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NUTRITIONAL POTENTIAL OF FIVE ACCESSIONS OF A SOUTH INDIAN TRIBAL PULSE, *Mucuna pruriens* var *utilis*

I. The effect of processing methods on the content of L-Dopa, phytic acid, and oligosaccharides

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SUMMARY

Five accessions of an indigenous tribal pulse, *Mucuna pruriens* var *utilis* (three with white, two with black seed color) were gathered from Western Ghats, South India and analyzed for three anti-nutritional factors: L-Dopa, phytic acid, and oligosaccharides (i.e., raffinose, stachyose, and verbascose). The accession "Valanad (black)" contained the lowest levels of all three factors. Additionally, the effect of various processing methods on the levels of the anti-nutritional factors was studied. Repeated boiling in water (seven times) resulted in the greatest reduction of L-Dopa and phytic acid (by 56-60% and 36-38%, respectively). Both autoclaving and crude ∝-galactosidase treatments were the most effective methods to reduce the levels of raffinose (reduction of 40-43%; 79-85%, respectively), stachyose (53-57% and 95-98%, respectively), and verbascose (52-54% and 82-83%, respectively).

Key words: *Mucuna* beans, accessions, antinutrients, Western Ghats, South India.

INTRODUCTION

Mucuna's use in South Asia and its anti-nutritional factors

The *Mucuna* bean, *Mucuna pruriens* (L.) DC. var *utilis* (Wall ex Wight) (Baker ex Burck) is an under-utilized legume species grown predominantly in Asia, Africa and in parts of the Americas (Vadivel and Janardhanan, 2000). Traditionally in India, the mature seeds of *Mucuna* bean are consumed by a South Indian hill tribe, the Kanikkars, after repeated boiling (Janardhanan and Lakshmanan, 1985). Recently, Dravidian tribes in the Tirunelveli district have started cultivating it for use as a pulse. (Janardhanan *et al.,* in press). Various preparations of the bean are also traditionally consumed in several parts of Sri Lanka by low-income groups (Ravindran and Ravindran, 1988). In South East Asia, the immature pods and leaves of *Mucuna* bean are used as vegetables. In parts of Asia and Africa, the seeds are roasted and eaten (Haq, 1983). The *Mucuna* bean is also used in indigenous Ayurvedic medicine (Shaw and Bera, 1993) and the L-Dopa extracted from it is used to provide symptomatic relief in Parkinson's disease (Nagashayana and Sankarankutty, 2000). The beans were also employed as a powerful aphrodisiac in Ayurveda (Amin, 1996) and have been used to treat nervous disorders (Jayaweera, 1981; Wijeyaratne, 1987) and arthritis (Wijeyaratne, 1987). The bean, if applied as a paste on scorpion stings, is presumed to absorb poison (Jayaweera, 1981).

Although there is some information available on the nutritional and anti-nutritional properties of *Mucuna* beans (Janardhanan and Lakshmanan, 1985; Ravindran and Ravindran, 1988; Udedibe and Carlini, 1998; Siddhuraju *et al*., 2000; Vadivel and Janardhanan, 2000), there has been relatively few systematic collections and little evaluation of diverse *Mucuna* bean accessions. This situation also characterizes Western Ghats, South India, where a number of *Mucuna* bean accessions exist, some of which are utilized by the local population. There is an urgent need for collection and conservation of all available accessions from this region due to the severe genetic erosion of this wild legume in the last 20-25 years in the Indian subcontinent (Janardhanan *et al.,* in press).

Although legumes constitute one of the richest and least expensive sources of protein in human diet, their utilization is limited by anti-nutritional factors which interfere with the assimilation of nutrients. Some of these anti-nutritional factors are heat labile (e.g., protease inhibitors [trypsin and chymotrypsin], phytoheamagglutinins [lectins], goitrogens and antivitamins), while others are heat stable (e.g., tannins, oligosaccharides [flatulence factors], phytic acid, L-Dopa, saponins, estrogens, lysinoalanine, and allergens) (Liener, 1980).

