Lipid Composition and Antimicrobial Activity of Raw, Boiled and Fermented Seed Extracts of *Pentaclethra macrophylla* (BENTH)

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ABSTRACT

Lipids possess versatile biological properties in human health and nutrition. The compositions of lipids were investigated on the raw, boiled and fermented seeds of *Pentaclethra macrophylla* n-hexane extracts. The seed extracts were esterified, and chemical composition of the fatty acids was evaluated using gas chromatography-mass spectrometry (GC-MS). Subsequent chromatographic purification and isolation on the lipids was carried out using column chromatography and thin layer chromatographic techniques. The antimicrobial activity of extracts was evaluated using disc diffusion and broth dilution techniques on selected bacterial and fungal species, Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi*, *Proteus mirabilis*, *Corynebacterium ulcerans*, *Escherichia coli*, *Candida krusei*, *Candida albicans* and *Candida tropicalis*. GC-MS analysis revealed that fatty acids such as linoleic acid, butyl 9,12- octadecadienoate acid, oleic acid, hexadecanoic acid, methyl 20-methyl heneicosanoate, tetracosanoate acid, ethyl tetracosanoate and methyl stearate were common in the raw, boiled and fermented seed extracts. However, linoleic acid content was higher (60%) in fermented seeds, indicating an increased production due to fermentation effect. The extracts have demonstrated growth inhibition on bacterial and fungal species with broad-spectrum activity for the fermented seeds (17-19 mm; MIC 5 mg/mL) and the raw and boiled seeds (16-18 mm; MIC 5 mg/mL). Monoglycerides were isolated and structures elucidated using ¹H-NMR and ¹³C-DEPT as 2,3-dihydroxypropyl tetracosanoate (**1**), 2,3-dihydroxypropyl pentacosanoate (**2**) and 2,3- dihydroxypropyl hentriacontylate (**3**). Compounds (**2**) and (**3**) are reported for the first time from the seeds. This report indicated the potential benefit of *P. macrophylla* seeds fermentation to human nutrition and health.

Keywords: *Pentaclethra macrophylla*, fermentation, lipids, fatty acids, monoglycerides, GC-MS

INTRODUCTION

Although there is no exact definition of lipids, they can be defined as a wide variety of natural products such as fatty acids and their derivatives, steroids, terpenes and carotenoids and substances related biosynthetically or functionally to these compounds [1, 2]. Reports from literature have shown that fermented foods may undergo rancidity and this may cause deterioration of the lipid composition [3, 4]. It has been stated that when fats and oils are exposed to factors such as air, light, temperature, moisture and microbial action, they undergo rancidity [3, 4]. Lipids have been used and are still used in pharmaceutical, cosmetic and food industries due to their versatile physiochemical

and biological activities [5, 6]. Hence, fermentation effects of food processing on bioactive compounds such as lipids and fatty acids are relevant and therefore deserve investigation. The aim of this research is to study the effect of fermentation on the lipophilic seed extracts of *P. macrophylla* with a view to assessing the fatty acid and lipid composition on the raw, boiled and fermented seeds and then evaluate their antimicrobial activity on selected bacterial and fungal species.

Fermentation method of processing food can be defined as a metabolic process which results from the activities of microorganisms that can change the chemical profiles of various compounds/metabolites present in food matrices

in relation to their health benefits [7, 8, 9]. Fermentation can change bioactive components and produce new bioactivities [10]. It has been reported that processing is expected to affect the content, activity and bioavailability of bioactive compounds and thus cannot be overlooked [11]. Generally, it has been stated that fermentation increases the lipid and fatty acid compositions in fermented foods [9,12]. During fermentation processes micro-organisms metabolize various lipids and fatty acids differently thus, resulting in variations in compositions [13].

The plant, *Pentaclethra macrophylla* (Benth) is a tropical tree crop that is mostly found in the Southern and Middle Belt Regions of Nigeria and also in other coastal parts of West and Central Africa. It belongs to the family Leguminosae (Mimosoideae) [14]. It is commonly known as African oil bean and the Nigerian local names are; *Ugba* (Igbo tribe),

METHODOLOGY

Extraction protocols

The seeds of the plants (1.5 kg) was collected from Umuezuo Umuokirika Ekwerazu Ahiazu Mbaise LGA, Imo State Nigeria, identified and authenticated at the Herbarium Department of National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja with voucher number NIPRD/H/6886.

