



EVALUATION OF *MUCUNA* SPECIES AS PROTEIN AND CARBOHYDRATE SOURCES: NUTRITIONAL QUALITY AND BIOPROSPECTING

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ABSTRACT:

Mucuna species are widely recognized for their nutritional and medicinal importance and are commonly used in traditional food and herbal formulations. The present study aimed to estimate the major nutritional components, namely proteins, fats, and carbohydrates, from different species of *Mucuna*. The samples were collected, dried, powdered, and analyzed using standard biochemical methods. Protein content was estimated by the Kjeldal method, fat content by Soxhlet extraction, and carbohydrate content by the Anthrone method. The results revealed significant variation in the levels of proteins, fats, and carbohydrates among the different *Mucuna* species analyzed. Certain species exhibited higher protein and carbohydrate content, indicating their potential as a nutritional supplement, while fat content varied moderately among samples. The findings of the study highlight the nutritional diversity of *Mucuna* species and support their potential use in food, nutraceutical, and pharmaceutical applications.

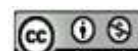
Keywords:- *Mucuna species*, Protein estimation, Fat estimation, Carbohydrate estimation, Nutritional analysis.

INTRODUCTION:

Leguminous crops are fundamental to global food security due to their dense nutritional profiles and their ecological role in enhancing soil fertility through atmospheric nitrogen fixation (Kaushik *et al.*, 2018; Dissanayaka *et al.*, 2024). Within the Fabaceae family, the genus *Mucuna* (L.) DC. has emerged as a focal point of research due to its multifaceted nutritional, medicinal, and functional food properties (Jorge *et al.*, 2007; Pathania *et al.*, 2020). Distributed across tropical and subtropical regions of Asia, Africa, and the Americas, *Mucuna* species have a long history of traditional use as food, fodder, and ethnomedicine, particularly among rural and indigenous communities (Adanson, 1763; Lampariello *et al.*, 2012).

The seeds of *Mucuna* species are characterized by high concentrations of proteins, carbohydrates, dietary fibers, and essential minerals, positioning them as a viable alternative to conventional protein

sources (Baby *et al.*, 2023; Sowdhanya *et al.*, 2023). Beyond basic nutrition, these seeds are rich in bioactive constituents, most notably L-DOPA (Levodopa)—the metabolic precursor to dopamine—alongside various phenolics and antioxidants (Longhi *et al.*, 2011; Rai *et al.*, 2020). Consequently, *Mucuna* is recognized for its neuroprotective, anti-parkinsonian, anti-inflammatory, and anti-diabetic activities (Rachsee *et al.*, 2021). This intersection of dietetic value and therapeutic efficacy underscores its potential as a sustainable resource for both human health and functional food formulation (Pathania *et al.*, 2020). Despite this potential, many *Mucuna* species remain underutilized. Their integration into mainstream diets is hindered by a lack of standardized processing techniques and the presence of anti-nutritional factors (ANFs) such as tannins, phytates, trypsin inhibitors, and cyanogenic glycosides (Pugalenthi *et al.*, 2005;



Ezegbe *et al.*, 2023). These compounds can significantly impair nutrient bioavailability and cause adverse physiological effects if consumed without adequate treatment (Laurena *et al.*, 1994). Furthermore, significant inter-species variation exists in nutrient composition, influenced by genetic diversity, environmental factors, and maturity stages at harvest.

There is, therefore, a critical need for systematic comparative studies to identify species with superior nutritional and bioactive profiles. This research evaluates the nutritional composition of various *Mucuna* (L.) DC. species, with a focus on macronutrients (protein, fat, carbohydrates). By providing a rigorous biochemical characterization, this study seeks to provide the scientific evidence required to promote *Mucuna* as an affordable, high-quality nutritional resource for modern food systems.

MATERIALS AND METHODS

.1 Determination of Crude Fat Content (Soxhlet Extraction Method)

The crude fat content of the sample was determined using the Soxhlet extraction method with hexane as the solvent. The procedure was conducted as follows:

1. Preparation: A 5 g sample (Ws) was accurately weighed and placed into a cellulose thimble. The initial weight of a clean, dry round-bottom flask (W1) was recorded.
2. Extraction: The thimble was inserted into the Soxhlet extraction tube. Hexane (boiling point: 69°C) was used as the solvent, and the extraction process was maintained for 4 hours.
3. Evaporation: Following extraction, the thimble was removed. The solvent containing the fat extract was evaporated by submerging the flask in a water bath maintained at 100°C.
4. Drying and Weighing: To ensure the removal of all residual moisture and

solvent, the extract was dried in a hot air oven at 100°C for 30 minutes. The flask was then cooled to room temperature in a desiccator, and the final weight of the flask containing the dried fat (W2) was recorded.

