



ASSESSMENT OF NUTRITIONAL AND ANTINUTRITIONAL
POTENTIAL OF UNDERUTILIZED LEGUMES OF THE GENUS
MUCUNA

[EVALUACIÓN DEL POTENCIAL NUTRICIONAL Y
ANTINUTRICIONAL DE LEGUMINOSAS SUBUTILIZADAS DEL
GENERO *MUCUNA*]

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SUMMARY

There is an increasing interest in finding new food sources to alleviate malnutrition in developing countries, due to lack of protein rich food. Protein supply can be broadened by exploration and exploitation of alternate legume sources. Eventhough copious wild legume landraces have been identified, their utilization is limited due to inadequate attention. *Mucuna pruriens* var. *utilis* (black and white coloured seed coat), *Mucuna pruriens* var. *pruriens* and *Mucuna deeringiana* are underutilized legume species having the credibility to be a rich protein source. This study was designed to assess the proximate composition, mineral profiles, vitamins (niacin and ascorbic acid), protein fractions, fatty acid profiles, amino acid profiles of total seed protein, *in vitro* protein digestibility and antinutritional potential of the above said *Mucuna* varieties/species. The major findings of the study were as follows: crude protein content ranged from 23.76-29.74%, crude lipid 8.24-9.26%, total dietary fibre, 6.54-8.76%, ash 4.78-5.30%, carbohydrates 56.56-63.88%, and calorific value 1783.02-1791.60 kJ/100g⁻¹ DM. The examined seed samples contained minerals such as Na, K, Ca, Mg and P in abundance. The fatty acid profiles revealed that, the seed lipids contained higher concentrations of linoleic and palmitic acid. The antinutritional fatty acid, behenic acid (1.87-3.04%) was also detected. The current investigation helps in understanding the nutritional and antinutritional versatility of the *Mucuna* varieties/species, thereby developing further tactics for optimum utilization.

Key words: *Mucuna* varieties/species; vitamins; IVPD; antinutrients.

RESUMEN

Hay un incremento en el interés por encontrar nuevas fuentes de alimento para disminuir la desnutrición en países en desarrollo, debido a la ausencia de alimentos ricos en proteína. El suministro de proteína puede ser ampliado por la exploración y explotación de leguminosas alternativas. A pesar de han sido identificadas varias especies de leguminosas, su utilización se encuentra limitada debido a una inadecuada atención. *Mucuna pruriens* var. *utilis* (Semilla de cubierta negra y blanca) *pruriens* var. *pruriens* y *Mucuna deeringiana* son especies de leguminosas subutilizadas las cuales se creen que que son ricas en proteína. Este estudio fue diseñado para evaluar la composición proximal, perfiles minerales, vitaminas (niacina y ácido ascórbico), fracciones de proteína, perfiles de ácidos grasos, perfiles de aminoácidos de la proteína total de la semilla, digestibilidad *in vitro* de la proteína y potencial antinutricional de las antes mencionadas especies/variedades de *Mucuna*. Los principales resultados del estudio fueron los siguientes: Contenido de proteína con un rango de 23.76-29.74%, lípidos crudos 8.24-9.26%, fibra dietética total 6-54-8.76%, cenizas 4.78-5.30%, carbohidratos 56.56-63.88% y valor calorífico 1783.02-1791.60 kJ100g⁻¹DM. La muestras de semillas examinadas contenían minerales como Na, K, Ca, Mg y P. Los perfiles de ácidos grasos revelaron que los lípidos de la semilla contenían altas concentraciones de ácido linoleico y ácido palmítico. También se detectó el ácido benico como ácido graso antinutricional. La investigación actual ayuda al entendimiento de la versatilidad nutricional y antinutricional de las variedades/especies de *Mucuna*, por lo tanto se deben desarrollar estrategias para su optima utilización.

Palabras clave: Variedades/Especies de *Mucuna*; Vitaminas; IVPD; antinutrientes.

INTRODUCTION

In most developing and underdeveloped countries of the world, seeds serve as the major sources of the nutrient needs of humans and animals. Experimental, epidemiological and clinical studies show correlations between the consumption of food legumes and decreasing incidence of several diseases such as, cancer, cardiovascular disease, obesity and diabetes (Bhathena and Velasquez, 2002; Kris-Etherton *et al.*, 2002 and Kushi *et al.*, 1999). A latest epidemiological study showed that among studied fruits and vegetables, only bean and lentil consumption was related to a lower incidence of breast cancer (Adebamowo *et al.*, 2005). Antioxidant activities and phenolic compounds in raw legumes have been reported in several earlier communications (Amarowicz *et al.*, 2003; Xu *et al.*, 2007). Generally, legumes have been reported to have low nutritive value because of low amounts of sulphur containing amino acids, low protein digestibility and the presence of antinutritional factors.

