

Nutritional Composition of *Mucuna pruriens* **var.** *utilis* **(Velvet bean) Seeds Grown in Nanyumbu District, Tanzania**

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Abstract

In tropical countries, *Mucuna* seeds have been utilized to promote better health and ensure a stable food supply. The use of *Mucuna pruriens* var. *utilis* seeds in Tanzania has prompted an evaluation of the nutritional composition of two common cultivars grown in the Nanyumbu district. *Mucuna* seed samples (black-reddish brown and black-greyish) were collected and analyzed for their nutritional content using standard extraction and spectrophotometric techniques. Data analysis was conducted using GraphPad InStat 3.0 software, with a t-test employed to compare means at a 95% confidence level. The results indicated that the differences in nutritional parameters between the black-reddish brown and black-greyish *Mucuna* seeds were not significant ($P > 0.05$), except for crude lipid and carbohydrate content. The protein, lipid, carbohydrate, fiber, ash, vitamin C, beta carotene, lycopene, and energy content ranged from 48.13% to 46.50%, 2.13% to 1.39%, 26.75% to 23.12%, 15.99% to 14.51%, 3.23% to 3.21%, 0.98 to 0.84 mg/g, 0.011 to 0.004 mg/100 mg, 0.006 to 0.0058 mg/100 mg, and 1303.7 to 1243.1 kJ/100 g, respectively. Both cultivars of *Mucuna* seeds exhibit dietary qualities that can be explored as alternative food sources for the malnourished population in the country.

Keywords: Black-greyish seeds, Black-reddish brown seeds, Cultivars, Nutritional quality

Introduction

Velvet bean, *M. pruriens* L., commonly known as 'Upupu,' is an annual climbing legume from the Fabaceae family (Buckles 1995). This plant species originated in Eastern India and China and has since spread throughout tropical regions worldwide (Wilmot-Dear 1984, Suryawanshi et al. 2020). *Mucuna* is widely recognized for its nutritional and bioactive contributions to human health (Timsina 2018, Pathania et al. 2020). During periods of food scarcity, *Mucuna* seeds serve as a crucial safeguard against famine (Huisden 2008). Various ethnic groups and undernourished

populations have traditionally used these seeds as a food source (Janardhanan et al. 2003; Lampariello et al. 2012). Studies have shown that *Mucuna* seeds can help alleviate malnutrition in infants when consumed by nursing mothers (Huisden 2008). Currently, the species is cultivated in Western Tanzania as green manure (Matata et al. 2017). In southern Tanzania, however, *Mucuna* is often undervalued and primarily used as food (Constantine et al. 2020, Sakamoto et al. 2020). Given that the plant's chemical composition can vary with growing conditions, nutritional analyses of *Mucuna*

seeds from Nanyumbu, Tanzania, are warranted (Kalidas and Mohan 2011).

The appeal of *Mucuna* as a functional food lies in its high nutritional and potential phytochemical content (Encalada and Campos 2021). *Mucuna* seeds demonstrate long viability, pest resistance, and high germination rates even in challenging conditions (Siddhuraju et al. 2000). *Mucuna* has a high yielding capacity across various environmental conditions (Buckles 1995; Carew and Gernat 2016), thriving best in warm, moist climates with sandy or loamy soils at altitudes of about 1600 m (Buckles 1995). The plant can tolerate drought, low soil fertility, and high acidity (Correia et al. 2014). The life cycle of common varieties typically ranges from 160 to 170 days, with flowering occurring between the 59th and 66th days (Sathyanarayana et al. 2012).

As the plant adapts to environmental stresses, it accumulates antinutritional substances that may impact its nutritional value and could lead to adverse effects when ingested (Lorenzetti et al. 2010, Banti & Bajo 2020). Moreover, the presence of high levels of these factors limits the acceptance of *Mucuna*. The pods contain brown hairs with mucunain protein, which can cause severe itching upon contact with skin (Mosissa et al. 2021). Phytochemicals identified as potential antinutritional factors in *Mucuna* seeds include L-DOPA, phenolics, flavonoids, and ursolic acid (Kala et al. 2010, Rakesh and Praveen 2020). L-DOPA can be harmful if consumed in improperly processed seeds (Kosower and Kosower 1967, Lorenzetti et al. 2010). However, when seeds are properly processed, they provide beneficial bioactive compounds associated with pharmacological and antioxidant properties (Rai et al. 2017; Rai et al. 2019). This highlights the necessity of including *Mucuna* in the diet, as these compounds cannot be synthesized in the human body (Kumar and Pandey 2013).

