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Extraction and Characterization of Linoleic Acid from the Leaves of the Traditional Medicinal Plant *Caloncoba Echinata* in Sierra Leone

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Abstract

Dried powdered organs of **Caloncoba echinata** plant were subjected to organoleptic evaluation and Fluorescence properties. The reagent which gave the most fluorescent character was used to extract a compound from the plant materials. 3.14% (2.50 g) of an oily substance was extracted from 79.62g of powdered leaves of **Caloncoba echinata** in 450 mL 10% of HNO_3 and allowed to stand for 72 hours. The extract was filtered using a Buchner funnel attached to portable Vacuum Pump and the acidic crude extracted with petroleum ether (10 mL x 3).The crude oily compound was separated from the mixture, purified weighed and labelled as **LKL01. Sample LK01** tested positive for terpenoids and unsaturation with Saponification and Iodine Values of **201.96** and **177.66** respectively indicating that the compound is very suitable soap production and cosmetic purposes. Chemical and spectroscopic analysis and from literature revealed the compound to be **Linoleic acid**. This is the first report of the presence of **Linoleic acid** in the leaves of **Caloncoba echinata**. Linoleic acid has been reported to be used as an emollient and thickening agent in cosmetics, antioxidant and an anti-inflammatory agent in the treatment of burns, cold sores and other minor wounds supporting the use of **Caloncoba echinata** plant in traditional medicine.

Keywords: Linoleic Acid; Organoleptic; Fluorescence; Pharmacognostical evaluation; Saponification value; Iodine value and McLafferty Rearrangement.

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1. Introduction

The local and direct utilization of plant parts (the bases of traditional medicine) has provided and continues to provide the resource base for remedial medication in developing countries. In Sierra Leone, for example, where over 80% of people live below the poverty line and with added problem of drug unavailability, the dependence on traditional herbal medicine is high. Some plants used in traditional medicine in Sierra Leone contain principles, which have been demonstrated to be biologically active, such as *Habropetalum dawei*, [1, 2] *Cymbopogon citratus* [3], *Aspilia africana* [4] etc. *Phenol*, present in *Habropetalum dawei* plant in a high concentration, was reported to kill fish at dilutions down to 10 ppm. *Inositol* and *Coumarin* isolated from the leaves of *Aspilia africana* were reported to be the compounds responsible for the haemostatic and wound healing ability of the plant [4].

The biodiversity of the country ensures, from ethno-botanical survey, enhanced resources to a large variety of chemotherapeutic agents – with less than 1% that has been exhaustively investigated [5] and necessitating the need for this study. Sadly though, many plants with worldwide claims to have medicinal properties show no bioactivity during laboratory tests [6]. The recent advances in bioassay screening, isolation techniques and structural elucidation have shortened and facilitated the process of drug discovery from medicinal plants. Quite a large number of drugs have been identified and synthesized in laboratories.

The aim of this research work is to ascertain the active principles in the leaves of *Caloncoba echinata*, one of the traditional plants used in Sierra Leone for the treatment of general diseases and specifically for the treatment of viral diseases such as smallpox, chickenpox and measles and Malaria [7, 8, 9, 10, 11, 12 and 13]. A decoction of leafy twigs is also used in Ivory Coast to wash sores and by enema and in baths for small-pox [14].

2. Constraints/Limitations of the Research Work

The constraints encountered in this research work are as follow;

- Collection of plant materials. Due to indiscriminate collection of fire wood, charcoal production and extensive shifting cultivation, ***Caloncoba echinata*** can only be found in protected forests in Sierra Leone. The Authors have to travel more than 20 Km into the Gola Forest to collect the plant materials.
- Instrumental analysis of plant Samples. Research works are usually hampered in Sierra Leone because of lack adequate Analytical Laboratories to characterize compounds isolated from traditional medicinal plants. Samples were sent to Germany and China for instrumental analysis.
- Finance. Lack of adequate finance has been one of the major factors limiting research activities is Sierra Leone. The Authors sort financial assistance from The Sierra Leone Commercial Bank in Kenema and from the Principal of Eastern Polytechnic, Kenema.

3. Materials and Methods

3.1. Collection and preparation of dried plant materials

Fresh Leaves, Stem barks and Root barks of ***Caloncoba echinata*** were collected from the Gola Forest in the

Eastern Province of Sierra Leone, reduced in size by cutting it into smaller pieces using a cutlass and dried under the shade and not the sun so as to protect the thermo labile components if present from being chemically transformed. After the plant material had been dried, it was each grounded into powder using a laboratory mill and kept in a proper container until the time of the extraction.

A voucher specimens of the plants organs investigated [Leaves (**101**), Stem bark (**102**) and Root bark (**103**)] of ***Caloncoba echinata*** were deposited in the Herbarium of the Botany Department, Fourah Bay College, University of Sierra Leone.

The dried and powdered plant materials were used for the following analysis;

i) Organoleptic evaluation of powdered plant materials.
ii) Fluorescence characters of powdered plant materials.
iii) Extract compound from the plant organ that gave the most significant fluorescent characteristics.
iv) Determination of the Iodine and Saponification values of the compound extracted from the plant identified in (iii) above
v) Characterize the compound isolated from the plant organ by wet chemical, instrumental analytical techniques and by McLafferty Rearrangement to confirm the structure of the compound extracted.