A recent research project sought to find ways to eliminate or reduce various anti-inutrients in two varieties of *Mucuna* beans procured from Tamil Nadu, India (Siddhuraju and Becker, 2001). In the present investigation, five accessions of *Mucuna* beans have been collected from the tropical forests of Western Ghats (Tamil Nadu and Kerala states), South India and screened for the profiles of anti-nutrients (L-Dopa, phytic acid, oligosaccharides, total free phenols, tannins, protease inhibitors and phytoheamagglutinins). In addition, *in vitro* protein digestibility (IVPD) of raw samples was also investigated. The present investigation was conducted to determine the content of the previously discussed anti-nutrient factors as well as the protein digestibility of *Mucuna* beans. Part I focuses on L-Dopa, phytic acid and oligosaccharides, while Part II reports on total free phenolics, tannins, trypsin and chymotrypsin inhibitors, phytohaemagglutinins (lectins) and *in vitro* protein digestibility. An additional objective for Part I is to investigate the extent to which L-Dopa, phytic acid and oligosaccharides are reduced or eliminated if the beans are soaked, autoclaved, repeatedly boiled in water or subjected to treatment with crude ∝-galactosidase enzyme.

L-Dopa, phytic acid, and oligosaccharides and the impact of processing

This article focuses on the content of L-Dopa, phytic acid, and oligosaccharides in *Mucuna* seeds. These three anti-nutrients are important in determining the nutritional impact and acceptability of the food:

- *L-Dopa:* Several toxic non-protein amino acids occur in higher plants (Fowden, 1976; Roy, 1981; Evans *et al.,* 1993). The non-protein amino acid L-Dopa was first isolated from the fruits of *Vicia faba* (Brain, 1976). Since then, its presence has been observed in several species of the genus *Mucuna*. Consumption of improperly boiled seeds of *Mucuna* bean is known to cause an increase in body temperature and skin eruptions among the Kanikkars, tribals that traditionally consume the beans in Kerala. These symptoms are attributed to the presence of L-Dopa (Shankaranarayan, 1978; Jabadhas, 1980). Other side effects of L-Dopa include states of confusion (Infante *et al*., 1990), vomiting and diarrhea (Duke, 1981; Afalobi *et al.,* 1985).

- *Phytic acid:* Phytic acid is found in most cereal grains, pulses, nuts, and oil seeds. It acts as the primary phosphorus reservoir accounting for up to 85% of total phosphorus in cereals and legumes. A great deal of research has focused on the unique structure of phytic acid that gives it the ability to bind minerals, proteins and starch, to lower bioavailability of minerals, to form complexes with proteins and starch, and to inhibit enzymatic digestion of both proteins and starch (Oatway *et al*., 2001).

- *Oligosaccharides:* Oligosaccharides, which are common in legume seeds, are thought to be the major producers of flatus when consumed (Reddy and Salunkhe, 1980). These saccharides contain one, two or three galactose units joined to ∝-1-6 linkages. They cannot be hydrolyzed and absorbed in monogastric animals because of the lack of ∝-galactosidase activity in the small intestine. Microorganisms in the large intestine utilize these sugars, leading to the production of flatus gases (H_2, CO_2 and small amounts of CH_4), abdominal rumbling, diarrhea and discomfort (Olson *et al.,* 1981; Liener, 1994).

It is known that various processing methods improve the protein digestibility of legume seeds by decreasing the levels of some anti-nutrients (Sathe and Salunkhe, 1984; Barampama and Simard, 1995). Processing methods such as soaking in salt water and boiling in water are routinely done in India not only to eliminate or reduce anti-nutrients but also to improve the protein digestibility of legumes. Autoclaving is a standard scientific method employed to evaluate the aforesaid parameters in food grains. Treatment of samples with crude ∝-galactosidase enzyme forms an advanced biotechnological tool for complete degradation of oligosaccharides. In the following study, three anti-nutritional factors and the impact of processing on them are explored. As mentioned, Part II of this series (Gurumoorthi *et al*., this volume) focuses on the content of total free phenolics, tannins, trypsin and chymotrypsin inhibitors, phytohaemagglutinins (lectins) as well as *in vitro* protein digestibility of *Mucuna* beans.

