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Nutritional and Antinutritional Assessment of *Mucuna atropurpurea* DC: An Underutilized Tribal Pulse

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Abstract: Two accessions of the underutilized legume, *Mucuna atropurpurea* DC, collected from two different locations of Western Ghats, Tamil Nadu were analyzed for proximate composition, mineral profiles, vitamins (niacin and ascorbic acid) fatty acid profiles, amino acid profiles of total seed protein, *in vitro* protein digestibility and certain antinutritional factors. Both the accessions of *Mucuna atropurpurea* contained higher amounts of crude protein and crude lipid when compared with most of the commonly consumed pulses. The fatty acid profiles of both the accessions revealed that the seed lipids contained higher concentration of palmitic and linoleic acid. Amino acid profiles of *Mucuna atropurpurea* revealed that the seed proteins contained relatively higher levels of essential amino acid, threonine, valine, isoleucine, tyrosine and phenylalanine compared with the FAO/WHO (1991) requirement pattern. Antinutritional substances like total free phenolics, tannins, L-DOPA, phytic acid, hydrogen cyanide, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity were investigated.

Key words: Underutilized legume · Proximate and mineral composition · Amino acid profiles · *in vitro* protein digestibility · Antinutrients

INTRODUCTION

The growing third world population and its domestic animals need more protein. The most cost-effective proteins are those derived from plant materials which, although in abundance in many developing countries, are underutilized. The search for novel high-quality but cheap sources of protein and energy has continued to be of major concern to governments and other bodies charged with the responsibility for food and nutrition in many parts of the developing world [1]. Leguminous seeds are important sources of protein in the diet of millions of people. There has been a constant search for new legumes with high protein contents and suggestions for utilization of unconventional legumes have been made from time to time [2]. In India, information on chemical composition of seeds of tribal pulses and wild progenitors of cultivated legumes is relatively meagre. While searching for new food sources, nutritionally-improved plants within the domesticated lines and wild plants are now receiving more attention. In view of this, in the present study an attempt was made to understand the nutritional and antinutritional profiles of two accessions of the underutilized tribal pulse *Mucuna atropurpurea* DC. In India, the roasted kernels of this tribal pulse are known to be consumed by the Palliyar tribals living in Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, South-Eastern slope of Western Ghats, Tamil Nadu [3].

MATERIALS AND METHODS

Source of Seed: Two accessions of *Mucuna atropurpurea* DC were gathered as mature pods from natural strands of two agroclimatic/ecological regions of Western Ghats Tamil Nadu, viz, Kalakad hills, Tirunelveli District and Sirumalai hills, Madurai District, Tamil Nadu. Pods of two accessions were collected from tropical forests of Western Ghats during April 2008. With the help of keys by Wilmot-Dear [4] , the accessions were botanically identified. After thoroughly drying in the sun, the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds, forgein material and mature seeds were stored in airtight plastic jars at room temperature (25°C).

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Proximate Composition: The moisture content was determined by drying 50 transversely cut seed in an oven at 80°C for 24 hr and is expressed on a percentage basis. The air-dried samples were powdered separately in a Wiley mill (Scientific Equipment, Delhi, India) to 60-mesh size and stored in screw capped bottles at room temperature for further analysis.

The nitrogen content was estimated by the micro-Kjeldahl method [5] and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus. The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr [6]. Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method proposed by Li and Cardozo [7]. The nitrogen free extract (NFE) was obtained by difference [8]. The energy value of the seed (kJ) was estimated by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, respectively [9].

Minerals and Vitamins Analysis: Five hundred milligrams of the ground legume seed was digested with a mixture of 10ml concentrated nitric acid, 4ml of 60% perchloric acid and 1ml of concentrated sulphuric acid. After cooling, the digest was diluted with 50ml of deionised distilled water, filtered with Whatman No. 42 filter paper and the filtrates were made up to 100ml in a glass volumetric flask with deionised distilled water. All the minerals except phosphorus were analysed from a triple acid-digested sample by an atomic absorption spectrophotometer-ECIL (Electronic Corporation of India Ltd., India) [10]. The phosphorus content in the triple acid digested extract was determined colorimetrically [11]. Ascorbic acid and niacin content were extracted and estimated as per the method given by Sadasivam and Manickam [12].

