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IDENTIFICATION AND QUANTIFICATION OF BIOACTIVE COMPOUNDS AND BIO-METALS OF *PTEROCARPUS MILDBRAEDII* DRIED SEED POWDER AND OIL

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ABSTRACT

The seeds of *Pterocarpus mildbraediis* are uncommon among many users of its leaves as vegetable in the preparation of Oha soup. This research work was carried out to ascertain the qualitative phytochemicals, minerals and fatty acid composition of *P. mildbraedii* seed oil. Qualitative phytochemical screening and mineral analysis were carried out on *P. mildbraedii* dried seed powder using standard procedures and Atomic Absorption Spectrophotometer (AAS) respectively. The oil was extracted using cool maceration method and n-Hexane as solvent. This was further subjected to characterization and, identification and quantification of its fatty acid profiling using GC-MS analysis. The result from extraction procedure indicates that n-hexane gave a relatively high percentage yield of 016.036 ± 0.160 (%w/w). Our observation on qualitative phytochemical profiling reveals a distinct partitioning of phytoconstituents between the extracted oil and the dried seed powder, reflecting the differential solubility of compounds in lipophilic and hydrophilic matrices. The extracted oil primarily contains non-polar lipophilic constituents**,** whereas the seed powder retains polar primary and secondary metabolites, including ketose functional groups and free amino acids. Mineral analysis showed the mineral concentrations in the following decreasing order; potassium (19.180 ± 0.100 ppm). sodium (0.880 ± 0.001 ppm), calcium (0.558 ± 0.091 ppm), iron (0.376 ± 0.106 ppm), magnesium (0.193 ± 0.005 ppm), copper (0.014 ± 0.111 ppm), selenium (0.022 ± 0.021 ppm) and zinc (0.020 ± 0.004 ppm). However, only manganese is beyond dictation limits. Our findings indicate a relatively high value of saponification value, percentage glycerine and ester values, but remarkably low levels of iodine, free fatty acid and acid values in comparison with soya beans oil. Our observations from Gas chromatography-mass spectrophotometric analysis of methylated *P. mildbraedii* seed oil indicates that it contains a complex mixture of fatty acid methyl esters, long-chain alcohols, diterpenes, hydrocarbons, and other phytochemicals. These compounds suggest that the seeds oil possesses both nutritional and pharmacological potential, making it a candidate for food, cosmetic, and therapeutic applications.

KEYWORDS: Pterocarpus mildbraediis, Bioactive compounds, Atomic Absorption Spectrophotometer (AAS).

INTRODUCTION

Pterocarpus mildbraedii, commonly known as "Oha" in Eastern Nigeria, is a semi-deciduous tree of the Fabaceae family, indigenous to several African countries including Nigeria, Ghana, Cameroon, Liberia, and Sierra Leone. Traditionally, the leaves are widely used as vegetables in soups, particularly in south eastern Nigeria, and have been the subject of various nutritional and medicinal studies. However, despite the cultural and economic importance of this plant, very little is known about its seed and, more specifically, its seed oil. The seed of *P. mildbraedii* is rarely available, as it is only produced by mature trees aged $\geq$ 20 years. Consequently, most existing research has focused on the more accessible leaves and stems. The lack of scientific data on the seed oil presents a significant research gap, particularly in exploring its potential nutritional, medicinal, or industrial applications. Compounding this scarcity of the seeds is the traditional vegetative propagation practices, although with its challenges. The major difficulty in vegetative propagation of *P. mildbraedii* using its stem cuttings is poor rooting ability. Although recent work by Okoli et al. (2022) introduced pre-treatment with coconut water as a method to enhance rooting success, effective large-scale vegetative propagation remains a bottleneck in making seeds more accessible for research and cultivation. This study aims to address one of these dual challenges; underexplored seed oil composition by investigating the physicochemical properties and nutritional potentials of *P. mildbraedii* dried seed powder and oil. The findings could contribute to both the scientific literature and the broader effort to conserve and commercialize this underutilized African oil rich seed plant species.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Precision weighing balance (Ohaus China), refrigerator (Sonik, Japan), water bath (U-Clear England), spectrophotometer (U-Clear England), incubator (U-Clear England), rotary evaporator (Eyla N-1000, Japan), desiccator, centrifuge (Hanil, MF-80, Korea), soxhlet extractor (U-Clear England), atomic absorption spectrophotometer (Agilent) and Shimadzu GC–MS QP2010 Ultra equipment (Japan), muffle furnace (Heraeus, Germany).

