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NUTRITIONAL AND ANTINUTRITIONAL POTENTIAL OF FIVE ACCESSIONS OF A SOUTH INDIAN TRIBAL PULSE *Mucuna atropurpurea* DC.

[CARACTERISTICAS NUTRITIVAS Y ANTINUTRITIVAS DE CINCO ACCESIONES DE *atropurpurea* DC PROVENIENTES DEL SUR DE LA INDIA]

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SUMMARY

Five accessions of the underutilized legume, *Mucuna atropurpurea* DC collected from five different agroclimatic regions in Western Ghats, Tamil Nadu, India were analysed for their proximate and mineral composition, vitamins (niacin and ascorbic acid) fatty acid profiles, amino acid profiles of total seed proteins, *in vitro* protein digestibility (IVPD) and certain antinutritional factors. The crude protein ranged from 21.10 – 24.72%, crude lipid 8.40 – 14.10%, total dietary fibre 6.48 – 8.82%, ash 3.78 – 4.76% and carbohydrates 48.20 – 58.51%. The energy level of the seed (1576.18 – 1620.43kJ100g^{-1}DM) was comparable with commonly consumed Indian pulses. The investigated seeds were rich in minerals such as Na, K, Ca and Mn. The fatty acid profiles of all accessions revealed that the seed lipids contained higher concentrations of linoleic and palmitic acid. The essential amino acid profile of total seed proteins compared favourably with FAO/WHO (1991) requirement pattern, except that there were deficiencies of sulphur containing amino acids in all the five accessions. The IVPD of the accessions ranged from 69.00 – 74.10%. Antinutritional substances, total free phenolics, tannins, L-DOPA, phytic acid, hydrogen cyanide, total and soluble oxalates, trypsin inhibitor activity, oligosaccharides and haemagglutinating activity were revealed that all the accessions contained the lowest levels of all the investigated antinutrients when compared with other *Mucuna* species except total and soluble oxalates. Verbascose was the major oligosaccharide in all the accessions. Lower levels of phytohaemagglutinating activity for human erythrocytes of 'O' blood group than for 'A' and 'B' blood groups were found in all accessions. The antinutritional fatty acid, behenic acid also was detected in the present study. Therefore, *M. atropurpurea* represents a potential food source if given adequate promotion and research attention.

Key words: *Mucuna atropurpurea*; accessions; anti-nutrients; L-DOPA; phytic acid; Oxalate; IVPD.

RESUMEN

Se analizaron cinco accesiones de *Mucuna atropurpurea* DC colectadas de cinco diferentes regiones agroclimaticas de Ghats occidentales, Tamil Nadu, India. Se evaluó composición proximal, contenido mineral, vitaminas (niacina y ácido ascorbico), perfil de ácidos grasos y amino ácidos, digestibilidad in vitro de la proteína y factores antinutricionales. El contenido de proteína cruda fluctuó de 21.1 a 24.7%, lípidos de 8.4 a 14.1%, fibra dietetica 6.4 a 8.82%, y carbohidratos de 48.2 a 58.5%. El contenido de energia fluctuo de 1.5 a 1.6 MJ 100g MS. Las semillas fueron ricas en Na, K, Ca y Mn. Se obtuvo una alta concentración de linoleico y palmitico. El perfil de amino ácidos fue comparable con el perfil FAO/WHO (1991) pero con una pequeña deficiencia en amino ácidos azufrados. La digestibilidad in vitro de la proteína fluctuó de 69 a 74%. El contenido de factores antinutricionales fue menor al de otras accesiones de Mucuna, excepto por el contenido de oxalatos. El principal carbohidrato fue Verbascosa. Mucuna atropurpurea representa un alimento potencial para el consumo humano que requiere mayor atención y promoción.

Palabras clave: *Mucuna atropurpurea*; variedades; compuestos antinutricionales; L-DOPA; ácido fítico; oxalato; digestibilidad in vitro de la proteína.

INTRODUCTION

The prevalence of hunger and protein malnutrition in tropical and subtropical areas of the world's well recognized and appreciated (Abatena, 1987; FAO, 1994; Univ, 1994). Several reports indicate protein deficiency as the commonest form of malnutrition in the developing countries particularly in regions where diets are mainly based on roots and tuber crops (Ashworth, 1985; Sankale and Barbotin–Larrieu, 1984). The problem becomes more obvious when per capita protein intake is considered (Pellet and Young, 1994). Providing sufficient protein should therefore be given the highest priority in every effort to increase national food supplies. In view of prevalent food shortages, attention is currently being focused on the exploitation of lesser – known and unconventional plant resources (Becker, 1986; Vadivel and Janardhanan, 2000; Arinathan *et al.,* 2007). In this content, a detailed investigation of all plant resources available worldwide, including little-known plant species, is the need of the hour to feed an ever – increasing population. The tribal sects of India live on a vast treasure of germplasm that remains untapped. Some of the underutilized legumes/tribal pulses may fit well into subsistence agriculture as alternative protein sources.

