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Nutritional and antinutritional assessment of *Mucuna pruriens* (L.) DC var. *utilis* (Wall ex. Wight) Bak. Ex Burck and *Mucuna deeringiana* (Bort) Merril: An underutilized tribal pulse

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Abstract

Two samples of seed materials of the Indian tribal pulse, *Mucuna pruriens* var. *utilis* were collected from Periamyllaru and the seeds of another tribal pulse, *M. deeringiana* were collected from Chinnamyllaru, Kanyakumari district, Western Ghats, Tamil Nadu. The mature seed samples were analysed for proximate and mineral composition, vitamins (niacin and ascorbic acid), fatty acid profiles, amino acid composition and antinutritional factors. The investigated seed samples contained higher amounts crude protein and crude lipid when compared with most of the commonly consumed pulses. The investigated seeds were rich in minerals such as calcium, potassium, phosphorus, magnesium, iron and manganese. The fatty acid profiles revealed that the seed lipids contained higher concentration of palmitic acid and linoleic acid. The seed proteins of both samples of *Mucuna pruriens* var. *utilis* and *M. deeringiana* contained higher level of the essential amino acids such as threonine, valine, isoleucine, tyrosine, phenylalanine and histidine. The *in vitro* protein digestibility (IVPD) of the studied seed samples ranged from 71.86-77.28%. The antinutritional factors ranges from: total free phenolics 2.78-4.38 g 100 g^{-1}, tannins 0.11-0.218 g 100 g^{-1}, L-Dopa 4.00-7.23 g 100 g^{-1}, phytic acid 463.20-526.12 mg 100 g^{-1}, hydrogen cyanide 0.22-0.26 mg 100 g^{-1}, trypsin inhibitor activity 44.68-46.10 $TIUmg^{-1}$ protein. Verbascose was the principle oligosaccharide of the presently studied underutilized tribal pulses. Low levels of phytohaemagglutinating activity for human erythrocytes of 'O' blood group than 'A' and 'B' blood groups were found in all the seed samples.

Introduction

Legumes play important role in traditional diets of many regions in the world, because they have low amount of fat and are excellent source of protein, dietary fiber, certain micronutrients and phytochemicals. Bridging the gap between the teeming population and food production is one of the most important tasks of developing countries. Most developing tropical countries have depended on major conventional legumes and animal based sources as key protein concentrates for livestock feeding as well as for human nutrition. The demand for these items has given rise to a disproportionate increase in their prices and consequently on the cost of livestock and feeds. Protein-Energy Malnutrition (PEM) has therefore been recognised as the most common form of malnutritions in regions where people depend on starch based diets for survival (FAO, 1994; Pellelier, 1994: Michaelson and Henrik, 1998; Nwuoguikpe *et al*., 2011), there is therefore, need for identification and exploitation of other novel legumes to fulfil the growing need of plant based protein and under utilized legumes as inexpensive and good sources of protein than the conventional sources of protein (Chel-Guerrero *et al*., 2002; Kala and Mohan, 2010; Kalidass and Mohan, 2011).

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*., 2003a). Various preparation of this bean is 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 under-utilized 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

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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 two samples of the tribal pulse *Mucuna pruriens* var. *utilis* (black and white coloured seed coat), and one sample of *Mucuna deeringiana*.

Materials and Methods

Collection of seed samples

The seed samples of *Mucuna pruriens* (L.) DC var. *utilis* (Wall ex. Wight) Bak. Ex Burck (black and white coloured seed coat) were collected from Periamyllaru and the seed of another tribal pulse, *Mucuna deeringiana* (Bort) Merril were collected from Chinnamyllaru, Kanyakumari district, south eastern slopes of Western Ghats, Tamil Nadu during August 2012. 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°±2°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 2 g of the dried sample in a silica dish at 600°C for 6 hr (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.

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

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, 4 ml 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 10 ml 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 3 ml with distilled H_2O 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 3 hr incubation at 37°C, 7 ml of 80.0 % H_2SO_4 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 H_2SO_4 for 30 min. After cooling, this suspension was made up to 50 ml with distilled H_2O 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 H_2SO_4 was added to the supernatant. The mixture was allowed to stand for 1 h and centrifuged. The 5 ml of 40.0 % $ZnSO_4$ 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).