2.1.2 Chemicals and Reagents

The following analytical grade chemicals were used.

a. Qualikems (India)

Acetic acid, Acetone, Aluminium chloride, ammonium hydroxide, Bismuth carbonate, Ethanol, Ferric chloride, Ferrous sulphate, Hydrochloric acid, Lead subacetate, Picric acid, Sodium chloride, Sodium hydroxide, Sulphuric acid (tetraoxosulphate VI acid).

b. Sigma-Aldrich Co. (St. Louis, USA)

A-Naphthol, Ammonia, Ammonium thiocyanate, Chloroform, Potassium bismuth iodide solution, Iodine, Potassium mercuric iodide, Methylene chloride, Potassium iodide, Potassium sulphate, Sodium nitroprusside, Potassium iodide.

c. LobaChemie, India)

Zinc Chloride $ZnCI_2$, Ethyl acetate, n-Hexane, Potassium hydroxide, Rochelle salt, Potassium dehydrogenate phosphate.

2.2 Methods

2.2.1 Reagent Preparations

2.2.1.1 Preparation of Alcholic KOH

A known quantity (22.40 g) of KOH was dissolved in 200 ml of analytical standard ethanol.

2.2.2 Collection of Oha seeds

A known quantity (2.41 kg) of *P. mildbraedii* seeds were collected from different locations within Umuodu village in Amokwe Town in Udi Local Government of Enugu State in Eastern Nigeria. The plant was identified and authenticated by Late Mr. Alfred Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka. However, the seeds were plugged out from winged seeds and 20 g of it were immediately used for proximate analysis, while the rest were dried at room temperature and kept for further use.

2.2.3 Determination of the mineral content of *P. mildbraedii* dried seed powder

(a). Digestion of the samples

P. mildbraedii dried seed powder was assayed for its mineral contents. The plant material was ashed in a Muffle Furnace at 600°C and 10 mL of HNO_3 (50%) was added to 1 g of ground ash each. The solutions were boiled on a hot plate for 30 min. After cooling, H_2O_2 (10 mL) and 10 mL of distilled water were added to each. The mixtures were placed back on hot plate and boiled until their colour changed or those acids boiled off. The whole contents were transferred into 50 mL volumetric flask each and made up to the mark with distilled water.

(b). Determination of mineral contents of the samples

The mineral contents of the digested dried seed powder were estimated using atomic absorption spectrophotometer (AAS). The digested samples aspirated by a tiny tube were heated to a high temperature, so that compound and molecules were converted into free atoms (a process called atomization). Light of specific wavelength (e.g. 450 nm for Ca) emitted from a hollow cathode tube lamp is passed through the flame which contains free atoms that have been generated by thermal-dissociation of the metal complexes/compounds present in the digested sample. Free atoms of element excited at that particular wavelength (450 nm) selectively absorbs monochromatic light set at that wavelength. The unabsorbed light beam then enters monochromator tuned to the same specific wavelength. The detector measures the light intensity after adjusting to zero with the blank. The absorption behaviour follows Beer's Law. Thus, absorbances (A) of standards and samples were measured and concentrations determined using the Beer-Lambert law (A = abc).

Where A is absorbance

a. is the molar absorbtivity with units of L mol^{-1} cm^{-1}
b. is the light path - that is, the path length of the cuvette in which the sample is contained.
c. is the concentration of the metal in solution, expressed in mol L^{-1}

The concentration of the metal was extrapolated from the standard graph of the standard compound used.

2.2.4 Qualitative Phytochemical Screening of *P. mildbraedii* dried seed powder and oil

P. mildbraedii dried seed powder and oil were subjected to qualitative phytochemical screening to detect the presence of major classes of bioactive constituents, the screening was carried out using standard methods described by Harborne (1998), Trease and Evans (1998), and Siddiqui and Ali (1997).

2.2.5 Extraction of oil from dried *P. mildbraedii* seeds

A known quantity (Q ± 0.00 g) of the dried, pulverized fine seed powder was extracted with n-hexane (%w/v) at room temperature using maceration method for 48 hours. The mixture was first filtered using muslin cloth, followed by filtration with Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at a reduced temperature not exceeding 40 °C, to give N ± 0.00 g of crude oil, corresponding to a percentage yield of Y ± 0.00 % (w/w). The extractive yield was calculated using the formula: Yield (%) = [Weight of extract (g)/Weight of plant material (g)] *100.

