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The Proximate Composition and Essential Oils of Processed *Pentaclethra macrophylla* Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Author EBE designed the study and supervised the work. Author UCN carried out the work. Author RCO designed the work and performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EBE and RCO managed the analyses of the study. Author UCN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The chemical composition of various stages of processed *Pentaclethra macrophylla* (PM) seeds were investigated using standard analytical procedures. The PM seeds were processed in different stages, of raw (uncooked), 1st cooking (cooked for 16-18 hours and dehulled), 2nd cooking (dehulled sliced seeds cooked for 3 hrs), fermented, salted and fermented. Proximate analysis showed high protein content of highest value of (25.94 ± 0.48%) in fermented sample and 22.42 ± 0.47% in raw sample. Moisture was highest in fermented sample (9.5 ± 0.10%) and lowest in raw sample (4.05 ± 0.57%). Ash content in fermented sample was (5.28 ± 0.35%) and is highest and least in the raw sample (1.08 ± 0.14%). Raw sample has the highest lipid content of (62.75 ± 0.47%) and lowest in salted and fermented sample (43.86 ± 0.58%). Fibre content ranges from 2.64 ± 0.33% in fermented sample to 20.07 ± 0.55% in raw samples. GC/MS analysis of essential oils showed that Raw had eleven essential oils with 9,12–Octadecadienoic acid (96.30%) highest; n-Hexadecanoic acid (0.185%) lowest, 1st cooking has 10 essential oils with methyl ester of 9,12 – Octadecadienoic acid, (95.481%) highest; Cyclopropaneoctanal, 2-Octyl (0.092%) lowest. 2nd cooking has 10 essential oils

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with Oleic acid (94.909%) highest; 9,12-Octadecadienoic acid (0.031%) lowest. Fermented has 9 essential oils with 9,12-Octadecadienoic acid (96.807%) highest; Cycloeicosane (0.064%) lowest. Salted and fermented sample has 6 essential oil with 9, 12-Octadecadienoic acid (55.598%) highest, n-Hexadecanoic acid (0.598%) lowest. The results showed that the seeds could thus served as a functional food and can be added to existing food supplements.

Keywords: Proximate; essentials; oils; processed; pentaclethra; macrophylla.

1. INTRODUCTION

Plants are mainly used as food and drugs for man since the beginning of time and have been in use for the eradication of human sufferings [1]. The link between food and health is not new, but a common theme across multiple cultures. Hippocrates considered a father of western medicine famously stated "let your food be your medicine and your medicine be your food". Ayurveda also taught that food is central to both health and healing. Several academics from the disciplines of ethnopharmacololgy, ehnobotany, anthropology and pharmacy are exploring the food-medicine interface from various perspectives.

Pentaclethra macrophylla is a leguminosae family and sub-family Mimosoideae. Commonly called the African oil bean, Oil bean seed is also known and called "ekpuru", "Ukpaka" or "Ugba" in the South-Eastern part of Nigeria, "Apara" in South- West Nigeria and "Ukana" in South-South Nigeria [2]. It is usually low branching with the seeds used as foods and decorations pinnae in homes and gardens. The flowers are creamy-yellow with spikes. January-May and July-December are periods of the year through which it springs.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

The seeds of *Pentaclethra macrophylla* were obtained from Nkwo-Orodo in Mbaitoli Local Government Area of Imo state, identified by Prof. B.E Okoli in the Plant Science and Biotechnology Department, University of Port Harcourt, Rivers State, Nigeria. The seeds were scrutinized and the bad ones were removed and the good ones were stored in air tight bags for subsequent use.

2.2 Processing the Seeds

The seeds were processed and the different products were into different stages. The raw samples of the seed were stored and used subsequently. For the second treatment which serves as first cooking, the raw seeds were boiled in water for 16-18 h and the rough testae were removed (dehulling). The dehulled seeds were sliced, dried and converted to powder form and kept intact for subsequent use. For the third treatment, it is the second cooking; the cotyledons were boiled again for about 30 minutes and left overnight. Processed seeds were dried and converted to powder by grinding and kept intact for subsequent use. For the fourth treatment, some samples from stage three were packaged for about three (3) days to ferment without salt. For the fifth treatment, salt was included to some samples from stage three and mixed together to taste. They were wrapped for about 7 days to ferment. The fermented samples were dried, ground and packaged for subsequent use.