The itching bean, *Mucuna pruriens* (L.) DC var. *pruriens* is an underutilized legume species grown predominantly in Asia and Africa and in parts of America (Vadivel and Janardhanan 2000a). Mature seeds, seeds from unripe pods and young pods of itching beans are soaked and boiled / roasted and eaten as such or mixed with salt by the north-east Indian tribes; Khasi, Naga, Kuki, Jaintia, Chakma and Mizo (Arora, 1991); north western part of Madhya Pradesh tribes; Abujh-Maria, Muria, Gond and Halba (Sahu, 1996); south Indian tribes; Mundari and Dravidian (Jain, 1981); Kani, Kader and Muthuvan (Radhakrishnan *et al.*, 1996) and Savera Jatapu, Gadebe and Kondadora (Rajyalakshmi and Geervani, 1994).

The mature seeds of velvet bean, *Mucuna pruriens* (L.) DC var. *utilis* (Wall.ex Wight) Bak. ex Burck are consumed by a South Indian hill tribe, the Kanikkars, after repeated boiling (Janardhanan and Lakshmanan, 1985]. Recently Dravidan tribes in the Tirunelveli district have started cultivating it for use as a pulse (Janardhanan *et al.*, 2003). Various preparations of this bean are also traditionally consumed in several parts of Srilanka by low-income groups (Ravindran and Ravindran, 1988). In parts of Asia, and Africa, the seeds are roasted and eaten (Haq, 1983).

Despite, the potential of this underutilized species as a source of less consumed food, to our knowledge, meager information is available on the germplasm collection from South Western Ghats, Tamil Nadu and its evaluation for chemical composition. In this context, in the present study, an attempt was made to understand the biochemical composition and assess the nutritional value of the tribal pulses *Mucuna pruriens* var. *pruriens*,

Mucuna pruriens var. *utilis* (black and white coloured seed coat) and *Mucuna deeringiana*.

MATERIALS AND METHODS

Collection of seed samples

The seed samples of *Mucuna pruriens* (L.) DC var. *pruriens* were collected from Devipattinum, Tirunelveli District, south eastern slopes of Western Ghats, Tamil Nadu, during June 2010. The seeds of *Mucuna pruriens* (L.) DC var. *utilis* (Wall ex. Wight) Bak. Ex Burck (black and white coloured seed coat) were collected from Periamyllum and the seeds of another tribal pulse, *Mucuna deeringiana* (Bort) Merrill were collected from Chinnamyllum, Kanyakumari district, south eastern slopes of Western Ghats, Tamil Nadu during August 2010. With the help of keys by Wilmot-Dear (1987) the seed samples were botanically identified. The collected pods were thoroughly dried in the sun; the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds, foreign materials and immature 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 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 (Humphries, 1956) and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus (AOAC 2005). The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr (AOAC 2005). 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 95.0 % 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 78.0 % ethanol, 10 ml of 95.0 % ethanol and 10 ml acetone. The crucible containing the residue was dried ≥ 2 h at 105° C and

then cooled ≥ 2 h in a desiccator and weighed. One crucible containing residue was used for ash determination at 525°C for 5 hr. The ash-containing crucible was cooled for ≥ 2 h 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.

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

Where Wr is the mg residue, P is the % protein in the residue; A is the % ash in the residue, and Ws is the 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).

Minerals and vitamins analysis

Five hundred milligrams of the ground legume seed was digested with a mixture of 10 ml concentrated nitric acid, 4ml of 60% perchloric acid and 1ml of concentrated sulphuric acid. After cooling, the digest was diluted with 50 ml 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 contents were extracted and estimated as per the method given by (Sadasivam and Manickam, 1996). For the extraction of ascorbic acid, 3 g air-dried powdered sample was ground with 25 ml of 4.0 % 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 25 ml with 4% oxalic acid and used for ascorbic acid estimation. Two millilitres of the extract was made up to 3ml with distilled H₂O in a test tube. One millilitre of 2.0 % 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, 7 ml of 80.0 % H₂SO₄ was added to dissolve the osazone crystals and the absorbance was

measured at 540 nm against a reagent blank (Sadasivam and Manickam, 1996).