Lipid Extraction and Fatty Acid Analysis: The total lipid was extracted from the seeds according to the method of Folch *et al.* [13] using chloroform and methanol mixture in ratio of 2: 1 (v/v). Methyl esters were prepared from the total lipids by the method of Metcalfe *et al.* [14]. Fatty acid analysis was performed by gas chromatography (ASHMACO, Japan; Model No: ABD20A) using an instrument equipped with a flame ionization detector and a glass column (2mX3mm) packed with 1% diethylene glycol succinate on chromosorb W. The temperature conditions for GC were injector 200°C and detector 210°C. The temperature of the oven was programmed from 180°C and the carrier gas was nitrogen at a flow rate of 30ml/min. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters; the relative weight percentage of each fatty acid was determined from integrated peak areas.

Amino Acid Analysis: The total seed protein was extracted by a modified method of Basha *et al.* [15]. The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). A protein sample of 30mg was hydrolysed by 6N HCL (5ml) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 hr. The sealed tube was broken and the acid removed completely by repeated flash evaporation after the addition of de-ionized water. Dilution was effected by means of citrate buffer pH 2.2 to such an extent that the solution contained 0.5 mg protein ml^{-1}. The solution was passed through a millipore filter (0.45µM) and derivitized with O-phthaldialdehyde by using an automated pre-column (OPA). Aminoacids were analysed by a reverse-phase HPLC (Method L 7400, HITACHI, Japan) fitted with a denali C_{18} 5 micron column (4.6X 150mm). The flow rate was 1 ml min^{-1} with fluorescence detector. The cystine content of protein sample was obtained separately by the Liddell and Saville [16] method. For the determination of tryptophan content of proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together with 1 ml 5M NaOH. The ampoules were flame sealed and incubated at 110°C for 18 hr. The tryptophan contents of the alkaline hydrolysates were determined colorimetrically using the method of Spies and Chambers [17] as modified by Rama Rao *et al.* [18]. The contents of the different amino acids were expressed as g100g-1 proteins and were compared with FAO/WHO [19]. reference pattern. The essential amino acid score was calculated as follows:

$$\text{Essential amino acid score} = \frac{\text{grams essential amino acid in 100g of total protein}}{\text{grams of essential amino acid in 100g of FAO/WHO (1991) reference pattern}} \times 100$$

Analysis of Antinutritional Compounds: The antinutritional compounds, total free phenolics [20] tannins [21], the non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine) [22], phytic acid [23] and hydrogen cyanide [24] were quantified. Trypsin inhibitor

activity was determined by the method of Kakade *et al.* [25] by using enzyme assay of benzoil-DL-arginin-*p*-nitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein. Extraction, TLC separation and estimation of Oligosaccharides were done following the method of Somiari and Balogh [26]. The eluted individual oligosacchrides were estimated by the method of Tanaka *et al.* [27]. Lectin activity was determined by the method of Almedia *et al.* [28]. One g of air-dried seed flour was stirred with 10ml of 0.15N sodium chloride solution for 2hr and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20min. and the supernatants were collected separately. The protein content was estimated by the Lowry *et al.* [29] method.

Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3min at low speed. Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24 drops of phosphate-buffered saline. Human blood (blood groups A, B and O) was procured from the blood bank of Jothi Clinical Laboratory, Tuticorin. The determination of lectine was done by the method of Tan *et al.* [30]. The phytoheamagglutinating activity was expressed as heamagglutinating units (HU)/mg protein.

Determination of *in vitro* Protein Digestibility (IVPD): This was determined using the multi-enzyme technique [31]. The enzymes used for IVPD were purchased from Sigma Chemical Co., St. Louis, MO, USA. Calculated amounts of the control (casein) and sample were weighed out, hydrated in 10ml of distilled water and refrigerated at 5°C for 1h. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37°C. The IVPD was determined by the sequential digestion of the samples containing protein with a multi-enzyme mixture [trypsin (porcine pancreatic trypsin-type IX with 14190 BAEE unites per mg protein), ∝-chymotrypsin (bovine pancreatic chymotrypsin-type II, 60 units per mg powder) and peptidase (porcine intestinal peptidase-grade III, 40 units per g powder)] at 37°C followed by protease (type IV from *Streptomyces griseus*) at 55°C. The pH drop of the samples from pH 8.0 was recorded after 20min of incubation. The IVPD was calculated according to the regression equation $Y = 234.84\text{-}22.56\ X$, where Y is the % digestibility and X the pH drop.