2.2.6 Characterization of *Pterocarpus mildbreadii* seed oil

2.2.6.1 Percentage Free fatty acid value

Free fatty acid value indicates the measure of fatty acids hydrolysed from esterification bond with glycerol were determined using the method of Association of Analytical Chemists (2005). A known weight of the oil (56.40 g) was accurately weighed into a neatly washed and dried 250 mL Erlenmeyer flask using a precision weighing balance. A known volume (2.00 mL) of phenolphthalein indicator and 50 mL of neutralized ethanol were added, with proper shaken to form homogenous mixture. The mixture was titrated with standard base (0.1N NaOH), with vigorous but constant shaking until the endpoint, indicated by a slight pink colour change that persists for 60s, was reached. The titrant volumes were recorded.

Data and Calculations

Calculate the FFA value of each sample as follows:

%FFA = Titre Value x 0.05

Acid Value = FFA x 1.99 (mg KOH/g oil) (AOAC, 2005).

2.2.6.2 Saponification Value

The saponification value, which represent the amount of alkali needed to saponify a given amount of oil, expressed as mg KOH to saponify 1 g sample, was determined using the method of AOCS (2009). A known weight of the oil 5.0 g was accurately weighed into a neatly washed and dried 250 mL Erlenmeyer flask using a precision weighing balance. This was followed by the addition of 50 mL of alcoholic potassium hydroxide into the flask. Duplicate blank samples were prepared with just 50 mL of alcoholic potassium hydroxide in 250 mL Erlenmeyer flasks without samples. Few boiling beads were added to the flasks with oil sample and were connected .to condenser. These were gently but steadily heated on a hot plate until the sample is clear and homogenous, indicating complete saponification, then the samples were allowed to cool to room temperature after disconnecting the flask from condenser. A known volume (1ml) of phenolphthalein indicator was added into the samples/blank and titrated with 0.5 *N* HCl until the pink colour disappeared. The volumes of titrants used were recorded.

$$\text{Saponification Value} = \frac{(B-S)\times N\times 56.1}{W}$$

2.2.6.3 Iodine Value

The iodine value (or number) which represent the level of unsaturation is the amount in grams of iodine absorbed per 100g sample, was determined using the method of AOCS (2009). A known weight of the oil 0.5g was accurately weighed into a neatly washed and dried 500 mL glass-stoppered conical flask using a precision weighing balance, followed by addition of 10 ml chloroform to dissolve the oil. Two blank samples were prepared by adding only 10 ml

chloroform to 500 ml glass-stoppered flasks. With pipette, 25 ml Wijs iodine solution was added into the sample and blank flasks and were left to stand for 30 min in the dark with occasional shaking. After incubation in the dark, 20 ml potassium iodide solution was added to each flask and were thoroughly shaken. Hundred millilitres (100 mL) of freshly boiled and cooled water was used to wash down any free iodine on the stopper. The final solution was gradually titrated with standard sodium thiosulfate, with constant and vigorous shaking until the yellow colour disappears, and finally followed by the addition of 1ml of starch indicator. The titration continued until the blue colour entirely disappeared. The volumes of titrants used were recorded.

$$\text{Iodine Value} = \frac{126.9\ (B-S)\times N}{W}$$

2.2.6.4 Peroxide values

Peroxide value, the mill equivalents of the amount of peroxide or hydroperoxide groups per kilogram of oil sample, was determined using the method of AOCS, 2009. A known weight of the oil 5.0 g was accurately weighed into a neatly washed and dried 250 mL Erlenmeyer flask using a precision weighing balance, followed by the addition of 30 mL of acetic acid-chloroform solution and was vigorously shaken to dissolve the oil. The blank sample containing only 30 mL of solvent solution, without sample was also prepared. A known volume 5 mL of saturated KI solution was added to both samples and the blank, followed by shaking for 1 min. Distilled/deionized water (30 mL) was added before titration with 0.1 N sodium thiosulfate solution, with vigorous shaking until yellow colour disappeared. A known volume 0.5 mL of 1% starch solution was added before continue the titration with vigorous shaking to release all iodine from chloroform layer, until blue colour disappeared. The volumes of titrants used were recorded.

$$\text{Peroxide Value} = \frac{(S-B)\times N}{W} \times 1000$$

2.2.6.5 Specific gravity/Density of oil sample

The density of the oil sample was measured using a clean dry 25ml capacity density bottle with empty weight of (Y_0). It was later filled with the oil sample, stopper inserted and reweighed to give (Y_1). It was later washed clean and dried using oven. The clean dry 25ml capacity density bottle was filled with sample oil, stopper inserted and reweighed to give (Y_2) Specific gravity = $(Y_1-Y_0)/(Y_2-Y_0)$ = weight of the sample/weight of equal volume of water.

Density = $(Y_1-Y_0)/25$

2.2.6.6 Calculation of Ester value of oil sample

Ester value is calculated as the difference between saponification and acid values. It represents the portion of the oil composed of neutral esters (triglycerides).