Despite the potential of under utilized species as a source of less consumed food and medicine, to our knowledge, meager information is available on the germplasm collection from South India and its evaluation for chemical composition. In south India, the tropical forests of Western Ghats, one of the 18 biodiversity hot-spots of the world (Gadgil, 1996), has a large groups underutilized food plants from leguminosae, whose chemical potential hitherto remains untapped. In the context, nutritional and antinutritional evaluation of five accessions of *Mucuna atropurpurea* collected from five different agroclimatic/ecological regions of South-Eastern slopes of Western Ghats, Tamil Nadu have been investigated. 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 (Arinathan *et al.*, 2007).

MATERIAL AND METHODS

Collection of seeds

Five accessions of *Mucuna atropurpurea* DC. were collected as mature pod (each accession nearly 5 Kg) from natural stands of five different agroclimatic regions of Western Ghats, Tamil Nadu, India (Table 1). With the help of keys by Wilmot-Dear (1987), the accessions were botanically identified. The pods were collected from the tropical forests of South Western Ghats. After thoroughly drying in the sun, the pods were thrashed to remove seeds. The seeds, after through cleaning and removal of broken seeds and foreign materials and mature seeds were stored in airtight plastic jars at room temperature (25°C).

Proximate composition

The moisture content (%) was determined by drying 50 transversely cut seed in an oven at 80°C for 24 h. 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 (Humphries, 1956) 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 at 600°C for 6h (AOAC, 1975). Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method (Li and Cardozo, 1994). To determine the TDF, duplicate 500 mg ground samples were taken in separate 250 mL beakers. To each beaker 25 mL water was added and gently stirred until the samples were thoroughly wetted, (i.e. no clumps were noticed). The beakers were covered with Al foil and allowed to stand 90 min without stirring in an incubator maintained at 37°C. After that, 100 mL 95% ethanol was added to each beaker and allowed to stand for 1 h at room temperature (25±2°C). The residue was collected under vacuum in a pre-weighed crucible containing filter aid. The residue was washed successively with 20 mL of 78% ethanol, 10 mL of 95% ethanol and 10 ml acetone. The crucible containing the residue was dried $\geq$2 h at 105°C and then cooled $\geq$ 2 h in a desiccator and weighed. One crucible containing residue was used for ash determination at 525°C for 5 h. The ash-containing crucible was cooled for $\geq$ 2h in a desiccator and weighed. The residue from the remaining duplicate crucible was used for crude protein determination by the micro-Kjeldahl method as already mentioned. The TDF was calculated as follows.

$$\text{TDF\%} = 100 \times \frac{Wr - [(P+A)/100]\,Wr}{Ws}$$

Where: Wr = mg residue, P = % protein in the residue; A = % ash in the residue, and Ws = mg sample.

The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). 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 (Siddhuraju *et al.*, 1996).

Table 1. Collection details of five accessions of *Mucuna atropurpurea DC.*

Botanical name, & Accession No.	Place of Collection	Agro-climatic region
Mucuna atropurpurea DC. VOC/Bot/VRM/0721	Thallainai hills, Tirunelveli Dist, Tamil Nadu.	Latitude: 09º 18" 44.43" N Longitude: 077º 20" 56.71" E Annul rain Fall: 1440 – 1905 mm Habitat: Deciduous forest, slightly elevated in 1004 ft altitude, sandy soil.
VOC/Bot/VRM/0817	Saduragiri hills, Virudhunagar Dist, Tamil Nadu.	Latitude: 09º 34" 20.43" N Longitude: 077º 39" 51.22" E Annul rain Fall: 1150 – 1680 mm Habitat: Deciduous forest, slightly elevated in 950 ft altitude, sandy soil.
VOC/Bot/VRM/0724	Sivagiri Reserve forest, Tirunelveli Dist, Tamil Nadu.	Latitude: 09º 22" 39.61" N Longitude: 077º 22" 15.28" E Annul rain Fall: 1435 – 1895 mm Habitat: Semi evergreen forest, slightly elevated in 833 ft altitude, sandy soil.
VOC/Bot/VRM/0711	Anaikatti Reserve Forest, Coimbatore Dist, Tamil Nadu.	Latitude: 11º 05" 26.85" N Longitude: 076º 50" 14.20" E Annul rain Fall: Habitat: Deciduous forest, slightly elevated in 1916 ft altitude, sandy soil.
VOC/Bot/VRM/0842	Ayyanarkoil Reserve Forest, Virudhunagar District, Tamil Nadu.	Latitude: 09º 19" 00.44" N Longitude: 077º 22" 27.12" E Annul rain fall: 1440 – 1905 mm Habitat: Deciduous forest, slightly elevated in 697 ft altitude, loamy soil.