MATERIALS AND METHODS

Samples

Five accessions of *Mucuna* bean were gathered in March 2001 as mature pods from natural stands of four ecological regions of Tamil Nadu and Kerala states in South India (Table 1; Figure 1). After drying in the sun, the pods were thrashed to separate mature seeds. After thorough cleaning and removal of broken seeds and foreign materials, the seeds were stored in plastic containers at room temperature (25°C) until further use.

Preparation of raw seed samples

Dry mature seeds of different accessions (10 g each) were powdered in a Wiley Mill to 60-mesh size with suitable precaution to avoid contamination of samples. The powders were stored in plastic containers at room temperature (25°C) until further use.

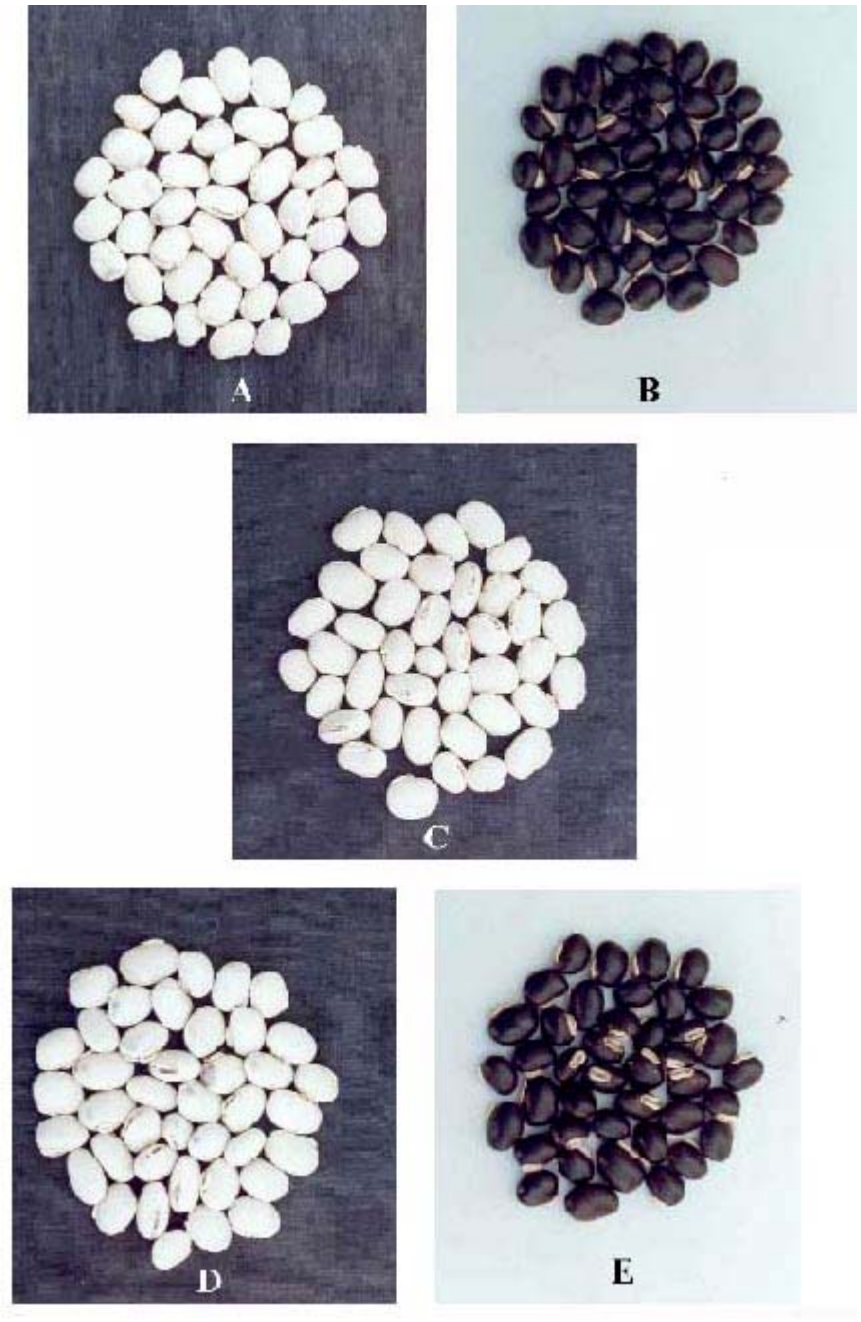

Figure 1. Five accessions of *Mucuna* beans collected from Western Ghats, South India: A. Thachenmalai accession (white-coloured seed coat), B. Thachenmalai accession (black-coloured), C. Kailasanadu accession (white-coloured), D. Mundandurai accession (white-coloured), and E. Valanad accession (black-coloured).