2.3 Proximate Analysis

The proximate composition of the sample was analyzed using methods described in AOAC 2006 [3] as follows:

2.3.1 Moisture content determination

The different processing stages were subjected temperatures of about 100-105°C to attain a constant weight referred to as dry matter. When these samples are heated the gradual water lost is equivalent to the moisture content .The initial weight of a crucible that was empty was recorded and after heating several times. This weight is known as the constant weight (W1) at a temperature of 105°C using an oven. Finely ground sample of weight 2 g was put into the crucible and weighed together again and recorded (W2), then oven dried and weighed again until constant weighed is obtained (W3).

$$\% \text{ Moisture} = W_2 - W_3/(W_2 - W_1) \times 100$$

2.3.2 Determination of ash content

Very high temperatures of 560 and 600°C were used to heat the sample after using solvent for

extraction. The ash ignited is weighed as ash content of the sample. Crucibles were dried again in order to attain a constant weight for 10 minutes weighed (W1). 2 g ground sample was transferred to the crucible that was previously reweighed (W_2). The content was operating at a temperature of 560°C in a furnace after it was ignited and about 7-8 h left in the furnace to achieve adequate ashing and weighed after cooling in a desiccators (W_3).

% Ash Content = W_3–W_1/(W_2-W_1) x 100

2.3.3 Determination of crude lipid content

This method measures lipid which is extracted by solvents such as petroleum ether or hexane. The extraction is based on the sparing solubility of lipids in water and their considerable solubility in non-polar organic solvents (i.e. solvents of hydrophobic nature). The solvent is evaporated off to get the lipid. The measured lipid consists of all the soluble materials. A clean capacity 500 ml flask with some granules was weighed and recorded (W_1). 300 ml of petroleum ether was added for the extraction (40-60°C) and fitted for soxhlet extraction. The extraction thimble which contains 2 g of the milled seeds and heated for 6 hrs. After solvent recovery, the oil was oven dried at 70°C for 1 hr cooled and then weighed (W_2).

% Lipid content = W_2 – W_1/Wt of sample x 100

2.3.4 Crude fibre content determination

In this principle, samples that are fat-free sample were treated with hot sulphuric acid and NaOH. The residue obtained after extraction and subtraction of the ash becomes the fibre. 2 grams of the sample was initially weighed and put into a cork fitted flask which had 100 ml of 0.25 M sulphuric acid which was boiled and under refluxing for 25 mins. After filtration under suction, the insoluble material was washed with hot water severally to make it acid free, turned into a second flak containing 100 ml of hot 0.31 M sodium hydroxide solution and boiled again under refluxing for 25 mins and quickly filtered under suction. The residue obtained was again washed severally with hot water to make it base free again. After drying, the contents were weighed (C_1) incinerated at 550°C for 2 hours in a furnace, cooled in the desiccators and reweighed (C_2).

% Crude fibre = C_1 – C_2/Wt of original sample x 100

Where C_1 - C_2 = the loss in weight on incineration

2.3.5 Determination of crude protein content

The procedure involves digesting the material in sulphuric acid that is highly concentrated, catalyst like copper sulphate. This is to convert all organic nitrogen present in the sample to ammonium ions. Alkali is used to release ammonia from the distilled into boric acid solution. The distillate titrated with hydrochloric acid is used to calculate the ammonia in the boric acid. For the digestion, exactly 1.5 grams defatted sample in an ash-less filter paper was dipped into 300 ml Kjeldahl flask containing 25 ml of concentrated H_2SO_4 and 2 g of metal catalyst were also introduced into the flask. The digestion stage is maintained for some time until allowed to form a clear green colour, cooled and diluted to 200 ml with distilled water. 200 ml of the distillate in the Kjeldahl flask which contains anti-bumping granules and NaOH 40%. A 250 ml conical flask also contains a mixture of 30 ml 2% boric acid and 4 drops combined indicator was used to collect the ammonia that was released. Distillation was stopped when the colour of the boric acid in the receiving flask changes from purple to pale green. The distillate in (boric-acid ammonia) was titrated against HCl 0.1M.