The accessibility and high yield of the two common cultivars of *Mucuna* seeds in the Nanyumbu district ensure a reliable food supply throughout the year. Despite its potential, there is limited information on the nutritional composition of *M. pruriens* var. utilis seeds cultivated in Nanyumbu, Tanzania. This study aims to evaluate the nutritional composition of two common cultivars of *M. pruriens* var. utilis seeds and compare the nutritional parameters between black-reddish brown and black-greyish *Mucuna* seeds grown in the Nanyumbu district.

Materials and Methods Description of the study area

The study was conducted in Nanyumbu District, covering four villages: Namatumbusi, Mikangaula, Kilosa, and Chang'ombe, which lie within the grid range of 10°49'54"S to 10°50'30"S and 38°38'36"E to 38°41'24"E. The four villages are situated at elevations between 330 and 340 meters, within Nanyumbu district, which lies in the lowlands at altitudes ranging from 0 to 501.343 meters above sea level. Two cultivars of *M. pruriens* var. utilis, with black-reddish brown and black-greyish seed coats, exhibit high performance in the study area. Nanyumbu district receives an average annual rainfall of 800 mm, with a single rainy season that lasts from November to May. The mean temperature is 27 °C during hot periods and 24 °C during cool periods. Figure 1 presents a map of Nanyumbu district, indicating the four sampling microsites of the two common cultivars of *M. pruriens* var. utilis. The natural soil of Nanyumbu district is fertile and composed of sandy, loamy, gritty, and gravelly structures.

Figure 1: A map of Nanyumbu District indicating four sampling sites

Collection and preparation of seed sample

Two seed samples of *M. pruriens* var*. utilis* (black-reddish brown and black-greyish) were collected from four microsites as mature dry pods during harvesting period of July, 2022 by adopting procedure described by Ade-Omowaye et al. (2015). With the help of keys by Gillett et al. (1971) the seeds were botanically identified. Dry pods were threshed to remove seeds and thorough winnowed. A 500 g of each seed sample were collected from every village, packed in a polythene bags, well labelled and transferred to the Botany department, University of Dar es Salaam for nutritional analysis. Sample preparation was done following the procedure described by Nwaoguikpe et al. (2011).

Laboratory analysis

For every cultivar, collected seeds sample were separately grounded using a laboratory mill into fine powder, labelled and kept in refrigerator at 4 °C for nutritional analysis (Siddiq et al*.* 2010). Each accession of *Mucuna* seeds was analysed in triplicates for crude protein, crude lipid, crude fiber, total

ash, vitamin C, β-carotene, lycopene and carbohydrate. For chemical extraction this study adopted the procedures described by James (1995). The study employed the use of UV-Visible spectrophotometer (Jenway 6560, Limited, U.K) in all readings. Chemical analysis to determine nutritional composition of each sample of *Mucuna* seeds was done using standard procedure.

Determination of protein content

Crude proteins derived from *Mucuna* seeds was done based on the total nitrogen (N) content through micro-Kjeldahl method as described by Buurman et al. (1996). An extract was made from 0.1 g of raw sample were digested in micro-Kjeldahl at 380 to 340 °C and oxidized by 97% concentrated sulphuric acid under copper catalyst and potassium sulphate that raised boiling point of a mixture. The cold digests and standard (NH4Cl) was mixed with a combined reagent (0.35 g of sodium potassium tartrate, 0.17 g sodium salicylate, 0.005 g nitroprusside and 0.4 ml of 50% sodium hydroxide) and 4 ml of 0.15% hypochlorite solution at the ratio of

5:40:4 ml which then reduced to 1:8:0.8 ml. Both treatments and standard read absorbance at 660 nm. A created calibration curve was used to calculate concentration of total nitrogen [x] from the standard using an equation:

$$
[x] = (y - b)/m
$$

Where $x =$ concentration of standard ammonium chloride, y = absorbances of sample extract, $b = y$ -intercept and $m = slope$. A [x] was used to obtain the percentage composition of total nitrogen (%N) by equation;

% N = (concentration $(x) \times 100$)/ $10 \times 8 \times 0.05$

Where 10, 8, 0.05 were dilution factors used for nitrogen

Then, protein (P) content was obtained by the equation $P = \%N \times 6.25$ (Tomé et al. 2022).