For the extraction of niacin, 5 g air-dried powdered sample was steamed with 30 ml concentrated H₂SO₄ for 30 min. After cooling, this suspension was made up to 50 ml with distilled H₂O and filtered. Five millilitres of 60.0 % basic lead acetate was added to 25 ml of the filtrate. The pH was adjusted to 9.5 and centrifuged to collect the supernatant. Two millilitres of concentrated H₂SO₄ was added to the supernatant. The mixture was allowed to stand for 1 h and centrifuged. The 5 ml of 40.0 % ZnSO₄ was added to the supernatant. The pH was adjusted to 8.4 and centrifuged 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 6 ml with distilled water in a test tube, 3 ml cyanogen bromide was added and shaken well, followed by addition of 1 ml of 4.0% aniline. The yellow colour that developed after 5 min was measured at 420 nm 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 100 g of powdered samples (Sadasivam and Manickam, 1996).

Extraction and estimation of total proteins and protein fraction

The total (true) protein was extracted by the method of Basha *et al* (1976) with slight modification (ethanol treatment was omitted to save prolamin fraction). The extracted proteins were purified by precipitation with cold 20.0 % trichloroacetic acid (TCA) and estimated by the method of Lowry *et al* (1951). The albumin and globulin fractions of the seed protein were extracted and separated according to the method of Murray (1979). The prolamin fraction was extracted from the residual pellet by treating the pellet with 80.0 % ethanol (1:10w/v) overnight. After centrifugation (20,000 g for 20 min at room temperature) the supernatant containing prolamins was air-dried and dissolved in 0.1N NaOH. The resulting pellet was extracted with 0.4N NaOH (1:10w/v) overnight and centrifuged as above. The supernatant was designated as glutelins. All four fractions so obtained were precipitated and washed with cold 10.0 % TCA. All samples were redissolved in 0.2M NaOH and protein content was determined by the Lowry *et al* (1951) method.

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 1.0 % 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 30 ml/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 20.0 % trichloroacetic acid (TCA). A protein sample of 30mg was hydrolysed by 6N HCL (5 ml) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 h. 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-phthalaldehyde by using an automated pre-column (OPA). Aminoacids were analysed by a reverse – phase HPLC (Method L 7400, HITACHI, Japan) fitted with a denali C₁₈ 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 (Liddelle 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 h. 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:

$$\text{EAAS} = \frac{\text{g AA} / 100\text{g TP}}{\text{gAA}/100\text{g FAO/WHO}(1991) \text{ reference}} \times 100$$

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) and hydrogen cyanide (Jackson, 1967) 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 10 ml 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 all the samples of seed flours were extracted with 50 ml of 70.0% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 hr and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25 ml of 70.0 % (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). 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 verbascose (procured from sigma chemical co., St. Louis, USA). 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 an 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 2 hours 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. 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 micro-titration 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 titre values were recorded. One haemagglutinating unit (HU) is defined as the least amount of haemagglutinin that will produce positive evidence of agglutination of 25µl of a blood group erythrocyte after 3hr incubation at room temperature. The phytohaemagglutinating activity was expressed as haemagglutinating 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 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, α -chymotrypsin and peptidase) at 37°C followed by protease 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 $Y = 234.84 - 22.56 X$, where Y is the % digestibility and X the pH drop.

RESULTS AND DISCUSSION

The results of proximate composition of *Mucuna pruriens* var. *pruriens*, *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) and *Mucuna deeringiana* are depicted in table 1. The crude protein (23.76-29.74%) and crude lipid (8.2-9.6%) contents were found to be higher than the pulse crops commonly consumed in India, such as black gram, green gram (crude protein: 22.30 – 23.30%, crude lipid: 1.12 – 1.25 %), pigeon pea, chick pea (crude protein: 22.0-24.0 %, crude lipid: 4.1 – 5.4 %) and cowpea (Gupta and Wagle, 1978; Jambunathan and Singh, 1980; Nwokolo and Oji, 1985; Nwokolo, 1987); Co₅ and TMV-1 varieties of *Vigna mungo* (crude protein: 26.22 % and 26.07 %, crude lipid: 2.94 % and 4.24 %) (Tresina *et al.*, 2010) which have been reported earlier. To meet the protein demands in developing countries where animal protein is grossly inadequate, considerable attention is being paid to less consumed protein sources, especially in legumes (Balogun and Fetuga, 1986) which are considered as protein tablets (Salunkhe, 1982). The crude protein levels of the studied samples suggest its usefulness as alternative source of protein. The total dietary fibre content of the samples ranged from 6.54-8.76% which are higher than the tribal pulses *Dolichos trilobus*, *Vigna radiata* var. *sublobata*, *V. unguiculata* subsp. *cylindrica* (3.4 - 6.6 %) (Arinathan *et al.*, 2009) and *V. unguiculata* subsp. *unguiculata* (4.80 %) (Tresina and Mohan, 2011a). Fibre is a very important component of food. It has been reported to have a major influence on metabolism in the gastrointestinal tract. According to Guthrie (1989), legumes contained high fibre which slows down the release of glucose into the blood stream; hence, high legume diets are recommended for diabetic patients (Gibney, 1989; Jenkins *et al.*, 1982). The ash content of investigated *Mucuna* varieties/species (4.78-5.30%) would be important to the extent that it contains nutritionally important mineral elements, which are presented in table 2. All the presently investigated *Mucuna* varieties/species registered high range of energy (1783.02-1791.60 kJ100g⁻¹DM) than the earlier reported accessions of *Mucuna pruriens* var. *pruriens* (Kala and Mohan, 2010); *Vigna mungo* varieties (Tresina *et al.*, 2010) and *Vigna unguiculata* subsp. *unguiculata*, *Vigna aconitifolia* (Tresina and Mohan, 2011 b) which are in the range of 1597.61-1691.81 kJ100g⁻¹DM.