3.2. Experimental

The Organoleptic characters of the various plant organs were evaluated based on the method described by Siddiqui and his colleagues [15]. It refers to evaluation of the powdered plant materials by colour, odour, taste and texture etc. The results are reported in **Table 1.**

Fluorescence characters of each of the powdered plant materials with different chemical reagents were determined under ordinary and ultraviolet light [16]. 100 mg of each of the powdered plant organs was placed on a watch glass and treated with various reagents (Conc. HCl, 10%HNO_3, 1M NaOH, CH_3COOH) for the presence of their fluorescence characters under ultra-violet lamp.

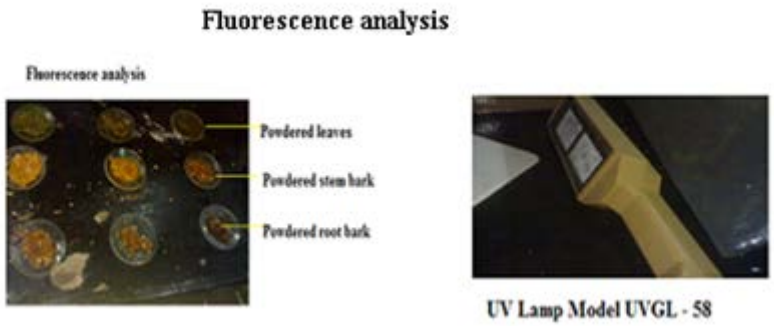

Figure 1: Plant samples in eye glasses with test reagents, **Figure 2:** Uv Lamp used to examine plant extracts

The results of fluorescent studies of the powdered plant material using different chemical reagents were studied

and reported in **Table 2**. Fat/oil was extracted from the plant organ that gave the most significant fluorescent activity as illustrated below;

79.62g of powdered leaves of *Caloncoba echinata* plant was transferred into a 1000ml beaker and 450ml of 10% HNO_3 added to it and allowed to stand for 72 hours. The extract was filtered using a Buchner funnel and portable Vacuum Pump. The filtrate was extracted with petroleum ether (x 3) and the crude oily liquid was separated from the mixture, purified weighed, labelled as **LKL01.** The percentage of oil in the leaves was determined using the formula below;

$$\text{Percentage of oil extracted} = \frac{\text{Mass of crude oil}}{\text{Mass of powdered leaves}} \times 100$$

Sample **LK01** did not crystallize when allowed to stand but was tested for terpenoids. It was then 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

- Wet chemical methods of analysis
- Instrumental methods of analysis and
- Medicinal value of Sample **LK01** extracted from the leaves of the plant

3.3. Test for Unsaturation of Sample LK01

1.0cm^3 of Sample **LK01** was placed in test tube and tested for unsaturation. 1% of acidified $KMnO_4$ solution was added drop wise to Sample **LK01** which was liquid in a test tube, stirred and colour change observed

3.4. Determination of Saponification value and iodine value of LK01

Saponification and iodine values of Sample **LK01** were determined using standard procedures [17, 18, 19, 20, 21, 22, 23,24] 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 25 mL 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 3a and b**

3.5. Instrumental Analysis

Elemental analysis was performed by wet chemical methods and confirmed by the Carlo Elba 1106 elemental analyzer. ^{1}H and ^{13}C NMR spectra were both measured in $CDCL_3$ as internal standards using a Bruker AM 400Model. 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 SFO1, 100.5876228 MHz for Channel 1 and at SFO2, 399.9916000 MHz for Channel 2 in Germany and the LC-MS system and GC/MS method was carried out on Sample **LK01** by the Sundia Meditech Co. LTD in China

4. Results and Discussion

The main aim of this research work was to investigate the secondary plant metabolites obtained from the ***Caloncoba echinata*** plant in order to ascertain the active compounds which could be responsible for the use and efficacy of the plant as a traditional pharmaceutical. The following activities were carried out during the investigation;

i. Organoleptic evaluation of powdered plant materials.
ii. Fluorescence characters of powdered plant materials.
iii. Determination of the total lipids content of the plant organ that gave the most significant fluorescent characteristics of ***Caloncoba echinata***
iv. Determination of the iodine and Saponification values of the extracted oil extracted from the leaves of ***Caloncoba echinata*** plant.
v. Characterize the compound isolated from the plant by wet chemical, instrumental analytical techniques and McLafferty Rearrangement.

4.1. Organoleptic Evaluation of Powdered Plant Materials [25]

The results of organoleptic characters of the powdered plant organs of ***Caloncoba echinata*** plant evaluated are

reported in **Table 1** below;

Table 1: Results of Organoleptic Evaluation on the Powdered Plant Parts of *Caloncoba Echinata*

PLANT PART	COLOUR	ODOUR	TASTE	TEXTURE	PARTICLE SIZE
Powdered leaves	Light Green	Characteristics	Bitter	Smooth	100 # wire gauge
Powdered stem bark	Brown	Spicy	Bitter	Smooth	100 # wire gauge
Powdered root bark	Brown	Spicy	Bitter	Smooth	100 # wire gauge

The bitter taste indicates that each of the powdered plant materials contain alkaloids.

4.2. Results of Fluorescence Analysis of Powdered Plant Materials

The results of fluorescent studies carried out on the various powdered plant organs (Stem bark, Root bark and the Leaves) of ***Caloncoba echinata*** are reported in table 2 below;

Table 2: Showing a Summary of Reagents and the Colours in Visible Light and Under UV Lamp.