Calculation: - The percentage of crude fat was calculated using the following formula:

$$\text{Crude fat \%} = \frac{(W2 - W1)}{W_s} \times 100$$

Where:

Ws: Weight of the sample (5 g)

W1: Weight of the empty round-bottom flask

W2: Weight of the flask plus the dried fat extract

1.2 Determination of Crude Protein (Kjeldahl Method)

The crude protein content of the samples was determined using the Kjeldahl method. This process involves three distinct stages: acid digestion, distillation, and back-titration.

- I. Digestion: A 2 g sample was transferred into a Kjeldahl digestion flask. To this, 20 mL of concentrated H₂SO₄ and 2 g of a catalyst mixture (K₂SO₄:CuSO₄ in a 1:1 ratio) were added. The flask was swirled to ensure thorough mixing and placed on a heating mantle. Digestion was maintained for approximately 3 hours until the solution became clear with a characteristic pale green colour. Once cooled, the digest was transferred to a 100 mL volumetric flask and diluted to the mark with double distilled water.
- II. Distillation: A 10 mL aliquot of the digest was introduced into the distillation unit. To this, 50 mL of 40% NaOH and 50 mL of distilled water were gradually added to

neutralize the acid and release ammonia gas (NH₃). The distillation was carried out for 45 minutes. The liberated NH₃ was collected as ammonium hydroxide (NH₄OH) in a receiving conical flask containing 30 mL of 4% boric acid solution and a modified indicator (methyl red and bromocresol green, 1:1 ratio). The appearance of a yellowish colour indicated the absorption of ammonia.

III. III. Titration: The distillate was titrated against a standard 0.1 N HCl solution until a pink endpoint was observed. The volume of titrant used for the sample was recorded as V_s. A blank titration (V_b) was performed using acetanilide or tryptophan with 1 g of saccharose to account for any residual nitrogen in the reagents.

Calculations: The nitrogen content (%N) was first determined, and the crude protein percentage (%P) was calculated by multiplying the nitrogen content by the protein conversion factor (6.25).

Formula for Nitrogen %:

$$\% N = \frac{(V_s - V_b) \times N \times 1.4007}{W}$$

Formula for Crude Protein %:

$$\text{Crude Protein \%} = \% N \times 6.25$$

Where:

V _s	:	Volume of HCl used for sample (ml)
V _b	:	Volume of HCl used for blank (ml)
N	:	Normality of HCl (0.1N)
W _s	:	Weight of the sample (g)

1.3 Determination of Total Carbohydrate Content

The total carbohydrate content was determined using the Anthrone method, following the protocols

described by Yemm and Willis (1954) and Sadasivam and Manickam (1992).

Sample Preparation and Extraction: To remove interfering soluble sugars and non-carbohydrate impurities, 200 mg of the sample was homogenized in 80% ethanol. The homogenate was centrifuged at 3000 rpm for 10 minutes, and the supernatant was discarded; this ethanol washing step was performed twice. The resulting pellet was air-dried to remove residual ethanol.

For the extraction of insoluble carbohydrates (starch/glycogen), 5 mL of distilled water was added to the dried pellet. The mixture was heated in a boiling water bath for 30 minutes to facilitate solubilization. After cooling, the mixture was centrifuged, and the clear supernatant was collected for the assay.

Anthrone Assay and Quantification: The assay utilized a freshly prepared 0.2% Anthrone reagent dissolved in ice-cold concentrated sulfuric acid (H₂SO₄). The reaction was carried out as follows:

- **Reaction:** 1 mL of the aqueous extract was mixed with 4 mL of the chilled Anthrone reagent.
- **Incubation:** The mixture was heated in a boiling water bath for 10 minutes, followed by rapid cooling in an ice bath to stabilize the colour development.
- **Measurement:** The absorbance was measured at 620 nm using a spectrophotometer.