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) (Issac and Johnson, 1975). The phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman and Bray, 1940).

Ascorbic acid and niacin content were extracted and estimated as per the method given by Sadasivam and Manickam (1996). For the extraction of ascorbic acid, 3g air-dried powdered sample was ground with 25mL of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10mL of the filtrate until it turned orange-yellow to remove the enolic hydrogen atoms. The excess of bromine was expelled by blowing in air. This filtrate was made up to 25mL with 4% oxalic acid and used for ascorbic acid estimation. Two millilitres of the extract was made up to 3mL with distilled H_2O in a test tube. One millilitre of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. The contents of the test tube were mixed thoroughly. After 3hr incubation at 37°C, 7mL of 80% H_2SO_4 was added to dissolve the osazone crystals and the absorbance was measured at 540nm against a reagent blank.

For the extraction of niacin, 5g air-dried powdered sample was steamed with 30mL concentrated H_2SO_4 for 30min. After cooling, this suspension was made up to 50mL with distilled H_2O and filtered. Five millilitres of 60% basic lead acetate was added to 25mL of the filtrate. The pH was adjusted to 9.5 and centrifuged (10,000g for 10 min at room temperature) to collect the supernatant. Two millilitres of concentrated H_2SO_4 was added to the supernatant. The mixture was allowed to stand for 1hr and centrifuged (10,000g for 10 min at room temperature). The 5mL of 40% $ZnSO_4$ was added to the supernatant. The pH was adjusted to 8.4 and centrifuged (10,000g for 10 min at

room temperature) again. Then the pH of the collected supernatant was adjusted to 7 and used as the niacin extract. For estimation, 1mL extract was made up to 6mL with distilled water in a test tube, 3mL cyanogen bromide was added and shaken well, followed by addition of 1mL of 4% aniline. The yellow colour that developed after 5min was measured at 420nm against a reagent blank. The ascorbic acid and niacin contents present in the sample were calculated by referring to a standard graph and expressed as milligrams per 100grams of powdered samples.

Lipid extraction and fatty acid analysis

The total lipid was extracted from the seeds according to the method of Folch *et al.*, (1957) 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.*, (1966). 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.*, (1976). 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 deionized 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). Amino acids 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 (1959) 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 (1949) as modified by Rama Rao *et al.*, (1974). The contents of the different amino acids were expressed as g100g-1 proteins and were compared with FAO/WHO (1991) reference pattern. The essential amino acid score (EAAS) was calculated as follows:

$$EAAS = \frac{EAAS\ (g/100g\ of\ total\ protein)}{EAAS\ (g/100g\ reference\ pattern)} \times 100$$

Analysis of antinutritional compounds

The antinutritional compounds, total free phenolics (Bray and Thorne, 1954), tannins (Burns, 1971), the non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine) (Brain, 1976), phytic acid (Wheeler and Ferrel, 1971), hydrogen cyanide (Jackson, 1967), total oxalate and soluble oxalate (AOAC, 1984) were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al.*, (1974) by using 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

Extraction of oligosaccharides was done following the method of Somiari and Balogh (1993). Five grams each of both raw seed flours of all the two accessions were extracted with 50 mL of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13hr 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 concentrationed sugar syrup was dissolved in 5mL 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 separatedly. 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 (1%, w/v). Plates were dried in a hot-air oven. The separated spots were compared with

standard sugar spots. A standard sugar mixture containing raffinose, stachyose and verbacose (Sigma chemical). Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped, eluted in 2 mL of distilled water kept overnight at room temperature 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 Elico UV-Spectrophotometer model SL 150 at 432 nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was expressed on dry weight basis

Quantitative determination of phytohaemagglutinating (Lectin) activity

Lectin activity was determined by the method of Almedia *et al.*, (1991). One g of air-dried seed flour was stirred with 10mL of 0.15N sodium chloride solution for 2hours 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.*, (1951) method.