No.	Reagent	Plant part	Colour in visible light	Colour under UV lamp
1	Conc. HCl	Leaves	Dark green	Green
2	10% $HNO_{3(aq)}$	Leaves	Grey	Greenish yellow
3	1M $NaOH_{(aq)}$	Leaves	Greenish yellow	Dark green
4	Conc. HNO_3	Leaves	Brown	Green
5	CH_3COOH	Leaves	Green	Pink
6	Conc. HCl	Stem bark	Brown	Yellow
7	10% $HNO_{3(aq)}$	Stem bark	Grey	Greenish yellow
8	1M $NaOH_{(aq)}$	Stem bark	Pale brown	Orange
9	Conc. HNO_3	Stem bark	Light yellow	Green
10	CH_3COOH	Stem bark	Colourless	Pink
11	Conc. HCl	Root bark	Brown	Dark brown
12	10% $HNO_{3(aq)}$	Root bark	Grey	Greenish yellow
13	1M $NaOH_{(aq)}$	Root bark	Pale brown	Orange
14	Conc. HNO_3	Root bark	Light yellow	Green
15	CH_3COOH	Root bark	Colourless	Pink

The 10% HNO_3 reagent gave a significant colour change compared with the other reagents during fluorescence analysis. It was used in extracting a compound from the various leaves of the plant. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material **[26].** If the substances themselves are not fluorescent, they are often converted into fluorescent derivatives by reagents which are one

of the ways of assessing crude drugs qualitatively in Pharmacognostical evaluation [27].

4.3. Extraction of Fat From the Powdered Leaves of Caloncoba echinata Plant using 10% HNO_3

Mass of empty plastic beaker = 37.629g, Mass of empty plastic beaker + powdered leaves = 117.249g, Mass of powdered leaves = 79.62g

Mass of powdered leaves = 79.62g

Mass of oil obtained = 2.50g

$$\text{Percentage of oil extracted} = \frac{\text{Mass of crude oil}}{\text{Mass of powdered leaves}} \times 100$$

$$= \frac{2.50g}{79.62g} \times 100$$

$$= 3.14\ \%$$

Hence 79.62g of powdered leaves of ***Caloncoba echinata*** plant gave 2.50g **(3.14%)** of fat and labeled as **LK01**

LK01 tested positive for triterpenoids with results shown below;

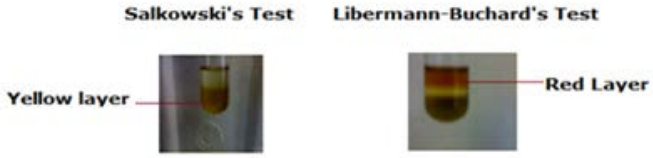

Figure 3: Test for terpenes

Sample LK01 tested positive for unsaturation as it absorbed 1% of acidified $KMnO_4$ solution in theoretical amounts to give a colourless solution. LK01 is a liquid at room temperature having a fairly low melting point.

4.4. Results and Discussions on the Determination of Saponification Value and Iodine Value of Sample LK01

a. Saponification Value for Sample LK01

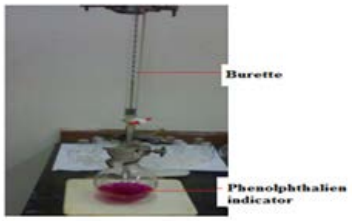

Figure 4: Titrating solubilized fat containing 0.5MkOH with 0.5MHCI

The results of the determination of Saponification and iodine values of Sample **LK01** are as shown below;

Table 3a: Showing the Results of Determination of Saponification Value

Experiment	**Burette Reading (Blank Va)**		**Titre (cm^3)**
	Initial Reading	**Final Reading**	
First readings	1.50	32.70	31.20
Second readings	2.00	33.15	31.15
Third readings	3.00	34.25	31.25
Average volume of titre		**31.20cm^3**	
Experiment	**Burette Reading (Blank Vb)**		**Titre (cm^3)**
	Initial Reading	**Initial Reading**	
First readings	2.00	33.56	31.56
Second readings	1.00	32.51	31.51
Third readings	2.50	34.11	31.61
Average volume of titre		**31.56cm^3**	

The Saponification value of **Sample LK01** from the above table was determined using the relation below;

$$\text{Saponification Value} = \frac{(Vb - Va) \times 0.02805 \times 1000}{0.05g}$$

$$= \frac{(31.56\ cm3 - 31.20\ cm3) \times 0.02805 \times 1000}{0.05gm}$$

$$= 201.96$$

Hence Saponification Value of Sample LK01 = 201.96

The importance of Saponification Value/Number

- Saponification Value/Number indicates the amount of fatty saponifiable material in a compounded oil
- It gives information concerning the character of fatty acids and the 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.

b. Iodine Value of Sample Lk01

Table 3b: Showing the Results of Determination of Iodine Value of Sample LK01

Experiment	Burette Reading (Blank Va)		Titre (cm^3)
	Initial Reading	**Final Reading**	
First readings	1.00	22.50	21.50
Second readings	2.50	24.10	21.60
Third readings	3.00	24.40	21.40
Average volume of titre (Va)		**21.50 cm^3**	
Experiment	**Burette Reading (Blank Vb)**		**Titre (cm^3)**
	Initial Reading	**Initial Reading**	
First readings	1.00	23.20	22.20
Second readings	2.50	24.80	22.30
Third readings	23.00	25.10	22.10
Average volume of titre (Vb)		**22.20cm^3**	

The Iodine value of **Sample LK01** from the above table was determined using the relation below;

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

$$V\,b = 22.20\ cm^3 \qquad V\,a = 21.50\ cm^3$$

$$\text{Iodine value} = \frac{(22.20\ cm^3 - 21.5\ cm^3) \times 0.01269 \times 1000}{0.05gm}$$

$$= 177.66$$

Hence iodine Value of Sample LK01 = 177.66

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.

