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Valorization of Underutilized Velvet Bean (*Mucuna pruriens* var. *utilis* and *Mucuna cochinchinensis*) Seeds: Biochemical Composition, Physicochemical Properties and GC-MS Profiling of Extracted Oils

Ozougwu Vincent Eric O¹, Enemmuo Maryann Chidimma^{1,2*}, Ezugwu Arinze L¹, Ogara, Amaechi L³.

¹Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.

²UNESCO International Center for Biotechnology, Nsukka 410001, Enugu State, Nigeria.

³Department of Science Laboratory Technology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Abstract

Underutilized legumes represent promising renewable resources for the discovery of plant-derived biomolecules with potential nutritional and industrial value. This study investigated the biochemical composition and physicochemical characteristics of oils extracted from two velvet bean species, *Mucuna pruriens* var. *utilis* and *Mucuna cochinchinensis*. Proximate and mineral analyses were conducted on the seeds, while the extracted oils were evaluated using physicochemical characterization and gas chromatography–mass spectrometry (GC–MS) profiling. The seeds contained appreciable levels of protein (26.15–27.49%) and carbohydrates (49.43–51.92%), together with essential minerals such as potassium, calcium, and magnesium. Oils extracted from the seeds exhibited high lipid purity (>96%) and moderate extraction yields (5.75–6.10%). Physicochemical analysis revealed differences between the two oils, with iodine values ranging from 15.25 to 22.17 g/100 g and saponification values from 48.92 to 61.32 mg KOH/g, indicating variations in fatty acid composition and degree of unsaturation. Peroxide values remained low, suggesting limited oxidative degradation. Principal component analysis further highlighted physicochemical parameters responsible for the differentiation of the oils. GC–MS profiling identified several lipid-derived compounds, including linoleic acid, palmitic acid, stearic acid, and their methyl esters, reflecting the presence of both saturated and unsaturated fatty acids typical of plant oils. These findings provide new biochemical insights into oils derived from two underutilized *Mucuna* species and support their potential as alternative plant-derived lipid resources for biochemical and industrial applications.

Keywords: Velvet bean; *Mucuna pruriens*; *Mucuna cochinchinensis*; Seed oil characterization; Physicochemical properties; GC–MS profiling; Fatty acid composition.

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*Corresponding Authors: Enemmuo, Maryann Chidimma (maryannenemmuo700@gmail.com)

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INTRODUCTION

Plant-derived oils remain essential raw materials for food, pharmaceutical, cosmetic, and industrial applications because of their biochemical composition and renewable origin [1,2]. The growing global demand for sustainable lipid resources has intensified the search for alternative oilseed crops capable of supplementing conventional sources such as soybean, palm, and rapeseed. In recent years, plant-based oils have gained particular attention due to their diverse fatty acid composition, which contributes to nutritional value, oxidative stability, and industrial functionality [3]. Studies have shown that seed oils rich in unsaturated fatty acids and bioactive compounds are increasingly explored for applications ranging from nutraceutical formulations to biofuels and biodegradable industrial products [1-3]. This renewed interest is driven by concerns over sustainability, supply chain vulnerability, and the need for renewable bioproduct feedstocks derived from plant biomass [4,5]. Legumes represent an important group of plant species with considerable biochemical and nutritional potential [6]. Beyond their role as dietary protein sources, leguminous seeds are recognized for their content of lipids, minerals, and bioactive compounds that may contribute to both nutritional and industrial applications [7,8]. In addition, many legumes contribute to sustainable agriculture through biological nitrogen fixation and adaptation to marginal environments, making them attractive candidates for diversified agricultural systems. Despite these advantages, numerous legume species remain underutilized, particularly in developing regions where traditional crops dominate agricultural production. Underutilized legumes are increasingly recognized as valuable biological resources capable of contributing to food security, biodiversity conservation, and the discovery of new functional biomolecules [6,9]. Among these underexplored legumes, species belonging to the genus *Mucuna* have attracted scientific interest because of their nutritional, medicinal, and biochemical characteristics. *Mucuna* species, commonly known as velvet beans, are tropical legumes widely distributed across Africa, Asia, and parts of the Americas. Their seeds contain substantial quantities of proteins, carbohydrates, minerals, and bioactive phytochemicals, including L-3,4-dihydroxyphenylalanine (L-DOPA), which has recognized pharmaceutical relevance. Previous investigations have also demonstrated that the seeds contain lipids composed of both saturated and unsaturated fatty acids, including palmitic, stearic, oleic, and linoleic acids. These fatty acids are biochemically significant because they influence the nutritional quality, physicochemical stability, and potential industrial applications of the oil fraction derived from the seeds [10,11]. Although *Mucuna pruriens* has received attention for its pharmacological properties, relatively limited research has focused on the oil composition and physicochemical properties of oils extracted