Ester Value {EV} = Saponification Value {SV} – Acid value {AV},

% Glycerine = Ester Value x 0.054664

2.2.7 Proximate Analysis

2.2.7.1 Determination of Percentage Moisture Content

The moisture content of *P. mildbraedii* fresh seeds was determined by oven-dry method (AOAC, 2007 and Chang, 2010). Freshly cut seed samples were dried in an oven at 105 ºC until constant weight were reached. Weight readings of the samples were taken before and after drying, the percentage weight loss on drying denoted the amount of moisture in the samples. The percentage moisture contents were expressed as.

Calculations

$$\% \text{ (w/w) LOD} = \% \text{ (w/w) moisture} = 100 \times \frac{\text{wt loss on drying, g}}{\text{wt test portion, g}}$$

% Dry matter = 100 - % LOD

Weight loss on drying = W_2-W_3

Where W_1 = weight of sample alone

W_2 = weight of crucible and sample before drying

W_3 = weight of crucible and sample after drying.

2.2.7.2 Determination of Percentage Ash Content

The ash content of *P. mildbraedii* fresh seeds was determined by direct-method (AOAC, 2005). A known quantity (2.00 g) of freshly cut seed samples were added into crucible that has been ignited, cooled in desiccator, and weighed soon after reaching room temperature. The current weight was noted. These were ignited in furnace at 600 ºC (dull red) until light gray ash results and were finally cooled in a desiccator and weighed soon after reaching room temperature. The ash content was expressed as.

$$\% \text{ (w/w) Ash} = \frac{\text{weigh of test portion, g} - \text{weight loss on ashing, g} \times 100}{\text{weight of test portion, g}}$$

Weight loss on ashing = $\mathbf{W_1}$- $\mathbf{W_2}$

2.2.7.3 Determination of Percentage Protein content

The crude protein contents of both samples were determined by using the micro- Kjeldahl method (AOAC, 2007). A known quantity (0.5 g) of fresh seeds powder of *P. mildbraedii* was added into digestion flask that contains; 1.0 g $CuSO_4$, 10 g powdered K_2SO_4 and 20 ml H_2SO_4. The digestive flask was placed in inclined position and gentle heated until frothing ceases; it was boiled briskly until solution clears and 30 min longer. After digestion, the digest was cooled to room temperature. A known volume of distilled deionized water (200 mL) was used to dissolve the digest. The Kjeldahl flask is connected to distilling bulb on condenser, and with the tip of condenser immersed in 5 ml of 1% Boric acid containing 2 drops of screened methyl red indicator in a conical flask. A known volume (10mL) of digested sample, few zinc granules and 10 ml of 40% NaOH solution were added to the Kjeldahl flask, through a vent on the Kjeldahl apparatus. Immediately, heat was applied on the Kjeldahl flask using a heating mantle set at 100° C until all NH_3 had distilled (≥10 mL distillate), at least for a period of 15 min. Finally, remove receiver, wash tip of condenser, and titrate the distillate with 0.1M of HCl.

$$\% \text{ Protein Content} = \frac{6.25 \text{ x } 20 \text{ x } 0.001412 \text{ x Titer vol x } 100}{\text{Weight of Sample}}$$

2.2.7.4 Determination of Percentage Oil content

The oil content of the sample was determined using the cold method of extraction of (Person, 1980). 10 grams of sample, fresh seeds powder of *P. mildbraedii* was weighed and mixed with 50ml of n-hexane and covered for 1hr. A clean and dry empty container (beaker) was weighed. After 4hours, the mixture of n-hexane and sample was filtered into the clean dried empty beaker using Whatman number filter paper. The filtrate was further heated to dryness in water bath at 50 °C, cooled and weighed. The oil content was calculated as follows:

$$\% \text{ Oil content} = \frac{((\textit{Weight of Beaker after heating to dryness}) - (\textit{Weight of empty Beaker})) \times 100}{\textit{Weight of sample}}$$

2.2.8 GC-MS Analysis

2.2.8.1 Methylation Procedure

Methylation method according to Wang at al. (2015) was used. Hundred microliter (100 µL) of the oil sample was vortex mixed (Biocote Stuart, model R00010, Korea) with 3 mL of methanolic potassium hydroxide (methanolic-KOH) and finally heated in a water bath at 70ºC for 15 min. Immediately, after heating three drops of H_2SO_4 was added, this was followed by 3 ml of distilled water and 3 ml of n-hexane. The whole mixture was centrifuged (Hanil, MF-80, Korea) for 15 min at 3000 rpm, the supernatant was collected and the vialed supernatant was injected into the GC–MS system (Schimadzu, Japan).