% N_2 = (14 x M x V_1 x Tv x 100)/(Wt of ground sample(mg) x V_2) x 100

% Crude protein = % nitrogen (N_2) × 6.25

Where

M = actual molarity of acid
T_v = titre volume
V_1= total volume of diluted digest
V_2 = aliquot volume distilled

2.4 Determination of Essential Oils

10 g of the dried milled sample was mixed in dichloromethane, intermittently shaking and after soaking for 5 days, extracted out. By filtering into a quartz beaker, the process was repeatedly carried out for two more consecutive times. The combined aliquot collected was evaporated to about 5 ml. This was purified by passing through a pasture pipette packed anhydrous sodium sulphate and a silica gel on a membrane and air dried to about 2 ml for gas chromatographic analysis GC/MS analysis, this group of powerful instruments interfaced helped to characterize the various compositions. The gas chromatographic Model: 7890A (GC) interfaced with Mass

Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion source temperature at 250°C. Highly pure helium gas (99.9% purity) served as carrier gas , while HP-5 (30 mm X 0.25 mm X 0.320 µm) served as the stationary phase. The oven temperature was at 60°C held for 0.5 minute and ramped to 140°C at the rate of 4°C/minutes holding for a minute, then ramped to 280 degrees while holding for 5 minutes at the rate of 8°C /minutes. 1µ/l was auto injected.

2.5 Statistical Analysis of Data

Data obtained from this study were expressed as mean ± S.E.M. one-way analysis of the variance (ANOVA) and turkey post hoc test was used for the establishment of significance differences set at ($p<0.05$).

3. RESULTS

3.1 Result of Proximate Composition of the Different Processed *Pentaclethra macrophylla* Seeds

Different processing stages of *Pentaclethra macrophylla* seeds and obtained proximate composition results are given in Table 1. The result showed the protein content of the seed was generally high with fermented stage having the highest composition of 25.9433% and the raw with the lowest value of 22.4267%. The raw seed has the lowest moisture value of 4.0500% and fermented stage has the highest value of 9.5167%. The fermented stage has the highest ash value of 5.2833% and the raw has the lowest value of 1.0833%. The lipid content in raw stage recorded (62.7533%) and lowest in salted and fermented stage (43.8667%). Fibre content of the raw seed was the highest (20.0733%) and that of the fermented stage the lowest (2.6400%). The raw stage has the highest carbohydrate content of 7.3833% and the fermented stage has the least value of 3.0733%.

3.2 Results of the Analysis by GC-MS of the Different Processed Samples of *Pentaclethra macrophylla* Seeds

GC/MS quantification of volatile compounds in the different stages of processed *Pentaclethra macrophylla* seeds is presented in Tables 2-6. Raw showed eleven essential oils with 9,12–Octadecadienoic acid (96.30%) highest; n-Hexadecanoic acid (0.185%) lowest, 1st cooking has 10 essential oils with 9,12 – Octadecadienoic acid, methyl ester (95.481%) highest; Cyclopropaneoctanal, 2-Octyl (0.092%) lowest. 2nd cooking has 10 essential oils with Oleic acid (94.909%) highest; 9,12-Octadecadienoic acid (0.031%) lowest. Fermented has 9 essential oils with 9,12-Octadecadienoic acid (96.807%) highest; Cycloeicosane (0.064%) lowest. Salted and fermented sample has 6 essential oil with 9,12-Octadecadienoic acid (55.598%) highest, n-Hexadecanoic acid (0.598%) lowest. Chromatogram generated for the different processed samples of *Pentaclethra macrophylla* Seeds is given in Figs. 1-5.