Calculation: A standard curve was generated using glucose concentrations ranging from 6.25 to 100 µg/mL. The carbohydrate concentration in the sample was determined by interpolating the absorbance values against this curve. Final results were expressed as milligrams of carbohydrate per gram (mg/g) of sample

RESULTS AND DISCUSSION

The proximate composition of the four *Mucuna pruriens* accessions was evaluated to determine their nutritional potential. The results revealed

significant variability ($p < 0.05$) in fat, protein, and carbohydrate concentrations among the wild and cultivated accessions.

3.1 lipid Profile and Total Fat Content

The total fat content across the samples ranged from approximately 2.1 g/100g to 6.3 g/100g.

Notably, sample S-4 (*Mucuna pruriens* Wild accession – Aurangabad) exhibited the highest lipid concentration (6.299 ± 0.624 g/ 100g), while S-1 and S-3 maintained lower relative concentrations (Table. 1).

Table 1: Total Fat Content of different *Mucuna* species and accessions

Sample ID	Species / Accession	Total Fat Content (g/ 100g)*
S-1	<i>Mucuna pruriens</i> Wild	4.066 ± 0.115
S-2	<i>Mucuna pruriens</i> var. <i>utilis</i>	5.566 ± 0.404
S-3	<i>Mucuna hirsuta</i>	4.099 ± 0.100
S-4	<i>Mucuna pruriens</i> Wild (Aurangabad)	6.299 ± 0.624

The data represents the mean values of triplicate measurements along with their standard deviation (\pm SD).

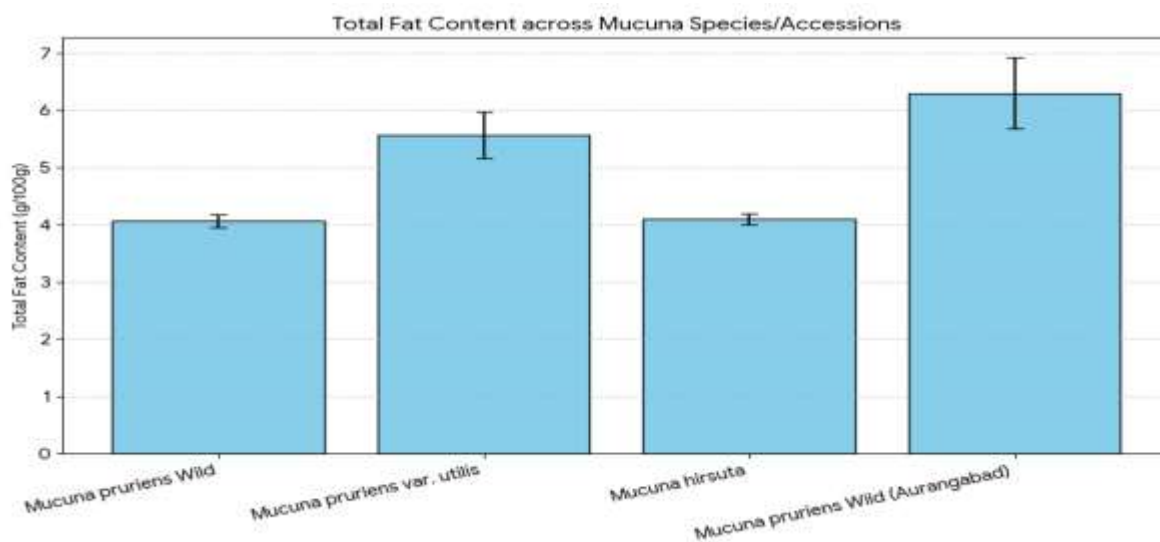


Fig.1. Variation in total fat content in different *Mucuna* species/ accessions

Collection of Zooplankton

These findings are consistent with the variability reported by Chinapolaiah et al. (2019), who noted that biochemical compositions in *Mucuna* germplasm are heavily influenced by geographic and genetic factors. Variation in total fat content in different *Mucuna* species/ accessions (Fig.1)

showed elevated fat content in the Aurangabad accession suggests its potential as a more energy-dense food source compared to other wild varieties.

3.2 Protein Concentration

Total Protein Content of different *Mucuna* species and accessions (Table 2) recorded protein analysis indicated that S-1 (Wild accession) recorded the

highest concentration (2.518 ± 0.022 g/ 100g), followed by S-2. Conversely, S-4, despite its high

fat content, showed the lowest protein concentration.