Processing methods

The seeds were soaked in 0.1% $NaHCO_3$, 0.1% NaCl, repeatedly boiled in distilled water and the seed flour of all the five accessions was subjected to crude ∝-galactosidase treatment. Details of the processing methods include:

- *Soaking:* Whole seeds were soaked in 0.1% $NaHCO_3$ solution and 0.1% NaCl solution for 16 hours at room temperature in the bean:water ratio of 1:10 l (w/v). The water was drained off and the seeds were dried at 55°C and powdered in a Wiley Mill to 60 mesh size.

- *Autoclaving:* The dry seeds were autoclaved at 1.05kg/cm^2 pressure (121°C) in distilled water (in bean:water ratio of 1:7 w/v) for 30 min. The seeds were rinsed with distilled water, dried at 55°C and powdered in a Wiley Mill to 60 mesh size.

- *Repeated boiling in water followed by decanting:* Separate batches of seeds were boiled in distilled water (95°C) in the bean:water ratio of 1:10 (w/v) for 15 min on a hot plate. At the end of 15 min, the water was decanted and boiling in water was repeated seven times. At the end of repeated boilings, the seeds were rinsed, dried at 55°C and powdered in a Wiley Mill to 60-mesh size.

- *Crude ∝-galactosidase treatment:* Partial purification of α-galactosidase from locally available guar seeds (*Cassia sericea*) was done following the method of Shivanna *et al*. (1989). The seeds were surface sterilized by treating with a 0.1% (w/v) mercuric chloride solution for 15 min. and then were washed with distilled water. Washed seeds were arranged at the bottom of moist filter paper, rolled and allowed to germinate at 27 °C for 3 days. After germination, seeds were homogenized with 0.2 M acetate buffer (pH 5) in a homogenizer for 10 min. at full speed. The homogenate was filtered through muslin cloth and allowed to settle down for few hours. The supernatant was decanted and centrifuged at 12,000 rpm for 30 min using a High Speed Refrigerated Centrifuge. The supernatant was precipitated with ammonium sulphate. Precipitate was subjected to centrifugation. After centrifugation, the residue was employed as crude enzyme and dissolved in acetate buffer (pH 5). The extracted crude α-galactosidase enzyme activity was determined as described by Mulimani *et al.* (1997). Treatment of 5 g of seed flour was done with 40 mL of crude α-galactosidase (0.45 units min^{-1}) at 50 °C for 4 hours with occasional shaking. For control, the volume of enzyme was replaced with 50 mM of acetate buffer (pH 5). After 4 hours of incubation, the contents were filtered through Whatman No. 1 filter paper. The residue was dried at 60°C for 24 hours. The dried samples were subjected to separation (by thin layer chromatography, TLC) and estimation of oligosaccharides

Table 1. Ecological regions and botanical characterization of the five collected accessions of *Mucuna* beans.

Accession	Place of collection	Ecological region	Botanical characterization				
			Germination (%)	Day of flower initiation	Pod setting (%)	Seeds per pod (No.)	100 seed wt (g)
Thachenmalai (white)	Kanyakumari district, Tamil Nadu	Deciduous forest, slightly elevated, sandy soil, near river	88.9	80	59.7	5.4	116.8
Thachenmalai (black)	Kanyakumari district, Tamil Nadu	Deciduous forest, slightly elevated, sandy soil, near river side	83.3	73	68.1	6.17	87.7
Mundandurai (white)	Tirunelveli district, Tamil Nadu	Ever green forest, red soil, altitude 500m	61.1	87	54.2	5.9	99.0
Kailasanadu (white)	Idukki district, Kerala	Semi ever green forest, red soil, altitude 500- 700m	87.2	78	61.2	5.3	115.8
Valanad (black)	Thiruvananthapuram district, Kerala	Moist deciduous forest, black clay soil, altitude 600-800m	88.87	71	73.2	6.00	65.87

Analysis of antinutritional factors

Extraction and estimation of L-Dopa

The non-protein amino acid, L-Dopa (3,4-dihydroxyphenylalanine), was extracted and quantified in raw and processed seed flour following the method of Brain (1976).