from *Mucuna* seeds. Existing studies indicate that *Mucuna* seed oils contain appreciable levels of unsaturated fatty acids, with linoleic acid frequently reported as the dominant component, followed by palmitic, oleic, and stearic acids [10,11]. The presence of these fatty acids is particularly relevant for industrial applications because unsaturated lipids contribute to oxidative reactivity and functional properties required for processes such as biodiesel production, lubricant formulation, and the synthesis of bio-based chemicals. Earlier investigations on the fatty acid composition of *Mucuna* species have suggested that these seeds may represent a viable alternative source of vegetable oil; however, the available data remain limited and often restricted to a few species or geographical regions [12,13]. Despite these observations, systematic biochemical characterization of oils derived from different *Mucuna* species remains limited. In particular, comparative evaluations of lipid composition, physicochemical parameters, and molecular constituents of oils from *Mucuna pruriens* var. *utilis* and *Mucuna cochinchinensis* are scarce in the literature. This lack of comparative biochemical data limits the understanding of their potential as alternative lipid feedstocks for industrial processing and bio-based product development. Moreover, comprehensive analytical investigations integrating physicochemical analysis with molecular profiling techniques such as gas chromatography–mass spectrometry (GC–MS) are still insufficient for many underutilized tropical legumes. Furthermore, the biochemical characterization of plant oils requires the integration of physicochemical analysis and advanced analytical techniques to understand their composition and potential applications. Analytical approaches such as gas chromatography–mass spectrometry (GC–MS) have become important tools for identifying fatty acids, esters, hydrocarbons, and other bioactive compounds present in plant oils [14,15]. These analytical techniques provide detailed information on the molecular composition of oils and allow the assessment of their suitability for industrial or nutritional applications. In combination with physicochemical parameters such as iodine value, saponification value, peroxide value, and viscosity, GC–MS profiling offers a comprehensive understanding of the chemical characteristics and functional behavior of plant-derived oils. Such integrated biochemical analyses are increasingly applied in the evaluation of oils obtained from underutilized plant resources [14,16]. Despite the agricultural availability of *Mucuna* species in many tropical regions, particularly in West Africa, the biochemical properties and industrial potential of their seed oils remain insufficiently characterized. In particular, limited comparative studies exist examining the oil extracted from different *Mucuna* species, including *Mucuna pruriens* var. *utilis* and *Mucuna cochinchinensis*. Both species are cultivated or consumed in parts of Africa and Asia, yet their lipid composition and physicochemical characteristics have not been extensively investigated in relation to potential industrial applications. A

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detailed biochemical assessment of these oils may therefore provide useful insights into their value as alternative lipid resources derived from underutilized legumes. Therefore, the present study investigated the biochemical and physicochemical characteristics of oils extracted from the seeds of *Mucuna pruriens* var. *utilis* and *Mucuna cochinchinensis*. The study combined proximate and mineral analyses of the seeds with physicochemical evaluation of the extracted oils and molecular characterization using GC–MS profiling. By providing a comparative biochemical assessment of two underutilized *Mucuna* species, this work contributes new data on their lipid composition and offers insights into their potential utilization as alternative plant-derived oils for industrial and biochemical applications.

Sample morphology

Seeds of the two *Mucuna* species investigated in this study are shown in Figure 1, illustrating the distinct seed coloration of *Mucuna pruriens* var. *utilis* (black seeds) and *Mucuna cochinchinensis* (white seeds).



Figure 1: Seeds of two *Mucuna* species used in this study: (A) *Mucuna pruriens* var. *utilis* (black seeds) and (B) *Mucuna cochinchinensis* (white seeds).

Materials and Methods

Sample collection and authentication

Mature seeds of *Mucuna pruriens* var. *utilis* (black seeds) and *Mucuna cochinchinensis* (white seeds) were purchased from Ogige Market, Nsukka, Enugu State, Nigeria (6.86° N, 7.40° E) in August 2024. Botanical identification and authentication were carried out by a taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Voucher specimens were deposited in the University of Nigeria Herbarium with accession numbers UNN/13041 and UNN/13042 for future reference. All chemicals and reagents used were of analytical grade and obtained from commercial suppliers including Randox Laboratories Ltd. (Crumlin, United Kingdom), Inteco Diagnostics Ltd. (London, United Kingdom), Spectrum Diagnostics (Germany), and Calbiotech Inc. (California, United States).

Sample preparation

Seeds were manually sorted to remove foreign materials and damaged seeds and were subsequently sun-dried for fourteen days to reduce moisture content. The dried seeds were milled into fine powder using a laboratory electric grinder (Model FW100 High-Speed Universal Grinder, Taisite Instruments Co., Tianjin, China) to increase surface area and improve extraction efficiency. The powdered samples were stored in airtight polyethylene containers at ambient laboratory temperature (approximately 25 ± 2 °C) until further analyses.

Oil extraction

Seed oil was extracted using Soxhlet solvent extraction according to the standard procedure of the Association of Official Analytical Chemists [17]. Approximately 200 g of powdered seed sample was wrapped in filter paper and placed in a Soxhlet extractor (Buchi Extraction System B-811, Buchi Labor Technik AG, Flawil, Switzerland). Extraction was performed using n-hexane as solvent for 8 h at approximately 68 °C. After extraction, the solvent was removed using a rotary evaporator (Rotavapor R-300, Buchi Labor Technik AG, Flawil, Switzerland), and residual solvent was removed by drying the extracted oil in a laboratory oven (Memmert UF55 Universal Oven, Memmert GmbH, Schwabach, Germany) at 45 °C. The extracted oils were stored in amber bottles in a desiccator until further analyses. Oil yield was calculated using the standard equation:

$$\text{OY (\%)} = (\text{weight of extracted oil} / \text{weight of seed sample}) \times 100.$$

Proximate composition analysis

Proximate composition of the seed samples and extracted oils was determined using standard methods of the Association of Official Analytical Chemists [17]. Moisture content was determined by oven drying at 105 °C to constant weight using a laboratory drying oven (Memmert UF55 Universal Oven, Memmert GmbH, Germany). Ash content was determined by incineration in a muffle furnace (Carbolite Gero AAF 1100 Furnace, Carbolite Gero Ltd., Derbyshire, United Kingdom) at 600 °C for 5 h. Crude protein content was determined using the Kjeldahl nitrogen determination method with a nitrogen-to-protein conversion factor of 6.25 using a Kjeldahl digestion and distillation unit (Kjeltec 2300 Analyzer, Foss Analytical A/S, Hillerød, Denmark). Crude fat content was determined by Soxhlet extraction using petroleum ether (boiling range 40–60 °C). Total carbohydrate content was calculated by difference.