The raw and boiled seeds of *P. macrophylla* (500 g each) were dehulled, sliced into smaller pieces, air-dried for 3 weeks and pulverized using a mortar and pestle prior to extraction. The pulverized plant seeds each were extracted using

Apapa (Yoruba tribe), *Kiriya* (Hausa tribe) [15]. It has variety of uses and applications in traditional medicine [14, 16]. The seeds are the edible part of the plant and are usually fermented before consumption [17].

Previous study on *P. macrophylla* reported the isolation of a secokaurane diterpenoid; secopentaclethrolide its O-glucoside Secopentaclethroside, a known alkaloid Caffeoylputrecine and glyceryl monotetracosanoate from the methanol extract of the raw seeds [18]. The following bioactive compounds have been reported to be present in the root bark; 1-monotetracosanoate glycerol, Bergenin, o-glucoside of beta-sitosterol and stigmaterol [19]. Here, we report for the first time the isolation and characterization of 2,3-dihydroxypropyl pentacosanoate (**2**) and 2,3-dihydroxypropyl hentriacontylate (**3**) from the seeds of *P. macrophylla*.

n-hexane (1000 mL) for 72 hr by cold maceration. The extracts were filtered and concentrated using a rotary evaporator at 40 °C. Percentage yield for the extracts was calculated as; 24.08 % (raw), 21.12 % (boiled) and 18.12 % (fermented).

Fermentation procedure

The raw seeds (500 g) were fermented locally using a previously method as illustrated in **Figure 1** [20, 21]. The fermented seeds were pulverized with a mortar and pestle and extracted using the same procedure as for the raw and boiled seed.

Figure 1: Steps of production process for fermented seeds (*Ugba*)

Esterification of lipophilic extracts

The crude n-hexane extracts of the seeds were esterified using 200 mL of 4 % concentrated sulphuric acid in anhydrous methanol [8]. To the mixture in a flask was added 10 mL acetic anhydride and 200 mL of dichloromethane as solvent. The mixture was put on a magnetic stirrer and left overnight. After the esterification procedure, esters were extracted with n-hexane (200 mL), followed by the addition of 200 mL distilled water and swirling. Two layers formed rapidly, the mixture was transferred to a separating funnel and the upper hexane phase containing the fatty acid esters was collected, dried with anhydrous sodium sulphate and filtered into clean glass ware for further analysis.

Gas chromatography-mass spectrometry (GC-MS)

An Agilent GC 7890 BGC gas chromatograph coupled with a mass selective detector 5977A was used (Agilent technologies) for the analysis of the esterified samples. Separations were performed on a HP-5Ms fused-silica capillary column (30 m × 0.25 mm, with a film thickness of 0.25 µm). The injector temperature was 320 °C. The temperature program was: 100 °C to 200 °C at 30 °C/min, 200 °C to 320 °C at 5 °C/min and hold 20 min at 320 °C. The carrier gas was helium at a flow rate of 1 mL/min. The temperatures of interphase, ionization source, and quadrupole were: 300, 230 and 150 °C respectively. The ionization energy was 70 eV. The mass spectrum was continuously acquired from 40 to 800 m/z with 3.12 scan/s in full scan mode. The injection volume was 1 µL of the esterified sample which was injected into the port of the GC. Compounds and the mass spectrum from each peak were identified by comparing to that of the National Institute of Standard and Technology (NIST) library 14 & Kovat's indices of previously isolated compounds [22, 23].