4. DISCUSSION

Proteins are biological molecules that are required to repair worn out tissues as well as synthesis [4,5]. In the seeds of processed *Pentaclethra macrophylla,* the protein content of the processed *Pentaclethra macrophylla* seeds was very high with the fermented sample having the highest protein content. The protein content increases with processing and this agrees with the work of [6,7] where the crude protein of dawadawa increases from 24.8% to 33.5% with processing. There were increase in protein content of fermented cereals and legume-based food products compared with unprocessed products. These high protein contents imply that the process seeds can contribute significantly to the daily human protein requirement. Moisture in seed was the highest in the fermented samples and lowest in the raw sample. This agrees with

Table 1. Proximate composition (%) of processed *Pentaclethra macrophylla* seeds

Parameters	Raw	1st Cooking	2nd Cooking	Fermented
Protein	22.42 ± 0.47^{ab}	22.50 ± 0.22^{ab}	23.40 ± 0.22^{ce}	25.94 ± 0.48^{d}
Moisture	4.05 ± 0.57^{ab}	4.46 ± 0.06^{abc}	4.99 ± 0.36^{bc}	9.51 ± 0.10^{d}
Ash	1.08 ± 0.14^{a}	1.95 ± 0.13^{bc}	2.09 ± 0.01^{bc}	5.28 ± 0.35^{d}
Lipid	62.75 ± 0.47^{ab}	49.45 ± 0.05^{ab}	48.19 ± 1.05^{c}	43.98 ± 0.18^{de}
Fibre	20.07 ± 0.55^{a}	17.84 ± 0.62^{bc}	16.84 ± 0.30^{bc}	2.64 ± 0.33^{d}
CHO	7.38 ± 0.49^{a}	6.41 ± 0.32^{b}	4.22 ± 0.15^{c}	3.07 ± 0.17^{de}

Values presented are mean ± standard deviation of four determinations. Mean values in each row with different small letter superscripts are statistically significant at $p \leq 0.05$

Table 2. Results of the analysis by GC-MS of raw samples of *Pentaclethra macrophylla* seeds

S/N	Compound	Retention time (min)	Percentage of the total	Molecular formula /weight
1	9,12-Octadecadienoic acid, (E,E)-methyl ester	18.434	0.054	$C_{19}H_{34}O_2$ 294.4721
2	11-Octadecenoic acid, methyl ester	18.491	0.057	$C_{19}H_{36}O_2$ 296.4879
3	Linoleic acid ethyl ester	19.078	0.331	$C_{20}H_{36}O_2$ 308.4986
4	n-Propyl 9-octadecenoate	19.133	0.256	$C_{21}H_{40}O_2$ 324.5450
5	n-Hexadecanoic acid	19.234	0.185	$C_{16}H_{32}O_2$ 256.4241
6	9,12-Octadecadienoic acid (Z,Z)-	19.123	96.301	$C_{18}H_{32}O_2$ 280.4455
7	i-Propyl 9-octadecenoate	19.410	0.471	$C_{21}H_{40}O_2$ 324.5411
8	9,17-Octadecadienal, (Z)-	19.484	0.259	$C_{18}H_{32}O$ 264.4461
9	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	23.575	1.190	$C_{16}H_{22}O_4$ 278.3435
10	Hexane, 2,5-dimethyl-	36.208	0.388	C_8H_{18} 114.2285
11	Trans-4-Oxo-2-pentenoic acid	36.270	0.507	$C_6H_8O_3$ 128.1259

Table 3. Results of the analysis by GC-MS 1st cooking samples of *Pentaclethra macrophylla* seeds

S/N	Compound	Retention time (min)	Percentage of the total	Molecular formula/weight
1	Benzene, 4-ethenyl-1,2-d imethoxy-	9.826	0.127	$C_{10}H_{12}O_2$ 164.2011
2	2',4'-Dimethoxyacetophenone	12.629	0.100	$C_{10}H_{12}O_3$ 180.2005
3	E,Z-5,7-Dodecadien-1-ol acetate	14.797	0.145	$C_{14}H_{24}O_2$ 222.3392
4	n-Hexadecanoic acid	17.382	0.617	$C_{16}H_{32}O_2$ 256.4241
5	9,12-Octadecadienoic acid, methyl Ester, (E,E)-	19.013	95.481	$C_{19}H_{34}O_2$ 294.4721
6	8-Octadecenoic acid, methyl ester	18.506	0.361	$C_{16}H_{30}O_2$ 254.4082
7	Linoleic acid ethyl ester	19.092	0.877	$C_{18}H_{30}O_2$ 278.4296
8	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	23.659	1.532	$C_{16}H_{22}O_4$ 278.3435
9	Cyclopropaneoctanal, 2-octyl-	26.035	0.092	$C_{19}H_{36}O$ 280.4885
10	Trans-13-octadecenoic acid	36.627	0.295	$C_{19}H_{36}O_2$ 296.4879
11	Cis-Vaccenic acid	23.659	0.374	$C_{18}H_{34}O_2$ 282.4614