Physicochemical characterization of oils

The physicochemical properties of the extracted oils were evaluated using established analytical methods for vegetable oil characterization. The parameters analyzed included viscosity, smoke point, specific gravity, refractive index, iodine value, saponification value, peroxide value, acid value, free fatty acid content, and ester value. Viscosity was measured using a digital rotational viscometer (Brookfield

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DV-I Prime Viscometer, AMETEK Brookfield, Middleboro, United States) according to ASTM D445 standard methods. Smoke point was determined by gradually heating the oil sample in a porcelain dish using a laboratory heating mantle while monitoring temperature with a digital thermometer (Hanna Instruments HI98501 Thermometer, Hanna Instruments Inc., Woonsocket, United States). The temperature at which continuous bluish smoke was observed was recorded as the smoke point. Specific gravity was determined using a glass pycnometer (50 mL density bottle; Pyrex®, Corning Inc., New York, United States) relative to distilled water. Refractive index was determined using an Abbe refractometer (Abbe Refractometer DR-A1, Atago Co. Ltd., Tokyo, Japan) at 30 °C. Iodine value, saponification value, peroxide value, and acid value were determined using standard titrimetric methods widely applied for characterization of vegetable oils and fats. Free fatty acid content was calculated as percentage oleic acid equivalent. Ester value was calculated as the difference between saponification value and acid value. These physicochemical parameters are routinely used to evaluate oil stability, degree of unsaturation, oxidation state, and industrial suitability of vegetable oils.

Mineral composition analysis

Mineral elements including calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and lead (Pb) were determined using atomic absorption spectrophotometry. Samples were dry-ashed in a muffle furnace at 550 °C, and the ash was dissolved in dilute hydrochloric acid before analysis using an atomic absorption spectrophotometer (PerkinElmer AAnalyst 400 Atomic Absorption Spectrometer, PerkinElmer Inc., Waltham, United States). Sodium (Na) and potassium (K) were determined using a flame photometer (Jenway PFP7 Flame Photometer, Bibby Scientific Ltd., Staffordshire, United Kingdom). Phosphorus (P) was determined spectrophotometrically using the vanadomolybdate method with absorbance measured at 420 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Shimadzu Corporation, Kyoto, Japan).

GC-MS analysis of fatty acids

Fatty acid composition of the extracted oils was determined using gas chromatography-mass spectrometry following the fatty acid methyl ester derivatization procedure. Oil samples were converted into fatty acid methyl esters prior to analysis. Approximately 0.015 g of oil was mixed with 0.20 mL toluene, 1.50 mL methanol, and 0.30 mL hydrochloric acid (8 % w/v), and the mixture was heated at 100 °C for 5 min to promote methyl ester formation. After cooling, 1 mL hexane and 1 mL distilled water were added, and the hexane layer containing the fatty acid methyl esters was separated for analysis. Gas chromatography-mass spectrometry analysis was performed using an Agilent 6890 gas chromatograph

coupled with a 5973-mass selective detector (Agilent Technologies Inc., Santa Clara, United States) equipped with an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL min⁻¹. One microliter of sample was injected with a split ratio of 15:1. The oven temperature program was initially set at 165 °C and increased at a rate of 4 °C min⁻¹ to a final temperature of 290 °C. Mass spectra were recorded at 70 electron volts over a mass range of 29–550 m/z. Compounds were identified by comparison of their mass spectra with those in the National Institute of Standards and Technology (NIST) mass spectral library and by comparison of retention times with available standards. Gas chromatography-mass spectrometry analysis of fatty acid methyl esters is widely used for identification and quantification of fatty acids in plant oils due to its sensitivity and accuracy [18].

Quality control and analytical validation

To ensure analytical accuracy and reproducibility, all experiments were conducted in triplicate and results were reported as mean ± standard deviation. Analytical instruments were calibrated according to manufacturer specifications prior to measurements. Reagents of analytical grade purity were used throughout the study, and all glassware was thoroughly cleaned and rinsed with distilled water before use. For gas chromatography-mass spectrometry analysis, instrument performance was verified using standard fatty acid methyl ester mixtures prior to sample analysis. Compound identification was based on comparison of mass spectra with those contained in the National Institute of Standards and Technology (NIST) Mass Spectral Library and confirmed by retention time comparison where reference standards were available. Only compounds with acceptable spectral matching scores were considered for identification. For physicochemical analyses, blank determinations were carried out where applicable to correct titration values. Replicate measurements were performed to minimize analytical errors and to ensure the reliability of the reported values. The reproducibility of measurements was evaluated using standard deviations obtained from triplicate analyses. These quality control procedures are consistent with recommended practices for analytical studies involving characterization of vegetable oils and lipid-derived compounds.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation. Statistical comparison between the two *Mucuna* species was performed using Student's t-test at a significance level of $p < 0.05$. In addition, multivariate statistical analyses were performed to explore relationships among measured variables. Pearson correlation analysis was used to evaluate relationships between physicochemical parameters, while principal

component analysis was used to identify patterns and clustering among oil samples based on their biochemical and physicochemical characteristics. Statistical analyses were performed using Statistical Package for the Social Sciences software version 26 (IBM Corp., Armonk, United States). Multivariate statistical approaches such as principal component analysis are widely used in food and lipid chemistry studies to interpret complex biochemical datasets and identify relationships among compositional variables.

Results

Proximate composition of Velvet bean seeds

The proximate composition of *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* seeds is presented in Table 1. Both species contained substantial levels of macronutrients, particularly protein and carbohydrates. Moisture content ranged from 10.53 ± 0.15 % to 11.53 ± 0.32 %, while ash content ranged from 3.63 ± 0.03 % to 3.73 ± 0.03 %. Crude fiber content was 4.30 ± 0.10 % in *M. cochinchinensis* and 4.04 ± 0.09 % in *M. pruriens* var. *utilis*. Protein content was slightly higher in *M. cochinchinensis* (27.49 ± 0.09 %) compared with *M. pruriens* var. *utilis* (26.15 ± 0.06 %). Carbohydrate content ranged from 49.43 ± 0.31 % to 51.92 ± 0.17 %, with the highest value observed in *M. pruriens* var. *utilis*. Statistical analysis indicated significant differences ($p < 0.05$) between the two species in moisture, ash, crude fiber, protein, and carbohydrate contents, whereas fat content did not differ significantly ($p > 0.05$).

Table 1: Proximate composition of *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* seeds.

