Extraction, Purification and Determination of Phyto-component of Fatty acids from *Irvingia gabonensis* Seeds

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ABSTRACT

This study was designed to extract, purify and determine the phyto-components in the petroleum ether extract of *Irvingia gabonensis seeds (IGS).* A multiple steps extraction procedure was employed using petroleum ether as a solvent. A specified quantity of the *IGS* was size reduced, dispersed in freshly distilled water by heating to a temperature of 80 ^{o}C. The dispersion was then filtered through a clean muslin cloth. The fats content was separated from the Biopolymer by first dispersing in 90 % alcohol, filtered and dried at 40 ^{o}C. The fat component of the residue was finally extracted with petroleum ether. GC-MS analysis of the petroleum ether extract of IGS was performed using a Agilent G1701 GC/MSD system. This investigation was done to determine the possible chemical components from IGS by GC-MS. This analysis revealed that the petroleum ether extract of IGS contained mainly fatty acid compounds– lauric acid (36.82%), myristic acid (31.52%), benzyl dodecanoate (9.06%) and palmitic acid (4.03%), n-Decanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester. The results from the present study show that IGS contains various bioactive compounds and is recommended as a plant of phyto-pharmaceutical importance.

Keywords: Extraction, Purification, GC-MS, Phytochemistry, Fatty acids, *Irvingia gabonensis* Seeds

INTRODUCTION

The *Irvingia gabonensis* seeds has been studied and said to have some industrial potential. Although the two species' seeds do differ in their composition, the differences are not always found to be significant and some studies fail to identify which species is being analysed. This is particularly so for those that were undertaken before I. wombolu was recognised as a separate species, therefore in some cases figures given for *I. gabonensis* are actually for I. gabonensis var. excelsa (I. wombolu).

Onyeike and co-worker[1] report that the crude fat content of *I. gabonensis* seeds is 62.25% ±0.55, proving them to be 'very good oil seeds'.

Amubode and Fetuga[2] analysed the amino acids in *I. gabonensis* and came up with the following:

Crude protein: Tryptophan, Lysine, Histidine, Arginine, Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Valine, Methionine, Cystine, Iso-leucine, Tyrosine, Phenylalanine.

Omogbai's[3] study of the lipid and fatty acid composition of Nigerian tropical seeds included *I. gabonensis.*

Fruits: The juicy fruit pulp of *I. gabonensis* is rich in vitamin C and is widely reported to be consumed as a dessert fruit or snack throughout Western and Central Africa.[3,4]

Seeds: The kernels of *I. gabonensis* and *I. wombolu* are classed as oilseeds. They are ground with a pestle and mortar or on a stone into a paste or cake called 'dika bread', which is used as a soup, stew or sauce additive, for flavouring and thickening.[5,6] The kernels are highly valued for the slimy consistency they produce. Okafor[7] notes that whilst kernels from both Irvingia spp. are used in soup making, *I. gabonensis* kernels can only be used when fresh since they become too slimy over time. Irvingia kernels form an important part of the West and Central African diet, providing carbohydrate and protein.[1] Fat extracted from the kernels can be used for food applications, such as in cooking oil or margarine, and is also suitable for pharmaceuticals, cosmetics, and soap.[8] The potential industrial applications of bush mango kernel fat listed by Joseph[9], include: cooking oil, margarine, perfume, soap and pharmaceuticals. Aside from its role as a thickener, the residual kernel cake could also be used as a binder in food or pharmaceutical products.[9] Ndjouenkeu and co-worker[10] extracted the polysaccharides from Irvingia kernels and from an analysis of their properties concluded that they have potential as an industrial gum.

Method

Extractions of *Irvingia gabonensis* (Ogbono) gum

One kilogram of *Irvingia gabonensis* seed was size reduced using electric blender. The granules were then transferred into a dried clean beaker (1000 ml capacity). Water was added up to 1000 ml mark and with the aid of the hot plate, it was heated until temperature of 80ºC was reached. The solution was constantly stirred and left to stand for 24hours for proper dispersion in **distilled** water.

After 24hours, the dispersion was filter through a clean muslin cloth, the filtrate obtained contains: biopolymer, fat, and water About ten (10) liters of 95 % ethanol was added to the filtrate until the mixture of biopolymer and fat crumbled in the ethanol indicating complete precipitation and total separation from water. The residue was collected, while the filtrate was discarded.

The residue extracted was properly spread on a clean brown paper and air dried for 24hours for complete drying, it was dried in an over at a temperature of 40 ºC for 20 minutes.