the work of [8] where processing increased this parameter of African locust bean. High moisture content is an index of spoilage [9]. This implies that the raw seed of *Pentaclethra macrophylla* seeds may have a relatively longer shelf-life than the fermented samples. The ash content was

highest in the fermented sample and least in raw sample an indication of the mineral materials. This implies that the fermented sample has more mineral content than the raw sample. In line with works of [10] that showed that fermentation increases ash content of Ugba. Highest in raw samples and least in the fermented sample was Crude fibre. Adequate intake of dietary fibre can contribute colon cancer, obesity prevention [6]. Processed *Pentaclethra macrophylla* seeds in the raw sample having the highest lipid content, salted and fermented sample having the least lipid content. Processed seeds show highest concentration in raw samples and least values in fermented samples [10,11,12] which show that fermentation lead to decrease in carbohydrate content.

Table 4. Results of the analysis by GC-MS of 2nd cooking samples of *Pentaclethra macrophylla* seeds

S/N	Compound	Retention time (min)	Percentage of the total	Molecular formula/ weight
1	n-Hexadecanoic acid	18.751	0.598	$C_{16}H_{32}O_2$ 166.2170
2	l-(+)-Ascorbic acid 2,6-dihexadecanoate	19.246	0.584	$C_{38}H_{68}O_8$ 652.9417
3	Octadecanoic acid	19.400	0.283	$C_{18}H_{36}O_2$ 284.4772
4	Oleic Acid	19.917	94.909	$C_{18}H_{34}O_2$ 282.4614
5	9,12-Octadecadienoic acid (Z,Z)- 11, 12, 13	21.464	0.031	$C_{19}H_{34}O_2$ 294.4721
6	Methyl 9,12-heptadecadienoate	21.777	0.220	$C_{18}H_{32}O_2$ 280.4450
7	Phthalic acid, cycloheptyl isohexyl ester	23.585	0.521	$C_{18}H_{24}O_4$ 304.3807
8	D-Galactose, 2-amino-2-deoxy-3,4,5,6-tetrakis-O-(trimethylsilyl)-	25.499	1.917	$C_{18}H_{45}NO_5Si_4$ 419.8974
9	Trans-13-Octadecenoic acid	36.295	0.935	$C_{19}H_{36}O_2$ 296.4879

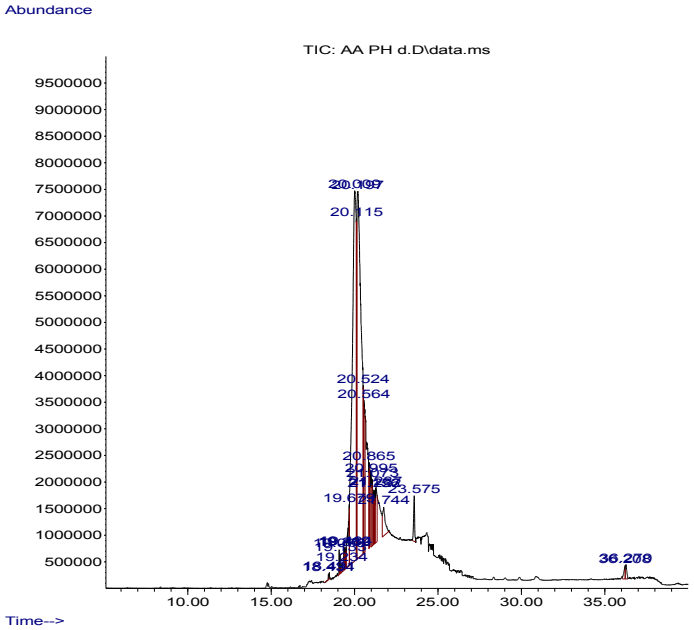

Fig. 1. Chromatogram of essential oils in raw sample

Table 5. Results of the analysis by GC-MS of fermented samples of *Pentaclethra macrophylla* seeds