Determination of antimicrobial activity

Bacterial and fungal isolates such as Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Shigella dysenteriae*,

Salmonella typhi, *Proteus mirabilis*, *Corynebacterium ulcerans*, *Escherichia coli*, *Candida krusei*, *Candida albicans* and *Candida tropicalis* were obtained from the Department of Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria. Disc diffusion method [24] was used for the antimicrobial screening of crude extracts of the raw, boiled and fermented seeds. Broth dilution method [25, 26] was used for the determination of minimum inhibitory concentration (MIC). Two-fold serial dilution of extract in sterile broth was done to obtain concentrations of 20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL and 0.63 mg/mL for the raw and boiled seeds. Concentrations of 10 mg/mL, 5 mg/mL, 1.25 mg/mL, 0.63 mg/mL and 0.31 mg/mL were obtained for the fermented seeds. The initial concentration was obtained by dissolving 0.2 g (raw and boiled seeds) and 0.1 g (fermented seeds) of the extracts in 10 mL of the sterile broth.

Chromatographic Separation/Isolation

Column chromatography was performed with silica gel (0.063-0.200 mm), 70-230 mesh (ASTM, Merck KGaA, Darmstadt, Germany). The n-hexane extracts (20 mL) each of the raw, boiled and fermented seeds was loaded on the column. Gradient elution was carried out using solvent mixtures of increasing polarity (95% hexane: 5% ethyl acetate with subsequent increases (5%) in the ethyl acetate and corresponding decreases (5%) in the n-hexane till 100% ethyl acetate was attained). A total of a hundred and seventy fractions each were collected from hexane extract of the raw and boiled seeds. For the fermented seeds, a hundred and forty fractions were collected. Similar fractions were combined on the basis of Thin Layer Chromatography (TLC) analysis which was carried out using different solvent mixture; hexane-ethyl acetate in the ratio 4:1, 7:3, 9:1 and 1:1. They were five sub categories each from the raw, boiled and fermented n-hexane extracts. In this paper, we report the isolation of **compound 1** [raw seed extract (from combined fractions 131-135; hexane: ethyl acetate in the ratio 4:1; R_f -0.5)], **compound 2** [boiled seed extract (fractions 131-135; hexane: ethyl acetate in the

ratio 4:1; R_f -0.4)] and **compound 3** [fermented seed extract (fractions 129-134; hexane: ethyl acetate in the ratio 1:1; R_f -0.4)]. The compounds were isolated from elution with n-hexane-ethyl acetate in the ratio 1:1.

Products Analysis

A Stuart automatic melting point apparatus (SMP40) was used to determine the melting

point of the isolated compounds. Characterization and structure elucidations were carried out using 1D and 2D NMR (Nuclear Magnetic Resonance) spectroscopic techniques. NMR spectra were processed using Topspin 3.2 (Bruker), MestRenova 12[®] and (ACDLABS) ChemsSketch[®] software.

RESULTS AND DISCUSSION

Chemical Composition of Extracts using GC-MS

The GC-MS profile of the esterified (methylation) hexane extracts of the raw, boiled and fermented seeds of *P. macrophylla* is presented in **Table 1**. Hexadecanoic acid methyl ester and methyl 20-methyl heneicosanoate were observed only in the fermented seeds extract. Methyl stearate was identified in the boiled and fermented seeds not present in the raw seeds. The major constituents of the raw and boiled seeds extract is linoleic acid ethyl ester while for the fermented it was the methyl ester linoleic acid. Docosanoic acid ethyl ester and tetracosanoic acid methyl are two constituents that have apparently been metabolised during the fermentation process. Increase in the composition of methyl stearate in the fermented

extract as opposed to the above-mentioned fatty acids could be attributed to the lipase activity of lactic acid bacteria metabolizing individual fatty acids differently [27]. A similar trend of increase in the composition of methyl stearate in fermented seeds has been reported [28]. Hexadecanoic acid ethyl ester, linoleic acid ethyl ester, butyl 9,12-octadecadienoate and ethyl tetracosanoate acid were present in the raw, boiled and fermented seeds in varying concentrations. The percentage composition of the unsaturated fatty acids in the extracts increases with processing, this being more pronounced in the fermented product than in the boiled seeds.

