-13(2-cyclopentenyl) tridecanoic acid. The results of antimicrobial activity (Escherichia coli, Staphylococcus aureus, Candida albicans and Epidermophyton floccosum) of the oil extracted from the seeds support the use of Sample LK03 for the healing of wounds and skin eruptions in Traditional Medicine [33]. Also the presence of double bond in the structure of Sample LK03 provide binding sites for substrates thus supporting its use as a traditional pharmaceutical

6 Acknowledgement

The authors are grateful to Prof. Peter, Bayer (Germany), Sundia Meditech Co. Ltd of 388 Jialilue Road, Zhangjiang Hightech Park, Shanghai, China for LCMS/GCMS for elemental and spectral analysis, the Bank Manager, Sierra Leone Commercial Bank, Kenema and the Principal Eastern Polytechnic, Kenema for providing financial assistance.

7 References

[1]. Burkill, H. M. The useful plants of west tropical Africa. (Use Pl WT Afr) Chapter 13. sinaver Association, sunder land, 1985

[2]. Burkill, H. M. The useful plants of west tropical Africa. (Use Pl WT Afr) Chapter 13. sinaver Association, Sunder land, 2004

[3]. Schlossberger, H.: Chaulmoograöl. Geschichte, Herkunft, Zusammensetzung, Pharmakologie, Chemotherapie. In: Handbuch der Pharmakologie, Ergänzungswerk. Heubner, W., Schüller, J. (eds.). Berlin: Springer, 1938, Vol. 5, pp. 1–141

[4]. (Cooper & Record, 1931).

[5]. (Kerharo & Bouquet, 1950).

[6]. Singer S. J., Nicolson G. L.(1972) The fluid mosaic model of the structure of cell membranes. Science. 1972;175(4023):720–731.

[7]. Burr G. O., Burr M. M. A new deficiency disease produced by the rigid exclusion of fat from the diet. Journal of Biological Chemistry. 1929;82:345–367.

[8]. Spector A. A., Yorek M. A. Membrane lipid composition and cellular function. Journal of Lipid Research. 1985;26(9):1015–1035.

[9]. Innis S. M.(1991) Essential fatty acids in growth and development. Progress in Lipid Research. 1991; 30(1):39–103.

[10]. Linda McGraw, (2000): 'Biodegradable Hydraulic Fliud Nears Market. US DA. http://www.ars.usda.gov/is/pr/2000/00419.htm. (April 19, 2000) Retrieved 2006-09-29

[11]. Kalu (Oil Presser). Banglapedia. http:/banglapedia.search.com.bd/HT/K 0050.htm. Retrieved 2006-11-12

[12]. Janet Bachmann. "Oilseed Processing for small-scale Producers".http://www.attra.org/attra-pub/oilseed.html. Retrieved 2006-07-31.

[13]. Fairman, R.M. "Illipe". Minor oil crops. FAO. http://www.fao.org/es/faodef/fdef14e.htm. Retrieved 2006-11-12.

[14]. Eisenmenger, Michael; Dunford, Nurhan T.; Eller, Fred; Taylor, Scott; Martinez, Jose. (2006) "Pilot-scale supercritical carbon dioxide extraction and fractionation of wheat germ oil". Journal of the American Oil Chemists' Society 83 (10): 863. 2006

[15]. Haryati T., Che Y.B. Man, Ghazali H. M., Asbi B.A., Buana L.(1998): Determination of iodine value of palm oil based on triglyceride composition. J. of the American oil Chemist's society, 1998, volume 75, Issue 7 pp 789 - 792

[16]. JIS K 0070 – 1992 Test method for acidity, Saponification value, Ester value, Iodine value and Hydroxy value of Chemical products and saponifiables

[17]. David T Plummer (1987) An Introduction to Practical Biochemistry, third edition by, McGraw-Hill, C. McGraw-Hill Book Compny (U.K.) Ltd, London. 1987

[18]. Vlab.amrita.edu. (2011) Estimation of Saponification Value of Fats/Oils

[19]. ISO 3657:2002 Animal and vegetable fats and oils – Determination of saponification value

[20]. Firestone D. (1994) "Determination of the iodine value of oils and fats: summary of collaborative study" J AOAC Int. 77 (3); 674-6. 1994

[21]. Thomas, Alfred (2002). "Fats and fatty oils" Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH

[22]. Morio Yasuda, 1931; The determination of the iodine number of lipids 1931, 94:401-409.

[23]. Stokes, J. E. and Ridgway, G.L. Clinical Bacteriology, 1980, Ed 5, chapter. 7 Arnold Publishers, London

[24]. Pierre, Blaise; Marie, Farines; Jacques, Soulier ; Identification of cyclopentenyl fatty acids by 1 H and 13 C nuclear magnetic resonance. Journal of the American Oil Chemists' Society – Springer Journals; Published: Jun 1, 1997 DOI: 10.1007/s11746-997-0209-7

[25]. Gerhard Knothe and James A. Kenar: Determination of the fatty acid profile by 1H-NMR spectroscopy, Eur. J. Lipid Sci. Technol. 106 (2004) 88–96

[26]. Hopkins, C.Y.: Nuclear magnetic resonance in fatty acids and glycerides. In: Progress in the Chemistry of Fats and Other Lipids. Holman, R.T. (ed.). Oxford: Pergamon Press, 1965, Vol. 8, pp. 213–252

[27]. Hsu FF, Turk J. Distinction among Isomeric Unsaturated Fatty Acids as Lithiated Adducts by Electrospray Ionization Mass Spectrometry Using Low Energy Collisionally Activated Dissociation on a Triple Stage Quadrupole Instrument. J Am Soc Mass Spectrom. 1999;10:600–612. [PubMed]

[28]. McLafferty FW. Mass Spectrometric Analysis: Molecular Rearrangements. Anal Chem. 1959;31:82–87.

[29]. Power FB, J Chem Soc 1907, 91, 563)

[30]. Shriner RL et al. Amer Chem Soc 1925, 47, 2727).

[31]. Badami RC et al., Prog Lipid Res 1981, 19, 119

[32]. PERKINS, G. A. Jour. American Chern. Soc.. 48 (1926) 1714.

[33]. Jacobsen, P.L., Levy, L.: Antibacterial spectrum of hydnocarpic acid, a component of chaulmoo-gra oil. Proc. West. Pharmacol. Soc. 1972 (15), 44–46