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RESEARCH PAPER

Nutritional and Antinutritional Assessment of *Mucuna pruriens* (L.) DC var. *pruriens* an Underutilized Tribal Pulse

K. R. Fathima, P. Tresina Soris, and V. R. Mohan*
Ethnopharmacology unit, Research Department of Botany
V.O. Chidambaram College, Tuticorin-628008, Tamil Nadu.
***Corresponding author e-mail: vrmohan_2005@yahoo.com

ABSTRACT

Three accessions of the under-utilized legume, itching bean (Mucuna pruriens var. pruriens), collected from three different locations of South-Eastern slopes of Western Ghats, Tamil Nadu, India were analyzed for proximate composition, mineral profiles, vitamins (niacin & ascorbic acid), fatty acid profiles, amino acid profiles of total seed protein, in vitro protein digestibility and certain antinutritional factors. All the three accessions of Mucuna pruriens var. pruriens contained higher amounts of crude protein and crude lipid when compared with most of the commonly consumed pulses. The fatty acid profiles revealed that the seed lipids contained higher concentration of palmitic and linoleic acids. Amino acid profiles of Mucuna pruriens var. pruriens revealed that the seed proteins contained relatively higher levels of certain essential amino acids compared with the FAO/WHO (1991) requirement pattern. The investigated seeds were rich in minerals such as potassium, calcium, magnesium and phosphorus. Antinutritional substances like total free phenolics, tannins, L-DOPA, phytic acid, hydrogen cyanide, trypsin inhibitor activity, oligosaccharides (raffinose, stachyose and verbascose) and phytohaemagglutinating activity also were investigated. The antinutritional, fatty acid, behenic acid, also were detected in the present study.
***KEY WORDS**: Itching beans, proximate composition, vitamins, IVPD, antinutrients.*

INTRODUCTION

Protein-energy malnutrition is among the most serious problems tropical developing countries are facing today. This can be attributed mainly to the ever-increasing population as well as to the enhanced dependence on a cereal – based diet, scarcity of fertile land and degradation of natural resources [1,2]. It has been estimated that 800 million malnourished people exist in some of the least developed countries [3]. This has necessitated exploration alternate sources of protein to bridge the gap for protein requirement of the various section of vegetarian population. In this context, alternate sources like untraditional legumes (under exploited/ tribal pulses) assume significance.

The itching bean *Mucuna pruriens* (L.) DC var. *pruriens* is an underutilized legume species grown predominantly in Asia, Africa, in parts of America [4]. Mature seeds, seeds from unripe pods and young pods of itching bean, *Mucuna pruriens* (L.) DC var. *pruriens* 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 (5); North-Western parts of Madhya Pradesh tribes; Abujh- Maria, Maria Muria, Gond and Halba (6); South Indian tribes; Mundari and Dravidan [7]; Kani, Kader and Muthuvan (8) and Savera Jatapu, Gadebe and Kondadora [9]. To make this less- known legume palatable, tribal people follow a special processing method of continuous boiling and draining for about eight times until the boiled water changes from black to milky white. Consumption of improperly boiled seeds of]itching bean is known to cause increase in body temperature and skin eruptions [10]. It is attributed to the presence of high levels of 3, 4- dihydroxy-L-phenylalanine, L-Dopa, the aromatic non-protein amino acid [11].

Hence, in the present study, the seeds of tribal pulse *Mucuna pruriens* (L.) DC var. *pruriens* were collected from three different agroclimatic regions in Southern Western Ghats, Tamil Nadu and their chemical composition was investigated with a view to assess their nutritional potential.

MATERIALS AND METHODS

Source of seed

Three accessions of itching bean (*Mucuna pruriens* (L.) DC var. *pruriens)* pods from natural strands of three agroclimatic / ecological regions of Souther- Western Ghats, Thallaianai and Saduragiri, Virudhunagar district and Siruvani, Coimbatore district, Tamil Nadu. With the help of keys by Wilmot-Dear [12], the accessions were botanically identified. After thoroughly drying in the sun, the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds, foreign material 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 hr and is expressed on a percentage basis. The air-dried samples were powdered separately in a Wiley mill (Scientific Equipment, Delhi, India) to 60-mesh size and stored in screw capped bottles at room temperature for further analysis.