Table 1. Chemical composition of esterified hexane extracts of raw, boiled and fermented seeds of *P. macrophylla* by GC-MS Analysis

Fatty acids	Retention Times			(%) Composition		
	Raw	Boiled	Fermented	Raw	Boiled	Fermented
Hexadecanoic acid, methyl ester	-	-	8.8	-	-	3.8
Hexadecanoic acid, ethyl ester	9.4	9.4	9.4	5.3	4.8	1.0
Linoleic acid methyl ester	10.5	10.5	10.5	3.1	2.4	45.7
Methyl stearate	-	10.8	10.8	-	0.1	1.7
Linoleic acid ethyl ester	11.4	11.3	11.3	47.6	48.2	14.7
Butyl 9,12-octadecadienoate	16.2	16.2	16.2	6.6	7.5	14.4
Methyl 20-methyl-heneicosanoate	-	-	17.3	-	-	2.8
Docosanoic acid ethyl ester	18.8	18.8	-	4.2	3.1	-
Ethyl tetracosanoate	25.3	25.2	25.2	18.3	14.1	2.1
Tetracosanoic acid methyl	36.4	36.2	-	5.7	3.2	-
Total				90.8	83.4	86.2

Composition (%) of saturated and unsaturated fatty acids in raw seeds = 33.5 % and 57.3 % respectively.

Composition (%) of saturated and unsaturated fatty acids in boiled seeds=25.3 % and 58.1 % respectively.

Composition (%) of saturated and unsaturated fatty acids in fermented seeds=11.4 % and 74.8% respectively.

Generally, linoleic acid as the methyl and ethyl ester (an omega-6 fatty acid) was the prominent fatty acid with highest concentration in the fermented seeds (60.4 %). This result agrees with literature reports that linoleic acid is the prominent unsaturated fatty acid present in the seed oil [28, 29]. Increase in the composition of unsaturated fatty acids in the fermented seed extract is likely due to increased hydrolysis of the glycerides (lipid hydrolysis) by the microorganisms which is one of the biochemical changes that fermented seeds of *P. macrophylla* has been reported to undergo [28]. It was observed that although methanol was used for the esterification, both methyl and ethyl esters of some of the fatty acids were identified. This could mean that ethyl esters are naturally present in the seeds of this plant [30]. Linoleic acid is an essential fatty acid required in diet and

known to have the following therapeutic implications such as; preventing high blood pressure, cancer prevention, in the treatment of cardiovascular diseases as found in atherosclerosis and coronary heart disease, as well as having anti-inflammatory and antieczemic properties [31]. Hexadecanoic acid have been reported to have the following medicinal properties; hypocholesterolemic, antifungal, antioxidant and antimicrobial activities [32]. Generally, unsaturated fatty acids in diets have been reported as being able to reduce total cholesterol thus causing a significant reduction in the risk of developing chronic heart diseases [31]. The presence of these bioactive compounds can contribute significantly to the dietary and medicinal potential of *P. macrophylla*.

Antimicrobial activity

The antimicrobial screening results (**Table 2**) showed that the following microbes; Methicillin resistant *S. aureus*, *S. aureus*, *S. dysenteriae*, *P. mirabilis*, *C. ulcerans*, *E. coli*, *C. krusei*, *C. albicans* and *C. tropicalis* were susceptible to the lipophilic extracts of seeds of *P. macrophylla*. This result is in agreement with previous report on the activity of *P. macrophylla* against *E. coli*, *S. aureus* and *C. albicans*. The extracts were however inactive against *S. typhi* despite previous report to the contrary [33]. The initial concentration of the extracts used was 20 mg/mL for the raw and boiled seeds and 10 mg/mL for the fermented seeds. It was however, observed that the hexane extract of the fermented seeds exhibited the highest antimicrobial activity against the microorganisms as compared to the raw and boiled seed extracts thus indicating the effect of fermentation. In addition, only fermented seeds extract was found to be active against *E. coli* and *C. tropicalis*. This increased activity could be due to new metabolites produced by the fermentation process. Metabolites commonly reported as products of the fermentation process

are bacteriocins, organic acids, hydrogen peroxide and ethanol [34].

Fatty acids and monoglycerides are known to exhibit antimicrobial properties such as inhibiting the growth of Gram-negative and Gram-positive bacteria, enveloped viruses, yeast, fungi and parasites that infect both the skin and the mucosa of animals [5, 35]. Thus, fatty acids and mono-glycerides are considered important target molecules for drug applications as in antimicrobial formulations and alternatives to antibiotics against antibiotic-resistant bacteria [5].