Determination of crude lipid content

Crude lipid content was determined using chloroform-methanol method (Nielsen 1998). An extract was obtained by dissolving 3 g of a grounded sample in chloroform, 78% methanol and distilled water of 1:2:0.8 ratio. A chloroform was added in a mixture to double its volume to the ratio of 2:2:0.8 and the content was homogenized for 30 seconds. Finally, distilled water was added to double its volume making a ratio of 2:2:1.6. The content was then filtered through a whatman No. 1 filter paper. The layered filtrate was separate and an eluted upper layer was placed in oven at 40 °C for 24 hours to evaporate chloroform leaving the lipid extract. Residual weight of lipid content was obtained by subtracting the weight of a beaker containing lipid with that of an empty beaker. Percentage composition of crude lipid was obtained using the equation below

% Crude lipids = [(volume of solvent \times residual weight)/ aliquot \times 15] \times 100

Where 15 is a dilution factor

Determination of carbohydrate content

Carbohydrate was determined by anthrone calorimetric method (Maness 2010). Anthrone reagent (760 ml concentrated sulphuric acid, 330 ml distilled, 1 g of Anthrone and 1 g of Thiourea) treated in cool and dark place for 30 min. A 2 g of grounded seeds was dissolved in hot water and later allowed to cool then filtered. Each of filtrates, standard (A freshly prepared sugar solution of 0.25 g/) and water blank in boiling tubes were treated in cool and dark place. A 10 ml Anthrone reagent was added while the tubes immersed in running cold water. The contents in tubes were warmed while placed in dark and boiled, thereafter cooled. Standards, sample extracts and a blank absorbances were read at 625 nm. A prepared calibration graph was used to obtain milligram of glucose in the sample of four aliquots which was expressed as glucose equivalent percent. Carbohydrate concentration was calculated using an equation;

$Y = m[x] + b$

Where $x =$ concentration of glucose in mg/ml, $Y =$ absorbances of the sample, $b = y$ intercept and m = slope or a gradient factor.

The percentage composition of was determined using equation;

% Carbohydrate = $(x \times 30)/10 \times 2 \times 0.05$

Where 2 was a mass of sample, 30, 10 and 0.05 were used as dilution factors for carbohydrates. The validity of results was justified with correlation coefficient (R^2) .

Determination of energy value

The calorific equation was used to estimate energy value of *Mucuna* seeds by multiplying the percentage of crude protein, crude lipid and crude carbohydrate by 16.7, 37.7 and 16.7, respectively (Siddhuraju et al. 1996).

Determination of crude fibre

Procedure described by James et al. (2020) was adopted to determine crude fibre content in *Mucuna* seeds. A 1 g of sample was acidified by boiling with HCl (1%) then, contents were filtered and boiled with an alkali (NaOH). The sample residue was dried, cooled and weighed. Dry residues were ashed in a muffle furnace at 550 °C then cooled and re-weighed. Percentage of weight loss of an incinerated residue was considered as a weight of crude fiber content as calculated using an equation:

% Crude fibre $=$ [(weight of sample – weight) of shed)/weight of original sample] \times 100 **Determination of total ash**

Total ash of the *Mucuna* seeds were determined following procedure described by James et al. (2020). Grounded samples of 5 g was incinerated in a muffle furnace at 500 °C. The percentage weight of ashes was calculated using the equation;