2.2.8.2 GC–MS analysis

Separation and molecular identifications of the analytes were carried out with Shimadzu GC–MS QP2010 Ultra equipment (Japan), with injector temperature at 250ºC and using an ultra-inert double taper liner in splitless mode. (RESCEK RXI^{TM} –MS Column, USA (60m x 0.23mmlD x 0.25µm df) was used with a carrier gas (helium) flow rate of 1.88 mL min^{-1}. The temperature started at 100ºC and increased, with a ramp of 100ºC min^{-1}, to 230ºC; this temperature was maintained for 8.0 min and then increased, using a 35ºC min^{-1} ramp, to a final temperature of 280 ºC, maintained for 9.50 min. The transfer line, set at 280ºC, connects the column to an electronic impact source in positive mode (EI^{+}) programmed at 250ºC and 70 eV.

2.2.9 Statistical Analysis

The data analysed by SPSS were recorded as means ± standard deviation. ANOVA procedures were used for One-way analysis of variance. $p < 0.05$ was regarded as significant and $p > 0.05$ was non-significant.

3.0 RESULTS

3.1 Percentage Extractive Yield

n-Hexane yielded a total oil extract of weight of 028.907 ± 0.130 g from an initial powdered seed material weight of 180.254 ± 0.050 g, corresponding to a percentage yield of 016.036 ± 0.160 % (w/w). This yield was calculated based on the weight of extracted oil relative to the initial dried powdered seed material.

3.2 Mineral Composition of *P. mildbreadii* seed powder

Table 1 presents the mineral composition of ***P. mildbreadii*** seed powder. The dried seed powder contained selenium (0.022 ± 0.021 ppm), iron (0.376 ± 0.106 ppm), zinc (0.020 ± 0.004 ppm), copper (0.014 ± 0.111 ppm), calcium (0.558 ± 0.091 ppm), magnesium (0.193 ± 0.005 ppm), sodium (0.880 ± 0.001 ppm) and potassium (19.180 ± 0.100 ppm). However, only manganese was beyond dictation limit.

3.4 Qualitative phytochemical constituents of dried seed powder and oil extracted from *P. mildbreadii* seeds

Qualitative phytochemical screening of dried seed powder and oil extracted from ***P. mildbreadii*** seeds reveals distinct partitioning of phytoconstituents between the extracted oil and the dried seeds powder. The oil extracted with n-hexane tested positive for non-polar lipophilic constituents (resins, saponins, terpenoids, steroids and phytosterols), whereas the seed powder retained primary and secondary polar metabolites, including ketose functional groups and free amino acids.

Figure 1: Dried *P. mildbreadii* seeds.

Figure 2: Oil extracted from *P. mildbreadii* seeds (yellow-green colour).

Table 1: Percentage Extractive Yield of oil from *P. mildbreadii* seeds.

Parameter	Value (g)
Weight of plant material	180.254 ± 0.050
Weight of oil extract	028.907 ± 0.130
Percentage yield (% w/w)	016.036 ± 0.160

Table 2: Mineral Composition of the Dried *P. mildbreadii* Seed Powder.

Mineral	Dried Seed Powder (ppm)
Selenium	0.022 ± 0.021
Iron	0.376 ± 0.106
Zinc	0.020 ± 0.004
Manganese	ND
Copper	0.014 ± 0.111
Calcium	0.558 ± 0.091
Magnesium	0.193 ± 0.005
Sodium	0.880 ± 0.001
Potassium	19.180 ± 0.100

Results are expressed in mean ± SD; n = 3

Table 3: Qualitative Phytoconstituents of dried seed powder and oil extracted from *P. mildbreadii* seeds.

Phytochemical Constituents	Relative presence	
	Extracted oil	Dried Seed Powder
Oil	+++	+++
Amino acids	ND	+++
Carbohydrate	ND	+++
Reducing Sugar	ND	ND
Polysaccharide	ND	ND
Reducing Monosaccharide (Barfoed's Test)	ND	ND
Ketose functional group (Seliwanoff's Test)	++	+++
Secondary metabolites		
Flavonoids	ND	+++
Cardiac Glycosides	++	++
Phenols	ND	+++
Resins	+++	+
Saponins	++	ND
Steroids	+++	
Tannins (Lead acetate Test) Ferric Chloride Test	ND ND	+++ ND
Terpenoids	+++	+++
Alkaloids	ND	++
Polyphenols	ND	+++
Phytosterols	+++	++
Quinones	ND	ND
Anthraquinones	ND	++

Key: +++ = Strong presence; ++ = Moderate presence; + = Mild presence; ND = Not Detected