Table 2: Antimicrobial activity of lipophilic hexane extracts of raw, boiled and fermented seed of *P. macrophylla*

Test organisms	Zone Raw	of Boiled	Inhibition (mm) Fermented	Standard drugs (µg/mL)	
				Ciprofloxacin	Fluconazole
MRSA	16(5)	0	18(5)	32	NT
<i>S. aureus</i>	17(5)	18(5)	18(5)	35	NT
<i>C. ulcerans</i>	0	16(5)	0	0	NT
<i>E. coli</i>	0	0	17(5)	39	NT
<i>S. dysenteriae</i>	16(5)	18(5)	0	40	NT
<i>S. typhi</i>	0	0	0	42	NT
<i>P. mirabilis</i>	18(5)	0	18(5)	30	NT
<i>C. albicans</i>	0	16(5)	0	NT	33
<i>C. krusei</i>	18(5)	17(5)	19(5)	NT	35
<i>C. tropicalis</i>	0	0	18(5)	NT	32

NT= not tested

Note: The numbers in brackets are the MIC values in mg/mL.

MRSA: Methicillin resistant *Staphylococcus aureus*

Isolation and characterization

Compound 1 was isolated as a yellowish waxy solid from the hexane extract of the raw seeds of *Pentaclethra macrophylla*.

NMR spectral data (ppm): (500 MHz, Chloroform-*d*): signal at 0.88 ppm (position H-24') was deduced as that of the methyl protons, signals at 1.26 ppm (H-4 to 22') were those of the methylene overlap protons in α or β position to the carboxylic group, signal at 1.63 ppm (H-3') was the β -CH₂ protons, signal at 2.35 ppm (H-2') was the α -CH₂ protons to the carboxylic group and signals in the range of 4.21-3.65 ppm (H-1,2,3) were protons of the glycerol moiety. Carbon signal at δ 14.25 ppm (C-24') was that of the methyl carbon, the methylene overlap carbons appeared in the region; δ 34.33-22.84 ppm, carbon signals at δ 63.53, 65.37 and 70.48 ppm (C-3,1,2) were those of the glycerol moiety and finally the ester carbonyl at δ 174.46 ppm (C-1'). The ¹H and DEPT 135 NMR spectral suggested the presence of a monoglyceride (MAGs) skeleton. The spectral also indicated a single linear alkyl chain. The DEPT 135 spectral showed an ester carbonyl at δ 174.46 ppm (**Table 3**). HSQC experiments allowed for assignments of protons to their corresponding carbons: the methyl protons at position δ 0.88 ppm (H-24') were correlated to methyl carbons at 14.25 ppm (C-24'), the bulk methylene protons including the α - and β -CH₂ were also correlated to their

corresponding carbons; [δ 1.26 to 29.86 ppm, 1.63 to 25.09 ppm, 2.35 to 34.33 ppm]. From the HSQC experiment, correlations between the protons and carbons of the glycerol moiety were unambiguously assigned [δ 3.65 to 63.54 ppm, 4.21 to 65.37 ppm and δ 3.93 to 70.48 ppm]. The HMBC experiment showed correlation of the proton signal at δ 2.35 ppm (H-2') to the carbon at δ 174.46 ppm (C-1') which is typical of CH₂ protons adjacent to the carboxyl group.

The ¹H-NMR and DEPT spectral data showed some signals that are evidenced of a 1-monoglyceride such as; proton signals of the glycerol group in the range δ 3.65-4.21 ppm and three carbon signals for the glycerol moiety at δ 63.53, 65.37 and 70.48 ppm. This conforms well to previous reports from literature [6, 36]. From the spectral data, **compound 1** was characterized as a 1-monoglyceride of tetracosanoic acid (**2,3-dihydroxypropyl tetracosanoate**) and was assigned the molecular formula; C₂₇H₅₄O₄. Both methyl and ethyl esters of tetracosanoic acid were identified in the raw, boiled and fermented seed extracts by GC-MS analysis. The extracts of the raw seed had the highest composition (24.0 %). We report the isolation of a 1-monoglyceride of tetracosanoic acid from the raw seed. Melting point of 93.6 °C-96.6 °C (Lit. m.p. = 94-95 °C) [19]. The HREI mass spectrum of **compound 1** was reported previously to show a molecular ion m/z

442 with an intense fragment at m/z 323 due to the loss of $C_4H_7O_4$ [37].