4.5. Results of the Characterization of Compounds Isolated from the Plant by Wet Chemical and Instrumental Analytical Techniques

i) 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 LK01		**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**. It also tested positive for Acid test and Unsaturation.

ii) Results Of Instrumental Analysis Of Samples Sent Abroad

Characterization of Sample LK01 extracted from the medicinal plant *Caloncoba echinata*

a. Physical Properties of Sample LK01

Appearance: Pale yellow clear oily liquid

Exact Mass: 280.24 g/mol

Monoisotopic Mass: 280.24 g/mol

Rotatable Bond Count: 14

Topological Polar Surface Area: 37.3 A^2

Heavy Atom Count: 20

Defined Atom Stereocenter Count: 0

Undefined Atom Stereocenter Count: 0

Refractive Index: 1.46970 @ 20.00 ^{O}C

Melting Point: -6.9^{O}C @ 760.00 mm Hg

Boiling Point: 230 ^{O}C at 16 mm Hg; 202 ^{O}C at 1.4 mm Hg

Solubility: In water, 1.59 mg/L at 25 ^{O}C. Very soluble in acetone, benzene, diethyl ether, and ethanol

Vapor Density: 9.7 (Air = 1)

Density: 0.9022 g/ cm^3 at 20 ^{O}C

Vapour Pressure: 8.68X10-7 mm Hg at 25 ^{O}C

Decomposition: When heated to decomposition it emits acrid smoke and irritating fumes.

Organoleptic Properties:

Odor Strength: None

Odor Description: Faint fatty at 100.00 %.

LK01 tested positive for triterpenoids with results shown below;

The structure of Sample **LK01** was confirmed using ^{1}H NMR, ^{13}C NMR and by LG/MS and GC/MS and by the use of McLafferty rule.

b. Analysis of NMR Spectrum of Sample LK01 – P. Bayer Germany

Analysis of ^{1}H spectra for LK01

Number of signals (Clusters) in the ^{1}H spectrum and their chemical shifts

The ^{1}H Spectrum has 4 signals as shown below.

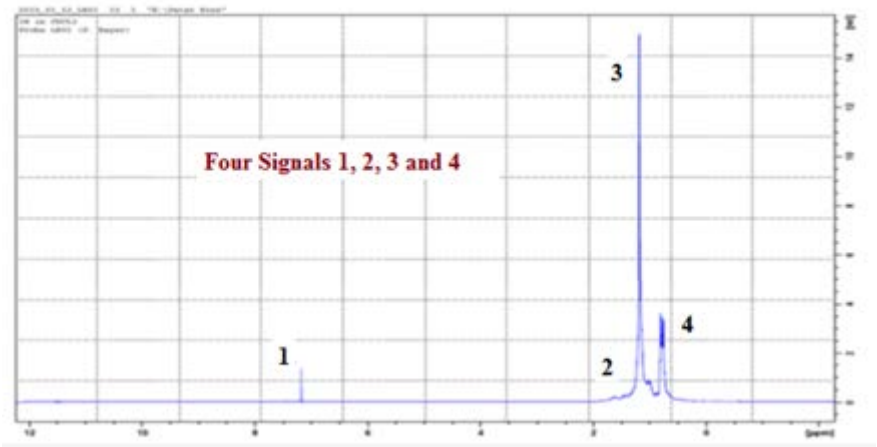

Figure 5: ^{1}H NMR for LK01

At the Low field (left) the spectrum has a single (^{1}H) at 7.4ppm. It can only indicate an aldehyde because this signal is a singlet (n – 1 = 1; n = 0) there cannot be any ^{1}H nuclei on the adjacent carbon. Hence an aldehyde group is entered to the left of the **Box A**

Figure 10

At the high field (right) there is a triplet (3H) at 0.8ppm; 3H at the high field is almost a methyl group. The signal is a triplet (n + 1 = 3); therefore the methyl group must have

(n = 2) i.e. 2 ^{1}H neighbours that must be a **CH_2- group**. Hence the **CH_2** signal at 1.25ppm is a quartet (n + 1 = 4), must have a (n = 3) ^{1}H neighbours that can only be a **CH_3 groups**.

The combination of a "triplet, 3H" with a "quartet, 2H" is always a **C_2H_5 group**. Hence an ethyl group is entered to the right of the **Box B** as shown below;

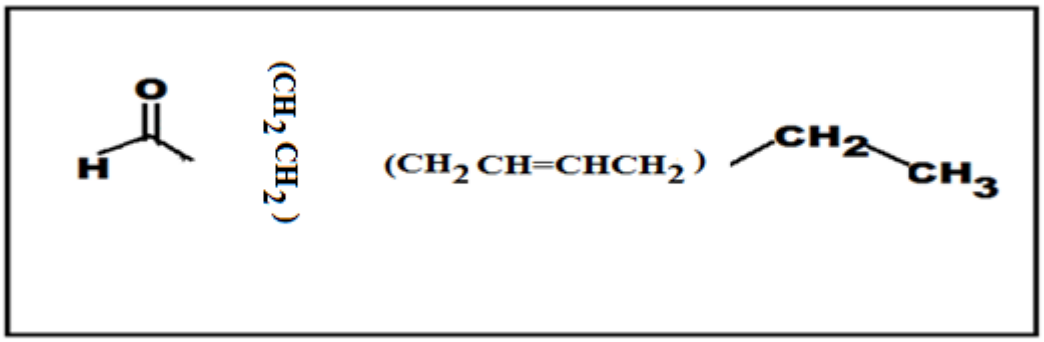

Figure 11

It does not matter in what order we probe the spectrum. If we had started with the high field region it would have been **C_2H_5** before **CHO**, the conclusion would be the same. The **--CH=CHCH$_2$CH=CH----** can be located in the middle of the box as shown above.