S/N	Compound	Retention time (min)	Percentage of the total	Molecular formula
1	Benzene, 4-ethyl-1,2-dimethoxy-	9.109	0.285	$C_{10}H_{14}O_2$ 166.2170
2	Cycloeicosane	18.485	0.064	$C_{20}H_{40}$ 280.5316
3	9,12-Octadecadienoic acid (Z,Z)-	20.630	96.807	$C_{18}H_{32}O_2$ 280.4455
4	1,2-Benzenedicarboxy lic acid, mono (2-ethylhexyl) ester	23.474	0.999	$C_{16}H_{22}O_4$ 278.3435
5	Benzene, 1-(chloromethyl)-4-(2-pro penyl)-	25.321	0.088	$C_{10}H_{11}Cl$ 166.6943
6	Benzamide, 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]-	35.895	0.388	$C_{20}HN_2O_2BrF$ 467.0107
7	Phenylserine, 2-fluoro-4,5-dimetho	35.927	0.188	$C_{12}H_2NO_5F$ 259.1268
8	3-(2-Thienyl)-4,5-dihydro-5-isoxazolemethanol	35.955	0.295	$C_8H_9NO_2S$ 183.2276
9	Dodecanoic acid, 1,2,3-propanetriyl ester	38.289	0.886	$C_{39}H_{74}O_6$ 639.0013