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 (2 m X 3 mm) 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 30 mg was hydrolysed by 6 N 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 (0.45 µM) 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 C18 5 micron column (4.6X 150 mm). 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 5 M 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 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 (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 70.0b% (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 Genesys 10S UV-VIS Spectrophotometer 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 10 ml of 0.15 N sodium chloride solution for 2 hours and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20 min. 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 (25 µl) 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 25 µl 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 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, µ-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.

Result and Discussion

The proximate composition of two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* are presented in Table 1. Among the samples studied *M. pruriens* var. *utilis* white coloured seed coat contained the higher amount of crude protein (29.53 g 100 g^{-1}) followed by black coloured seed coat *M. pruriens* var. *utilis* (28.25 g 100 g^{-1}). It seems to be higher than

Table 1. Proximate composition of two accessions of *Mucuna pruriens* var. *utilis* and *Mucuna deeringiana* (g 100 g^{-1})*

Components	*M. pruriens* var. *utilis* (white coloured seed coat)	*M. pruriens* var. *utilis* (black coloured seed coat)	*Mucuna deeringiana*
Moisture	10.25±0.14	10.85±0.08	9.68±0.14
Crude protein(Kjeldahl N x 6.25)	29.53±0.18	28.75±0.17	24.50±0.31
Crude lipid	8.04±0.07	8.26±0.05	8.24±0.10
Total Dietary Fibre (TDF)	8.66±0.06	8.68±0.06	9.06±0.11
Ash	4.22±0.10	5.72±0.02	5.64±0.07
Nitrogen Free Extractives (NFE)	49.55	48.59	52.56
Calorific values (kJ100g^{-1} DM)	1623.74	1602.98	1597.55

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

the some of the commonly cultivated legumes and tribal pulses like green gram (23.69-23.96 g 100 g^{-1}), bengal gram (18.22-23.64 g 100 g^{-1}), horse gram (23.5 g 100 g^{-1}) (Nwokola, 1987; Alajaji and El- Adway, 2006; Khatoon and Prakash, 2004; Kakati *et al*., 2010). The crude protein content of two accessions of *Mucuna pruriens* var. utilis is found to be higher than the earlier investigations of Kanjarampettai and Shenbathoopu accessions of *Mucuna pruriens* var. *pruriens* (24.50 – 26.98 g 100 g^{-1}) (Kala and Mohan, 2010); *Mucuna pruriens* var. utilis (23.21 g 100 g^{-1}) (Bhat *et al*., 2008) and *M. atropurpurea* (21.10 g 100 g^{-1}) (Kala *et al*., 2010a) and comparable to an earlier report on *M. pruriens* var. *utilis* Marthandam and Salem accessions. (Siddhuraju *et al.*, 2000). The two accessions of *M. pruriens* var. *utilis* (8.04 and 8.26 g 100 g^{-1}) and *M. deeringiana* (8.24 g 100 g^{-1}) are found to be higher source of crude lipid content. It seems to be higher than the previously studied common/ tribal pulses such as *Vigno mungo* (2.94-4.24 g 100 g^{-1}) (Tresina *et al*., 2010); *Lablab purpureus* (2.28-4.17 g 100 g^{-1}) (Kala *et al.*, 2010b); *Rhynchosia cana* (3.3 g 100 g^{-1}), *R. suaveolens* (3.2 g 100 g^{-1}), *Tamarindus indica* (5.5 g 100 g^{-1}), *Teramnus labialis* (4.4 g 100 g^{-1}), *Vigna radiata* var. *sublobata* (5.3 g 100 g^{-1}) and *Vigna unguiculata* subsp *cylindrica* (3.4 g 100 g^{-1}) (Arinathan *et al*., 2009). When compared with two accessions of *M. pruriens* var. *utilis* (8.66-8.68 g 100 g^{-1}), seeds of *M. deeringiana* (9.06 g 100 g^{-1}) appeared to be relatively higher amount in total dietary fibre content. The total dietary fibre content of all the presently investigated samples is found to be higher than the tribal pulses *Canavalia gladiata* (5.28 g 100 g^{-1}), *Lablab purpureus* var. *lignosus* (3.2 g 100 g^{-1}) *Vigna unguiculata* subsp. *unguiculata* (4.56 g 100 g-1) (Arinathan *et al*., 2003); *Mucuna pruriens* var. *pruriens* (6.58 g 100 g^{-1}) (Kala and Mohan, 2010), *Vigna aconitifolia* (5.86 g 100 g^{-1}) and *V. unguiculata* subsp *unguiculata* (4.80-5.58 g 100 g^{-1}) (Tresina and Mohan, 2011a). The ash content of these legumes (4.22-5.64 g 100 g^{-1}) would be important to the extent that it contains the nutritionally important mineral elements, which are present in Figure 1 and 2. Due to the lipid rich nature, the seeds of *M. pruriens* var. utilis and *M. deeringiana* registered high food energy values than those of cowpea, green gram, horse gram, moth beans and peas (Rao *et al*., 1989) which are in the range of 1318-1394 KJ 100g^{-1}DM.