Table 2: Total Protein Content of different *Mucuna* species and accessions

Sample ID	Species / Accession	Total Protein Content (g/100g)*
S-1	<i>Mucuna pruriens</i> Wild	2.518 ± 0.022
S-2	<i>Mucuna pruriens</i> var. <i>utilis</i>	2.256 ± 0.044
S-3	<i>Mucuna hirsuta</i>	2.067 ± 0.041
S-4	<i>Mucuna pruriens</i> Wild (Aurangabad)	1.583 ± 0.209

The data is presented as the Mean Standard Deviation (SD) of triplicate determinations.

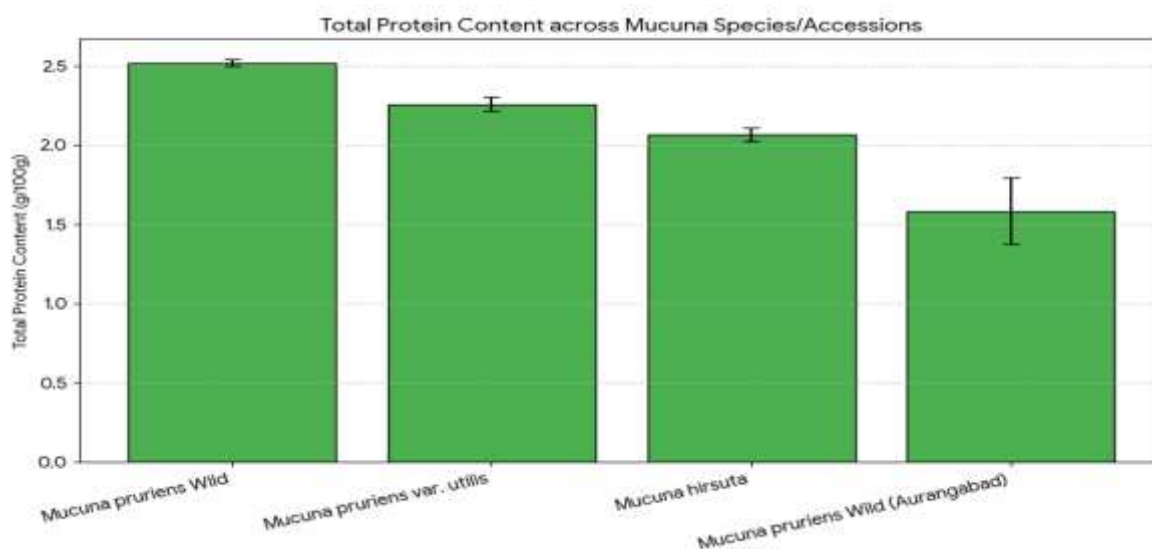


Fig.2. Variation in total seed protein in *Mucuna* species/ Accessions

While *Mucuna* is generally recognized as a sustainable protein source (Baby et al., 2023), the values observed in this study are lower than some processed seed flours, which often range higher depending on the extraction method (Ezegbe et al., 2023). This suggests that while S-1 is superior in protein among the tested samples (Fig. 2), processing techniques such as fermentation or soaking may be required to enhance protein

bioavailability and reduce anti-nutritional factors (Álvarez et al., 2024; Nwaoguikpe et al., 2011).

3.3 Carbohydrate Content and Calorific Value

Carbohydrate content was the most dominant macronutrient in all samples (Table 3). S-4 exhibited a significantly higher carbohydrate concentration (76.488 ± 0.026 g/100g) compared to S-2, which showed the lowest.

Table 3: Total Carbohydrate Content of different *Mucuna* species and accessions

Sample ID	Species / Accession	Total Carbohydrate Content (g/100g)*
S-1	<i>Mucuna pruriens</i> Wild	50.595 ± 0.129
S-2	<i>Mucuna pruriens</i> var. <i>utilis</i>	38.943 ± 0.229
S-3	<i>Mucuna hirsuta</i>	45.446 ± 0.249
S-4	<i>Mucuna pruriens</i> Wild (Aurangabad)	76.488 ± 0.026

The values represent the mean of triplicate analyses (SD) standard deviation.