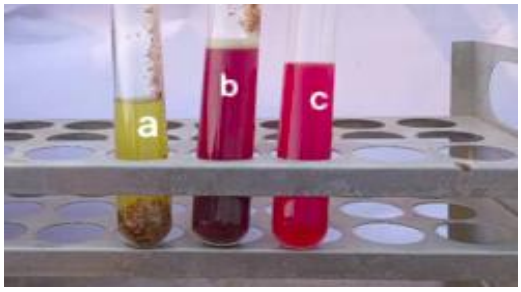


Figure 3: Qualitative test for ketose functional group using Seliwanoff's Test.

a = Negative control sample (seed powder dissolved in ethanol);

b= Test sample (seed powder dissolved in ethanol + Seliwanoff's reagent) and

c = Standard sample (Fructose solution + Seliwanoff's reagent. All heated in boiling water bath for 10min.

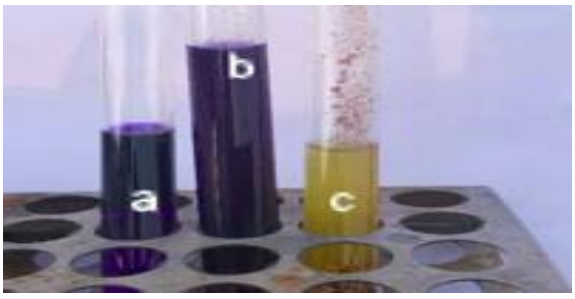


Figure 4: Qualitative test for free amino acids using Ninhydrin Test.

a = Standard sample (Glycine solution + Ninhydrin reagent)

b= Test sample (seed powder dissolved in ethanol + Ninhydrin reagent) and

c = Negative control sample (seed powder dissolved in ethanol). All heated in boiling water bath for 10min.

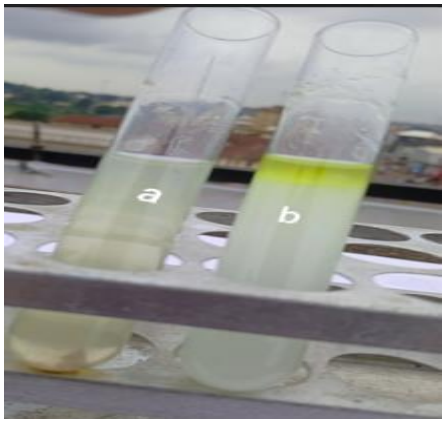


Figure 5: Qualitative Turbidity test for resins.

a = Test sample (seed powder dissolved in 2mL of 98% ethanol + 5 mL of distilled water) and

b = Oil sample (seed oil dissolved in 2mL of 98% ethanol + 5 mL of distilled water).

Table 4: Characterization of Oil extracted from *P. mildbreadii* Seeds.

S/N	Parameters	Value ± SD
1.	**Density (**g/cm³)	1.057 ± 0.00
2.	**Free Fatty Acid** (%)	0.351 ± 0.01
3.	**Acid Value** (mg KOH/g oil)	0.699 ± 0.00
4.	**Peroxide Value**	Nil
5.	**Saponification Value** (mg KOH per g of sample)	550.18 ± 0.31
6.	**Iodine Value** (g iodine absorbed per 100 g of sample)	14.580 ± 0.20
7	**Ester Value**	368.070± 0.03
8.	**% Glycerine**	20.120± 0.15

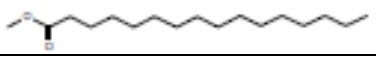
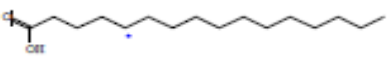
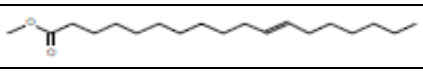
Results are expressed in mean ± SD; n = 3

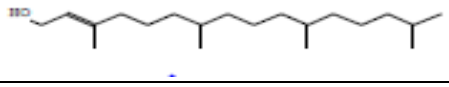
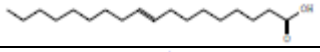
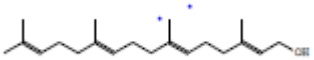
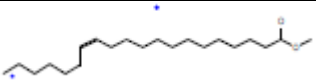
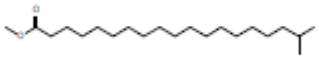
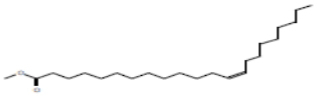
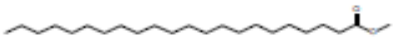
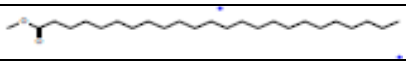
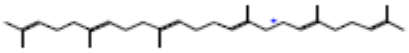
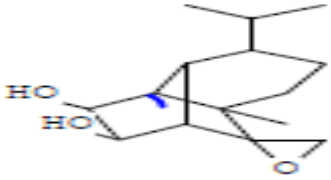
Table 5: Proximate values of Dried *P. mildbreadii* Seed powder