The data on mineral composition (Figure 1a, b) indicated that the two accessions of *M. pruriens* var. utilis and *M. deeringiana* registers higher level of potassium, calcium, zinc, copper and magnesium. Food legumes are a good source of minerals such as calcium, iron, copper, zinc, potassium and magnesium (Salunkhe *et al.*, 1985). The two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* registers a higher level of potassium and their levels of potassium seem to be higher compared with recommended dietary allowance values (1550 mg) (NRC/ NAS, 1980). The high content of potassium can be utilized beneficially in the diets of people who take diuretic to control hypertension and suffer from excessive excretion of potassium through the body fluid (Siddhuraju *et al.*, 2001). The calcium content of two accessions of *M. pruriens* var. *utilis* is found to be higher than that of recommended dietary allowances

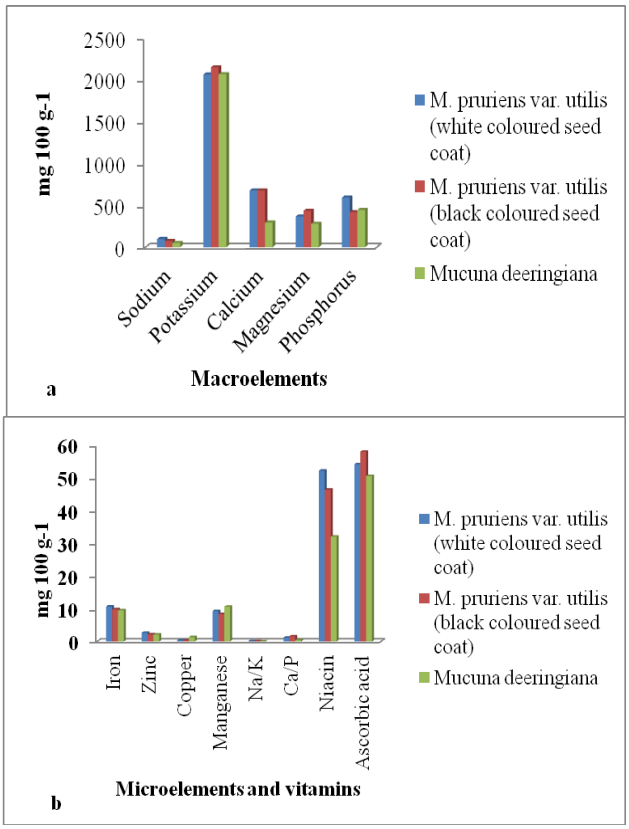

Figure 1a, b. Mineral composition and vitamins of two accessions of *Mucuna pruriens* var. *utilis* and *M. deeringiana* (mg 100 g^{-1})*.