Human blood (blood groups A, B and O) was procured from the blood bank of Jothi Clinical Laboratory, Tuticorin. Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3min at low speed (3,000g for 10 min at room temperature). 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.

The determination of lectin was done by the method of Tan *et al.*, (1983). Clear supernatant (50µl) was poured into the depression (pit) on a microtitration plate and serially diluted 1:2 with normal saline. The human blood erythrocyte (A, B and O blood groups) suspensions (25µl) were added to each of the twenty depressions. The plates were incubated for 3 hours at room temperature. After the incubation period, the titer values were recorded. One Haemagglutinating unit (HU) is defined as the least amount of heamagglutinin that will produce positive evidence of agglutination of 25µl of a blood group erythrocyte after 3hr incubation at room temperature. 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 (Hsu *et al.*, 1977). 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 10 mL 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 20 min of incubation. The IVPD was calculated according to the regression equation $Y = 234.84 - 22.56\ X$, where Y is the % digestibility and X the pH drop.

Statistical analysis

Analysis of Variance (ANOVA) and Duncan multiple range test (DMRT) were used for analysis (SPSS software for windows release 11.5; SPSS Inc., Chicago IL, USA) of any significant different in chemical compositions among the five accessions collected from the five locations. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of proximate analysis are shown in table 2. The *Mucuna atropurpurea* seeds contain 21.10 – 24.72% of protein, a range that is higher than those reported for food legumes such as *Cajanus cajan* (Nwokolo, 1987) and *Cicer arietinum* (Janardhanan and Singh, 1980). These two legumes are used extensively in tropical Indian diets and are expected to play a significant role in improving protein nutrition in India. The Anaikatti accession had the lowest level and the Ayyanarkoil accession the highest crude protein. The significant ($p \leq 0.05$) difference in protein content was probably due to different growing conditions for the plants from which the seeds were colled. The range of protein obtained in the present study is also lower than those reported for the beans of other *Mucuna* species namely *M. gigentea* (Rajaram and Janardhanan, 1991), *M. utilis* (=*M. pruriens* var. *utilis*) (Mohan and Janardhanan, 1995a) *Mucuna pruriens* var. *utilis* (four accessions) (Vadivel and Janardhanan,

2000a) and *M. pruriens* (=*Mucuna pruriens* var. *pruriens*) (Mary Josephine and Janardhanan, 1992; Vadivel and Janardhanan, 2000b). The crude lipid content detected in the samples of the present investigation was found to be higher than the pulses crops commonly consumed in India, such as black gram, green gram, pigeon pea, chick pea and cowpea, which have been reported earlier (Gupta and Wagle, 1978; Jambunathan and Singh, 1980, Nwokolo and Oji, 1985, Nwokolo, 1987). The Saduragiri accession had the highest level of crude lipid than the other accession. Nonetheless, the occurrence of higher contents of crude lipid, compared to the values of the present samples, in *M. utilis, M. monosperma* (Mohan and Janardhanan, 1995a); *M. gigantea, M. hirsuta* (Rajaram and Janardhanan, 1991; 1992) *Parkia roxburghii* (Mohan and Janardhanan, 1993) and *Tamarindus indica* (Arinathan *et al*., 2009) has already been detected and reported. The dietary fibre ranged from 6.48 to 8.82 %. The dietary fibre content of *M. atropurpurea* was higher than that of other commonly cultivated pulses such as chick pea, horse gram, peas, red gram and black gram (Premakumari *et al.*, 1984). Due to the lipid rich nature, the seeds of all the accessions of *M. atropurpurea* registered high food energy values than that of *Phaseolus vulgaris, P. limensis, Vigna unguiculata, Cicer aritinum, Pisum sativum* and *Lens culinaris* (Meiners *et al*., 1976a).

Robinson (1987) reported that a diet that meets two-thirds of the RDA (Recommended Dietary Allowances) values is considered to be adequate for an individual. Food legumes have been recognized as important sources of several mineral in India diets (Gopalan *et al.*, 1978). In the present investigation, all the accessions of *M. atropurpurea* registered a higher level of potassium (Table 3) when compared with recommended dietary allowance value (RDA) of infants and children (<1550mg) (NRC/NAS, 1980). 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 (Siddhuraju *et al.*, 2001). The seeds of all 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 aritinum, Pisum sativum* and *Lens culinaris* (Meiners *et al.*, 1976b). The manganese content of *M. atropurpurea* was found to be high than that of Estimated Safe and Adequate Daily Dietary Intake of minerals (ESADDI) (NRC/NAS, 1989). Generally, the statistically significant difference in values, except zinc, presented in Table 3 may have been related to the soil types in which the legume plant was found growing and/or to the efficiency of the uptake from the soil by the plants (Vadivel and Janardhanan, 2002).