Food legumes are a good source of minerals such as calcium, iron, copper, zinc, potassium and magnesium (Salunkhe *et al.*, 1985). The data on mineral composition of presently investigated *Mucuna* varieties/species are furnished in table 2. In the present investigation, *Mucuna* varieties/species registered a higher level (1836.34 – 2138.12 g 100g⁻¹) of potassium and their levels of potassium seem to be higher when compared with recommended dietary allowance values (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 calcium content of *Mucuna pruriens* var. *pruriens*, *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) is found to be higher than that of the recommended dietary allowances of calcium (400 mg) for children by the Indian Council of Medical Research (ICMR, 1992); whereas, the levels of micro-minerals (zinc and copper) are lower than the recommended dietary allowance (ICMR, 1992). The ICMR recommended 15mg zinc and 2.2mg copper for Indian children. The manganese content of all the *Mucuna* varieties/species of the present investigation is found to be higher than that of recommended dietary allowance of manganese (5.5 mg) by the ICMR (1992).

The ratios of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are also shown in table 2. Na/K ratio in the body is in great concern for prevention of high blood pressure. Na/K ratio less

than one is recommended. Hence, in the present study, all the *Mucuna* varieties/species would probably reduce high blood pressure, because, they had Na/K ratio less than one. Modern diets which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine (Shills and Young, 1988). This had led to the concept of the Ca/P ratio. If the Ca/P ratio is low (low calcium, high phosphorus intake), more than the normal amount of calcium may be lost in the urine, decreasing the calcium level in bones. Food is considered ‘good’ if the ratio is above one and ‘poor’ if the ratio is less than 0.5 (Nieman *et al.*, 1992). The Ca/P ratio in the present study ranged between 0.59 to 1.28 indicating that, they would serve as good sources of minerals for bone formation.

In the currently investigated tribal pulses, *Mucuna* varieties/species exhibited highest level of niacin content (Table 3), which is found to be higher than that of an earlier report in *Cajanus cajan*, *Dolichos biflorus*, *Mucuna pruriens*, *Phaseolus mungo*, *Vigna catjang* and *Vigna* spp. (Rajyalakshmi and Geervani, 1994); *Rhynchosia filipes*, *Vigna trilobata*, *V. unguiculata* subsp. *unguiculata*, *V. unguiculata* subsp. *cylindrica* (Arinathan *et al.*, 2003; 2009) and *Vigna mungo* varieties (Tresina *et al.*, 2010). In the present investigation, *Mucuna* varieties/species registered a high level of ascorbic acid content than *Cicer arietinum* (Fernandez and Berry, 1988), *Atylosia scarabaeoides*, *Dolichos trilobus*, *Teramnus labialis* (Arinathan *et al.*, 2003; 2009) and three varieties of *Vigna mungo* (Tresina *et al.*, 2010).

Table: 1 Proximate composition of *Mucuna* varieties/species (%)^a

Components	<i>Mucuna pruriens</i> var. <i>utilis</i> (Black coloured seed coat)	<i>Mucuna pruriens</i> var. <i>utilis</i> (White coloured seed coat)	<i>Mucuna pruriens</i> var. <i>pruriens</i>	<i>Mucuna deeringiana</i>
Moisture	9.38 ± 0.08	10.68 ± 0.11	10.48 ± 0.17	8.40 ± 0.06
Crude protein	29.74 ± 0.12	28.36 ± 0.18	29.36 ± 0.34	23.76 ± 0.21
Crude lipid	9.26 ± 0.06	9.04 ± 0.05	8.24 ± 0.06	8.48 ± 0.11
Total Dietary Fibre	8.76 ± 0.04	8.34 ± 0.03	6.54 ± 0.04	7.12 ± 0.14
Ash	4.94 ± 0.06	5.30 ± 0.03	4.78 ± 0.03	5.24 ± 0.02
Nitrogen Free Extractive	56.56	58	59.32	63.88
Calorific value kJ100g ⁻¹ DM	1790.31	1783.02	1791.60	1783.28

^a All values are of means of triplicate determination expressed on dry weight basis; ± denotes standard error.