The above structures predicted as fragments of the suspected structure are put together in the ^{1}H spectrum below. The ^{1}H Spectrum does not give information on how the two groups are connected. To do so we shall now

consider the ^{13}C Spectrum for LK01 shown below;

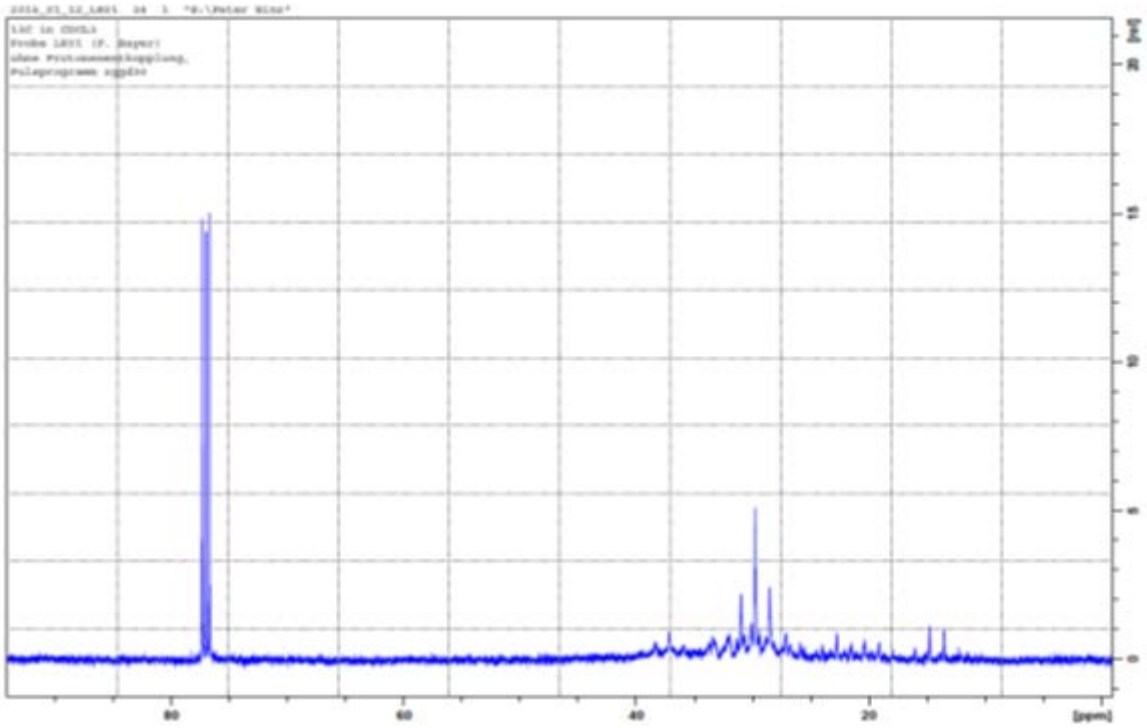

Figure 6: ^{13}C NMR Result for Sample LK01

In support for the structure of Sample **LK01** we also consider the structure of the fragments in the ^{13}C Spectrum shown above. The ^{13}C Spectrum for Sample **LK01** as shown in Figure 6, has ten major signals moving from low field (left) to high field (right). The possible fragments and their interpretations with respect to structure confinement [29, 30] are shown below;

PeakList1DHeader

Peak	Positions F1 (ppm)	Intensity	Type	Possible interpretation
Peak1D	**77.3172**	**43.56**	**0**	= **--CHCOOH**
Peak1D	**76.9995**	**43.54**	**0**	
Peak1D	**76.6823**	**44.15**	**0**	
Peak1D	**38.3890**	**1.70**	**0**	
Peak1D	**37.1856**	**2.68**	**0**	
Peak1D	**35.8992**	**1.41**	**0**	**--CH_2CH=$CHCH_2CH_2$---**
Peak1D	**33.7686**	**1.68**	**0**	
Peak1D	**33.4023**	**2.36**	**0**	
Peak1D	**31.9925**	**2.35**	**0**	
Peak1D	**31.3745**	**2.00**	**0**	**--CHO**
Peak1D	**31.0074**	**6.42**	**0**	

Peak1D	**30.7104**	**2.42**	**0**	
Peak1D	**30.0536**	**3.57**	**0**	
Peak1D	**29.7477**	**15.00**	**0**	
Peak1D	**29.4220**	**2.76**	**0**	**--OH**
Peak1D	**28.4973**	**7.09**	**0**	**--COOH**
Peak1D	**27.1569**	**2.57**	**0**	**--CH_2COOH**
Peak1D	**26.7757**	**1.48**	**0**	
Peak1D	**25.9020**	**1.53**	**0**	
Peak1D	**25.5227**	**1.23**	**0**	
Peak1D	**23.9829**	**1.44**	**0**	
Peak1D	**23.2487**	**1.07**	**0**	**--$COCOCH_3$**
Peak1D	**22.7306**	**2.66**	**0**	
Peak1D	**22.0580**	**1.01**	**0**	**--CH=CH---**
Peak1D	**21.5041**	**1.51**	**0**	
Peak1D	**20.3678**	**1.83**	**0**	
Peak1D	**19.1059**	**1.70**	**0**	
Peak1D	**17.9035**	**0.93**	**0**	
Peak1D	**15.9943**	**1.22**	**0**	**$CH_3CH_2CH_3$**
Peak1D	**14.7517**	**3.35**	**0**	**-- $(CH_2)_6CH_3$**
Peak1D	**13.5150**	**3.19**	**0**	
Peak1D	**12.2698**	**1.16**	**0**	