Compound 2 was isolated from the hexane extract of the boiled seeds as a yellowish waxy solid.

NMR spectral data (ppm): (400 MHz, 126 MHz, Chloroform- d): signal at 0.88 ppm (H-25') were those of the methyl protons, signals at 1.25 ppm (H-4-23') were those of the methylene overlap protons in α or β position to the carboxylic group, signal at 1.62 ppm (H-3') was the β -CH₂ protons, signal at 2.35 ppm (H-2') was the α -CH₂ protons to the carboxylic group and signals in the range of δ 4.21-3.60 ppm were protons of the glycerol moiety. From the DEPT spectral, the methyl carbon signal appeared at δ 14.27 ppm (C-25'), the methylene overlap carbons appeared in the region; δ 34.31-22.85 ppm, carbon signals at δ 63.49, 65.34 and 70.44 ppm (C-3,1,2) were those of the glycerol moiety and the ester carbonyl was seen at δ 174.49 ppm (C-1'). As in the case of **Compound 1**, the ¹H and DEPT 135 NMR spectral suggested the presence of a monoglyceride (MAGs) skeleton (Table 3). ¹H COSY correlations between the alkoxy protons in the region δ 4.21-3.60 ppm, together with the HSQC correlations of these protons and their corresponding carbons revealed the presence of a glycerol moiety. HSQC experiments allowed for the following correlations: the methyl protons at position δ 0.88 ppm (H-25') were correlated to methyl carbons at 14.25 ppm (C-25'), the bulk methylene protons including the α - and β -CH₂ were also correlated to their corresponding carbons; [δ 1.25 to 29.92 ppm, 1.29 to 22.97 ppm, 2.35 to 34.33 ppm]. HSQC experiment allowed for correlations between the protons and carbons of the glycerol moiety to be unambiguously assigned [δ 4.17 to 65.34 ppm, 3.93 to 70.44 ppm]. The HMBC experiment showed correlation of the proton signal at δ 2.35 ppm (H-2') to the carbon at δ 174.49 ppm (C-1'). **Compound 2** had a melting range of 73.3 °C to 84.8 °C, it was characterized as a 1-monoglyceride of pentacosanoic acid (**2,3-dihydroxypropyl pentacosanoate**) and

assigned the molecular formula; $C_{28}H_{56}O_4$. Generally, waxy solids tend to have a wide range of melting point. The melting points differs widely, even beyond the expected range, according to factors such as oil content and crystalline structure [38, 39]. This could account for the wide melting point range observed for compounds 2 and 3.

Compound 3 was isolated from the hexane extract of the fermented seeds as a faint yellowish waxy solid. NMR spectral data (ppm): (400 MHz, Chloroform- d): From the ¹H NMR spectral of the **Compound 3**, the methyl protons were at 0.88 ppm (H-31') and the methylene overlap protons in α or β position to the carboxylic group appeared at 1.26 ppm (4-30). Signals at 1.62 ppm (H-3') and 2.35 ppm (H-2') were the β -CH₂ protons and α -CH₂ protons to the carboxylic group respectively. Signals of the glycerol moiety appeared at 3.70 and 3.60 ppm (H-3a and b), position 3.93 ppm (H-2) and 4.21 and 4.15 ppm (H-1a and b). From the DEPT spectral, the methyl carbon signal appeared at position δ 14.25 ppm (C-31'), the methylene overlap carbons appeared in the region; δ 29.29-29.80 ppm (C-4 to 30'), carbon signals at δ 63.49 ppm (C-3), at 65.34 ppm (C-1) and 70.44 ppm (C-2) were those of the glycerol moiety (Table 3). The ester carbonyl was seen at δ 174.49 ppm (C-1'). HSQC experiments allowed for the following correlations: the methyl protons at δ 0.88 ppm (H-31') were correlated to methyl carbons at 14.25 ppm (C-31'), also correlations between the protons and carbons of the glycerol moiety were assigned [δ 4.21 to 65.36 ppm, 3.93 to 70.47 ppm and 3.70 to 63.53 ppm]. The HMBC experiment showed correlation of the proton signal at δ 2.35 ppm (H-2) to the carbon at δ 174.49 ppm (C-1'). **Compound 3** was characterized as a 1-monoglyceride of hentriacontylic acid (**2,3-dihydroxypropyl hentriacontylate**) with the molecular formula $C_{34}H_{68}O_4$. Compound 3 had a melting range of 85.6 °C-91.4 °C (Lit. m.p. = 64-66 °C [40]). The molecular mass of **compound 3** has previously been reported as, HREIMS: m/z 540.5110 [M]⁺ [40].