Table 2. Mineral composition of *Mucuna* varieties/species (mg 100g⁻¹)^a

Components	<i>Mucuna pruriens</i> var. <i>utilis</i> (Black coloured seed coat)	<i>Mucuna pruriens</i> var. <i>utilis</i> (White coloured seed coat)	<i>Mucuna pruriens</i> var. <i>pruriens</i>	<i>Mucuna deeringiana</i>
Sodium	73.20 ± 0.31	68.56 ± 0.48	62.10 ± 0.28	54.10 ± 0.28
Potassium	2068.00 ± 1.76	2138.12 ± 2.48	1836.34 ± 1.34	2096.17 ± 2.76
Calcium	738.14 ± 0.54	689.20 ± 0.66	536.26 ± 0.36	292.10 ± 0.59
Magnesium	412.12 ± 0.38	456.42 ± 0.54	576.30 ± 0.54	268.14 ± 0.78
Phosphorus	652.30 ± 0.76	538.12 ± 0.38	476.06 ± 0.63	498.21 ± 0.54
Iron	13.30 ± 0.11	10.48 ± 0.08	9.34 ± 0.07	11.56 ± 0.09
Zinc	3.56 ± 0.03	2.98 ± 0.01	1.34 ± 0.03	2.84 ± 0.03
Copper	0.66 ± 0.01	0.42 ± 0.01	0.78 ± 0.01	0.96 ± 0.01
Manganese	10.72 ± 0.13	8.46 ± 0.11	8.88 ± 0.11	10.10 ± 0.08
Na/K	0.04	0.03	0.03	0.03
Ca/P	1.13	1.28	1.13	0.59

^aAll values are of means of triplicate determination expressed on dry weight basis; ± denotes standard error.

Table 3 Vitamins (niacin and ascorbic acid) content of *Mucuna* varieties/species (mg 100g⁻¹)^a

Components	<i>Mucuna pruriens</i> var. <i>utilis</i> (Black coloured seed coat)	<i>Mucuna pruriens</i> var. <i>utilis</i> (White coloured seed coat)	<i>Mucuna pruriens</i> var. <i>pruriens</i>	<i>Mucuna deeringiana</i>
Niacin	48.10 ± 0.12	38.22 ± 0.14	54.36 ± 0.24	22.10 ± 0.09
Ascorbic acid	55.14 ± 0.36	58.32 ± 0.24	33.21 ± 0.32	42.16 ± 0.17

^aAll values are of means of triplicate determination expressed on dry weight basis ± denotes standard error.

Among the studied *Mucuna* varieties/species, *Mucuna pruriens* var. *pruriens* showed highest level of total proteins than the other varieties and species (Table 4). *Mucuna pruriens* var. *pruriens* and *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) are found to contain more total protein than that of different varieties of *Vigna mungo* (Tresina *et al.*, 2010); other tribal pulses such as *Vigna capensis*, *Vigna sinensis*, *Parkia roxburghii*, *Entada phaseoloides*, *Canavalia gladiata* (Mohan and Janardhanan, 1993a, b; 1994); *Tamarindus indica* and *Sesbania bispinosa* (Pugalenthi *et al.*, 2004). In general, globulin constitutes the major seed storage protein in legumes. In all the presently investigated *Mucuna* varieties/species globulins constitute the major storage protein fraction (Table 4). This is in consonance with some earlier reports (Mohan and Janardhanan, 1993a, b; 1994; Pugalenthi *et al.*, 2004; Tresina *et al.*, 2010).

Fatty acid profiles (Table 5) reveal that, all the presently investigated *Mucuna* varieties/species contained high levels of palmitic and linoleic acids as in the case of some edible legumes such as *Vigna radiata*, *Vigna mungo* (Salunkhe *et al.*, 1982a); *Vigna unguiculata* and *Phaseolus vulgaris* (Omogbai, 1990). 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 including lowering of blood pressure and constriction of smooth muscle (Aurang *et al.*, 1987). Linoleic and linolenic acids are the most important essential fatty acids required for the growth, physiological functions and maintenance (Pugalenthi *et al.*, 2004). In the present study, most of the fatty acids reported were unsaturated fatty acids. In the fatty acid composition, high amounts of unsaturated fatty acids make *Mucuna* varieties/species a special legume, suitable for nutritional applications. The antinutritional fatty acid, behenic acid is detected in *Mucuna pruriens* var. *pruriens*, *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) and *Mucuna deeringiana*. Earlier reports indicate the detection of behenic acid in ground nut (Kritchevsky *et al.*, 1973); winged bean (Bean *et al.*, 1984; Fernando and Bean, 1985; 1986), *Parkia roxburghii*, *Entada phaseoloides*, *Mucuna utilis*, *Mucuna monosperma* (Mohan and Janardhanan, 1993a; 1995); *Mucuna pruriens* var. *pruriens* (Fathima *et al.*, 2010); and *Mucuna pruriens* var. *utilis* and *Mucuna deeringiana* (Kala and Mohan, 2010).