Parameter (%)	<i>M. cochinchinensis</i> (white seed)	<i>M. pruriens</i> var. <i>utilis</i> (black seed)
Moisture	11.53 ± 0.32 ^a	10.53 ± 0.15 ^b
Ash	3.73 ± 0.03 ^a	3.63 ± 0.03 ^b
Crude fiber	4.30 ± 0.10 ^a	4.04 ± 0.09 ^b
Fat	3.84 ± 0.08 ^a	3.75 ± 0.02 ^a
Crude protein	27.49 ± 0.09 ^a	26.15 ± 0.06 ^b
Carbohydrate	49.43 ± 0.31 ^b	51.92 ± 0.17 ^a

Values are mean ± standard deviation (n = 3). Values within the same row with different superscript letters differ significantly ($p < 0.05$).

Proximate composition of extracted oils

The proximate composition of oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* seeds is presented in Table 2. Both oils were characterized by a very high lipid content, exceeding 96% of the total composition, reflecting efficient extraction of the lipid fraction from the seeds. Moisture content ranged from 0.91 ± 0.02 % to 0.96 ± 0.02 %, indicating low residual water in the extracted oils.

Ash content varied between 0.32 ± 0.03 % and 0.41 ± 0.02 %, while crude protein ranged from 0.94 ± 0.02 % in *M. cochinchinensis* oil to 1.15 ± 0.02 % in *M. pruriens* var. *utilis* oil. Carbohydrate content was very low in both oils, ranging from 0.92 ± 0.04 % to 1.00 ± 0.09 %. Statistical comparison indicated that moisture and fat contents were significantly higher ($p < 0.05$) in *M. cochinchinensis* oil, whereas ash and protein contents were significantly higher ($p < 0.05$) in *M. pruriens* var. *utilis* oil. No significant difference ($p > 0.05$) was observed in carbohydrate content between the two oils.

Table 2: Proximate composition of oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis*.

Parameter (%)	<i>M. cochinchinensis</i> oil	<i>M. pruriens</i> var. <i>utilis</i> oil
Moisture	0.96 ± 0.02 ^a	0.91 ± 0.02 ^b
Ash	0.32 ± 0.03 ^b	0.41 ± 0.02 ^a
Fat	96.86 ± 0.02 ^a	96.56 ± 0.05 ^b
Crude protein	0.94 ± 0.02 ^b	1.15 ± 0.02 ^a
Carbohydrate	0.92 ± 0.04 ^a	1.00 ± 0.09 ^a

Values are mean ± standard deviation (n = 3). Values within the same row with different superscript letters differ significantly ($p < 0.05$).

Mineral composition of Velvet bean seeds

The mineral composition of *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* seeds is presented in Table 3. Both species contained appreciable levels of essential macro- and micro-minerals. Potassium was the most abundant mineral detected in both seeds, followed by calcium and sodium. Comparative analysis revealed species-dependent variations in mineral distribution. *M. pruriens* var. *utilis* showed significantly higher concentrations ($p < 0.05$) of potassium, calcium, phosphorus, and zinc, whereas *M. cochinchinensis* contained significantly higher levels ($p < 0.05$) of sodium and magnesium. Iron content did not differ significantly between the two species ($p > 0.05$).

Table 3: Mineral composition of *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* seeds.

Mineral (mg/100 g)	<i>M. cochinchinensis</i> (white seed)	<i>M. pruriens</i> var. <i>utilis</i> (black seed)
Sodium	121.73 ± 0.31 ^a	115.15 ± 0.05 ^b
Potassium	147.18 ± 0.08 ^b	151.48 ± 0.10 ^a
Calcium	130.36 ± 0.08 ^b	138.74 ± 0.05 ^a
Magnesium	39.46 ± 0.05 ^a	35.42 ± 0.08 ^b
Iron	3.82 ± 0.03 ^a	3.45 ± 0.05 ^a
Phosphorus	10.95 ± 0.05 ^b	13.20 ± 0.10 ^a
Zinc	1.33 ± 0.06 ^b	1.55 ± 0.05 ^a

Values are mean ± standard deviation (n = 3). Values within the same row with different superscript letters differ significantly ($p < 0.05$).

Mineral composition of extracted oils

The mineral composition of oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* is presented in Table 4. Overall, the concentrations of minerals in the oil fraction were substantially lower than those observed in the seeds, reflecting the limited solubility of mineral elements in lipid matrices. Among the detected elements, sodium was the most abundant mineral in both oils. Comparative analysis showed that *M. pruriens* var. *utilis* oil contained significantly higher concentrations ($p < 0.05$) of sodium, potassium, magnesium, calcium, and phosphorus than *M. cochinchinensis* oil. In contrast, zinc content was significantly higher ($p < 0.05$) in *M. cochinchinensis* oil. Iron content did not differ significantly between the two oils ($p > 0.05$).

Table 4: Mineral composition of oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis*.

Mineral (mg/100 g)	<i>M. cochinchinensis</i> oil	<i>M. pruriens</i> var. <i>utilis</i> oil
Sodium	4.31 ± 0.03 ^b	4.63 ± 0.03 ^a
Potassium	2.34 ± 0.04 ^b	2.54 ± 0.02 ^a
Magnesium	0.35 ± 0.02 ^b	0.40 ± 0.02 ^a
Calcium	1.73 ± 0.02 ^b	1.80 ± 0.02 ^a
Iron	1.13 ± 0.03 ^a	1.17 ± 0.02 ^a
Phosphorus	0.25 ± 0.03 ^b	0.32 ± 0.03 ^a
Zinc	0.70 ± 0.02 ^a	0.11 ± 0.02 ^b

Values are mean ± standard deviation ($n = 3$). Values within the same row with different superscript letters differ significantly ($p < 0.05$).