The nitrogen content was estimated by the micro-Kjeldahl method [13] and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus [14]. The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr [14]. Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method [15]. 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 hr 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 hr at 105°C and then cooled $\geq$ 2 hr 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 $\geq$ 2hr 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 [16]. 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 [17].

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) [18]. The phosphorus content in the triple acid digested extract was determined colorimetrically [19].

Ascorbic acid and niacin content were extracted and estimated as per the method given by Sadasivam & Manickam [20]. 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 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. The 5ml of 40% $ZnSO_4$ 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 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* [21]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* [22]. 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* [23]. The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). A protein sample of 30mg was hydrolysed by 6N HCL (5ml) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 hr. The sealed tube was broken and the acid removed completely by repeated flash evaporation after the addition of de-ionized water. Dilution was effected by means of citrate buffer pH 2.2 to such an extent that the solution contained 0.5 mg protein ml^{-1}. The solution was passed through a millipore filter (0.45µM) and derivitized with O-phthaldialdehyde by using an automated pre-column (OPA). Aminoacids were analysed by a reverse – phase HPLC (Method L 7400, HITACHI, Japan) fitted with a denali C_{18} 5 micron column (4.6X 150mm). The flow rate was 1 ml min^{-1} with fluorescence detector. The cystine content of protein sample was obtained separately by the Liddell and Saville [24] 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 [25] as modified by Rama Rao *et al* [26]. The contents of the different amino acids were expressed as $g100g^{-1}$ proteins and were compared with FAO/WHO (27) 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 [28], tannins [29], the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) [30], phytic acid [31] and hydrogen cyanide [32] were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al* [33] 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 [34]. Five grams seed flours of three accessions were extracted separately with 50mL of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130rpm for 13hr and then filtered through Whatman No. 1

filter paper. Residue was further washed with 25mL of 70% (v/v) ethanol. The filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in five mL of double-distilled water.

Separation of oligosaccharides was done by TLC. Thirty g of cellulose-G powder were dissolved in 45mL 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* [35]. 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 oligosacchrides were estimated by the method of Tanaka *et al* [35]. 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 oligosacchrides was expressed on dry weight basis.

Quantitative determination of phytoheamgglutinating (lectin) activity

Lectin activity was determined by the method of Almedia *et al* [36]. One g of air-dried seed flour was stirred with 10ml of 0.15N sodium chloride solution for 2hr and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20min. and the supernatants were collected separately. The protein content was estimated by the Lowry *et al* [37] 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* [38]. 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)

In vitro protein digestibility was determined using the multi-enzyme technique [39]. The enzymes used for IVPD were purchased from Sigma Chemical Co., St. Louis, MO, USA. Calculated amounts of the control (casein) and sample were weighed out, hydrated in 10ml of distilled water and refrigerated at 5°C for 1h. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37°C. The IVPD was determined by the sequential digestion of the samples containing protein with a multi-enzyme mixture [trypsin (porcine pancreatic trypsin–type IX with 14190 BAEE unites per mg protein), ∝-chymotrypsin (bovine pancreatic chymotrypsin–type II, 60 units per mg powder) and peptidase (porcine intestinal peptidase–grade III, 40 units per g powder)] at 37°C followed by protease (type IV from *Streptomyces griseus*) at 55°C. The pH drop of the samples from pH 8.0 was recorded after 20min of incubation. The IVPD was calculated according to the regression equation $Y = 234.84 – 22.56\ X$, where Y is the % digestibility and X the pH drop.

RESULTS AND DISCUSSION

The results of proximate analysis are presented in table 1. The crude protein content of all the three accessions of *Mucuna pruriens* var. *pruriens* show little variation and contain higher crude protein