Table 4 Data on total (true) protein and protein fractions of seed flour of three different varieties/species of *Mucuna*^a

Components	<i>Mucuna pruriens</i> var. <i>utilis</i> (Black coloured seed coat)		<i>Mucuna pruriens</i> var. <i>utilis</i> (White coloured seed coat)		<i>Mucuna pruriens</i> var. <i>pruriens</i>		<i>Mucuna deeringiana</i>	
	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein
Total protein	23.78 ± 1.21	100	23.30 ± 1.11	100	24.40 ± 0.78	100	21.60 ± 1.24	100
Albumins	8.65 ± 0.14	36.37	8.30 ± 0.15	35.62	8.74 ± 0.21	35.82	5.14 ± 0.07	23.80
Globulins	12.36 ± 0.36	51.98	12.56 ± 0.54	53.91	13.10 ± 0.11	53.69	13.74 ± 0.37	63.61
Prolamins	0.84 ± 0.04	3.53	0.79 ± 0.03	3.39	0.68 ± 0.03	2.78	0.82 ± 0.02	3.80
Glutelins	1.93 ± 0.07	8.12	1.65 ± 0.05	7.08	1.88 ± 0.02	7.71	1.90 ± 0.04	8.79

^aAll values are of means of triplicate determination expressed on dry weight basis; ± denotes standard error.

The amino acid profiles of the purified seed proteins and the essential amino acid score are presented in table 6. The content of sulphur containing amino acids and tryptophan of the presently investigated seed samples seem to be deficient; whereas, threonine, valine, isoleucine, tyrosine, phenylalanine and histidine in *Mucuna pruriens* var. *pruriens*, *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) and *Mucuna deeringiana* and leucine in *Mucuna pruriens* var. *pruriens*, *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) of the present investigations are found to be higher when compared with FAO/WHO (1991) requirement pattern.

Among the presently investigated *Mucuna* varieties/species, *Mucuna pruriens* var. *utilis* (white coloured seed coat) registered the highest level of IVPD (74.36%) than the other samples (Table 7) and their protein digestibility was found to be higher than *Cajanus cajan* (Singh and Eggum, 1984), *Cicer arietinum* (Attia *et al.*, 1994) *Lablab purpureus*, *Vigna mungo* and *V. aconitifolia* (Kala *et al.*, 2010a; Tresina *et al.*, 2010; Tresina and Mohan, 2011b).

The presence of antinutritional factors is one of the major drawbacks limiting the nutritional and food qualities of the legumes (Salunkhe *et al.*, 1982b). 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, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity (Table 7). The total free phenolics occurred within the range of 2.74 to 5.24% and tannins ranged from 0.16 to

0.31%. Tannins have been claimed to affect adversely protein digestibility (Sathe and Salunkhe, 1984). In *Mucuna* bean, Ravindran and Ravindran (1988) and Mary Josephine and Janardhanan (1992) reported that, most of the tannins are located in the seed coat and with only traces in the cotyledons. Since the seed coats are usually removed by soaking prior to consumption, the tannins in the *Mucuna* beans are of little significance from the nutritional point of view. Besides, Siddhuraju *et al.* (1996) and Vijayakumari *et al.* (1996) reported that, in *Mucuna pruriens* the levels of phenolics and tannins are reduced significantly during dry and wet heat treatments, and their reduction improves the protein digestibility. Recently, phenolics have been suggested to exhibit health related functional properties such as anticarcinogenic, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activities (Shetty, 1997).