The presently investigated seeds of *M. atropurpurea* exhibited the highest level of niacin content (Table 4). This was found to be higher than that of an earlier report in *Cajanus cajan, Dolichos lablab, D. biflorus, Mucuna pruriens, Phaseolus mungo, Vigna catjans* and *Vigna* species (Rajyalakshmi and Geervani, 1994) and *Rhynchosia suaveolen*s and *Vigna unguiculata* subsp. *cylindrica* (Arinathan *et al.*, 2009). The presently investigated tribal pulse also registered higher level of ascorbic acid content than *Cicer arietinum* (Fernandez and Berry, 1988); *Atylosia scarabaeoides* and *Teramnus labialis* (Arinathan *et al*., 2003; 2009).

Table 2. Proximate composition of five accessions of *Mucuna atropurpurea*. (g 100 g^{-1} seed flour).*

Components	Thallaianai	Saduragiri	Sivagiri	Anaikatti	Ayyanarkoil
Moisture	10.69±0.58[a]	10.47±0.57[a]	9.91±0.35[a]	11.41±0.01[a]	10.23±0.78[a]
Crude protein (Kjeldhal N × 6.25)	22.44±1.19[b]	24.53±0.04[b]	23.43±0.17[b]	21.10±0.58[a]	24.72±1.13[b]
Crude lipid	10.48±0.01[b]	14.10±0.58[d]	9.72±0.01[b]	8.42±0.01[a]	12.24±0.01[c]
TDF (Total Dietary Fibre)	7.52±0.57[b]	8.49±0.34[b]	6.48±0.57[a]	7.81±0.15[b]	6.79±0.01[a]
Ash	4.75±0.58[b]	4.38±0.01[b]	4.76±0.03[b]	4.21±0.06[b]	3.78±0.01[a]
Nitrogen Free Extractives (NFE)	54.87	48.20	55.62	58.51	52.47
Calorific value (kJ100g^{-1} DM)	1685.13	1745.66	1686.41	1646.17	1750.52

*All values are means of triplicate determinations expressed on a dry weight basis ± denotes standard error.
→Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

Table 3. Mineral composition of five accessions of *Mucuna atropurpurea*. (mg 100 g^{-1} seed flour).* ✈

Components	Thallaianai	Saduragiri	Sivagiri	Anaikatti	Ayyanarkoil
Sodium	44.94±0.59^{d}	38.30±1.72^{c}	23.41±2.16^{a}	40.11±2.89^{c}	32.47±0.58^{b}
Potassium	1945.01±3.28^{a}	2148.10±5.74^{c}	2230.24±0.59^{d}	2048.09±1.17^{b}	2336.69±0.57^{e}
Calcium	220.01±0.58^{d}	193.48±0.59^{c}	151.10±0.58^{a}	178.40±0.57^{b}	223.45±1.20
Magnesium	101.01±0.57^{e}	94.72±1.00^{d}	82.14±0.57^{b}	72.41±0.01^{a}	90.40±0.58^{c}
Phosphorus	154.78±1.16^{b}	176.97±0.34^{d}	184.30±1.16^{e}	134.17±0.57^{a}	164.94±2.19^{c}
Iron	6.49±0.02^{b}	5.48±0.01^{a}	5.84±0.01^{a}	6.01±0.57^{a}	6.22±0.02^{a}
Zinc	2.02±0.58^{a}	1.87±0.01^{a}	2.12±0.01^{a}	2.38±0.01^{a}	2.20±0.01^{a}
Copper	0.98±0.01^{d}	0.78±0.04^{c}	0.84±0.01^{c}	0.54±0.01^{b}	0.36±0.01^{a}
Manganese	5.75±0.01^{a}	6.18±0.01^{b}	7.48±0.02^{e}	6.79±0.01^{d}	6.58±0.01^{c}

*All values are of means of triplicate determination expressed on dry weight basis ± denotes Standard error.
✈Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

Table 4. Vitamins (niacin and ascorbic acid) content of five accessions of *Mucuna atropurpurea*. (mg 100g^{-1}).* ✈