% Ash $=$ (weight of ash/weight of sample) \times 100

Determination of vitamin C

Vitamin C was extracted from 2 g of grounded *Mucuna* seeds and determined by 2, 4-Denitrophenylhydrazine assay as explained by Desai and Desai (2019). A sample was dissolved in 5 ml of 4% oxalic acid then homogenized and centrifuged at 5000 rpm for 10 min. Supernatant contents were filtered through a whatman no. 1 filter paper. The standard was prepared in a ratio of 1:2 (ascorbic acid-distilled water). Each of sample aliquot and standard were oxidized with bromine water. In every content of sample and standard a 1 ml of 2, 4- Denitrophenylhydrazine reagent was added followed by two drops of thiourea water and incubated at 37 °C for 3 hours in dark cupboard. Both contents of sample and standard were crystalised which were dissolved in 5 ml of 80% sulphuric acid. The resulted red colour solutions read absorbance at 540 nm. The graph created was used to calculate concentration of ascorbic acid (mg/l) from the equation;

 $C (mg/l) = (A_{540} - y)/m$

Where \overline{C} = concentration of the standard, A_{540} = Absorbance of the sample read at 540 nm, $y = y$ -intercept, $m =$ gradient factor

Finally ascorbic acid content in mg/g of sample dry weight were calculated by a formula;

Concentration $(mg/g) =$ Concentration of standard (mg/l) \times 10 factor

Determination of beta-carotene and lycopene

A petroleum ether method described by Barros *et al.* (2007) was used to determine βcarotene and lycopene content of *Mucuna* seeds. A 2 g of grounded *Mucuna* seeds was dissolved in 5 ml of 0.5 N ethanoic potassium hydroxide solution and 15 ml of petroleum ether then filtered. An upper petroleum ether layer formed by addition of distilled water were eluted and evaporated to dryness on a hot plate at 70 °C. A 100 g of cool dried ether extract was dissolved in 10 ml acetonehexane mixture (4:6) and filtered through whatman No.1 filter paper. The absorbance of the filtrates was measured at 663, 505 and 453 nm. The content of beta carotene and lycopene were calculated according to the equations below suggested by Barros et al. (2007).

Beta carotene $(mg/100mg) = (0.216 \times A_{663}) (0.304\times A_{505}) + (0.452\times A_{453})$ Lycopene (mg/100mg) = $(-0.0458 \times A_{453}) +$ $(0.372\times A_{505}) - (0.0806\times A_{663}).$

Data analysis

Data were analyzed using GraphPad InStat software version 3.0 (Motulsky et al. 2003). Descriptive statistics were presented in tables. Variations in means for each tested parameter were compared between the blackreddish brown and black-greyish *Mucuna* seeds using an unpaired t-test at a 95% confidence level. All determinations were carried out in triplicate, and data were expressed as mean ± standard deviation.

Results and Discussion Nutritional composition

Research on the nutritional composition of neglected food plants plays a significant role in enhancing the food industry. *M. pruriens* var. utilis seeds exhibit nutritional potential that promotes their use as a functional food source for undernourished populations. The nutritional composition of two cultivars of *M. pruriens* var. utilis seeds (black-reddish brown and black-greyish) is summarized in Table 1. The findings indicate that the nutritional composition of black-reddish brown and black-greyish seeds did not differ significantly $(P > 0.05)$ in all tested parameters, except for crude lipid and carbohydrate content.

Protein content

Both cultivars of *M. pruriens* var. utilis grown in the Nanyumbu district recorded high protein levels ranging from 46.50% to 48.13%. These levels are significantly higher than the 43.12% to 43.40% reported for *M. pruriens* seeds (Renata et al. 2015). Additionally, the protein content is nearly double that of the white variety (29.53%) and black variety (28.75%) of *M. pruriens* (Daffodil et al. 2016). Lower protein levels were also reported for the white variety (28.82%) and black seeds (26.26%) (Kalidass and Mahapatra 2014). The protein content in *Mucuna* seeds is about twice that found in commonly consumed legume seeds in the tropics, such as *Phaseolus vulgaris* (common bean) at 19.45% (Grela et al. 2017). Even lower protein levels have been noted in bambaranut, red bean, pigeon pea, cowpea, and groundnut (James et al. 2020). In comparison, the protein content of the *Mucuna* seeds in this study exceeds the 41.81% reported for soybean seeds (Mnembuka and Eggum 1995). The protein levels found in *Mucuna* seeds exceed the daily protein needs for adults, which range from 10% to 35% as recommended by the USA National Research Council (1989).