Defatting of the gum

The extracted biopolymer contains fat. It was defatted by the use of petroleum ether. This was done by adding 100 ml of petroleum ether onto the 100 g of extracted biopolymer with stirring, after which granular sediments (gum) were filtered from the petroleum ether. Six batches of the defatting was done using 100ml each until the biopolymer was free of fat (600ml of petroleum ether was used). The biopolymer obtained was air dried for 24hours, after which it was dried in the oven at a temperature of 40ºC for 30minutes. The dried purified biopolymer was then size reduced using a dry porcelain mortar and pestle. The weight of the dried granules obtained was 30 g and the percentage yield was determined.

Recovering the fat from petroleum ether

The several batches of the petroleum ether used for the purification of the biopolymer were collected together in a 1000 ml capacity beaker and left open air for 72 h, after which

pet ether had evaporated leaving behind a semisolid mass which is fat.

$$\textbf{\textit{Percentage yield}}\ (\%) = [\textbf{\textit{final weight}}/\ \textbf{\textit{Initial weight}}] \times \mathbf{100}$$

GC-MS analysis

GC-MS analysis of the petroleum ether extract of IGS was performed using a Agilent G1701 GC/MSD system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 μl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 100 °C (isothermal for 2 min), with an increase of 4 °C/min to 300°C, ending with a 10 min isothermal at 300 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 60 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was Agilent G1701 GC/MSD ChemStation and the library used was Agilent IO Libraries version 15.5.

Identification of phytocomponents

Interpretation on mass-spectrum GC-MS was conducted using the database of Agilent IO Libraries version 15.5
National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

Results and Discussion

GC chromatogram analysis of the petroleum ether extract of IGS (Figure 1) showed 12 peaks which indicating the presence of 12 phytochemical constituents (Table 1). On comparison of the mass spectra of the constituents with the Agilent IO library, eight (8) phytocompounds were characterized and identified (Figure 2). The various phytochemicals which contribute to the medicinal activities of the plant were shown in Figure 2. The mass spectra of all the phytochemicals identified in the petroleum ether extract of *IGS* were presented in Figure 2. Of the 8 compounds identified, the most prevailing compounds were lauric acid (36.82%), myristic acid (31.52%), benzyl dodecanoate (9.06%) and palmitic acid (4.03%). Among these compounds, three compounds ‘lauric acid, myristic acid and palmitic acid’ were reported to have antimicrobial activity.

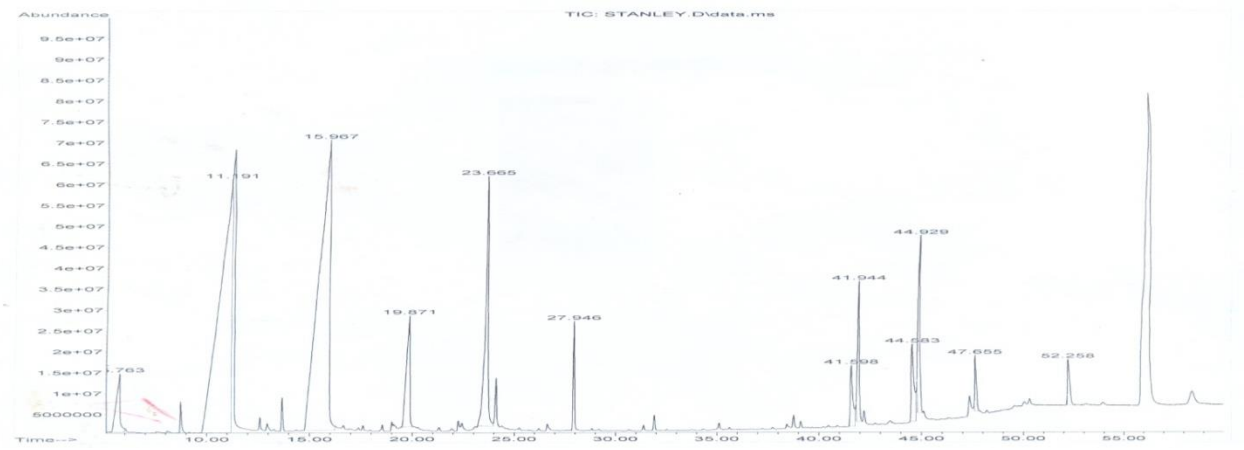
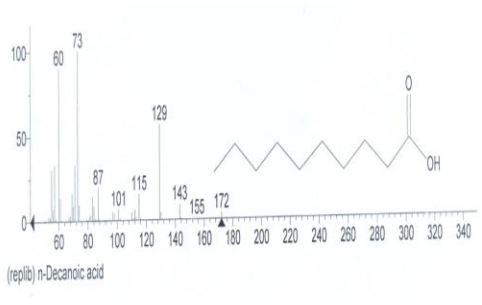


Figure 1. Mass spectrum of phytocomponents identified by GC-MS in the petroleum ether extracts of IGS.