*All values are means of triplicate determination expressed on a dry weight basis

of calcium (400 mg) for children by the Indian Council of Medical Reserch (ICMR, 1992); whereas the levels of micro-minerals (zinc and copper) are lower than the recommended dietary allowance figures (ICMR, 1992). The ICMR recommended 15 mg zinc and 2.2 mg copper for Indian children. The manganese content of presently investigated *Mucuna* species/accession is found to be higher than that of recommended dietary allowances 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 Figure 1b. 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* varities/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 0.44 and 1.56 indicating that, they would serve as good sources of minerals for bone formation.

The presently investigated tribal pulses, *M. pruriens* var. *utilis* (two accessions) and *M. deeringiana* exhibit the highest level of niacin content (figure 1b). It is found to be higher than that of an earlier report in *Cajanus cajan, Dolichos lablab, D. biflorus, M. pruriens, Phaseolus mungo, Vigna catjang* and *Vigna* spp. (Rajyalakshmi and Geervani, 1994), *Rhynchosia filipes, V. trilobata, V. unguiculata* subsp. *Unguiculata, Entada rheedi, R. suaveolens and V. unguiculata* subsp. *Cylindrica* (Arinathan *et al*., 2003; 2009); *V. mungo* varities (Tresina *et al*., 2010) and *Lablab purpureus* varieties (Kala *et al*., 2010b). Two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* registers a higher level of ascorbic acid content than *Atylosia scarabaeoides* (Arinathan *et al*., 2003) *D. trilobus* and *Teraminus labialis* (Arinathan *et al*., 2009); *V. mungo* varieties (Tresina *et al*., 2010) and *Lablab purpureus* (Kala *et al*., 2010b).

The fatty acid profiles (Table 2) revealed that the antinutritional fatty acid, behenic acid is detected in two accessions of *M. pruriens* var. *utilis* and *M. 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* (Mohan and Janardhanan, 1993) and *M. pruriens* var. *pruriens* (Kala and Mohan, 2010). The presence of betenic acid has been implicated with antherogenic properties (Kritchevsky *et al*., 1973). Two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* exhibited high levels of palmitic, linoleic acids and stearic acid as in the case of some edible legumes such as *Vigna radiata, V. mungo* (Salunkhe *et al*., 1982),*V. unguiculata* and *Phaseolus vulgaris* (Omojbai, 1990). Oleic acid and stearic acid in the investigated samples are found to be higher than that of earlier study in *Cajanus cajan, V. radiata, V. mungo* (Salunkhe *et al*., 1982), *V. unguiculata* and *Phaseolus vulgaris* (Omojbai, 1990) *V. mungo* varieties (Tresina *et al*., 2010) and *Lablab purpureus* (Kala *et al*., 2010b). Linoleic acid was the dominating fatty acid, followed by palmitic and oleic acids. The nutritional value of linoleic acid is due to its metabolism at tissue levels which produce the harmone like prostaglandins. The activity of these prostaglandins includes lowering of blood pressure and constriction of smooth muscle (Aurand *et al*., 1987). Linoleic acids are the most important essential fatty acids required for the growth, physiological

Table 2. Fatty acid composition of lipids of two accessions of *Mucuna pruriens* var. *utilis* and *Mucuna deeringiana* (g $100g^{-1}$)[a].

Fatty acid (%)	*M. pruriens* var. *utilis* (white coloured seed coat)	*M. pruriens* var. *utilis* (black coloured seed coat)	*M. deeringiana*
Palmitic acid (C16:0)	25.80	28.43	21.40
Stearic acid (C18:0)	13.38	15.38	17.36
Oleic acid (C18:1)	20.20	21.70	19.39
Linoleic acid (C18:2)	30.68	24.48	29.40
Linolenic acid (C18:3)	9.10	8.71	9.71
Behenic acid (C22:0)	1.64	1.30	2.74

[a]All values are of two determination.

Table 3. Amino acid profiles of acid-hydrolysed, purified total seed proteins of two accessions of *Mucuna pruriens* var. *utilis* and *Mucuna deeringiana* (g 100 g^{-1})*.