Carbohydrate content

The black-reddish brown seeds in this study exhibited a higher carbohydrate level (26.747%) compared to the black-greyish seeds (23.12%). The carbohydrate content in the black-reddish brown seeds aligns with the black (27.19%) and white (28.27%) varieties of *M. pruriens* var. utilis (Siddhuraju and Becker 2001). In contrast, the black-greyish seeds showed a lower carbohydrate content (23.121%) than the 28% found in *Mucuna* bean seeds (Siddhuraju and Becker 2005). These findings are consistent with carbohydrate levels in other pulses, including pigeon pea (23.66%) (Ade-Omowaye et al. 2015). The carbohydrate content is significantly higher than that of groundnut (7.34%) (James et al. 2020), soybean (1.33%), and winged bean (3.00%) (Mnembuka and Eggum 1995). However, both cultivars recorded lower carbohydrate levels compared to other popular legumes, such as chickpea (31.8%) and green gram (39.9%) (Bravo et al. 1999). Other legumes like green gram (38.54%), bambaranut (45.02%), pigeon pea (40.53%), field bean (36.30%), and cowpea (41.34%) (Mnembuka and Eggum 1995) also displayed higher carbohydrate content than the current findings. The carbohydrate levels of 26.747% to 23.121% in both studied *Mucuna* seeds exceed the recommended intake of 21.2% for managing cardiovascular disease risk (Shan et al. 2019).

Crude lipid content

The black-greyish seeds exhibited a higher lipid content (2.13%) compared to the blackreddish brown seeds (1.39%). The lipid content range of 2.13% to 1.39% in all cultivars of *M. pruriens* var. *utilis* aligns with the 2.69% found in *M. pruriens* seeds (Nwaoguikpe et al. 2011), but is lower than that reported for the white and black varieties (Daffodil et al. 2016, Kalidass and Mahapatra 2014, Tresina and Mohan 2013). Similar lipid levels were noted in other preferred legumes, such as *P. vulgaris* (1.190%) (Shimelis and Rakshit 2005), kidney bean (1.4%), lima bean (1.3%), pigeon pea (2.8%), and jack bean (2.5%) (Apata and Ologhobo 1994). Some commonly cultivated legumes, such as bambaranut, red bean, pigeon pea, and cowpea, reported higher crude lipid contents (5.87%, 6.50%, 6.73%, and 6.75%, respectively) (James et al. 2020). The crude lipid content does not categorize *M. pruriens* var. utilis grown in the Nanyumbu district as an oil-rich legume, especially when compared to groundnuts (34.63%) (James et al. 2020), soybeans (22.88%), and winged beans (21.75%) (Mnembuka and Eggum 1995).

Energy value

The energy value estimated for both cultivars ranged from 1243.1 to 1303.7 kJ/100 g (dry matter). These values are lower than the 1570.34 to 1607.66 kJ/100 g reported by Renata et al. (2015) for *M. pruriens* seeds, but higher than the 572.95 kJ/100 g of *M. pruriens* var. utilis (Rane et al. 2019). Compared to other preferred legumes, Mnembuka and Eggum (1995) reported higher energy values in soybean (2383.21 kJ/100 g), green gram (1945.14 kJ/100 g), bambara nut (1973.17 kJ/100 g), pigeon pea (1881.55 kJ/100 g), field bean (1877.78 kJ/100 g), cowpea (1925.48 kJ/100 g), and winged bean (2372.33 kJ/100 g).