when compared with commonly consumed pulse crops such as black gram, green gram, pigeon pea, chick pea and cow pea [40,41,42,43] and other *Mucuna* species which have been reported earlier [44, 45, 46, 4, 47]. The crude lipid content of all the accessions of *M. pruriens* var. *pruriens* seems to be higher than the previously studied common/tribal pulses such as *Vigna radiata* [48]; *Cicer arietinum* [41], *Cajanus cajan* [43], *Vigna capensis* and *V. sinensis* [49], *V. trilobata* [50], *Atylosia scarabaeoides*, *Neonotonia wighii* var. *coimbatorensis*, *Rhynchosia filipes* and *Vigna unguiculat*a subsp. *unguiculata* [51]. All the currently investigated samples contain high levels of total dietary fibre when compared with the other tribal pulses reported earlier *Canavalia gladiata*, *Lablab purpureus* var. *lignosus* and *Vigna unguiculuta* subsp. *unguiculata* [51]. The ash content of the investigated tribal pulse (Table 1) would be important to the extent that it contains the nutritionally important mineral elements, which are presented in table 2. The range in calorific values exceeds the energetic values of cowpea, green gram, horse gram, moth bean and peas [52], which are in the range of 1318 – 1394 kJ$100g^{-1}$ DM.

Table 1: Proximate composition of the seeds of three accessions of *Mucuna pruriens* var. *pruriens* (g $100g^{-1}$) [a].

Components	Saduragiri	Siruvani	Thallaianai
Moisture	10.21 ± 0.01	9.48 ± 0.23	9.78 ± 0.23
Crude protein (Kjeldahl Nx6.25)	32.48 ± 0.47	28.80 ± 0.46	29.40 ± 2.54
Crude lipid	8.50 ± 0.41	7.66 ± 0.47	8.94 ± 0.24
TDF(Total Dietary Fibre)	7.41 ± 0.01	6.20 ± 0.56	6.78 ± 0.01
Ash	4.10 ± 0.01	4.30 ± 0.01	4.52 ± 0.01
Nitrogen Free Extractives (NFE)	47.51	53.04	50.36
Calorific value (kJ$100g^{-1}$DM)	1656.28	1655.51	1669.03

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

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

Components	Saduragiri	Siruvani	Thallaianai
Sodium	54.12 ± 0.42	88.20 ± 0.46	69.21 ± 0.08
Potassium	1527.94 ± 0.04	1638.40 ± 0.48	1421.35 ± 0.44
Calcium	562.51 ± 0.23	710.42 ± 0.46	630.20 ± 0.38
Magnesium	410.10 ± 0.47	512.42 ± 0.25	478.82 ± 0.46
Phosphorus	408.52 ± 0.46	530.82 ± 0.46	448.12 ± 0.47
Iron	8.16 ± 0.47	7.15 ± 0.94	6.84 ± 0.23
Zinc	1.98 ± 0.01	3.34 ± 0.01	3.60 ± 0.19
Copper	0.66 ± 0.02	0.54 ± 0.01	0.72 ± 0.09
Manganese	7.10 ± 0.27	5.28 ± 0.04	6.48 ± 0.09
Niacin	34.20 ± 0.46	48.10 ± 0.50	42.64 ± 0.61
Ascorbic acid	45.70 ± 0.01	52.66 ± 0.55	38.12 ± 0.48

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

Table 2 shows the elemental composition of the sample. In the present investigation, the Sivagiri accession register a higher level of potassium than the other two accession and their levels of potassium seem to be higher compared to that of *Mucuna monosperma* [45], *M. pruriens* [17] and *M. utilis* [46]. Among the three accessions, Sivagiri accession registers the highest levels of calcium, magnesium and phosphorus and its calcium level is found to be higher than that of the recommended dietary allowances of calcium (400g) for children by the Indian Council of Medical Research [53], and its magnesium and phosphorus levels seem to be higher compared to that of *M. atropurpurea* and *M. utilis* [46]. The contents of iron, copper and zinc reported in all the three accessions are higher than that of the previous study in the *M. utilis* [46]. Similarly, the manganese content of all the three accessions seems to be low compared to that of *M. utilis* [46]. The variability in the content of minerals for the same species may be related to genetic origin, geographical source and the levels of soil fertility. The low sodium range makes the legume a good food source for people on low sodium diets [4].

The presently investigated tribal pulse, itching bean exhibits the highest level of niacin content (Table 2). This is found to be higher than that of an earlier reports in *Cajanus cajan, Dolichos lablab, D. biflorus, Mucuna pruriens, Phaseolus mungo, Vigna catjang* and *Vigna* sp [9], *Rhyncosia filipes, R. suaveolens, Vigna unguiculata* subsp. *unguiculata, V. unguiculata* subsp. *cylindrica* [51,47]. The investigated tribal pulse also registers higher level of ascorbic acid content than *Cicer arietnum* [54]; *Atylosia scarabaeoides, Lablab purpureus* var. *lignosus, Dolichos trilobus* and *Teramnus labialis* [51,47].