Physicochemical properties of extracted oils

The physicochemical properties of oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* are

summarized in Table 5. The oils differed slightly in visual appearance, with *M. cochinchinensis* oil exhibiting a light-yellow color, while *M. pruriens* var. *utilis* oil appeared yellowish-brown. Oil yield ranged from 5.75 ± 0.42 % to 6.10 ± 0.02 %, with *M. pruriens* var. *utilis* producing a significantly higher yield ($p < 0.05$). Viscosity values were similar for both oils, and no statistically significant difference ($p > 0.05$) was observed. Likewise, refractive index and specific gravity values were identical for both samples. Several chemical indices differed significantly between the oils. *M. pruriens* var. *utilis* oil exhibited significantly higher iodine value, saponification value, peroxide value, acid value, free fatty acid content, and ester value compared with *M. cochinchinensis* oil ($p < 0.05$). In addition, the smoke point was higher in *M. pruriens* var. *utilis* oil. These variations indicate differences in the degree of unsaturation and chemical composition between the oils extracted from the two *Mucuna* species.

Multivariate analysis of physicochemical properties

Principal component analysis (PCA) was applied to examine patterns in the physicochemical characteristics of the oils extracted from the two *Mucuna* species. The PCA score and loading plot. Figure 2, shows a clear separation between *Mucuna cochinchinensis* oil and *Mucuna pruriens* var. *utilis* oil along the first principal component, indicating differences in their physicochemical profiles. Variables associated with lipid reactivity and compositional characteristics, including iodine value, saponification value, peroxide value, acid value, free fatty acid content, and ester value, contributed strongly to the observed separation of the oils. In contrast, viscosity and smoke point contributed less to the differentiation pattern, reflecting the similarity of these parameters between the two species

Table 5: Physicochemical properties of oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis*.

Property	<i>M. cochinchinensis</i> oil	<i>M. pruriens</i> var. <i>utilis</i> oil
Color	Light yellow	Yellowish-brown
Oil yield (%)	5.75 ± 0.42 ^b	6.10 ± 0.02 ^a
Viscosity (mPa·s)	55.40 ± 0.10 ^a	56.07 ± 0.42 ^a
Smoke point (°C)	112.61 ^b	116.30 ^a
Specific gravity	0.92 ± 0.00 ^a	0.92 ± 0.00 ^a
Refractive index	1.46 ± 0.01 ^a	1.46 ± 0.00 ^a
Iodine value (g/100 g)	15.25 ± 0.02 ^b	22.17 ± 0.29 ^a
Saponification value (mg KOH/g)	48.92 ± 1.43 ^b	61.32 ± 2.89 ^a
Peroxide value (meq/kg)	3.42 ± 0.03 ^b	3.58 ± 0.08 ^a
Acid value (mg KOH/g)	1.34 ± 0.02 ^b	1.42 ± 0.03 ^a
Free fatty acid (mg KOH/g)	0.67 ± 0.01 ^b	0.71 ± 0.01 ^a
Ester value (mg KOH/g)	47.93 ± 1.46 ^b	60.37 ± 2.80 ^a

Values are mean ± standard deviation ($n = 3$). Values within the same row with different superscript letters differ significantly ($p < 0.05$). mPa·s: millipascal-second (dynamic viscosity unit); KOH: potassium hydroxide; g/100 g: grams per 100 grams of oil; meq/kg: milliequivalents of active oxygen per kilogram of oil; °C: degrees Celsius.

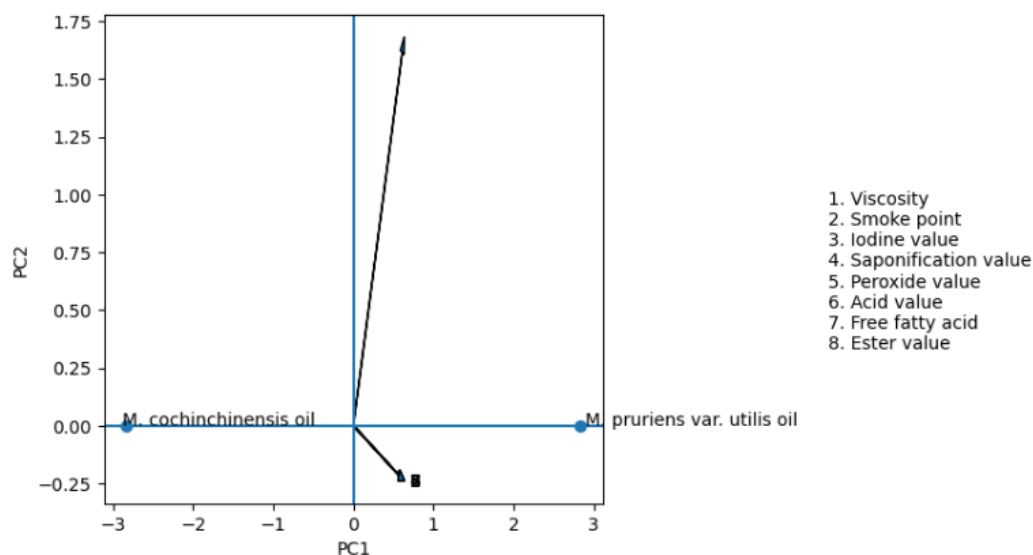


Figure 2: Principal component analysis (PCA) score and loading plot showing the separation of *Mucuna cochinchinensis* oil and *Mucuna pruriens* var. *utilis* oil. Loading vectors represent the contribution of physicochemical variables to the principal components: viscosity, smoke point, iodine value, saponification value, peroxide value, acid value, free fatty acid, and ester value. PC1 and PC2 denote the first principal component and second principal component, respectively.