Amino acid	*M. pruriens* var. *utilis* (white coloured seed coat)	EAAS	*M. pruriens* var. *utilis* (black coloured seed coat)	EAAS	*Mucuna deeringiana*	EAAS	FAO/WHO (1991) requirement pattern
Glutamic acid	12.60		12.28		13.30		
Aspartic acid	11.28		13.30		12.06		
Serine	4.64		4.83		3.73		
Threonine	3.54	104.11	3.68	108.24	4.14	121.76	3.4
Proline	3.19		3.56		2.64		
Alanine	4.26		4.06		5.18		
Glycine	5.12		5.94		4.28		
Valine	3.63	103.71	4.72	134.86	3.54	101.14	3.5
Cystine	1.11	75.6	1.24		0.78		2.5
Methionine	0.78		0.64	75.2	0.66	57.6	
Isoleucine	6.68	238.57	7.24	258.57	6.31	225.36	2.8
Leucine	5.24	79.39	6.04	91.52	5.78	87.58	6.6
Tyrosine	3.31		4.41		3.76		6.3
Phenylalanine	4.03	116.51	4.48	141.11	4.56	132.06	
Lysine	5.20	89.66	5.78	99.66	5.14	88.62	5.8
Histidine	2.94	154.74	3.33	175.26	2.74	144.21	1.9
Tryptophan	1.01	91.82	0.76	69.09	0.48	43.64	1.1
Arginine	7.74		7.06		5.06		

EAAS- Essential amino acid score
*All values are of single determination

functions and maintenance (Pugalenthi *et al*., 2004). In the present study, most of the fatty acids were unsaturated fatty acid. The fatty acid composition and high amounts of unsaturated fatty acids make *M. pruriens* var. *utilis* and *M. deeringiana* a special legume, suitable for nutritional application.

The amino acid profile of the purified seed proteins and the essential amino acid score are presented in Table 3. The content of lysine, leucine, tryptophan and sulphur containing amino acid in the investigated two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* seem to be deficient; whereas threonine, valine, isoleucine, tyrosine, phenylalanine and histidine in the present investigations are found to be higher compared with the FAO/ WHO (1991) requirement pattern.

Among the studied *Mucuna* species, *M. pruriens* var. *utilis* (black coloured seed coat) registers the highest level of IVPD (77.28%)than the other species and their protein digestibility was found to be higher than that of *V. aconitifolia* and *V. unguiculata* subsp *unguiculata* (Tresina and Mohan, 2011a; Tresina and Mohan, 2011b) and comparable with some edible legumes such as *Dolichos biflorus* (Rajyalakshmi and Geervani, 1990), *Vigna radiata* (Reddy and Gowramma, 1987), *Vicia faba* (Moneam, 1990), *Glycine max* (Gross, 1982) and *Vigna umbellata* (Laurena *et al.*, 1990).

Although legumes provide 20% of all plant protein in the human diet and are even more important

Table 4. Data on antinutritional factors of two accessions of *Mucuna pruriens* var. *utilis* and *Mucuna deeringiana*

Components	*M. pruriens* var. *utilis* (white coloured seed coat)	*M. pruriens* var. *utilis* (black coloured seed coat)	*M. deeringiana*
Total free phenolics [a] (g 100 g^{-1})	4.38±0.12	4.18±0.21	2.78±0.06
Tannins [a] (g 100 g^{-1})	0.11±0.01	0.21±0.02	0.16±0.01
L-DOPA[a](g100g^{-1})	6.38±0.11	7.23±0.09	4.00±0.13
Phytic acid [a](mg 100 g^{-1})	463.20±0.70	526.12±0.09	512.46±0.77
Hydrogen cyanide [a] (mg 100 g^{-1})	0.26±0.01	0.24±0.01	0.22±0.01
Trypsin inhibitor activity [b] (TIU mg^{-1} protein)	45.60	44.68	46.10
Oligosaccharides[a] (g 100 g^{-1})			
Raffinose	1.040.02	0.960.01	0.980.02
Stachyose	1.210.02	1.180.02	1.080.02
Verbascose	4.180.06	3.780.12	4.240.05
Phytohaemagglutinating activity [b] (HU mg^{-1} protein)			
A	172	168	178
B	72	76	82
C	14	12	16