The fatty acid profiles (Table 3) revealed that all the three accessions of *Mucuna pruriens* var. *pruriens* seeds contain high levels of palmitic, oleic and linoleic acids as in the case of some edible legumes. Such as *Vigna radiata, V. mungo* [55] and *Phaseolus vulgaris* [56]. The antinutritional fatty acid, behenic acid, is present in the investigated samples as in groundnut [57], winged bean [58, 59, 60], *Parkia roxburghii, Entada phaseoloides, M. utilis* and *M. monosperma* [50, 46].

Table 3: Fatty acid profile of the seeds of three accessions of *Mucuna pruriens* var. *pruriens* .

Fatty acids (%)	Saduragiri	Siruvani	Thallaianai
Palmitic acid (C16:0)	27.14	25.34	29.32
Stearic acid (C18:0)	14.82	12.31	15.11
Oleic acid (C18:1)	17.15	21.42	17.14
Linoleic acid (C18:2)	28.12	30.14	23.40
Linolenic acid (C18:3)	11.14	9.48	13.10
Behenic acid (C22:0)	1.63	1.31	1.93

All values are of means of two determinations.

The amino acid profiles of the purified seed proteins and the essential amino acid score are presented in Table 4. The essential amino acid profiles of total seed proteins compared favourably with the FAO/WHO [27] requirement pattern, except that there are deficiencies of sulphur containing amino acid in all the three accessions and also in the leucine in Thallaianani accession, lysine in Siruvani accession and tryptophan contents in all the accessions.

All the three accessions of seed materials of itching beans, the Thallaianai accession registers the highest level of *in vitro* protein digestibility (75.16%) compared to other accessions and its protein digestibility is found to be higher than that of an earlier study in the other species of *Mucuna* [61] and Soya beans [62]. In the present study, the high level of trypsin inhibitor activity in the Saduragiri accession (48.30 TIU mg^{-1} proteins) might be attributed for low protein digestibility.

The presence of antinutritional factors is one of the major drawbacks limiting the nutritional and food qualities of the legumes [55]. For this reason, a preliminary evaluation of some of these factors in raw itching bean is made (Table 5). Total free phenolics occurred within the range of 4.38 – 5.02% and tannins ranged from 0.24 to 0.30%. Tannins have been claimed to affect adversely protein digestibility [63]. In *Mucuna* beans, Ravindran and Ravindran and Mary Josephine and Janardhanan

[61, 64] reported that most of the tannins are located in the seed coat with only traces in the cotyledons.

Table 4: Amino acid profiles of acid- hydrolysed, purified total seed proteins of the seeds of three accessions of *Mucuna pruriens* var. *pruriens* (g $100g^{-1}$)[a].

Amino acid	Saduragiri	EAAS	Siruvani	EAAS	Thallaianai	EAAS	FAO/WHO(1991) requirement pattern
Glutamic acid	14.11		13.50		10.38		
Aspartic acid	12.98		12.44		13.11		
Serine	4.40		3.50		4.24		
Threonine	3.78	111.76	4.12	121.17	3.56	104.70	3.4
Proline	2.80		4.04		2.44		
Alanine	4.24		4.94		5.12		
Glycine	5.95		4.44		4.90		
Valine	3.90	111.42	4.06	116.00	3.56	101.71	3.5
Cystine	0.54	71.20	0.73	56.40	1.01	70.00	2.5
Methionine	1.24		0.68		0.74		
Isoleucine	5.94	212.14	6.94	247.85	6.24	222.85	2.8
Leucine	7.24	109.69	6.30	95.45	5.94	90.00	6.6
Tyrosine	4.94	141.58	5.01	134.12	4.24	130.95	6.3
Phenylalanine	3.98		3.44		4.01		
Lysine	6.01	103.62	5.68	97.93	6.61	113.96	5.8
Histidine	4.44	233.68	3.60	189.47	2.90	152.63	1.9
Tryptophan	0.88	80.00	0.92	83.63	0.56	50.90	1.1
Arginine	5.06		5.94		6.66		