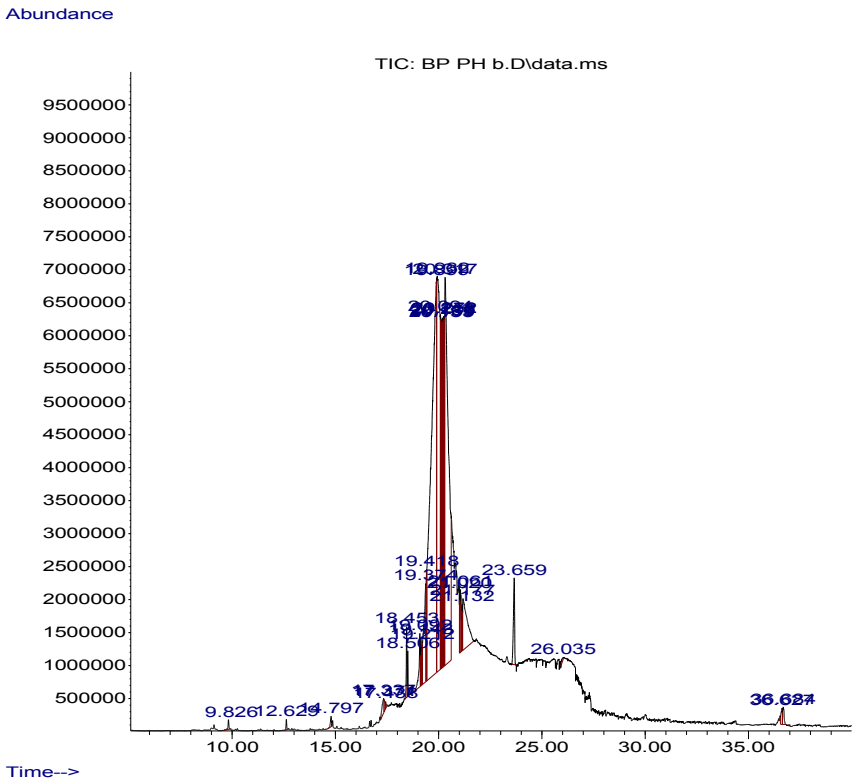

Fig. 2. Chromatogram of essential oils in 1st cooking sample

The raw sample contained eleven essential oils with 9,12-Octadecadienoic acid having the highest concentration and n-Hexadecanoic acid lowest. 1st cooking has ten (10) essential oils with the composition of methyl ester in the 9, 12-Octadecadienoic acid, having highest and cyclopropaneoctanal, 2-Octyl lowest. 2nd cooking has ten essential oils with oleic acid highest in concentration and 9,12-Octadecadienoic acid lowest. Fermented sample has nine essential oils with 9, 12-Octadecadienoic acid highest in concentration and cycloeicosane lowest. Salted and fermented sample has 6 essential oils with 9, 12-Octadecadienoic acid highest in concentration and n-Hexadecanoic acid lowest. Several works studied indicate all the essential oils as antioxidant, anti-inflammatory and anti-cancer agents [13,8,14].

Table 6. Results of the analysis by GC-MS of salted and fermented samples of *Pentaclethra macrophylla* seeds

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula
1	n-Hexadecanoic acid	17.530	0.598	$C_{16}H_{32}O_2$ 256.4241
2	9,12-Octadecadienoic acid (Z,Z)-	19.311	55.598	$C_{18}H_{32}O_2$ 280.4455
3	Phthalic acid, 2-ethylhexyl tetrad ecyl ester	23.895	8.173	$C_{30}H_{50}O_4$ 474.7156
4	5,9-Undecadien-2-one, 6,10-dimethyl-	30.199	7.435	$C_{13}H_{22}O$ 194.3132
5	Octadecane, 1-(ethenyloxy)-	37.252	15.531	$C_{20}H_{40}O$ 296.5310
6	Oleic Acid	37.303	3.800	$C_{18}H_{34}O_2$ 282.4614