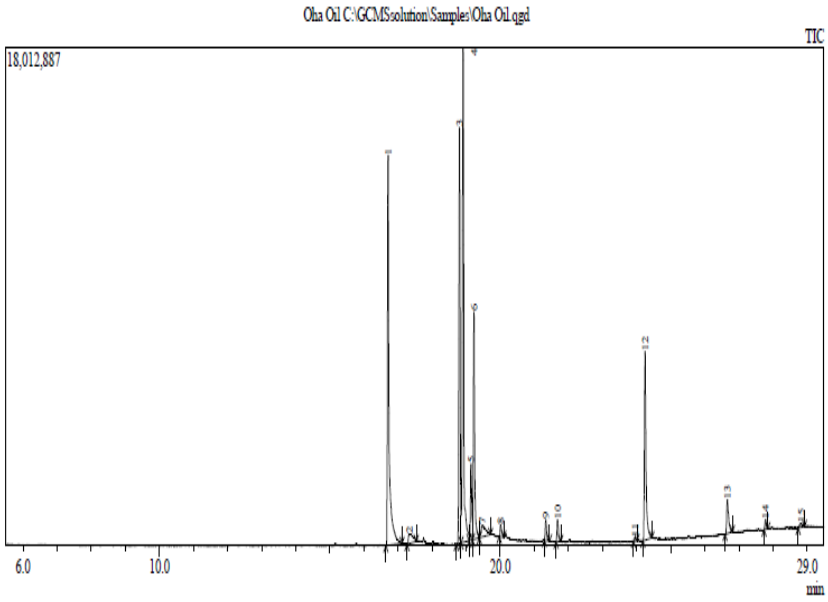
S/N	Parameters	Value ± SD
1.	% Moisture	35.025 ± 0.051
2.	% Dry Matter	64.976 ± 0.003
3.	% Ash	02.335 ± 0.001
4.	% Oil	16.036 ± 0.160
5.	% Protein Content	35.300 ± 0.004
6	% Carbohydrate	11.304 ± 0.026
7.	Total Caloric value	1,383.817 kj/g

Results are expressed in mean ± SD; n = 3

Table 6: Identified Bioactive Molecular Compounds **from GC-MS Chromatogram of *P. mildbreadii* Seed Oil.**

Retention Time	% Area	Molecular Formula	Compound's Name	Molecular Structure
16.708	22.76	($C_{17}H_{34}O_2$)	Hexadecanoic acid Methyl ester	
17.336	1.54	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	
18.804	17.75	$C_{19}H_{34}O_2$	11,14-Octadecadienoic acid, methyl ester	
18.911	24.90	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	

19.140	3.66	$C_{20}H_{40}O$	Phytol 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	
19.228	10.42	$C_{19}H_{38}O_2$	Methyl stearate	
19.468	1.91	$C_{18}H_{34}O_2$	9-Octadecenoic acid, (E)-	
20.011	0.72	$C_{20}H_{34}O$	trans-Geranylgeraniol	
21.334	1.01	$C_{21}H_{40}O_2$	Methyl 13-eicosenoate	
21.680	1.15	$C_{21}H_{42}O_2$	Methyl 18-methylnonadecanoate	
23.947	0.17	$C_{23}H_{44}O_2$	13-Docosenoic acid, methyl ester, (Z)-	
24.248	10.80	$C_{23}H_{46}O_2$	Docosanoic acid, methyl ester	
26.659	2.34	$C_{25}H_{50}O_2$	Tetracosanoic acid, methyl ester	
27.778	0.48	$C_{30}H_50$	Squalene	
28.818	0.41	$C_{15}H_{24}O_3$	Spiro [tricyclo [4.4.0.0(5,9)] decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	