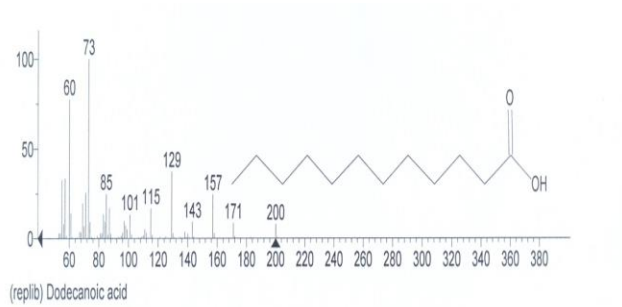
Table 1. Phytocomponents a identified in the IGS petroleum ether extract by GC-MS

No	RT	Name of compound	Molecular formula	MW	Peak area %
1	5.76	n-Decanoic acid	$C_{10}H_{20}O_2$	172	2.09
2	11.19	n-Dodecanoic acid (lauric acid)	$C_{12}H_{24}O_2$	200	36.82
3	15.97	n-Tetradecanoic acid (myristic acid)	$C_{14}H_{28}O_2$	228	31.52
4	19.87	n-hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256	4.03
5	23.67	benzyl dodecanoate	$C_{19}H_{30}O_2$	290	9.06
6	27.95	benzyl dodecanoate	$C_{19}H_{30}O_2$	227	2.11
7	41.60	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	456	1.75
8	41.94	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	456	2.01
9	44.58	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	456	2.24
10	44.93	Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{31}H_{60}O_5$	512	5.28
11	47.66	Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{31}H_{60}O_5$	512	1.96
12	52.26	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_5$	638	1.13

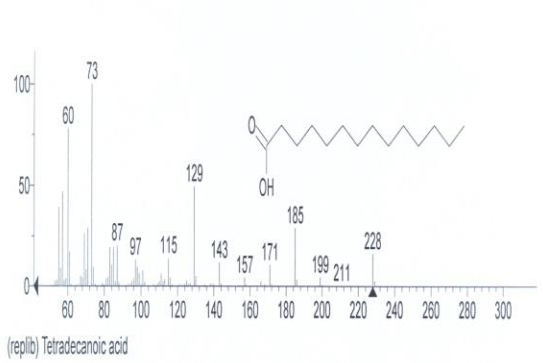
Phytocomponents identified in the petroleum ether extracts of IGS by GC-MS are shown in Figure 2