Components	Thallaianai	Saduragiri	Sivagiri	Anaikatti	Ayyanarkoil
Niacin	25.10±0.57^{a}	31.57±0.87^{c}	38.10±1.00^{d}	28.50±1.26^{b}	32.14±0.58^{c}
Ascorbic acid	38.14±0.58^{c}	34.28±0.57^{b}	29.20±0.58^{a}	30.79±0.56^{a}	35.26±1.16^{b}

* All values are of means of triplicate determination expressed on dry weight basis ± denotes Standard error
✈ Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

The fatty acid profiles (Table 5) revealed that all the accessions of *M. atropurpurea* seeds contained high levels of linoleic acid, as in the case of some edible legumes such as *Vigna radiata, V. mungo* (Salunkhe *et al.*, 1982), *V. unguiculata* and *Phaseolus vulgaris* (Omogbai, 1990). Oleic acid was found to be higher than the pulse crop commonly consumed in India such as *Cajanus cajan* (Salunkhe, 1982). Nonetheless, the palmitic acid level of an earlier investigation in *Bauhinia racemosa* (Mohan and Janardhanan, 1994a) was higher than the present samples. However, its level was comparable with that of other investigations, such as *Vigna trilobata* (Siddhuraju *et al.*, 1992) and *Mucuna monosperma* (Mohan and Janardhanan, 1995a) and *Canavalia ensiformis* (Mohan and Janardhanan, 1994b). Similarly, the level of stearic acid detected in the samples investigated in the present study seems to be higher than the samples of *Parkia roxburghii* and *Entada phaseoloides* of an earlier investigation (Mohan and Janardhanan, 1993) and comparable with that of another tribal pulse, *Cassia obtusifolia* (Mohan and Janardhanan, 1995b). The antinutritional fatty acid, behenic acid was detected in *Mucuna atropurpurea* (all the accessions). Earlier reports indicated the detection of behenic acid in ground nut (Kritchevsky *et al.*, 1973), winged bean (Bean *et al.*, 1984; Fernando and Bean, 1985, 1986) *Parkia roxbughii, Entada phaseoloides* (Mohan and Janardhanan, 1993) *Mucuna utilis* and *M. monosperma* (Mohan and Janardhanan, 1995a). The presence of behenic acid has been implicated with atherogenic property (Kritechevsky *et al*., 1973).

The amino acid profiles of the purified seed proteins and the essential amino acid score was presented in Table 6. The contents of cystine, methionine, leucine and tryptophan seem to be deficient in all the accessions of *Mucuna atropurpurea*; whereas threonine, valine, isoleucine, tyrosine and phenylalanine in all the accessions of present investigation were found to be higher compared to the FAO/WHO (1991) requirement pattern. Among the five accessions of seed materials of *Mucuna atropurpurea*, the Ayyanarkoil accession registered the highest level of *in vitro* protein digestibility (74.10%) than that of an earlier investigation in the *Mucuna pruriens* var. *utilis* (black coloured seed coat) accession (Vadivel and Janardhanan, 2000a).

Table 5. Fatty acid profile of lipid of five accessions of *Mucuna atropurpurea* [a].

Fatty acid (%)	Thallaianai	Saduragiri	Sivagiri	Anaikatti	Ayyanarkoil
Palmitic acid [C16:0]	22.10	25.14	23.34	23.79	24.70
Stearic acid [C18:0]	17.20	13.10	14.74	12.54	15.30
Oleic acid [C18:1]	19.14	16.50	15.60	18.80	17.29
Linoleic acid [C18:2]	27.30	29.30	30.24	28.16	26.30
Linolneic acid [C18:3]	10.20	13.26	12.15	13.25	14.10
Behenic acid [C22:0]	4.06	2.70	3.93	3.46	2.31

[a]All values are of two determination.

Table 6. Aminoacid profiles of acid-hydrolysed, purified total seed proteins of five accessions of *Mucuna atropurpurea*[a].