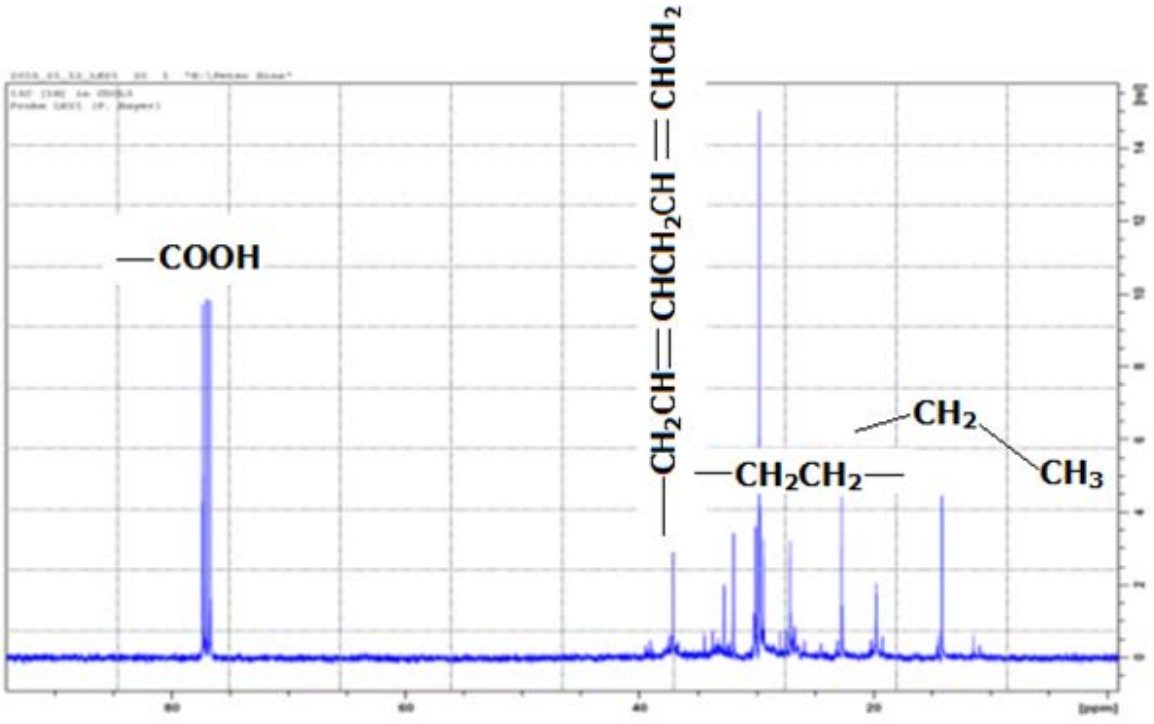

Figure 7: IIlustrating the finger print regions

The position of the fragments of the expected structure with the carbon positions indicated above also gave a clue to the expected structure **Sample LK01.** This is also found to be in line with the expected structure predicted in the ^{1}H Spectrum for **Sample LK01.**

The common unsaturated fatty acids present in many vegetable oils that can be obtained from the leaves of plants are Oleic, Linoleic and Linolenic acids [31]. They are identified by Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR and ^{13}C-NMR) and combined GMS and LCMS – Chromatography. A key feature is that the signals of the terminal methyl group of linoleic acid are shifted downfield from the corresponding signals in the other fatty acids [31], permitting the identification of LK01 and by the fragmentation patterns from GCMS and LCMS Chromatography as Linoleic acid.

IUPAC Name***: (9Z, 12 Z)-octadeca-9, 12-dienoic acid.***

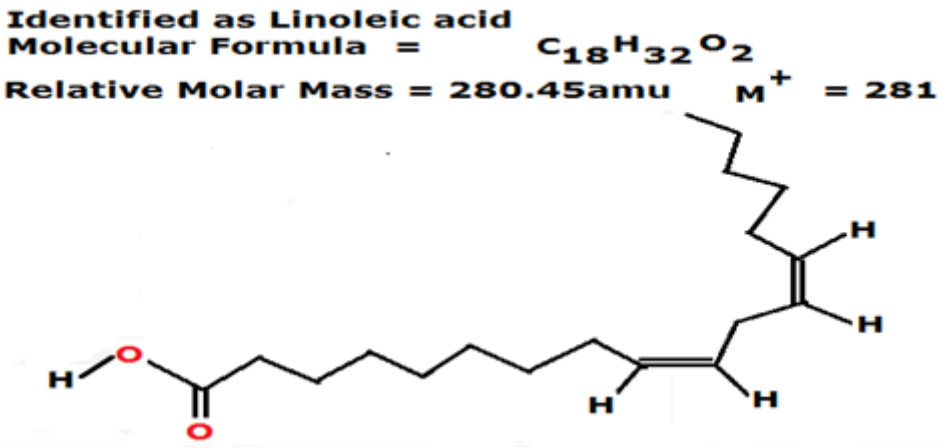

Figure 8: Expected structure of LK01

The exact structure of Linoleic acid was clarified by Hilditch T.P. and his colleagues in 1939, and was synthesized by Raphael R.A. and Sondheimer F. in 1950 and now confirmed in this research work by LC-MS system and GC/MS analysis carried out on Sample **LK01** by the Sundia Meditech Co. LTD in China and ^{1}H and ^{13}C NMR spectroscopy by Prof. Peter Bayer in Germany.

c. Liquid – Chromatography Results for Sample LK01

The LC-MS system fragmentations and difference in peak height gave the following fragmentation below;