The concentration of the non-protein amino acid L-Dopa in *Mucuna pruriens* var. *pruriens* and *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) was high when compared with those of the other earlier reports in *Mucuna gigantea*, *Mucuna monosprema*, all the accessions of *Mucuna atropurpurea* and Ayyanarkoil and Anaikatti accessions of *Mucuna pruriens* var. *pruriens* (Rajaram and Janardhanan, 1991; Mohan and Janardhanan, 1995; Kala *et al.*, 2010b; Kalidass and Mohan, 2011). The high range of L-Dopa is encouraging from the point of view of pharmaceutical industries. Cultivator difference (Jayaweera, 1981) and accessions variations (Mary Josephine and Janardhanan, 1992) are known to exist in the L-Dopa content of *Mucuna* beans. Our current results are in good agreement with aforesaid reports and the differences should be exploited to select

low L-Dopa lines for human consumption. Subsequently, by repeated boiling and decanting of the seed for seven times with water, a substantial reduction in the quantity of L-Dopa has been shown and consumption of such seeds has been shown safe (Janardhanan and Lakshmanan, 1985). It has been demonstrated in *Mucuna pruriens* that

the level of L- Dopa is significantly eliminated by dry heat treatment (Siddhuraju *et al.*, 1996) and cooking and autoclaving (Vijayakumari *et al.*, 1996).

Table 5. Amino acid profiles of acid- hydrolysed, purified seed proteins of *Mucuna* varieties/species (g 100g⁻¹)

Amino acid	<i>Mucuna pruriens</i> var. <i>utilis</i> (Black coloured seed coat)	EAAS	<i>Mucuna pruriens</i> var. <i>utilis</i> (White coloured seed coat)	EAAS	<i>Mucuna pruriens</i> var. <i>pruriens</i>	EAAS	<i>Mucuna deeringiana</i>	EAAS	FAO/WHO 1991
Glutamic acid	12.24		13.48		10.08		13.21		
Aspartic acid	14.21		13.76		13.46		12.44		
Serine	4.24		4.74		4.12		3.68		
Threonine	3.68	108.24	3.54	104.12	4.22	124.12	4.24	124.71	3.4
Proline	3.26		3.12		3.30		3.04		
Alanine	4.24		4.38		5.26		4.32		
Glycine	5.72		5.46		4.76		4.96		
Valine	3.78	108	3.56	101.71	3.56	101.71	3.66	104.57	3.5
Cystine	1.04	68.80	1.12	78.40	1.54	90.40	0.98	68.80	2.5
Methionine	0.68		0.84		0.72		0.74		
Isoleucine	7.24	258.57	6.96	248.57	6.21	221.79	6.56	234.29	2.8
Leucine	6.21	94.09	6.48	98.18	6.34	96.06	5.36	81.21	6.6
Tryosine	4.24	132.38	4.56	141.59	4.78	133.65	3.51	131.27	6.3
Phenylalanine	4.10		4.36		3.64		4.76		
Lysine	5.48	94.48	5.76	99.31	5.98	103.10	5.10	87.93	5.8
Histidine	3.13	164.74	3.74	196.84	2.54	133.68	2.98	156.84	1.9
Tryptophan	0.96	87.27	0.78	70.91	1.01	91.82	0.56	50.91	1.1
Arginine	5.48		6.32		6.39		5.14		

EAAS-Essential amino acid score

Table 6. Fatty acid profile of lipids of *Mucuna* varieties/species^a

Fatty acid (%)	<i>Mucuna pruriens</i> var. <i>utilis</i> (Black coloured seed coat)	<i>Mucuna pruriens</i> var. <i>utilis</i> (White coloured seed coat)	<i>Mucuna pruriens</i> var. <i>pruriens</i>	<i>Mucuna deeringiana</i>
Palmitic acid (C16:0)	25.44	28.10	29.14	24.20
Stearic acid (C18:0)	12.46	14.20	12.36	17.14
Oleic acid (C18:1)	19.40	18.24	18.13	19.58
Linoleic acid (C18:2)	32.08	28.12	26.48	28.94
Linolenic acid (C18:3)	8.71	8.30	10.78	7.42
Behenic acid (C22:0)	1.87	3.04	2.11	2.72

^aAverage values of two determinations.

Phytic acid has an antinutritional property because of its ability to lower the bioavailability of essential minerals and to form a complex with proteins, thereby, inhibiting the enzymatic digestion of ingested protein (Nolan and Duffin, 1987). The phytic acid content in the seeds of presently investigated *Mucuna* varieties/species is low when compared with the different varieties of *Vigna mungo* (Tresina *et al.*, 2010). It is worthwhile to note that, the phytate content in *Mucuna* beans could be substantially eliminated by processing methods such as soaking and cooking (Vijayakumari *et al.*, 1996). Hydrogen cyanide is known to cause acute or chronic toxicity. The content of HCN level in the presently investigated *Mucuna* varieties/species is far below than the lethal level (i.e. 36mg/100g) (Oke, 1969) and comparable with those of *Atylosia scarabaeoides*, *Neonotonia wightii* var. *coimbatorensis*, *Rhynchosia filipes*, *Vigna trilobata* and *Vigna unguiculata* subsp. *unguiculata* (Arinathan *et al.*, 2003); *Vigna unguiculata* subsp. *unguiculata* and *Vigna aconitifolia* (Tresina and Mohan, 2011b).