Aminoacid	Thall	EAAS	Sadu	EAAS	Siva	EAAS	Anai	EAAS	Ayya	EAAS	FAO/ WHO
Glutamic acid	11.14		9.48		8.78		13.10		9.56		
Aspartic acid	12.70		16.40		14.20		13.48		15.37		
Serine	4.44		4.93		3.34		4.50		3.78		
Threonine	4.08	120.00	4.84	142.35	5.28	155.29	5.10	150.00	4.50	132.35	3.4
Proline	3.10		2.50		3.14		4.54		3.74		
Alanine	4.68		5.70		5.84		4.15		3.60		
Glycine	4.35		4.17		4.40		4.53		5.12		
Valine	3.33	95.14	4.31	123.14	5.48	156.57	6.30	180.00	5.78	165.14	3.5
Cystine	1.62	}86.40	1.34	}97.60	2.20	}118.40	1.12	}86.40	1.69	}105.20	}2.5
Methionine	0.54		1.10		0.76		1.04		0.94		
Isoleucine	3.34	119.29	3.56	127.14	2.54	90.71	5.30	189.29	5.14	183.57	2.8
Leucine	5.74	86.97	6.48	98.18	6.50	98.48	5.17	78.33	5.80	87.88	6.6
Tyrosine	4.32	}118.25	2.43	}114.76	5.11	}139.84	3.40	}124.44	3.78	}134.29	
Phenylalanine	3.13		4.80		3.70		4.44		4.68		}6.3
Lysine	5.21	89.83	5.40	93.10	4.85	83.62	5.17	89.14	5.08	87.59	5.8
Histidine	2.23	117.37	3.28	172.63	4.31	226.84	4.12	216.84	3.40	178.95	1.9
Tryptophan	1.14	103.64	1.04	94.55	0.78	70.91	1.21	110.00	0.92	83.64	1.1
Arginine	5.38		3.50		4.34		4.02		2.52		

Thall: Thallaianai, Sadu: Saduragiri, Siva: Sivagiri, Anai: Anaikatti, Ayya: Ayyanarkoil, EAAS: Essential aminoacid score. FAO/WHO (1991) requirement pattern [a]: All values are of single determination.

Although legumes provide 20% of all plant protein in human diet and are even more important in the diets of livestock, their usefulness is limited by toxic or antinutritional compound associated with large contents of protein in their seeds and vegetative parts (Nowacki, 1980). In view of this, in the present investigation an attempt has been made to detect the presence of certain antinutritional factors such as total free phenolics, tannins, L-DOPA, phytic acid, hydrogen cyanide, total and soluble oxalates, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity (Table 7). Among the different accessions of *Mucuna atropurpurea*, the content of total free phenolics of Ayyanarkoil accession and tannins of Thallaianai accession were significantly ($p<0.05$) higher than other accessions. The content of total free phenolics of investigated seed samples of *Mucuna atropurpurea* was found to be low when compared with four accessions of *Mucuna pruriens* var. *pruriens* (Vadivel and Janardhanan, 2000b) and *M. pruriens* (=*M. pruriens* var. *pruriens*) (Vijayakumari *et al*., 1996a). The contents of tannins present in the seeds of all the accessions of *M. atropurpurea* appeared to below when compared with the commonly consumed legume seeds such as green gram, cowpea, pigeon pea and black gram (Khan *et al*., 1979; Rao and Deosthale, 1982). Among the five accession, Sivagiri accession contained the highest level of L-DOPA (4.10%); the lowest amount of L-DOPA (2.94%) were found in the Ayyanarkoil

accession of the seed. The concentration of non-protein amino acid L-DOPA in *M. atropurpurea* (all the accessions) had been found to be low when compared with values reports earlier in *M. pruriens* var. *utilis* (Mohan and Janardhanan, 1995a; Vadivel and Janardhanan, 2000a; Janardhanan *et al.,* 2003). It has been demonstrated that in *M. pruriens*, the level of L-DOPA is significantly eliminated by dry heat treatment (Siddhuraju *et al.*, 1996) and cooking and autoclaving (Vijayakumari *et al*., 1996a). Regarding the content of phytic acid, seeds of Ayyanarkoil accession has been found to contain significantly higher amounts than the other four accession. However, all the accessions of *M. atropurpurea* was found to be low level of phytic acid when compared with commonly consumed grain legumes like *Vigna mungo* (Kataria *et al*., 1988), *V. radiata* (Kataria *et al*., 1989) and also compared with tribal pulses such as *Mucuna monosperma* (Vijayakumari *et al.*, 1996b); *M. pruriens* var. *utilis* (Janardhanan *et al*., 2003); *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al*., 1995) and *Mucuna pruriens* (Vijayakumari *et al*., 1996a). The level of hydrogen cyanide in *M. atropurpurea* seems to be negligible when compared with the lethal level HCN (35mg/100g) (Oke, 1969).