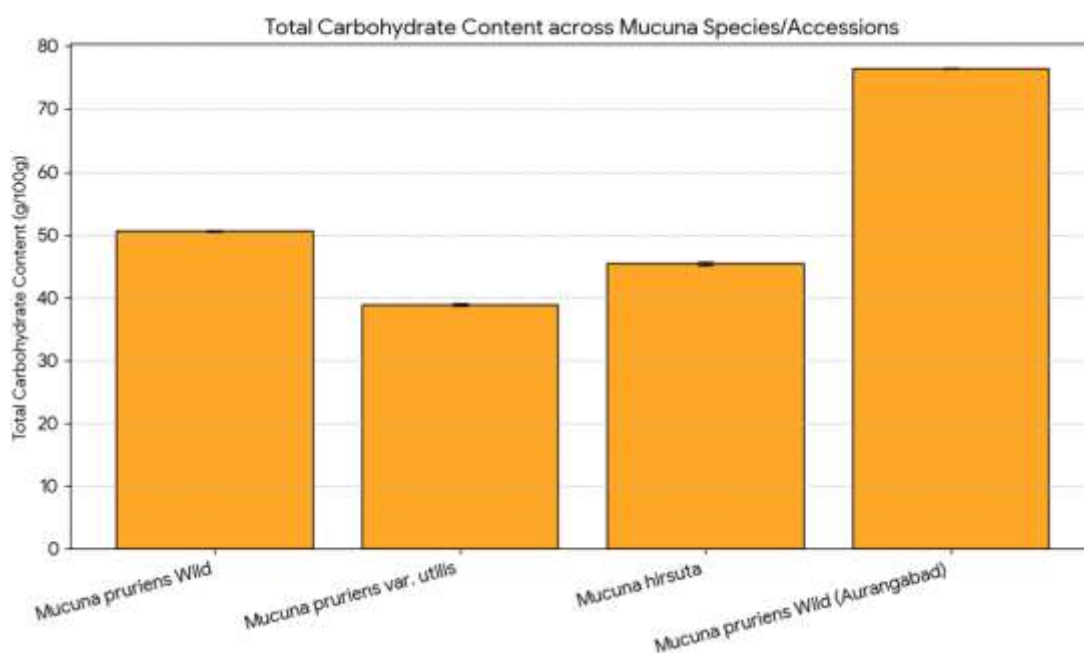


Fig.3. Total carbohydrate content variation in *Mucuna* species/ Accessions.

The high carbohydrate levels in S-4 align (Fig. 3) with caloric source, S-1 is more suited for protein the findings of Pugalenthil et al. (2005), reinforcing the supplementation. This diversity is critical for role of *Mucuna* as a primary energy source in pharmacological applications, particularly given underutilized tropical legumes. The low standard *Mucuna*'s known role in treating neurodegenerative deviation observed across measurements confirms conditions due to its L-Dopa content (Rai et al., 2020; high experimental reproducibility and precision, a Pathania et al., 2020). Future studies should focus necessity for validating the nutritional claims of on how these specific proximate compositions under-exploited vegetable legumes (Kaushik et al., 2018).

3.4 Comparative Analysis and Practical Implications

The inverse relationship between protein and carbohydrate content observed in S-4 versus S-1

accessions may differ in their bioactive efficacy (Longhi et al., 2011; Rachsee et al., 2021).

CONCLUSIONS

highlights the "accession-specific" nature of *Mucuna* nutritional profiles. While S-4 serves as an excellent

The comparative nutritional analysis of the four *Mucuna pruriens* accessions underscores the

significant biochemical diversity within the species, particularly between wild and cultivated variants. Our findings demonstrate that Sample S-4 (Aurangabad Wild accession) serves as a superior source of dietary energy, characterized by its significantly higher carbohydrate (76.488 ± 0.026 g/100g) and total fat (6.299 ± 0.624 g/100g) concentrations. In contrast, Sample S-1 (Wild) emerged as the most viable protein source (2.518 ± 0.022 g/100g), highlighting its potential in addressing protein-energy malnutrition.

The high precision and reproducibility of these results, indicated by low standard deviation values, validate the nutritional profiles of these underutilized legumes. The distinct variations observed across samples suggest that specific accessions may be targeted for different industrial applications: S-1 for protein-fortified food products and S-4 for high-energy feed or caloric supplementation.

Ultimately, this study reinforces the status of *Mucuna pruriens* as a versatile, sustainable, and nutrient-dense resource. Given its established medicinal properties and antioxidant potential, these accessions represent a promising frontier for both functional foods and pharmacological development. Future research should prioritize the impact of domestic processing methods on these specific accessions to maximize nutrient bioavailability while further mitigating anti-nutritional factors.

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