GC–MS profiling of *Mucuna* seed oils

Gas chromatography–mass spectrometry (GC–MS) analysis was performed to identify the lipid-derived compounds present in oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis*. The chromatographic profiles revealed several fatty acids and lipid-related compounds characteristic of plant-derived oils. In *Mucuna cochinchinensis* oil, linoleic acid (9,12-octadecadienoic acid) was the most abundant fatty acid detected, followed by palmitic acid (n-hexadecanoic acid). Other identified fatty acids included stearic acid (octadecanoic acid) and myristic acid (tetradecanoic acid). In addition, monoacylglycerol derivatives such as monopalmitin and monolinolein were detected, indicating the presence of glyceride-related lipid constituents. The complete list of compounds detected in *Mucuna cochinchinensis* oil is provided in Supplementary Table S1. In *Mucuna pruriens* var. *utilis* oil, several fatty acid methyl esters were identified, including methyl palmitate,

methyl linoleate, methyl elaidate, and methyl stearate. These compounds correspond to methylated derivatives of common fatty acids found in plant oils and are typically observed following fatty acid methyl ester derivatization prior to GC–MS analysis. The full list of compounds detected in *Mucuna pruriens* var. *utilis* oil is provided in Supplementary Table S2. A summary of the major fatty acids and lipid-related compounds identified in the oils of both *Mucuna* species is presented in Table 6, while additional minor lipid-related compounds and phytochemicals detected in the GC–MS analysis are listed in Supplementary Table S3. Representative GC–MS chromatograms of the oils extracted from *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* are shown in Figures 3 and 4, respectively. The chromatograms illustrate the distribution of lipid-derived compounds detected in the oils, including fatty acids, fatty acid methyl esters, monoacylglycerols, and other naturally occurring lipid-related molecules.

Table 6: Major fatty acids and lipid-related compounds identified in oils of *Mucuna cochinchinensis* and *Mucuna pruriens* var. *utilis* by GC–MS.

Compound	Chemical class	<i>M. cochinchinensis</i> oil (% peak area)	<i>M. pruriens</i> var. <i>utilis</i> oil (% peak area)
Linoleic acid (9,12-octadecadienoic acid)	Polyunsaturated fatty acid	9.37	–
Palmitic acid (n-hexadecanoic acid)	Saturated fatty acid	8.79	–
Stearic acid (octadecanoic acid)	Saturated fatty acid	1.88	–
Myristic acid (tetradecanoic acid)	Saturated fatty acid	1.88	–
Monopalmitin (2-monopalmitin)	Monoacylglycerol	4.22	–
Monolinolein	Monoacylglycerol	0.88	–
Methyl palmitate	Fatty acid methyl ester	–	2.01
Methyl linoleate	Fatty acid methyl ester	–	1.25
Methyl elaidate	Fatty acid methyl ester	–	0.54
Methyl stearate	Fatty acid methyl ester	–	0.48

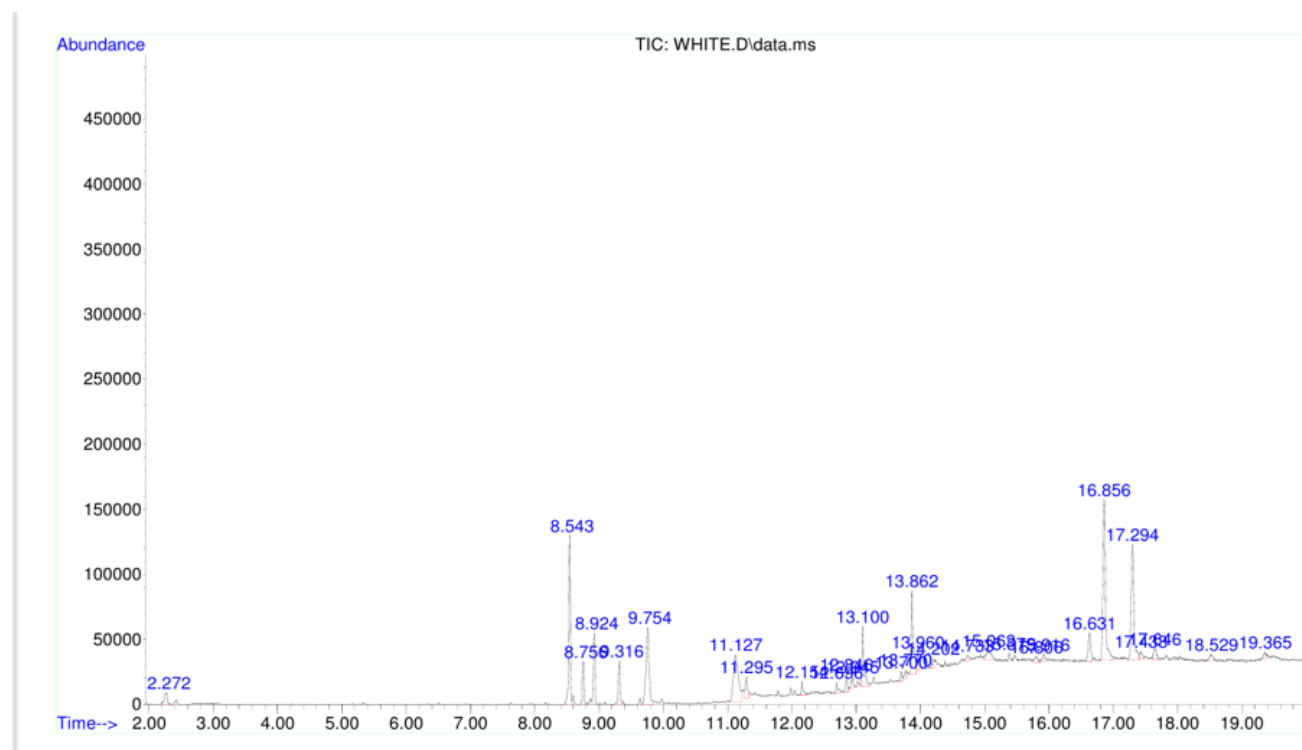


Figure 3: Gas chromatography–mass spectrometry (GC–MS) chromatogram of oil extracted from *Mucuna cochinchinensis* seeds.