Extraction and estimation of phytic acid

The content of phytic acid was determined following the method of Wheeler and Ferrel (1971). The phytate phosphorous was calculated from iron results, assuming a 4:6 Fe:P molecular ratio. The phosphorous was estimated by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$.

Extraction, TLC separation and estimation of oligosaccharides

Extraction of oligosaccharides was done following the method of Somiari and Balogh (1993). Five grams each of both raw and processed seed flours of all the five accessions were extracted with 50 mL of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 hours and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25 mL of 70% (v/v) ethanol. The filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in five mL of double-distilled water.

Separation of oligosaccharides was done by TLC. Thirty g of cellulose-G powder were dissolved in 45 mL of double distilled water and shaken well until the slurry was homogeneous. TLC plates were coated with the slurry and air-dried. Spotting of the sugar samples was done by using micropipettes. Five µl aliquots of each sample were spotted thrice separately. The plates were developed by using a solvent system of n-propanol, ethyl acetate and distilled water (6:1:3), and dried (Tanaka *et al.,* 1975). The plates were sprayed with α-naphthol reagent (1%, w/v): α-naphthol was dissolved in 95% (v/v) ethanol containing 10% (w/v) orthophosphoric acid (Albon and Gross, 1952). Plates were dried in a hot-air oven. The separated spots were compared with standard sugar spots. Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped, eluted in 2 mL of distilled water kept overnight and filtered through Whatman No. 1 filter paper. The filtrates were subjected to quantitative estimation.

The eluted individual oligosaccharides were estimated by the method of Tanaka *et al.* (1975). One mL of the eluted and filtered sugar solution was treated with one mL of 0.2 M thiobarbituric acid and one mL of concentrated HCl. The tubes were boiled in a water bath for exactly 6 min. After cooling, the oligosaccharide contents were quantified in a Spectronic 20 D spectrometer at 432 nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was expressed on dry weight basis.

Statistical analysis

Anti-nutritional factors, L-Dopa, phytic acid and oligosaccharides were estimated in triplicate determinations. Estimates of mean and standard error for the aforesaid parameters were calculated.

RESULTS AND DISCUSSION

Profiles of antinutrients

In the five accessions, L-Dopa content ranged from 5.60 to 6.56 g 100 g^{-1} (Table 2). These values were lower than those reported in earlier studies from India (Janardhanan and Lakshmanan, 1985; Mohan and Janardhanan, 1995; Siddhuraju *et al*., 1996; Vadivel and Janardhanan, 2000) but higher than those found in a recent study (Siddhuraju and Becker, 2001). Accession "Valanad" had the lowest L-Dopa level (5.60 g 100 g^{-1}), while that of the accession "Thachen-malai (black)" was the highest (6.56 g 100 g^{-1}). Variation in L-Dopa concentration in different accessions of *Mucuna* beans has been reported recently (Vadivel and Janardhanan, 2000; Siddhuraju and Becker 2001) and is presumably of both genetic and environmental origin.

The content of phytic acid ranged from 0.48 g 100g^{-1} to 0.70 g 100g^{-1} with Valanad accession registering the lowest value (Table 2). These values were lower than those reported in a recent investigation (Siddhuraju and Becker, 2001). The phytic acid level in Kailasanadu accession is comparable to that reported in an earlier study (Vijayakumari *et al*., 1996).

Slight variation in the levels in the raffinose family of sugars was detected with raffinose ranging from 0.95 g 100 g^{-1} to 1.10 g100 g^{-1}, stachyose ranging from 1.05 to 1.22 g 100 g^{-1} and verbascose ranging from 3.95 to 4.34 g 100 g^{-1} of seed flour (Table 2). Accession "Valanad" exhibited the lowest levels for all the three α-galactosides with verbascose having the highest concentration of the three.