RESULTS AND DISCUSSION

The contents of crude protein and crude lipid detected in the two accessions of *Mucuna atropurpurea* of the present investigation (Table 1) were found to be higher than the pulse crops commonly consumed in India, such as black gram, green gram, pigeonpea, chickpea and cowpea, which have been reported earlier [32-35]. The dietary fibre content of *Mucuna atropurpurea* was higher than that of other commonly cultivated pulses such as chickpea, horse gram, peas, red gram and black gram [36]. Due to the lipid rich nature, the seeds of both the accessions of *M. atropurpurea* registered high food energy values than those of *Phaseolus vulgaris, P. limensis, Vigna unguiculata, Cicer arietinum, Pisum sativum* and *Lens culinaris* [37].

Table 2 shows the mineral composition of the two accessions of *M. atropurpurea*. The seeds of both the accessions of *M. atropurpurea* contained higher levels of sodium, potassium and calcium when compared with other legumes, *Phaseolus vulgaris, P. limensis, Vigna unguiculata, Cicer arietinum, Pisum sativum* and *Lens culinaris* [38]. In the present investigation, both the accessions of *M. atropurpurea* registered higher level of potassium when compared with recommended dietary allowance value (RDA) of infants and children (<1550mg) [39]. The high content of potassium can be utilized beneficially in the diets of people who take diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid [40]. The manganese content of *M. atropurpurea* was found to be higher than that of Estimated Safe and Adequate Daily Dietary Intakes of minerals (ESADDI) [41]. The presently investigated tribal pulse exhibited the highest level of niacin content (Table 2). This was found to be higher than that of an earlier report in *Cajanus cajan, Dolichos lablab, D. biflorus, Mucuna pruriens, Phaseolus mungo,Vigna catjang* and *Vigna sp.* [42]. The investigated tribal pulse also registered higher level of ascorbic acid content than *Atylosia scarbaeoides* and *Lablab purpureus* var. *lignosus* [43].

The data on fatty acid composition of the total lipids of both the accessions of *M. atropurpurea* are summarized in Table 4. Fatty acid profile of two accessions of *M. atropurpurea* revealed that lipids as a

Table 1: Proximate composition of the seeds of *Mucuna atropurpurea*[a] along with some of the most common pulses (g100g^{-1})

	Component of 100g^{-1}						
Accessions	Moisture	Crude protein (Kjeldahl Nx6.25)	Crude lipid	Total dietary fiber	Ash	NFE (Nitrogen Free Extractive)	Calorific values (KJ100g^{-1}DM)
M. atropurpurea Kalakad	10.50±0.10	24.38±0.27	12.40±0.31	7.80±0.05	4.41±0.03	51.01	1726.49
M. atropurpurea Sirumalaihills	11.47±0.07	23.50±0.51	14.10±0.21	8.40±0.14	3.56±0.01	50.44	1766.37
Phaseolus mungo[b]	-	23.3	1.25	4.04	-	67.80	-
Phaseolus aureus[b]	-	22.3	1.12	4.83	-	68.30	-
Cicer arietinum[c]	-	20.7	4.50	3.50	2.70	57.1	-
Cajanus cajan[d]	-	19.4	3.24	5.56	4.05	57.2	-
Vigna unguiculata[e]	-	22.5	1.60	5.33	3.81	56.9	-

[a]All values are means of triplicate determinations expressed on a dry weight basis ± denotes standard error.
Sources: [b]Gupta and Wage (1978); [c]Jambunathan and Singh (1980); [d]Nwokolo (1987); [e]Nwokolo and Oji (1985)

Table 2: Mineral composition and vitamins (niacin and ascorbic acid) of the seeds of *Mucuna atropurpurea*. (mg 100 g^{-1})[a]

Components	*M. atropurpurea* Kalakad	*M. atropurpurea* Sirumalai hills
Sodium	44.19±0.32	38.34±0.21
Potassium	2148.00±1.24	2289.30±2.20
Calcium	196.36±0.27	178.41±0.14
Magnesium	82.00±0.17	64.70±0.07
Phosphorus	154.78±0.11	164.00±0.05
Iron	6.44±0.05	6.70±0.33
Zinc	4.2.40±0.01	1.69±0.05
Copper	0.58±0.03	0.78±0.01
Manganese	6.31±0.14	6.01±0.03
Niacin	56.34±0.24	74.32±0.64
Ascorbic acid	44.39±0.38	66.30±0.031

[a] All values are of means of triplicate determination expressed on dry weight basis ± denotes Standard error.