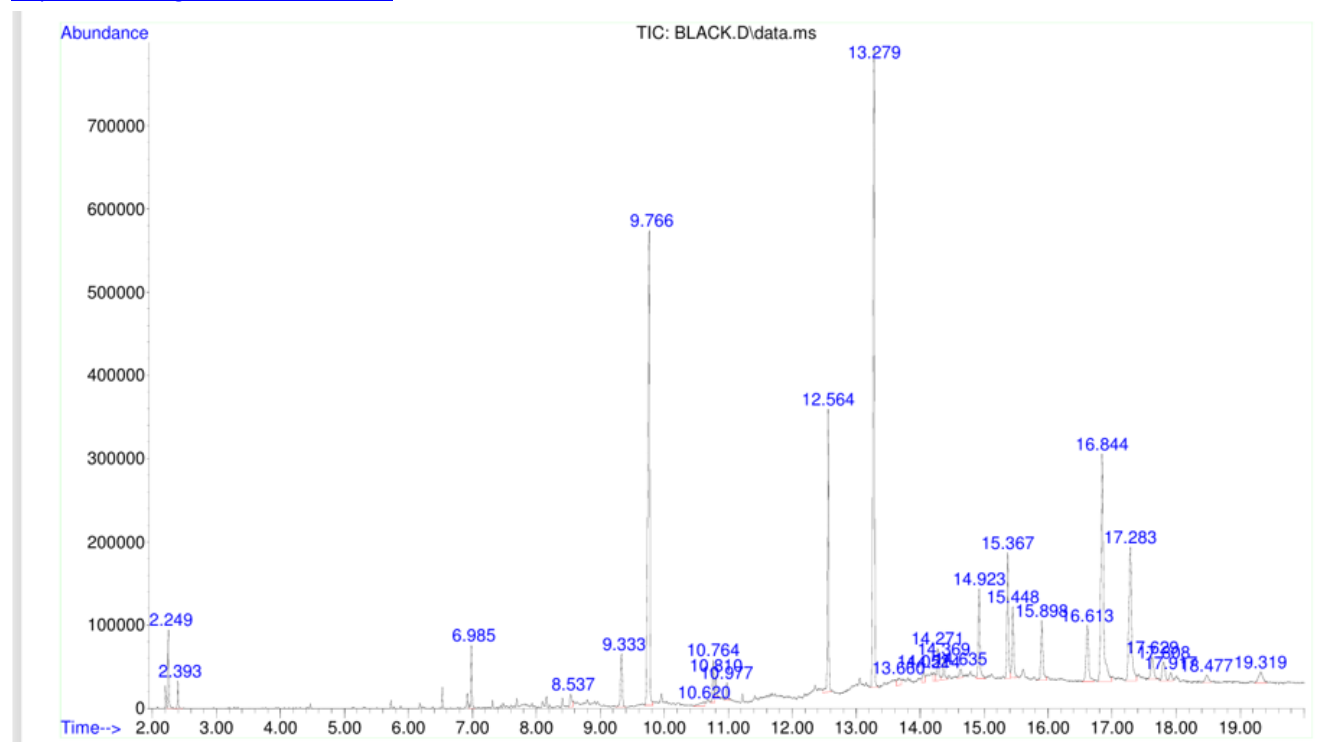


Figure 4: Gas chromatography–mass spectrometry (GC–MS) chromatogram of oil extracted from *Mucuna pruriens* var. *utilis* seeds.

Discussion

Plant-derived oils obtained from underutilized legumes are increasingly being investigated as potential sources of nutritionally valuable and industrially relevant biomolecules. The present study provides a comparative biochemical characterization of seeds and oils obtained from *Mucuna pruriens* var. *utilis* and *Mucuna cochinchinensis*, two velvet bean species that remain relatively underexplored despite their agricultural availability in tropical regions. The results presented here integrate proximate composition, mineral profiling, physicochemical characterization of extracted oils, and molecular identification of lipid-derived compounds using GC–MS. Interpreting these findings in the context of existing literature provides insight into the biochemical characteristics of these underutilized seeds and their potential relevance as alternative plant-derived lipid resources.

Nutritional composition of velvet bean seeds

The proximate composition results indicate that both *Mucuna* species contain substantial amounts of protein and carbohydrates, suggesting that the seeds may represent nutritionally valuable plant materials. Protein content ranged between approximately 26–27%, which is comparable to values reported for several legume crops. A similar study reported comparable protein levels in *Mucuna pruriens* seeds, indicating that velvet bean seeds contain nutritionally significant macromolecules that may contribute to dietary protein supply [10,11]. The carbohydrate content observed in

the present study further suggests that velvet bean seeds may serve as energy-rich plant resources. Previous studies have reported that velvet bean seeds contain appreciable carbohydrate fractions that contribute to their caloric value and functional food properties [11]. Beyond their nutritional relevance, the combined presence of proteins, carbohydrates, and lipids highlights the multifunctional biochemical composition of velvet bean seeds. Underutilized legumes with balanced macronutrient profiles are increasingly recognized as promising biological resources capable of contributing to food security and diversified agricultural systems.

Mineral composition and micronutrient relevance

The mineral analysis revealed that both *Mucuna* species contain measurable levels of essential macro- and microelements, including potassium, calcium, magnesium, iron, and zinc. Potassium was the most abundant mineral detected in both seeds, followed by calcium and sodium. These minerals play important physiological roles in human nutrition, including maintenance of electrolyte balance, bone metabolism, and enzymatic activity. Previous studies investigating mineral composition in underutilized legumes have reported similar mineral distributions. A study on the nutritional composition of tropical legume species reported potassium and calcium as dominant mineral elements in several leguminous seeds, highlighting their contribution to dietary micronutrient intake [7]. The differences observed between the two *Mucuna* species in mineral concentrations may reflect species-specific physiological characteristics or

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environmental factors affecting nutrient uptake. Previous studies have shown that the mineral composition of legume seeds can vary depending on genetic background, soil properties, and environmental conditions during cultivation [19,20], indicating that both genotype and growing environment influence mineral accumulation in plant seeds. The presence of these micronutrients suggests that velvet bean seeds may contribute to nutritional mineral intake while also serving as potential raw materials for nutritionally relevant plant products.