DISCUSSION

Our analysis detected selenium**,** zinc and copper in *P. mildbraedii* seed oil, key micronutrients essential for some metabolic pathways and functioning as cofactors for antioxidant enzymes. Their presence strategically positions *P. mildbraedii* seed oil as a valuable dietary component that may help support oxidative balance and immune health. Our observed high-density value of oil extracted from *P. mildbraedii* seeds, which is slightly greater than the typical range

of 0.91–0.93 g/cm³ for common vegetable oils could be attributed to a higher fraction of long-chain hydrocarbons, presence of more complex lipids (e.g., sterols, alcohols) or potential non-lipid components. This observation could be explained from the correlations between the qualitative phytochemical analysis that indicates strong presence of (resins, saponins, phytosterol and steroids) and physical yellow-green colouration of the extracted oil. The phytochemical profiling reveals a distinct partitioning of constituents between the extracted oil and the dried seed powder, reflecting the differential solubility of these compounds in lipophilic and hydrophilic matrices. The extracted oil primarily contains non-polar lipophilic constituents**,** whereas the seed powder retains polar primary and secondary metabolites**.** The GC-MS data confirms a lipid-rich oil matrix dominated by long-chain saturated and unsaturated fatty acid methyl esters**,** aligning with the positive reactions of qualitative phytochemical tests for oil, steroids, and phytosterols**.** Moreover, our data on low free fatty acid content of *P. mildbraedii* seed oil reflect minimal hydrolytic degradation, indicating freshness and high-quality, which aligns with its high saponification value**,** high ester value, and high glycerine content. These confirm that the oil retains a rich composition of intact triglycerides**,** making it suitable for edible, cosmetic, and industrial applications such as soap and biodiesel production**,** where triglyceride integrity is critical. Low iodine index observed in this current research work signifies a highly saturated oil with few unsaturated fatty acids, implying robust oxidative and thermal stability and a low risk of rancidity. This correlates with our observed zero peroxidation value, which could be further linked to the inherent richness of *P. mildbraedii* seed oil in antioxidants such as squalene and phytol. However, the GC-MS analysis revealed a spectrum of saturated fatty acids including palmitic acid**,** methyl palmitate**,** methyl stearate**,** behenic acid methyl ester**,** lignoceric acid methyl ester**,** and branched-chain fatty acids such as methyl 18-methylnonadecanoate. These long-chain saturated fatty acids contribute to its structural and energetic functions in the body. Observed behenic and lignoceric acids are particularly renowned in industrial and cosmetic applications for their emollient properties (Banov, 2014). In skincare, behenic acid helps to restore the skin's natural oils and improve overall levels of hydration, because it has lubricant, emollient, and soothing properties, and it is favourably soluble against the skin's surface, making it a desirable ingredient to infuse into skincare formulas (Banov, 2014; Pennick, et al., 2012; Raghallaigh, et al., 2012). *P. mildbraedii* seed oil also contains unsaturated fatty acids like methyl linoleate**,** oleate isomers**,** trans-oleate, as well as C20:1 and erucic acid derivatives. Unsaturated lipids, especially essential ones like linoleic acid play protective and structural roles in human health. However, erucic acid should be monitored in dietary formulations due to known toxicity (myocardial lipidosis a reversible accumulation of fat in heart muscle) concerns at elevated levels (Katrine et al., European Food Safety Authority, 2016). Notably, *P. mildbraedii* seed oil contains phytol and geranylgeraniol, compounds integral to biosynthetic pathways of nutritionally important molecules like carotenoids and tocopherols; these compounds are recognized for their antioxidant**,** anti-inflammatory, and antimicrobial activities. Squalene**,** a triterpenoid hydrocarbon, well-documented for its antioxidant and cholesterol-modulating effects in vegetable oils was also detected (Mingke, et al., 2023). Additionally, the detection of a unique spiro-oxirane terpenoid hints at distinctive secondary metabolites not commonly found in conventional seed oils. Squalene, although present in varying concentrations across oils, is widely acknowledged as a potent antioxidant. Its levels and synergistic effects with other antioxidants like tocopherols and sterols can critically influence oxidative stability, especially at elevated temperatures or during storage (Ma, et al., 2023). Relative to conventional seed oils such as soybean or sunflower, *P. mildbraedii* oil stands out for its blend of long- and branched-chain fatty acids, diverse terpenoids, and notable antioxidant profile. This eclectic composition highlights its promise beyond nutrition toward nutraceutical**,** cosmetic, and industrial applications. Our comprehensive chemical and physical assessment of *P. mildbraedii* seed oil portrays it as a nutrient-rich, stable, and bioactive lipid

source. Its favourable profile encourages exploration in functional foods, cosmetics, and therapeutic products, pending further safety evaluations and standardization.

CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

Further research should be particularly directed at verification of ***P. mildbreadii*** vegetable oil toxicity, cytotoxic screening and possibly any new cytotoxic aldehydes and carbonyl on wide panel of cells of different organs, particularly focused on erucic acid content. Scale-up feasibility studies to align with industrial applications. Clinical validation of its identified bioactive molecular compounds health-promoting effects.

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Conflicts of Interest

The author declares no conflict of interest.

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