Table 3: Fatty acid composition of lipids of *Mucuna atropurpurea* seeds[a] compared with common legume seed lipids

	Fatty acid %									
Pulses	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
M. atropurpurea Kalakad	-	24.10	-	14.12	18.79	28.32	11.48	-	-	3.19
M. atropurpurea Sirumalaihills	-	27.14	-	16.02	13.26	24.34	14.69	-	-	3.55
Cajanus cajan[b]	-	20.50	-	6.90	10.50	56.30	5.00	0.80	-	-
Vigna radiata[b]	-	14.10	-	4.30	20.80	16.30	35.70	-	-	9.30
Vigna mungo[b]	-	17.80	-	5.90	17.30	11.60	47.50	-	-	-
Vigna unguiculata[c]	2.00	14.60	9.30	7.30	18.90	20.70	18.20	-	7.90	1.10
Phaseolus vulgaris[c]	-	15.10	6.30	9.10	19.80	20.60	17.30	-	3.80	8.00

[a]All values are of two determinations. Sources: [b]Salunkhe *et al.*(1982); [c]Omogbai (1990)

Table 4: Amino acid profiles of acid-hydrolysed, purified seed proteins of *Mucuna atropurpurea* (g100g^{-1})

Amino acid	*M. atropurpurea* Kalakad	EAAS	*M. atropurpurea* Sirumalai hills	EAAS	FAO/WHO (1991) requirement pattern
Glutamic acid	11.70		13.84		
Aspartic acid	13.24		14.40		
Serine	ND		0.78		
Threonine	3.34	98.24	4.30	126.47	3.4
Proline	1.94		1.78		
Alanine	3.36		3.54		
Glycine	4.94		5.50		
Valine	3.78	108.00	4.68	133.71	3.5
Cystine	0.90	}67.60	0.58	}48.80	2.5
Methionine	0.79		0.64		
Isoleucine	3.59	128.21	5.56	198.57	2.8
Leucine	4.11	62.27	4.54	68.79	6.6
Tyrosine	3.17	}111.43	3.78	}125.40	6.3
Phenylalanine	3.85		4.12		
Lysine	4.59	79.14	4.24	73.10	5.8
Histidine	2.56	134.74	1.24	65.26	1.9
Tryptophan	0.79	71.81	0.54	49.09	1.1
Arginine	4.34		4.14		

EAAS: Essential amino acid score

Table 5: Data on IVPD and antinutritional factors of seeds of *Mucuna atropurpurea*

Components	*M. atropurpurea* Kalakad			*M. atropurpurea* Sirumalai hills		
in vitro protein digestibility (%)[a]	71.40			74.54		
Total free phenolics[b] g $100g^{-1}$	2.48±0.03			354±0.09		
Tannins[b] g $100g^{-1}$	0.24±0.01			0.16±0.03		
L-DOPA[b] g $100g^{-1}$	4.20±0.11			3.84±0.17		
Phytic acid[b] mg $100g^{-1}$	472.03±1.21			452.42±0.28		
Hydrogen cycnide[b] mg $100g^{-1}$	0.24±0.03			0.33±0.01		
Trypsin inhibitor activity[a] (TIU mg^{-1} protein)	43.20			40.10		
Oligosacchride[b] g $100g^{-1}$	Raff	Stac	Verb	Raff	Stac	Verb
	0.54±0.01	1.61±0.03	5.55±0.08	0.94±0.02	1.36±0.04	4.58±0.09
Phytohaemagglutinating activity [Hu mg^{-1} protein[a]]	A group	B group	O group	A group	B group	O group
	128	59	14	118	45	17

Raff: Raffinose; Stac: Stachyose; Verb: Verbascose

[a] All values of two independent experiments.