228.2 – 211.1	=	17.1	(--OH – group)
286.2 – 228.2	=	58	(CH_3C (OH) CH_2—
286.2 – 211.1	=	75.1	CH_3C (OH) $_2CH_2$—

318.3 – 228.3 = 90 $CH_3C(OH)_2CH_2$—CH_3

d. Gas – Chromatography Results for Sample Lk01

Fragmentation Patterns in Support of the Expected Structure of LK01 from Mass Spectrometry

Fragmentation ions	**Molar mass**	**Abundance**
M^+	281	500
[M – CO]	253	500
[M – CO – C_4H_9]	196	15000
[M – CO – C_4H_9–CHOH]	166	4500
[M – CO – C_4H_9 + CHOH – C_3H_8]	122	7500
[M – 2CO – C_4H_9 + CHOH – C_3H_8]	94	16000
[M – CO – C_4H_9 + CHOH – C_3H_8– C_3H_7O]	63	3000

Where CO = 28; C_4H_9 = 57; CHOH = 30; C_3H_8 = 44; C_3H_7O = 59.

The fragmentation patterns indicate the presence of conjugation in the actual structure of Sample **LK01**.

e. Confirmation of Expected Structure of Lk01 using McLafferty Rule

The McLafferty rearrangement illustrates the characteristic fragmentation of the molecular ion of a carbonyl compound containing at least one gamma hydrogen e.g.:

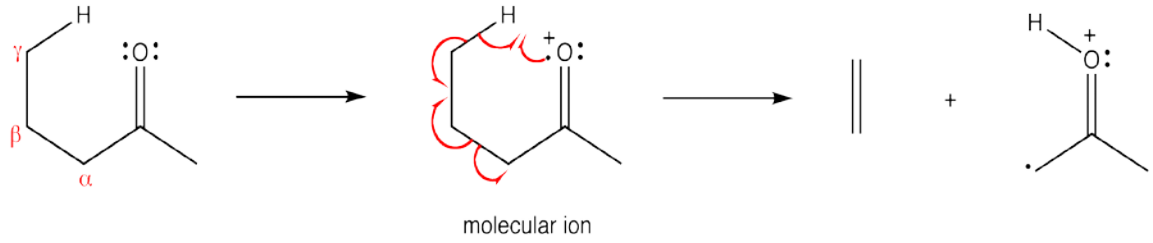

Figure 12

The characteristic peaks of ester carbonyl (–COO–) and C–O are the distinct peaks for confirmation of methyl esters present in the Sample **LK01**. In ^{13}C NMR spectrum, the characteristic peaks of carbonyl (–COO–) and C–O are observed at **77.3172** and **31.3745** ppm, respectively. The peaks between **35.8992** and **38.3890** ppm showed unsaturation of the methyl esters. Other peaks around **14.7517**and **15.9943** ppm are related to terminal

carbon of methyl groups and **22.56–34.09** ppm signals are related to methylene carbons of long carbon chain as shown in Figure 7.

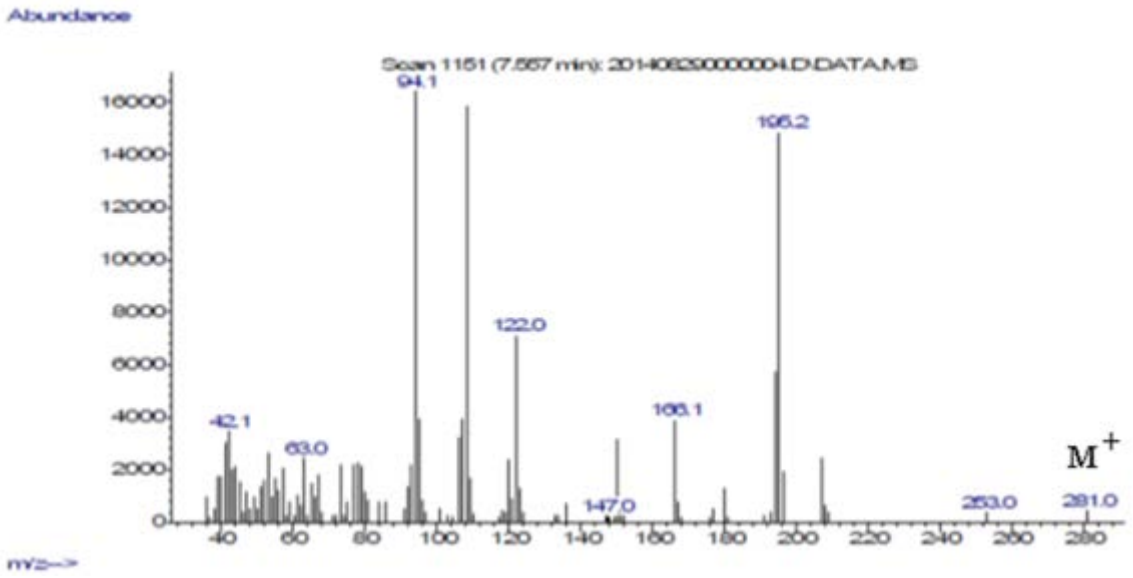

Figure 9: LG/MS spectrum for LK01

The fragmentation patterns proposed by McLafferty rearrangement are in support of the fragmentation given in the **Figure 9** of the LG/MS spectrum for LK01.