EAAS-Essential amino acid core

Since the seed coats are usually removed by soaking prior to consumption, the tannins in *Mucuna* beans are of little significance from the nutritional point of view. Besides, Siddhuraju *et al* and Vijayakumari *et al* [17, 65] reported that in *M. pruriens*, the levels of phenolics and tannins are reduced significantly during dry and wet heat treatments and their reduction improves the protein digestibility. In the present study, the Siruvani accession is found to contain more L-DOPA (8.13%) than the other two accessions. This value is found to be lower than that of an earlier study in the same species [65]. It has been demonstrated that in *M. pruriens*, the level of L-DOPA is significantly eliminated by dry heat treatment [17] and cooking and autoclaving [65]. All the accessions of *M. pruriens* var. *pruriens* is found to be low levels of phytic acid when compared with white coloured accessions of *M. pruriens* var. *utilis* Janardhanan *et al* and Vijayakumari *et al* [66,65]. The phytate molecule is negatively changed at the physiological pH and is reported to bind nutritionally important essential divalent cations, such as iron, zinc, magnesium and calcium. This binding forms insoluble complexes, thereby making minerals unavailable for absorption and utilization. The levels of hydrogen cyanide in *M. pruriens* var. *pruriens* seems to be negligible when compared with the lethal level of HCN (36mg/100g) [67] and safe varieties of *Phaseolus lunatus* [68]. The range in trypsin inhibitors activity (42.10 – 48.30 TIU mg^{-1} protein) is found to be low compared to *Cajanus cajan* var. part A-2 and UPAS -120 [69]. Slight variation in the levels of oligosaccharide is detected with raffinose ranging from 0.86 to 1.12g $100g^{-1,}$ stachyose ranging from 1.38 to 1.78g $100g^{-1}$ and verbascose ranging from 3.96 to 4.78g $100g^{-1}$ (Table 5). Verbascose is found to be the major oligosaccharide in all the accession of *M. pruriens* var. *pruriens* as has been reported earlier in *M. pruriens* var. *utilis* [66]. Regarding phytohaemagglutinating activity, all the accessions of *M. pruriens* var. *pruriens* register higher haemagglutinating activity with respect to 'A' blood group of human erythrocytes. All accessions have low levels of phytohaemagglutinating activity with respect to

Table- 5: Data on *in vitro* protein digestibility and antinutritional factors of the seeds of three accessions of *Mucuna pruriens* var. *pruriens.*

Components	Saduragiri	Siruvani	Thallaianai
In vitro protein digestibility (%)[a]	71.48	74.33	75.16
Total free phenolics ($g100g^{-1}$)[b]	4.40 ± 1.21	4.38 ± 0.89	5.02 ± 1.05
Tannins ($g100g^{-1}$)[b]	0.30 ± 0.03	0.26 ± 0.04	0.24 ± 0.03
L-DOPA ($g100g^{-1}$)[b]	7.48 ± 1.34	8.13 ± 2.36	7.06 ± 1.78
Phytic acid ($mg100g^{-1}$)[b]	568.14 ± 0.78	438.10 ± 0.97	476.72 ± 0.67
Hydrogen cyanide ($mg100g^{-1}$)[b]	0.33 ± 0.03	0.26 ± 0.02	0.31 ± 0.03
Trypsin inhibitor activity (TIU mg^{-1} protein)[a]	48.30	45.20	42.10
Oligosaccharide ($g100g^{-1}$)[b]	0.86 ± 0.11	1.12 ± 0.20	0.92 ± 0.10
Raffinose	1.78 ± 0.31	1.52 ± 0.21	1.38 ± 0.17
Stachyose	3.96 ± 0.17	4.78 ± 0.11	4.24 ± 0.26
Verbascose			
Phytohaemagglutinating activity (HU mg^{-1} protein)[a]			
	166	178	156
A group			
B group	84	72	78
O group			
	16	12	18

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

erythrocytes of 'O' blood group. This is in good agreement with earlier reports in same species [65]. However, dry heat and autoclaving are known to inactivate completely the trypsin inhibitors and phytohaemagglutinating activity in *Mucuna* beans [17].

From these chemical investigations, it is concluded that the presently investigated tribal pulse *Mucuna pruriens* (L.) DC var. *pruriens* 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.

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