[a]All values are means of triplicate determination expressed on a dry weight basis; ±denotes standard error.
[b]All values are of two independent experiments

in the diets of livestock, their usefulness is limited by toxic or antinutritional compounds 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, trypsin inhibitor activity, oligosaccharides and phytohaemoglutinating activity (Table 4). The content of total free phenolics of currently investigated two accessions of *M. pruriens* var. *utilis* appears to be higher than the earlier reported in other *Mucuna* species such as *M. atropurpurea* (Kala *et al.*, 2010); Sivagiri, Aliyar, Anaikatti and Seithur accessions of *M. pruriens* var. *pruriens* (Kalidass and Mohan, 2011). The total phenolic content of *M. deeringiana* is high compared with those in *V. aconitifolia* and *V. unguiculata* supsp. *unguiculata* (Tresina and Mohan, 2011b). The tannin content of the investigated pulses was relatively lower than the red gram, bengal gram, lentil (Salunke *et al.*, 2006) different chick pea cultivars (Zia Ul-Hug *et al.*, 2007) and *Phaseolus vulgaris* (Shimelis and Rakshit, 2007). Phenolics and tannins are known to inhibit activities of digestive enzymes and hence, the presence of even low levels of tannins and phenolics is not desirable from a nutritional point of view. However, in legumes the soaking and cooking process is known to reduce phenolics and tannins significantly (Vadivel and Puglendhi, 2008). Recently plant phenolics are increasingly gaining importance in relation to human health as wellness since they exhibit anticarcinogenic, antioxidant, antiviral, antimicrobial, anti-inflammatory and hypotensive properties (Shetty, 1997).

Concentration of non protein amino acid, L-Dopa in *M. pruriens* var. *utilis* (both the accessions) are found to be higher when compared with other *Mucuna* species/ accessions such as Thachanmalai accession of *M. pruriens* var. *utilis* (Vadivel and Janardhanan, 2000), Ayyanarkoil, Anaikatti accessions of *M. pruriens* var. *pruriens* (Kalidass and Mohan, 2011) *M. monosperma* (Mohan and Janardhanan, 1995) and *M. atropurpurea* (Kala *et al.*, 2010a). The level of L-Dopa in *M. deeringiana* is found to be low when compared with earlier reports in *M. pruriens* var. *pruriens* (Kala and Mohan, 2010; Fathima *et al.*, 2010). However, the pharmacologically active factor, L-Dopa (Pieris *et al.*, 1980) is potentially toxic (Duke, 1981; Afolabi *et al.*, 1985) if ingested in large amounts. L-DOPA a compound chiefly used in the treatment of Parkinson's disease, has been used reported to cause hallucinations, in addition to causing gastrointestinal disturbances such as nausea, vomiting and anorexia (Reynolds, 1989). This compound has also been shown to be toxic to

individuals with glucose-6-phosphate dehydrogenase deficiency in their erythrocytes and, as a result, to induce favism (Nechama and Edward, 1967). Takasaki and Kawakishi (1997) have reported that the oxidation products of L-DOPA conjugate with SH compound (cysteine) of proteins to form a protein-bound 5-S-cysteinyldopa cross link which leads to polymerization of proteins and/ or other protein, although the amount of formation is low. This might also be one of the factors which could be responsible for lowering of protein and starch digestibilities. However, it has been demonstrated that in M. pruriens, the level of L-Dopa is significantly eliminated by dry- heat treatment (Siddhuraja *et al*., 1996) and cooking and autoclaving (Vijayakumari *et al.*, 1996a).