The range of trypsin inhibitor activity (42.02-46.16 TIU/mg protein) is found to be low when compared with *Cajanus cajan* var. part A-2 and UPAS-120 (Singh and Eggum, 1984) and *Glycine max* (Salunkhe *et al.*, 2006). Slight variation in the levels of oligosaccharides was detected with raffinose ranging from 0.98-1.12%, stachyose ranging from 1.14-1.41% and verbascose ranging from 3.68-4.26% of seed flour. The oligosaccharide content of the seeds of *Mucuna* varieties/species is comparable with that of three accessions of *Mucuna pruriens* var. *pruriens* (Kala and Mohan, 2010). Verbascose is found to be the major

oligosaccharide in all the presently investigated *Mucuna* varieties/species as had been reported earlier in *Mucuna pruriens* var. *pruriens* (Kala and Mohan, 2010).

Regarding phytohaemagglutinating activity, *Mucuna* varieties/species registered high haemagglutinating activity with respect to 'A' blood group of human erythrocytes. All the presently investigated *Mucuna* varieties/species have low levels of phytohaemagglutinating activity with respect to erythrocytes of 'O' blood group. This is in good agreement with an earlier report in *Mucuna pruriens* var. *pruriens* (Kalidass and Mohan, 2011). However, dry heat and autoclaving are known to inactivate completely the trypsin inhibitors and phytohaemagglutins in *Mucuna* beans (Siddhuraju *et al.*, 1996).

CONCLUSION

Based on the nutritional and antinutritional assessment of the tribal pulses *Mucuna pruriens* var. *pruriens*, *Mucuna pruriens* var. *utilis* (black and white coloured seed coat) and *Mucuna deeringiana*, it is concluded that, the tribal pulses investigated seem to be a good source of protein, essential amino acids, essential fatty acid, minerals and vitamins. It can be used as protein sources to curtail with problem of protein deficiency in most of the developing countries which may result in many child killer diseases. The presence of antinutritional factors identified in the current report should not pose a problem for humans, if the beans are properly processed.

Table 7. Data on IVPD and antinutritional factors of *Mucuna* varieties/ species

Components	<i>Mucuna pruriens</i> var. <i>utilis</i> (Black coloured seed coat)	<i>Mucuna pruriens</i> var. <i>utilis</i> (White coloured seed coat)	<i>Mucuna pruriens</i> var. <i>pruriens</i>	<i>Mucuna</i> <i>deeringiana</i>
<i>In vitro</i> protein digestibility (%) ^a	73.78 ± 0.76	74.36 ± 0.26	72.14 ± 0.36	70.21 ± 0.14
Total free phenolics ^b g 100 g ⁻¹	4.24 ± 0.21	3.98 ± 0.17	5.24 ± 0.38	2.74 ± 0.14
Tannins ^b g 100g ⁻¹	0.31 ± 0.03	0.21 ± 0.02	0.23 ± 0.01	0.16 ± 0.01
L-DOPA ^b g 100g ⁻¹	6.35 ± 0.31	7.97 ± 0.28	7.26 ± 0.18	6.55 ± 0.42
Phytic acid ^b g 100g ⁻¹	496.14 ± 1.28	536.20 ± 2.16	568.72 ± 2.38	510.12 ± 1.24
Hydrogen cyanide ^b mg 100g ⁻¹	0.21 ± 0.01	0.24 ± 0.01	0.26 ± 0.03	0.18 ± 0.03
Trypsin inhibitor (TIU mg ⁻¹ protein)	43.20 ± 0.65	44.26 ± 0.78	42.02 ± 0.56	46.16 ± 1.12
Oligosaccharide ^b g100g ⁻¹				
Raffinose	1.12 ± 0.04	1.04 ± 0.03	1.08 ± 0.04	0.98 ± 0.03
Stachyose	1.36 ± 0.05	1.21 ± 0.01	1.41 ± 0.06	1.14 ± 0.04
Verbascose	3.68 ± 0.14	4.06 ± 0.11	4.26 ± 0.12	4.24 ± 0.09

Phytohaemagglutinating activity^a (Hu mg⁻¹ protein)

A group	172	184	132	168
B group	84	76	82	68
O group	18	14	16	12

^a All values of two independent experiments; ^b All values are of means of triplicate determination expressed on dry weight basis; ± Standard error.

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