Table 3. NMR Spectral Characteristic Peaks for the Isolated Compounds 1, 2 and 3.

Positions			¹ H	DEPT 135					
Compound	Compound 2	Compound 3	Compound 1	Compound 2	Compound 3	Compound 1	Compound 2	Compound 3	
1									
1-Ha			4.21(dd, $J=11.7\text{Hz}$, 1H)	4.21(dd, $J=11.7\text{Hz}$, 1H)	4.21(dd, $J=11.7\text{Hz}$, 1H)	65.37	65.34	65.36	
1-Hb			4.15 (dd, $J=11.7\text{Hz}$, 1H)	4.15(dd, $J=11.7\text{Hz}$, 1H)	4.15(dd, $J=11.6$, 6.1 Hz, 1H}				
2			3.93(q, $J=5.1\text{Hz}$, 1H)	3.93 (tt, $J=6.0\text{Hz}$, 1H)	3.93 (m, 1H)	70.48	70.44	70.47	
3-Ha			3.65(ddt, $J=47.8\text{Hz}$, 2H)	3.70 (dd, $J=11.4$, 1H)	3.70(dd, $J=11.4\text{Hz}$, 1H)	63.53	63.49	63.53	
3-Hb				3.60	3.60				
1'						174.46	174.49	174.46	
2'			2.35(t, $J=7.6\text{Hz}$, 2H)	2.35 (t, $J=7.6\text{Hz}$, 2H)	2.35(t, $J=7.6\text{Hz}$, 2H)	34.33	34.31	34.33	
3'			1.63 (p, $J=7.4$, 2H)	1.62 (p, $J=7.4\text{Hz}$, 3H)	1.62(q, $J=7.2\text{Hz}$, 2H)	25.09	25.08	25.09	
4'-22'	4'-23'	4'-29'	1.26 (s, 41H)	1.25 (s, 46H)	1.26 (s, 55H)	29.3-32.1	29.3-32.1	29.29-29.80	
23'	24'	30'	1.26	1.25	1.26	22.84	22.85	22.84	
24'	25'	31'	0.88(t, $J=6.9\text{Hz}$, 3H)	0.88 (t, $J=6.9\text{Hz}$, 4H)	0.88 (t, $J=5\text{Hz}$)	14.25	14.27	14.25	

All three compounds are saturated monoglycerides of fatty acids. Differences were however observed in terms of carbon chain length (**Table 3**). Increase in the fatty acid chain length for the fermented seeds is likely due to hydrolysis by the fermenting microbes. The microorganisms were probably able to metabolize the shorter chain glycerides leading to more long chain monoglycerides in the fermented extract.

CONCLUSION

Bioactive compounds were isolated from the raw, boiled and fermented seeds of *Pentaclethra macrophylla*. Extract of the fermented seeds had the highest composition of unsaturated fatty acids compared to the raw and boiled extracts thus indicating the effect of fermentation. This paper report for the first time

the isolation of 2,3-dihydroxypropyl pentacosanoate and hentriacontylate from this plant to the best of our knowledge.

SUPPLEMENTARY INFORMATION

We have attached a supplementary information on NMR spectra of compounds 1, 2 and 3 in a MS Word format.

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