basis ± denotes standard error,

[b] All values are means of triplicate determination expressed on dry weight

good source of the nutritionally essential linoleic and oleic acids. Linoleic acid was the dominating fatty acid, followed by palmitic acid and oleic acid. The nutritional value of linoleic acid is due to its metabolism at tissue levels which produce the hormone-like prostaglandins. The activity of these prostaglandins includes lowering of blood pressure and constriction of smooth muscle. Linoleic and linolenic acids are the most important essential fatty acid required for growth, physiological functions and maintenance. In the present study, most of the fatty acids were unsaturated fatty acids. The fatty acid composition and high amounts of unsaturated fatty acids make *M. atropurpurea* a special legume, suitable for nutritional application. The fatty acid composition of the presently investigated tribal pulse is comparable in the some edible legumes (Table 4), such as *Vigna radiata, V.mungo* [44] and *Phaseolus vulgaris* [45]. The anti-nutritional fatty acid, behenic acid was present in the investigated samples as in groundnut (46) and winged bean [47]. The presence of behenic acid has been implicated with atherogenic property [46]. The amino acid profiles of the purified seed proteins and the essential amino acid score are presented in table 5. The contents of cystine, methionine, leucine, lysine and tryptophan seem to be deficient in both the accessions of *M. atropurpurea*; whereas, threonine, valine, isoleucine, tyrosine and phenylalanine in both the accessions of present investigation were found to be higher compared to the FAO/WHO [19] requirement pattern. Among the two accessions of seed materials of *M. atropurpurea*, the Sirumalai hills accession registered the highest level of *in vitro* protein digestibility (74.54%) than that of an earlier study in the *Mucuna pruriens* var. *utilis* (black coloured seed coat) accession [48]. In the present study, the high levels of trypsin inhibitor activity in the Kalakad hills accession (40.10 TIU mg^{-1} protein) might be attributed for low protein digestibility.

The problem of plant protein digestibility has been suggested to be because of the interplay of several factors such as protease inhibitors, phytates, oxalates, lectins, goitrogens and other antinutritional factors. In societies where legumes are consumed rather than much more expensive animal foods, there was found to be great concern over the level of antinutritients in the diets. For this reason, a preliminary evalution of some of these factors in the seeds of *M. atropurpurea* was made (Table 5). The content of total free phenolics of investigated seed samples of *M. atropurpurea* was found to be low when compared with four accessions of *M. pruriens* var. *utilis* [48]. The contents of tannins present in the seeds of both the accessions of *M. atropurpurea* appeared to be low when compared with the commonly consumed legume seeds such as green gram, cowpea, pigeon pea and black gram [49]. The concentration of non-protein amino acid L-DOPA in *M. atropurpurea* (both the accessions) has been found to be low when compared with values reported earlier in *M. pruriens* var. *utilis* [48,50]. It has been demonstrated that in *M. pruriens*, the level of L-DOPA was significantly eliminated by dry heat treatment [9] and cooking and autoclaving [51]. The two accessions seed material of *M. atropurpurea* was found to be low level of phytic acid when compared with commonly consumed grain legumes like *Vigna mungo* [52] and *Vigna radiata* [53]. The phytate molecule is negatively changed at the physiological pH and is reported to bind nutritionally important essential divalent cations, such as iron, zinc, magnesium and calcium. This binding forms insoluble complexes, thereby making minerals unavailable for absorption and utilization. The level of hydrogen cyanide in *M. atropurpurea* seems to be negligible when compared with the lethal level of HCN (35mg/100g) [54]. The trypsin inhibitors activity was found to be low compared to *Cajanus cajan* var. pant A2 and UPAS-120 [55].

The oligosaccharide content of the seeds of *M. atropurpurea* was comparable with those five accessions of other species of *M. pruriens* var. *utilis* [56]. Verbascose was found to be the major oligosaccharide in both the accessions of *M. atropurpurea* as has been reported earlier in *M. pruriens* var. *utilis* [56]. Regarding phytohaemagglutinating activity, both the accessions of *M. atropurpurea* registered higher phytohaemagglutinating activity with respect to 'A' blood group of human erythrocytes. Both the accessions had low levels of phytohaemagglutinating activity with respect to erythrocytes of 'O' blood group. This is in good agreement with earlier reports in the other *Mucuna* species [51]. However, dry-heat and autoclaving are known to inactivate completely the trypsin inhibitors and phytohaemagglutins in *Mucuna* beans [9].

The observations made in the present study show that both the accessions of *M. atropurpurea* are rich in crude protein, most of the essential amino acids, fatty acid such as palmitic and linoleic acid and some minerals. This study reveals that the nutritional profiles of both the accessions of *M. atropurpurea* seems to be similar to or higher than that of the other *Mucuna* species/accessions reported earlier and can also be alleviate protein-energy-malnutrition, among the economically weaker sections of peoples in developing countries. The presence of antinutritional factors identified in the current report should not pose a problem for humans, if the beans are properly processed.

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