Table 2. Content of L-Dopa, phytic acid and oligosaccharides in raw seeds of five accessions of *Mucuna* bean. The data are means and standard errors of triplicate determinations.

Accession	L- Dopa (g $100g^{-1}$)	Phytic acid (g $100g^{-1}$)	Oligosaccharides (g $100g^{-1}$)		
			Raffinose	Stachyose	Verbascose
Thachenmalai (white)	5.93± 0.01	0.61± 0.06	1.05±0.03	1.15±0.01	4.25±0.02
Thachenmalai (black)	6.56±0.03	0.52±0.04	1.10±0.05	1.22±0.02	4.31±0.04
Mundandurai (white)	5.85±0.03	0.58±0.04	1.00±0.02	1.20±0.08	4.18±0.07
Kailasanadu (white)	5.85±0.05	0.70±0.01	1.00±0.01	1.10±0.08	4.34±0.06
Valanad (black)	5.60±0.05	0.48±0.02	0.95±0.04	1.05±0.05	3.95±0.04

Effect of processing methods

Tables 3 and 4 present the data on the impact of soaking on the anti-nutritional factors studied. Clearly, soaking in $NaHCO_3$ was more effective in reducing anti- nutritional factors than soaking in NaCl. L-Dopa content was reduced by 17-20% and phytic acid was reduced by 16-17% in samples soaked in $NaHCO_3$. This is in agreement with the results of Vijayakumari *et al.* (1996) and Siddhuraju and Becker (2001). Of all the oligosaccharides, soaking in $NaHCO_3$ reduced stachyose content the most (by 35- 38%).

The loss in levels of L-Dopa due to autoclaving was insignificant compared to the reduction in contents of phytic acid and oligosaccharides (Table 5). Autoclaving reduced the concentration of phytic acid by 27-29%, which agrees with the results of Khan *et al.* (1988) for a white variety of *Cicer arietinum* and Vijayakumari *et al.* (1996) for *Mucuna pruriens*. Autoclaving resulted in a reduction of raffinose by 40-43%, of stachyose by 53-57%, and of verbascose by 52-54%. Autoclaving reduced oligosaccharides more than soaking.

In this study, repeated boiling in water and decanting resulted in substantial reduction in levels of L-Dopa (56-60%). This is in agreement with the findings of Siddhuraju and Becker (2001) for the three varieties of *Mucuna* beans. Boiling reduced phytate content by 36-38% in the present study.

This is in good agreement with the previous findings for different pulses (Vijayakumari *et al*., 1996; Srivastava and Khokhar, 1996; Siddhuraju and Becker, 2001). Of all the three ∝-galactosides analyzed, maximum reduction was observed in the level of stachyose (58-62%) followed by verbascose (48-55%) and raffinose (38-42 %). These findings are in agreement with those of Jood *et al.* (1985) in *Phaseolus mungo* and *Phaseolus vulgaris*; Vijayakumari *et al.* (1996) in *Mucuna pruriens* and Mulimani and Devendra (1998) in red gram. Onigbinde and Akinyele (1983) have proposed that decrease in levels of raffinose, stachyose and verbascose during cooking might be attributed to the heat hydrolysis of disaccharides and monosaccharides or to the formation of other compounds.

Partially purified ∝-galactosidase from *Cassia sericea* almost completely removed the ∝-galactosides, viz., raffinose (79-85%), stachyose (95-98%) and verbascose (82-83%) (Table 7). There are several reports available in the literature on the use of ∝-galactosidase from plant and fungal sources for the removal of oligosaccharides from soymilk and legume flour (Somiari and Balogh, 1993; Mulimani and Ramalingam, 1995; Mulimani *et al.,* 1997; Mulimani and Devendra, 1998). Earlier, Mulimani and Devendra (1998) reported the use of ∝-galactosidase from *Cassia sericea* for eliminating oligosaccharides from red gram flour.

Table 3. Effect of soaking in 0.1%$NaHCO_3$ solution on the levels of L-Dopa, phytic acid and oligosaccharides in raw seeds of five accessions of *Mucuna* beans. The data are means and standard errors of triplicate determinations.