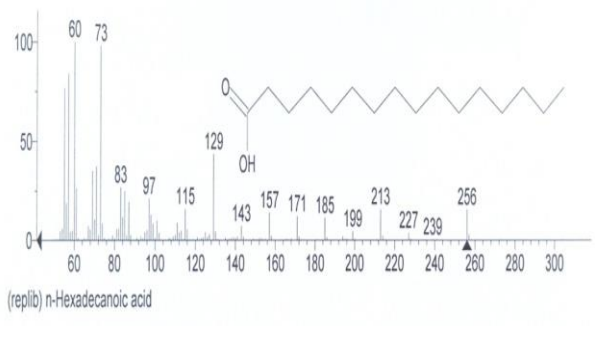
Decanoic acid



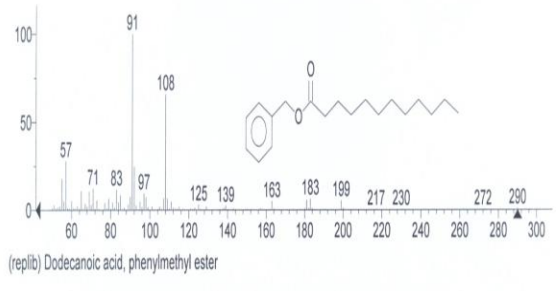
dodecanoic acid

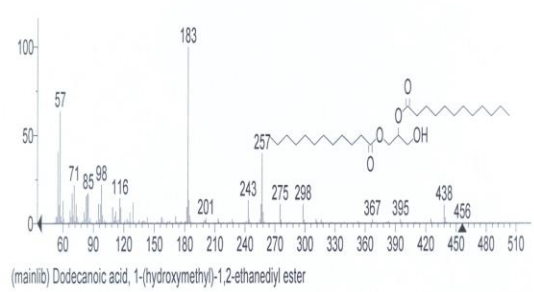


tetradecanoic acid

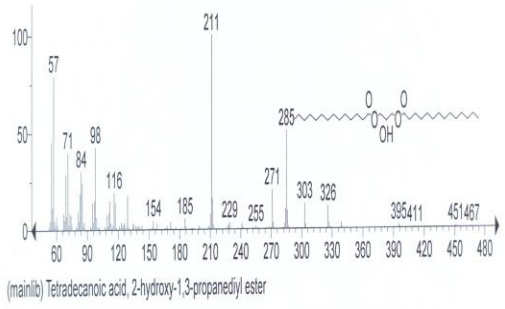


palmitic acid

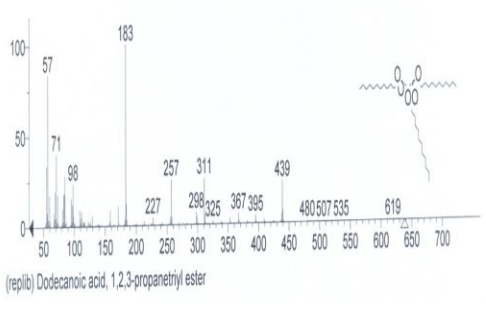




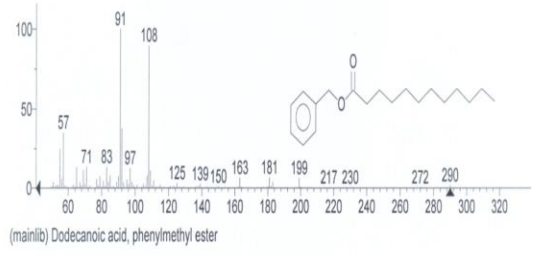
Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester benzyl dodecanoate



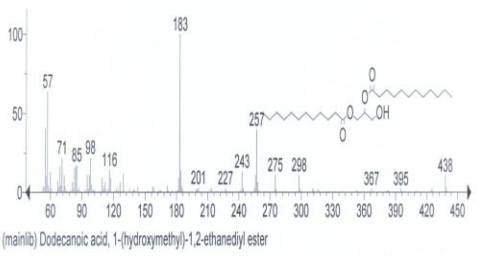
Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester



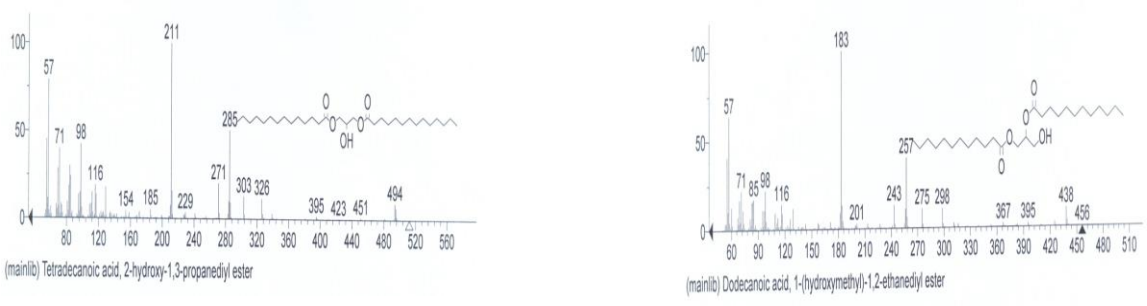
Dodecanoic acid, 1,2,3-propanetriyl ester



benzyl dodecanoate



Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester



Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester Dodecanoic acid, 1,2,3-propanetriyl ester

Figure 2. Mass spectrum and structure of contaminated phytocomponents identified by GC-MS in the petroleum ether extracts of IGS

The GC of the petroleum ether extract of IGS showed the presence of 12 compounds as shown in figure 1. Most of the phytocomponents found were fatty acid which include n-Decanoic acid, n-Dodecanoic acid (lauric acid), n-Tetradecanoic acid (myristic acid), n-hexadecanoic acid (palmitic acid), benzyl dodecanoate, Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester (figure 2). Some of the fatty acid could not be resolved but their mass spectrum indicated the presence of a benzyl dodecanoate, Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester and Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester as shown in figure 3.
The most abundant of the compounds include lauric acid followed by myristic acid (table 1)

Lauric, palmitic and myristic acids are known to have potential antibacterial and antifungal properties[11,12] thus suggesting the possible antibacterial and antifungal activity of this extract. Myristic acids have also been reported to have Larvicidal and repellent activity[13] and palmitic acid reported to have Anti-inflammatory,[14] Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor,[15] potent mosquito larvicide activities.[16] Decanoic acid being a fatty acid can form salt or ester with a drug which will increase its affinity for fatty tissue and its lipophilicity. This thus makes this extract good in formation of prodrug and Depot injection.) by using its decanoate form.

The activities of these compounds are a function of the lipophilic properties of their constituent, the properties of their functional groups, and their aqueous solubility.[17]

Conclusion

The various bioactive compounds presence in the petroleum ether extract of IGG justifies its use for various ailments by traditional practitioners. That notwithstanding, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. From the results, it could be concluded that Irvingia gabonensis fats contains various bioactive compounds. Therefore, it is recommended as a plant of phytopharmaceutical and medicinal importance. Future work shall be directed on isolation and designing a stable formulation for the bioactive compounds.

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