Table 7: Data on IVPD and antinutritional factors of five accessions of *Mucuna atropurpurea.* ✈

Components	Thallaianai	Saduragiri	Sivagiri	Anaikatti	Ayyanarkoil
in vitro protein digestibility (%)[a]	70.20	69.01	72.94	73.09	74.10
Total free phenolics[b] $g100g^{-1}$ ✈	3.13±0.01[c]	2.84±0.01[b]	3.04±0.01[e]	2.62±0.02[a]	3.50±0.01[d]
Tannins[b] g $100g^{-1}$ ✈	0.34±0.01[d]	0.26±0.02[b]	0.18±0.01[a]	0.24±0.01[b]	0.31±0.01[c]
L-DOPA[b] g $100g^{-1}$ ✈	3.24±0.01[b]	3.64±0.01[c]	4.10±0.01[e]	3.90±0.01[d]	2.94±0.01[a]
Phytic acid [b] $mg100g^{-1}$ ✈	423.00±0.57[b]	444.14±0.58[c]	456.34±0.58[d]	384.94±0.59[a]	464.09±1.16[e]
Hydrogencycnide[b] $mg100g^{-1}$ ✈	0.21±0.01[a]	0.31±0.01[c]	0.29±0.01[c]	0.24±0.01[b]	0.33±0.01[d]
Total oxalate [b] $mg100g^{-1}$ ✈	0.08±0.02[a]	0.06±0.01[a]	0.08±0.02[a]	0.04±0.01[a]	0.08±0.01[a]
Soluble oxalate[b] $mg100g^{-1}$ ✈	0.06±0.01[a]	0.04±0.01[a]	0.04±0.01[a]	0.02±0.01[a]	0.05±0.02[a]
Trypsin inhibitor activity[a] (TIU mg^{-1} protein)	43.50	44.10	42.70	40.94	39.24
Oligosaccharide[b] (g $100g^{-1}$)					
Raffinosa	0.64±0.01[b]	0.78±0.02[c]	0.54±0.01[a]	1.01±0.01[d]	0.96±0.03[d]
Stachyosa	1.34±0.01[b]	1.64±0.01[d]	1.48±0.03[c]	1.94±0.03[e]	1.21±0.01[a]
Veragosa	4.30±0.01[b]	4.55±0.02[c]	5.04±0.02[e]	4.64±0.02[d]	3.94±0.01[a]
Phytohaemagglutinating activity (Hu mg^{-1} protein[a])					
Group A	138	126	114	124	132
Group B	68	48	34	54	32
Group O	21	16	14	13	17

Raff: Raffinose; Stac: Stachyose; Verb: Veragose;
[a] All values of two independent experiments,
[b] All values are of means of triplicate determination expressed on dry weight basis
± Standard error,
✈ Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

The total and soluble oxalates content of *M. atropurpurea* was found to be higher than the other *Mucuna* species investigated earlier, (Ezeagu *et al*., 2003). Soluble oxalates are considered to have more deleterious effect on Ca^{2+} and Mg^{2+} absorption from foods undergoing digestion simultaneously, whereas insoluble oxalates binds Ca^{2+} within the food and renders it unavailable to the body. Soluble oxalates are early leached out during the soaking or boiling process (Ezeagu *et al*., 2003). Though the trypsin inhibitor activity has been studied in a number of pulses, the results obtained in the present investigation cannot be compared because the expression of trypsin inhibitor activity, nature and concentration of the substrate etc, are different. However, based on investigations that have been reported and where the same experimental conditions were used, the trypsin inhibitor activities in cultivated legumes like pigeon pea (67.1 – 71.3 TIU mg^{-1}protein) (Singh and Eggum, 1984) was higher than that of *M. atropurpurea*. Trypsin inhibitor activity has the greater impact on the IVPD of the legumes where the trypsin inhibitor activity was known to be heat labile. The oligosaccharide content of the seeds of *M. atropurpurea* was comparable with those five accessions of other species of *M. pruriens* var. *utilis* (Janardhanan *et al*., 2003). Verbascose was found to be the major oligosaccharide in all the accession of *M. atropurpurea* as has been reported earlier in *M. pruriens* var. *utilis* (Janardhanan *et al*., 2003).

Regarding phytohaemagglutinating activity (Table 7), all the accessions of *M. atropurpurea* registered higher phytohaemagglutinating activity with respect to 'A' blood groups human erythrocytes. All 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 (Vijayakumari *et al*., 1996a). However, dry heat and autoclaving are known to inactivate completely the trypsin inhibitors and phytohaemagglutins in *Mucuna* beans (Siddhuraju *et al*., 1996).

CONCLUSION

The observation made in the present study show that all 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 and vitamins. The overall nutrient quality is similar to that of most edible food legumes. Therefore, *M. atropurpurea* represents a potential food source if given adequate promotion and research attention. The presence of antinutritional factors identified in the current report should not poses problem for human, if the beans are properly processed.

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