Accession	L-Dopa (g 100 g^{-1})	% loss	Phytic acid (g 100 g^{-1})	% loss	Oligosaccharides (g 100 g^{-1})					
					Raffinose	% loss	Stachyose	% loss	Verbascose	% loss
Thachenmalai (white)	4.87 ± 0.02	- 18	0.51 ± 0.04	-16	0.88 ± 0.02	-16	0.75 ± 0.02	-35	3.06 ± 0.06	-28
Thachenmalai (black)	5.33 ± 0.02	-19	0.44 ± 0.07	-16	0.90 ± 0.05	-18	0.78 ± 0.08	-36	3.19 ± 0.05	-26
Mundandurai (white)	4.6 8± 0.05	-20	0.48 ± 0.05	-17	0.83 ± 0.05	-17	0.78 ± 0.04	-35	3.05 ± 0.08	-27
Kailasanadu (white)	4.86 ± 0.02	-17	0.58 ± 0.05	-17	0.82 ± 0.04	-18	0.71 ± 0.06	-35	3.22 ± 0.01	-25
Valanad (black)	4.65 ± 0.02	-17	0.40 ± 0.04	-17	0.80 ± 0.05	-16	0.67 ± 0.05	-38	3.12 ± 0.07	-21

Table 4. Effect of soaking in 0.1%NaCl solution on the levels of L-Dopa, phytic acid and oligosaccharides in five accessions of *Mucuna* beans. The data are means and standard errors of triplicate determinations.

Accession	L-Dopa (g 100 g^{-1})	% loss	Phytic acid (g 100 g^{-1})	% loss	Oligosaccharides (g 100 g^{-1})					
					Raffinose	% loss	Stachyose	% loss	Verbascose	% loss
Thachenmalai (white)	5.03 ± 0.04	- 15	0.56 ± 0.02	-8	1.00 ± 0.02	-5	1.10 ± 0.05	-4	4.04 ± 0.02	-5
Thachenmalai (black)	5.64 ± 0.04	-14	0.47 ± 0.05	-9	1.04 ± 0.07	-5	1.14 ± 0.04	-4	4.12 ± 0.05	-7
Mundandurai (white)	5.00 ± 0.07	-15	0.52 ± 0.04	-10	0.94 ± 0.07	-6	1.14 ± 0.02	-5	3.98 ± 0.05	-6
Kailasanadu (white)	4.95 ± 0.05	-15	0.64 ± 0.02	-8	0.95 ± 0.06	-5	1.05 ± 0.02	-5	4.16 ± 0.03	-4
Valanad (black)	4.82 ± 0.05	-14	0.44 ± 0.05	-8	0.90 ± 0.04	-5	0.98 ± 0.04	-7	3.75 ± 0.04	-5

Table 5. Effect of autoclaving on the levels of L-Dopa, phytic acid and oligosaccharides in five accessions of *Mucuna* beans. The data are means and standard errors of triplicate determinations.

Accession	L-Dopa (g 100 g^{-1})	% loss	Phytic acid (g 100 g^{-1})	% loss	Oligosaccharides (g 100 g^{-1})					
					Raffinose	% loss	Stachyose	% loss	Verbascose	% loss
Thachenmalai (white)	5.05 ± 0.04	-15	0.44 ± 0.03	-28	0.63 ± 0.02	-42	0.53 ± 0.05	-53	1.96 ± 0.04	-52
Thachenmalai (black)	5.58 ± 0.02	-15	0.37 ± 0.03	-29	0.66 ± 0.02	-40	0.56 ± 0.05	-54	1.98 ± 0.01	-54
Mundandurai (white)	4.98 ± 0.03	-15	0.42 ± 0.02	-28	0.60 ±0.03	-40	0.55 ± 0.05	-54	1.92 ± 0.01	-54
Kailasanadu (white)	4.98 ± 0.02	-15	0.51 ± 0.04	-27	0.60 ± 0.03	-40	0.51 ± 0.05	-54	2.00 ± 0.02	-54
Valanad (black)	4.76 ± 0.02	-15	0.35 ± 0.04	-27	0.54 ± 0.02	-43	0.48 ± 0.04	-57	1.81 ± 0.04	-54

Table 6. Effect of repeated boiling in water on the levels of L-Dopa, phytic acid and oligosaccharides in five different accessions of *Mucuna* beans. The data are means and standard errors of triplicate determinations.