Physicochemical properties and potential industrial relevance of the oils

The physicochemical properties of vegetable oils provide important indicators of their chemical composition, oxidative stability, and potential industrial applicability. In the present study, differences observed in iodine value between the two *Mucuna* oils suggest variations in the degree of unsaturation of their lipid fractions. Oils with higher iodine values generally contain greater proportions of unsaturated fatty acids, which influence oxidative reactivity and chemical functionality in lipid systems. Previous studies investigating vegetable oils have shown that iodine value is commonly used as an indicator of the degree of unsaturation and can reflect variations in fatty acid composition of plant oils [21,22]. The saponification values observed in the present study also provide insight into the molecular characteristics of fatty acids present in the oils. Saponification value reflects the average molecular weight of fatty acids in lipid materials and is frequently used to evaluate oils for industrial applications such as soap production and biodiesel processing. Previous research has reported that vegetable oils with higher saponification values generally contain shorter-chain fatty acids that contribute to enhanced soap-forming capacity and improved processing characteristics [23], indicating that this parameter can provide information on potential industrial utility. The relatively low peroxide values observed in the oils suggest that limited oxidative degradation occurred prior to analysis. Peroxide value is commonly used as an indicator of primary oxidation products formed during lipid oxidation. Peroxide value is widely used as an indicator of primary oxidation products formed during the early stages of lipid oxidation in vegetable oils. Previous studies have shown that low peroxide values in freshly extracted vegetable oils generally indicate minimal oxidation and acceptable oxidative stability during the early stages of storage [24,25], suggesting that the oils characterized in this study were not extensively oxidized during extraction or handling.

Multivariate interpretation of physicochemical variation

Multivariate statistical analysis provides an effective approach for examining relationships among physicochemical variables in complex biochemical datasets. In the present study, principal component analysis revealed a clear separation between oils extracted from *Mucuna*

cochinchinensis and *Mucuna pruriens* var. *utilis*, indicating that specific physicochemical parameters contributed to differences between the two oils. The PCA results suggest that iodine value, saponification value, and ester value contributed strongly to the separation observed between the oils, suggesting that the primary variation between the oils was associated with differences in lipid composition and reactivity. It has been demonstrated that principal component analysis can be applied to differentiate vegetable oils based on their physicochemical properties and fatty acid composition. A study on multivariate analysis of food chemical datasets reported that PCA is particularly useful for identifying variables responsible for sample differentiation and for interpreting relationships among compositional parameters [26], indicating that multivariate statistical tools can enhance the interpretation of biochemical datasets. The PCA results obtained in the present study therefore provide additional insight into the physicochemical relationships between the oils and highlight the parameters that contribute most strongly to their differentiation.

Fatty acid composition revealed by GC–MS profiling

GC–MS profiling identified several fatty acids and fatty acid methyl esters, including linoleic acid, palmitic acid, and stearic acid derivatives. Linoleic acid and palmitic acid were among the major compounds detected, indicating that the lipid fraction of these oils contains both saturated and unsaturated fatty acids commonly found in plant oils. Fatty acids such as linoleic acid are widely distributed in vegetable oils and contribute to nutritional value as well as physicochemical functionality. A study examining the chemical composition of velvet bean seeds reported that palmitic acid, stearic acid, and linoleic acid are among the dominant fatty acids present in *Mucuna pruriens* seed oil [13], indicating that the fatty acid profile observed in the present study is consistent with earlier reports. A previous review of *Mucuna pruriens* phytochemistry reported that seed lipids commonly contain palmitic, stearic, oleic, and linoleic acids [12], highlighting the characteristic fatty acid composition of this genus. The presence of both saturated and unsaturated fatty acids suggests that oils derived from these *Mucuna* species may possess physicochemical properties suitable for a range of applications. However, further studies evaluating large-scale extraction processes, oxidative stability, and industrial performance would be required to confirm their suitability for specific industrial uses.

Limitations of the study

Several limitations should be considered when interpreting the findings of this study. First, the seed samples analyzed were obtained from a single geographical location, and environmental factors such as soil composition, climate conditions, and agronomic practices may influence the biochemical composition of plant seeds. Previous studies on legume crops have demonstrated that environmental conditions and genotype-environment interactions can

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significantly affect nutrient composition and mineral accumulation in plant seeds [19,20]. Second, the GC–MS analysis performed in this study primarily provided qualitative identification of fatty acid methyl esters and related lipid compounds. Complementary analytical techniques such as gas chromatography with flame ionization detection (GC–FID) or nuclear magnetic resonance spectroscopy could provide more detailed quantitative information on lipid composition. Finally, the potential industrial applications suggested for the oils were inferred from physicochemical characteristics rather than validated through direct industrial processing experiments. Future investigations evaluating large-scale extraction efficiency, refining processes, and long-term oxidative stability would therefore provide a more comprehensive assessment of their potential utility.

Conclusion

This study provides a comparative biochemical characterization of seeds and oils obtained from two underutilized velvet bean species, *Mucuna pruriens* var. *utilis* and *Mucuna cochinchinensis*. The seeds contained appreciable levels of proteins, carbohydrates, and minerals, suggesting potential nutritional value. The extracted oils exhibited physicochemical characteristics typical of plant-derived lipids, including measurable iodine and saponification values that reflect differences in fatty acid composition between the two species. GC–MS profiling revealed the presence of several fatty acids and lipid-derived compounds, including linoleic acid, palmitic acid, and stearic acid, which are commonly associated with vegetable oils. Multivariate statistical analysis further highlighted physicochemical parameters that contribute to differences between the oils. Overall, the findings contribute new biochemical data on oils derived from two underutilized *Mucuna* species and suggest that these seeds may represent potential alternative plant-derived lipid resources. Further investigations focusing on large-scale processing, oil stability during storage, and industrial performance would be necessary to evaluate their practical applications.

Authors contribution

Maryann Chidimma Enemmuo: Conceptualization, methodology, investigation, writing – original draft, Visualization, formal analysis., **Vincent Ozougwu:** Conceptualization, methodology, investigation, manuscript editing and review, supervision., **Arinze Ezugwu:** Conceptualization, methodology and supervision., **Augustina Chinyere Amaefula:** Formal analysis, manuscript writing and editing, data curation and validation, **Okechukwu Kalu Iroha:** Formal analysis, manuscript writing and editing, data curation and validation., **Emeka John Dingwoke:** Formal analysis, validation, writing original manuscript draft, review and editing.

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