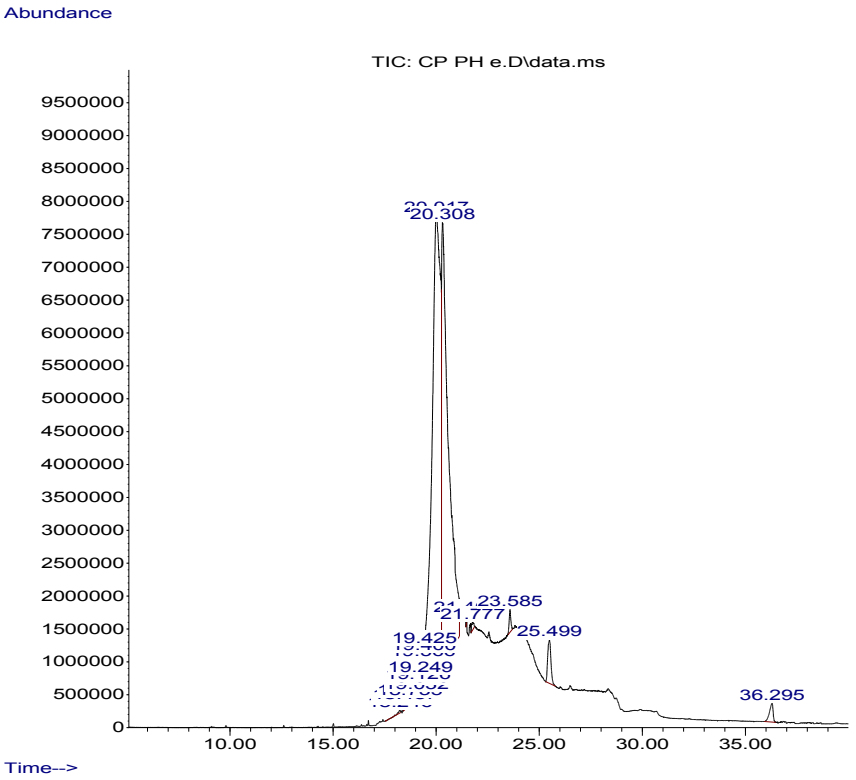

Fig. 3. Chromatogram of essential oils in 2nd cooking sample

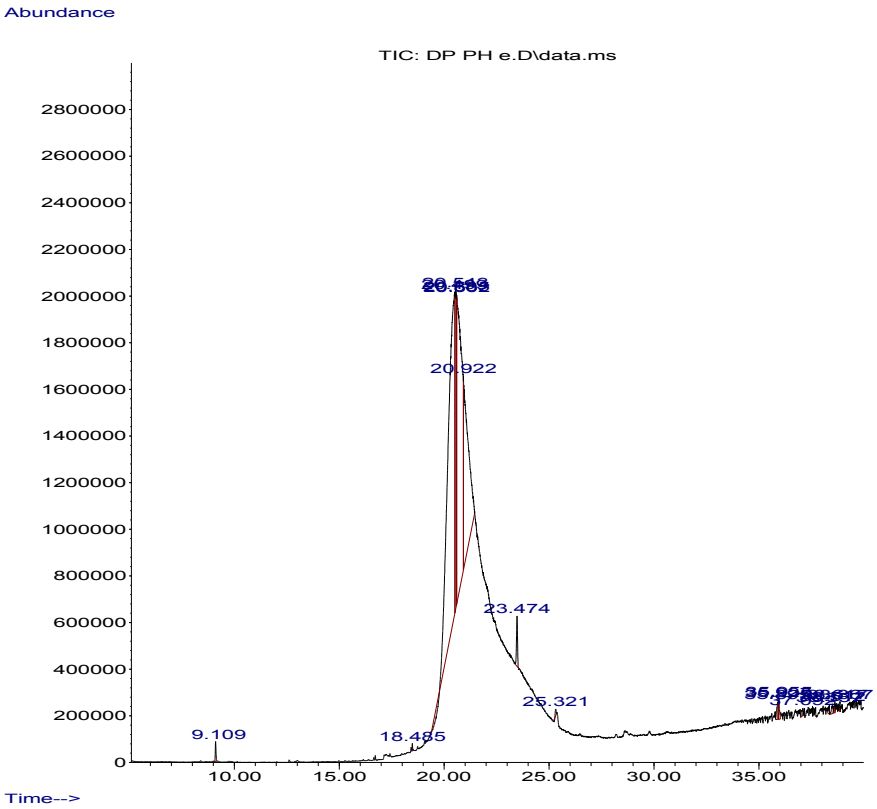

Fig. 4. Chromatogram of essential oils in fermented samples

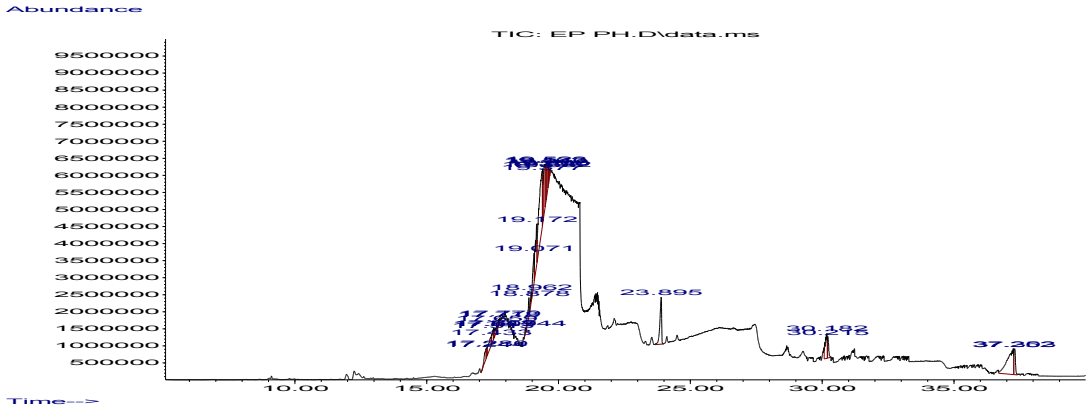

Fig. 5. Chromatogram of essential oils in salted and fermented samples

5. CONCLUSION

From this study, the nutritional and neutraceutical potentials of the processed *Pentaclethra macrophylla seeds* are potentially good sources of protein energy source. Therefore, they can serve as sources of nutrients, especially for the production of food supplements or nutrient concentrates. The composition of essential oils in the seeds can be utilized as functional foods to prevent the occurrences of diseases in the body emanating from varying pathogenesis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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