Crude fiber content

The findings indicate a crude fiber content ranging from 14.51% to 15.99%, which is higher than the levels reported for the white and black varieties of *M. pruriens* var. utilis (Daffodil et al. 2016, Kalidass and Mahapatra 2014, Tresina and Mohan 2013). These

findings also surpass the fiber content of common pea, common bean, and chickpea (4.571%, 4.434%, and 2.216%, respectively) (Grela et al. 2017). Lower fiber content has been reported in bambaranut, red bean, pigeon pea, cowpea, and groundnut (2.23%, 2.50%, 3.13%, 3.16%, and 6.11%, respectively) (James et al. 2020). The fiber content in both cultivars indicates that only 2 g of *Mucuna* pulses would meet the recommended daily fiber intake of 25–30 g for adults (Reynolds et al. 2019). Although fiber does not contribute directly to the nutritive value of foods, it plays a vital role in managing peristalsis and metabolism in the gastrointestinal tract (Daffodil et al. 2016, Tresina and Mohan 2013).

Total ash content

The total ash content of all studied *Mucuna* seeds ranged from 3.21% to 3.23%, aligning with the 3.10% found in *M. pruriens* seeds (Renata et al. 2015) and 3.3% in *Mucuna* accessions (Vadivel and Janardhanan 2000). These values are significantly lower than the 6.47% reported by Alaye et al. (2020) and the 4.94% and 5.30% for the black and white varieties, respectively (Tresina and Mohan, 2013). Comparatively, similar results were observed in chickpea (3.13%) (Grela et al., 2017), jack bean (3.0%) (Apata and Ologhobo 1994), and *P. vulgaris* (3.024%) (Shimelis and Rakshit 2005). Lower levels were reported in common pea (2.746%) (Grela et al., 2017) and lentils (2.8%) (Iqbal et al. 2006). The available ash content suggests that *Mucuna* seeds contain nutritionally significant mineral components (Kalidas and Mohan 2011; Tresina and Mohan 2013).

Vitamin C content

The vitamin C levels observed in both cultivars ranged from 0.982 to 0.8406 mg/g, significantly higher than the 0.55 and 0.5832 mg/g found in the black and white varieties of *M. pruriens* var. utilis (Tresina and Mohan 2013). Lower vitamin C levels were reported in mung gram, black gram, pigeon pea, and cowpea (Tomar et al. 2018). Despite the relatively low levels found in this study, the vitamin C content underscores the potential of *Mucuna* seeds as an important functional food source rich in antioxidants.

Beta-Carotene and Lycopene levels

The mean beta-carotene levels in both cultivars ranged from 0.011 to 0.004 mg/100 mg, higher than the 0.00015 to 0.00346 mg/100 mg reported for *M. flagellipes* (Nwajagu et al. 2021). Lower beta-carotene levels were noted pigeon pea and cowpea (Tomar et al. 2018), as well as in lupin seeds (11.98–50.43 µg/g) (Wang et al. 2008) and soybean seeds (Gebregziabher et al. 2021, 2022). Lycopene levels in this study ranged from 0.0062 to 0.0058 mg/100 mg, similar to the 12.7 mg/100 g (0.0127 mg/100 mg) found in lima beans (Farinde et al. 2017). In this context, *Mucuna* seeds appear to be a promising crop with antioxidant properties capable of combating free radicals.

All values are expressed as a Mean \pm SD, n = 3. Mean values in the following row sharing a common letter in superscript are not statistically significant at 95% confidence

level. DM = Digestible and Metabolized energy

Conclusion

The primary constituents of *Mucuna* seeds identified in this study were protein, carbohydrates, and fiber. The black-reddish brown seeds exhibited the highest levels of crude protein, carbohydrates, ash, beta carotene, lycopene, and energy. In contrast, the black-greyish seeds contained higher amounts of crude lipids, crude fiber, and vitamin C. The findings suggest that the nutritional profile of *M. pruriens* var. utilis (both black-reddish brown and black-greyish seeds) is comparable to, and even exceeds, that of other *Mucuna* seeds from similar or different varieties. The high protein content in these seeds may help combat proteinenergy malnutrition, while the levels of carbohydrates, lipids, and fiber can effectively address cholesterol and cardiovascular issues. Although *Mucuna* seeds contain anti-nutritional factors, these are not expected to pose significant risks to humans and may even provide antioxidant benefits at levels deemed healthy. There is a pressing need for further research on *Mucuna* species, as they possess the potential to serve as functional foods and natural medicines for large populations.

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