Accession	L-Dopa (g 100 g^{-1})	% loss	Phytic acid (g 100 g^{-1})	% loss	Oligosaccharides (g 100 g^{-1})					
					Raffinose	% loss	Stachyose	% loss	Verbascose	% loss
Thachenmalai (white)	2.38 ± 0.06	-60	0.38 ± 0.05	-38	0.65 ± 0.05	-38	0.48 ± 0.04	-53	2.21± 0.04	-48
Thachenmalai (black)	2.76 ± 0.05	-58	0.33 ± 0.04	-36	0.67 ± 0.05	-39	0.50 ± 0.04	-59	1.94 ± 0.05	-55
Mundandurai (white)	2.57 ± 0.03	-56	0.37 ± 0.07	-37	0.60 ± 0.04	-40	0.48 ± 0.08	-60	2.00 ± 0.06	-52
Kailasanadu (white)	2.46 ± 0.05	-58	0.43 ± 0.02	-38	0.59 ± 0.01	-41	0.42 ± 0.04	-62	2.13 ± 0.03	-51
Valanad (black)	2.46 ± 0.04	-56	0.30 ± 0.02	-37	0.55 ± 0.04	-42	0.40 ± 0.01	-62	1.98 ± 0.05	-50

Table 7. Effect of crude α - galactosidase treatment on the levels of oligosaccharides in five different accessions of *Mucuna* beans. The data are means and standard errors of triplicate determinations.

Accession	Oligosaccharide	Raw seeds (g 100 g^{-1})	Treated seeds	
			(g 100 g^{-1})	% loss
Thachenmalai (white)	Raffinose	1.05 ± 0.06	0.20 ± 0.06	81
	Stachyose	1.15 ± 0.01	0.03 ± 0.01	97
	Verbascose	4.25 ± 0.02	0.76 ± 0.04	82
Thachenmalai (black)	Raffinose	1.10 ± 0.05	0.18 ± 0.04	83
	Stachyose	1.22 ± 0.02	0.02 ± 0.10	98
	Verbascose	4.31 ± 0.04	0.76 ± 0.03	82
Mundandurai (white)	Raffinose	1.00 ± 0.02	0.15 ± 0.02	85
	Stachyose	1.20 ± 0.08	0.05 ± 0.02	95
	Verbascose	4.18 ± 0.07	0.72 ± 0.03	83
Kailasanadu (white)	Raffinose	1.0 ± 0.01	0.21 ± 0.01	79
	Stachyose	1.10 ± 0.08	0.02 ± 0.03	98
	Verbascose	4.34 ± 0.06	0.78± 0.04	82
Valanad (black)	Raffinose	0.95 ± 0.04	0.19 ± 0.04	80
	Stachyose	1.05 ± 0.05	0.03 ± 0.01	97
	Verbascose	3.95 ± 0.05	0.71± 0.05	82

CONCLUSION

It may be concluded that the presently investigated accessions of *Mucuna* beans from Tamil Nadu and Kerala, South India, exhibit variations in the levels of L-Dopa, phytic acid and oligosaccharides. Of the various common processing methods employed, repeated boiling in water followed by decanting greatly reduced all the investigated anti-nutrients except oligosaccharides. Incidentally, an indigenous hill tribe, Kanikkars, are known to consume the seeds after repeated boiling (seven times) in water followed by decanting. The most remarkable finding of the present study is that partially purified ∝-galactosidase can almost completely eliminate oligosaccharides. *Cassia sericea* is used as a source of ∝-galactosidase because of its high content of the substance, and its relatively lower cost in comparison to sources of fungal origin (Mulimani and Devendra, 1998). The use of crude enzyme, ∝-galactosidase, from *Cassia sericea* for the hydrolysis of raffinose family of sugars in different accessions of *Mucuna* beans is reported for the first time. Other cost-effective novel methods might be attempted to greatly reduce L-Dopa and other heat stable anti-nutrients include alkaloids and methylated and non-methylated tetrahydroquinoline compounds. After inactivation or elimination of these anti-nutrients, the *Mucuna* beans can be promoted as an alternative source of both food and feed not only in the third world countries but also at the global level.

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