Phytic acid, a major phosphorus storage form in plants and its salts known as phytates regulate various cellular functions such as DNA repair, chromatin remodeling, endocytosis, nuclear messenger RNA export and potential hormone signaling important for plant and seed development (Zhou and Erdman, 1995) as well as, animal and human nutrition (Vucenik and Shamsuddin, 2006). It is ofen regarded as antinutrient because of strong mineral, protein and starch binding properties thereby decreasing their bioavailability (Weaver and Kannan, 2002). The phytic acid content in the seeds of *M. pruriens* var. *utilis* and *M. deeringiana* is low when compared with earlier reports in *Mucuna* species (Vijayakumari *et al*., 1996a; Siddhuraju *et al.*, 2000; Kala and Mohan, 2010); and comparable with *V. mungo* (Kataria *et al*., 1988); *V. radiata* (Kataria *et al*., 1989); *M. monosperma* (Vijayakumari *et al.,* 1996b) and *Phaseolus vulagaris* (Yasmin *et al*., 2008).

Hydrogen cyanide is known to cause acute or chronic toxicity. The content of HCN level in the presently investigared two accessions of *M. pruriens* var. utilis and *M. deeringinana* is far below he lethal level (i.e. 0.36 mg/100 g) (Oke, 1969) and comparable with those of *V. sinensis* and *Pisum sativum* (Montgomery, 1980). *Dolichos lablab* var. *vulgaris, Bauhinia purpurea* (Vijayakumari *et al*., 1995; 1997), *Atylosia scarabaenoides, Neonotonia wightii* var. *coimbatorensis, Rhynchosia filipes, V. trilobata* and *V. unguiculata* subsp.*unguiculata* (Arinathan *et al.*, 2003).

The range of trypsin inhibitor activity (44.68-46.10 TU/mg protein) (Table 6) is found to be low compared with *Cajanus cajan* var. part A-2 and UPAS-120 (Singh and Eggum, 1984) *Glycine max* (Salunke *et al*., 2006) and *Vigna mungo* (Tresina *et al.*, 2010). Trypsin inhibitor activity has the greatest impact on the IVPD of the legumes where the former is known to be heat-labile. Alajaj and El-Adway (2006) reported that microwave cooking treatment decrease the trypsin inhibitor activity in the seeds of *Cicer arietinum*. The oligosaccharide content of the presently investigated seeds of *M. pruriens* var. *utilis* and *M. deeringiana* is comparable with that of five accessions of other species of *M. pruriens* var. *pruriens* (Kalidass and Mohan, 2011). Verbascose is found to be the major oligosaccharide in the two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* has been reported earlier in *M. pruriens* var. *utilis* (Janardhanan *et al.*, 2003b). Lectins combine with the cells that line the intestinal mucosa and cause a nonspecific interference with the absorption of available nutrients and also reduce feed intake (Liener, 1994). Phytohaemogglutinating activity of two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* registers higher activity with respect to ‘ A’ blood group of human erythrocytes. In the presently investigated *Mucuna* species/ accessions had low levels of phytohaemogglutinating activity with respect to erythrocytes of ‘O’ blood group. This is the good argument with earlier reports in the other *Mucuna* species (Kala and Mohan, 2010; 2012; Kalidass and Mohan, 2011). However dry heat and autoclaving are known to inactivate completely the trypsin inhibitors and phytohaemogglutinating in *Mucuna* beans (Siddhuraju *et al*., 1996). Khalil and Mansour (1995) reported that, boiling and autoclaving of faba bean completely eliminated the phytohaemogglutinating activity.

Conclusion

The observation made in the present study show that two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* are rich in crude protein, most of the essential amino acids, fatty acids such as palmitic acid and linoleic acid, some minerals and vitamins like niacin and ascorbic acid. Among the investigated *Mucuna* species, *Mucuna pruriens* var. *utilis* (white coloured seed coat) showed maximum amount of crude protein, calorific value, minerals such as sodium, calcium phosphorus, iron and zinc, vitamin niacin and linoleic acid whereas *Mucuna pruriens* var. *utilis* (black coloured seed coat) showed high amount of crude lipid, total dietary fibre and essential amino acid. The study also reveals that the nutritional profile of two accessions of *M. pruriens* var. *utilis* and *M. deeringiana* seems to be similar or higher than that of the other *Mucuna* species/ accessions reported earlier and can also be explored as alternate protein source to alleviate protein-energy malnutrition among the economically weaker sections of peoples in

developing countries. The presence of antinutritional factors identified in current report should not pose a problem for humans if the beans are properly processed before consumption.

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