Fragmentation ions	**Molar mass**	**Abundance**
M^+	281	500
[M – CO]	253	500
[M – CO – C_4H_9]	196	15000
[M – CO – C_4H_9–CHOH]	166	4500
[M – CO – C_4H_9 + CHOH – C_3H_8]	122	7500
[M – 2CO – C_4H_9 + CHOH – C_3H_8]	94	16000
[M – CO – C_4H_9 + CHOH – C_3H_8– C_3H_7O]	63	3000

Where CO = 28; C_4H_9 = 57; CHOH = 30; C_3H_8 = 44; C_3H_7O = 59.

In methylene interrupted dienes a series of diunsaturated aliphatic radicals with masses 42, 63, 94 and 122 dominates the spectrum at low masses. Most of the dienoic spectra found in this study showed molecular ions in the GC/MS at m/z 63 and generally look more like monoenes in the lower mass region. This can be explained

by suppression of the reaction leading to m/z 67 in non-methylene interrupted dienes. The m/z 63 ion is illustrated in **Figure 9**. When the two double bonds are separated by more than two single bonds, the ion cannot be formed without double bond migration prior to fragmentation. **[33] [34].** The intensities of the peaks in the LCMS with higher molecular mass than the target molecule indicate dimerization as a major role in the disintegration process of the molecule. The above fragments derived from the McLafferty Rearrangement contribute to the structure of **Linoleic acid** in **Figure 8.** The rearrangement involved fragments whose origin cannot be described by simple cleavage of bonds in the molecular ion, but as a result of intermolecular rearrangement during fragmentation.

f. Medicinal use of Linoleic Acid Which Support the use of the Leaves of *Caloncoba Echinata* in Traditional Medicine

Linoleic acid has been shown to be an unsaturated omega-6- fatty acid found in corn, safflower, and sunflower oils and used as an emollient and thickening agent in cosmetics. It is an Essential fatty acid (EFA) which cannot be synthesized by the human body. There are a number of researches showing it to be effective in cell regulation and skin-barrier repair, as well as being an antioxidant and an anti-inflammatory agent [35, 36, 37]. It plays a crucial role in tandem with omega-3 EFAs – in brain function, normal growth, skin and hair regeneration, bone health and metabolic function.

In the world of aesthetics, vitamin F is typically found in skin-nourishing formulations as Linoleic acid. Not only is it useful in accelerating the healing during the post-corrective treatment, it also can help treat burns, cold sores and other minor wounds. Essential Free fatty acids (EFAs) are also the building blocks of healthy cells, making them a vital part of any healthy aging regimen. They are vital to the synthesis of tissue lipids, and in the life and death of cardiac cells. These characteristics make EFAs important to healthy skin and hair – it helps maintain shine and strength in hair. In skin care, linoleic acid provides anti-inflammatory, moisturizing and healing support. It also helps fight acne, softens the skin, and keeps it supple and youthful. EFAs can also help facilitate the penetration of other active ingredients such as antioxidants, because of their ability to permeate the skin barrier. Deficiencies are more likely to occur with omega-3 than omega-6 EFAs simply because omega-6 EFAs are more abundant in our food sources **[38], [39] and [40].** Regardless, a deficiency in EFAs can result in dry hair and skin, hair loss, poor wound healing, and decreased cell regeneration. All of the above medicinal use of **LK01** identified as Linoleic acid confirmed the use of the extracts of the leaves of the ***Caloncoba echinata*** plant in traditional medicine.

5. Conclusion

Organoleptic evaluation and Fluorescence properties have been carried out on dried powdered organs of ***Caloncoba echinata***. The reagent which gave the most fluorescent character was used to extract compound **LK01** from the plant materials in which 79.62g of powdered leaves of *Caloncoba echinata* plant was transferred into a 1000ml beaker and 450ml of 10% HNO_3 added to it and allowed to stand for 72 hours. The mixture was stirred every 24 hours. The extract was filtered using a Buchner funnel attached to portable Vacuum Pump and the crude acid*ic* filtrate extracted with petroleum ether (10ml x 3).The crude oily compound was separated from

the mixture, purified weighed, labelled as **LK01. Sample LK01** tested positive for terpenoids and unsaturation with Saponification and Iodine Values of **201.96** and **177.66** respectively indicating that the compound is very suitable soap production and cosmetic purposes. Chemical and spectroscopic analysis revealed the compound to be **Linoleic acid**. This is the first report of the presence of **Linoleic acid** in the leaves of ***Caloncoba echinata*** **[41]**.

Linoleic acid has been reported to be used as an emollient and thickening agent in cosmetics and an Essential fatty acid (EFA) which cannot be synthesized by the human body. A number of researchers have shown it to be effective in cell regulation and skin-barrier repair, as well as being an antioxidant and an anti-inflammatory agent. It plays a crucial role in tandem with omega-3 EFAs – in brain function, normal growth, skin and hair regeneration, bone health, and metabolic function. Linoleic acid is not only useful in accelerating the healing during the post-corrective treatment, but also helps in the treatment of burns, cold sores and other minor wounds. This supports the use of the plant in traditional medicine. Animals can't synthesize it because they lack of Δ12-desaturase, the enzyme that catalyzes its synthesis, and are obliged to obtain it from plant foodstuff: so it is an essential fatty acid (EFA).

6. Recommendations

Linoleic acid isolated from the leaves of Caloncoba echinata is reported to be used as an emollient and thickening agent in cosmetics, antioxidant and an anti-inflammatory agent in the treatment of burns, cold sores and other minor wounds supporting the use of Caloncoba echinata plant in traditional medicine. The compound isolated from the plant has three main functions, *i.e.* as medicine, raw material for cosmetic production and as food supplement. It is therefore recommended that further work on the plant be carried out in order to isolate more compounds from the leaves of the plant and to cultivate the plant in our back yard gardens as another source of fresh vegetables.

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