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NUTRITIONAL AND ANTINUTRITIONAL COMPOSITION OF ITCHING BEAN (*Mucuna pruriens* (L.) DC var. *pruriens*): AN UNDERUTILIZED TRIBAL PULSE IN WESTERN GHATS, TAMIL NADU

[CONTENIDO DE COMPUESTOS NUTRICIOS Y ANTINUTRICIOS DEL FRIJÓL URTICANTE (*Mucuna pruriens* (L.) DC var. *pruriens*): UN GRUPO DE FRIJOLES SUBUTILIZADO DE LOS GHATS OCCIDENTALES, TAMIL NADU, INDIA]

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SUMMARY

Five accessions of the underutilized legume itching bean (*Mucuna pruriens* (L.) DC.) var. *pruriens*), collected from five different agroclimatic regions in Western Ghats, Tamil Nadu, India were analysed for their proximate and mineral composition, vitamins (niacin and ascorbic acid), fatty acid profiles, amino acid profiles of total seed proteins, *in vitro* protein digestibility (IVPD) and certain antinutritional factors, to determine their potential as an alternative source to alleviate protein-energy-malnutrition among the people of Tamil Nadu. The crude protein ranged from 27.5 – 31.2%, crude lipid 6.6 – 8.8%, total dietary fibre 6.5 – 9.7%, ash 4.3 – 6.0% and carbohydrates 46.1 – 52.5%. The energy level of the seed (1576 – 1620 kJ$100g^{-1}$ DM) was comparable with commonly consumed Indian pulses. The investigated seeds were rich in minerals such as Na, K, Ca, Mg and P. The fatty acid profiles revealed that the seed lipids contained higher concentrations of linoleic acid and palmitic acid. The essential amino acid profile of total seed proteins compared favourably with FAO/WHO (1991) requirement pattern, except that there were deficiencies of sulphur containing amino acids in all the five accessions. The IVPD of the five accessions ranged from 70.1 – 76.5%. The range of antinutritional factors were as follows: total free phenolics, 3.4 – 4.8%, tannins, 0.14 – 0.23%, L-Dopa, 5.4 – 7.0%, phytic acid, 478 – 648 mg$100g^{-1}$, hydrogen cyanide, 0.24 – 0.38 mg$100g^{-1}$ and trypsin inhibitor activity, 40.4 – 48.2 TIU mg^{-1} protein. Verbascose was the principal oligosaccharides in all accessions. Lower levels of phytohaemagglutinating activity for human erythrocytes of 'O' blood group than for 'A' and 'B' blood groups were found. The antinutritional fatty acid, behenic acid (1.28 – 2.74%) was also detected.

Key words:.

RESUMEN

Se evaluaron cinco accesiones del frijol urticante (*Mucuna pruriens* (L. DC) var. *pruriens*), collectadas de cinco diferentes regiones agroclimpaticas de los Ghats occidentales de la India. Se analizó su contenido de minerales, vitaminas (niacina y ácido ascórbico), perfil de ácidos grasos y amino ácidos, digestibilidad *in vitro* de la proteína (IVPD) y algunos factores antinutricionales. Su contenido de proteína cruda fluctuó entre 27.5 y 31.2%, grasa cruda 6.6 – 8%, fibra dietética 6.5 – 9.7%, ceniza 4.3 – 6.0% ycarbohidratos 46.1 – 52.5%. La energía bruta fluctuó entre 1576 y 1620 kJ $100g^{-1}$ DM. Fueron también ricos en Na, K, Ca, Mg y P. Se observó una alta concentración de ácido linoleico y pálmitico. El perfil de amino ácidos fue apropiado según el patrón FAO/WHO (1991) excepto en los amino ácidos azufrados donde se encontró deficiencias. La IVPD fluctuó de 70.1 a 76.5%. Se encontró fenoles libres (3.4-4.8%), taninos (0.14-0.23%), L-Dopa (5.4-7.0%), ácido fítico 478-648 mg $100g^{-1}$ cianuro de hidrógeno (0.24-0.38 mg $100g^{-1}$) y actividad inhibidora de tripsina (40.4-48.2 TIU mg^{-1} proteína). Verbascosa fue el principal oligosacárido. Se encontraron niveles más bajos de actividad fitohemaglutinadora para eritrocitos O que los del tipo A y B. Se detectó también el ácido behenico (1.28 – 2.74%) que es un ácido graso antinutricional.

Palabras clave: Frijól urticante; digestibilidad *in vitro;* amino ácidos; factores antinutricionales.

INTRODUCTION

Large segments of the human population and animals in developing countries suffer from protein malnutrition. About 800 million people are consuming less than 2000 calories a day and are living under conditions of permanent or intermittent hunger so that they are chronically undernourished. Most of the hungry are women and young children (Conway and Toenniessen, 1999). The populations of most developing countries are increasing rapidly: by the year 2020 there will be an additional 1.5 billion mouths to feed, mostly in the developing world. The search for alternative food and feed ingredients for man and livestock continues to attract the attention of researchers all over the world (Janardhanan *et al.*, 2003; Pugalenthi *et al.*, 2005). The underutilized plants, which have tremendous potential for commercial exploitation but remain ignored, offer a good scope in this context (Mal, 1992). Accounts of important underutilized plant species which await exploration for food, fodder, energy and industrial purposes have been given (Siddhuraju *et al.*, 2000; Arinathan *et al*., 2007; 2009).

Mature seeds, seeds from unripe pods and young pods of itching bean, (*Mucuna pruriens* (L.) DC var. *pruriens*), evaluated in the present study, 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, 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 Kondadddora (Rajyalakshmi and Geervani, 1994). South Indian tribes called this species "Naikurana". 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 by the tribe, Kanikkars, in Western Ghats, Tamil Nadu, India, is known to cause ill effects like increase in body temperature and skin eruptions (Jebadhas, 1980).

For medicinal purposes, the non protein amino acid, L-DOPA is extracted from the seeds for the production of commercial drugs for treatment of Parkinson's disease (Haq, 1983). The seed powder is known to exhibit faster hypothermic (Rajendran *et al.*, 1996) and anti-Parkinson activity than synthetic L-DOPA (Hussain and Manyam, 1997). The seed powder is known to stimulate more sexual activity in male albino rats than L-DOPA and also is reported to arouse sexual desire in patients suffering from Parkinson's disease (Kumar *et al.*, 1994). Vigorex-SF, an ayurvedic herbo-mineral formulation consisting of the seed powder reportedly improves libido disturbed by psychological fear, emotional imbalance and other allied ailments (Shaw and Bera, 1993). Seeds of this wild legume are widely used for treating male sexual dysfunction in Unani medicine (Amin *et al.*, 1996). The blocking effect of King cobra venom at the neuro-muscular junction is removed by the aqueous extract of the seed of this plant species, (Aguiji *et al.*, 1997). Rhinax, an herbal formulation comprising this wild pulse possesses anti-hepatotoxic activity (Dhuley and Naik, 1997). The tribe Garos of Maghalaya, India consume the seeds for increasing potency and the hairs of the pod are used as vermifuge (Vasudeva and Shanpru, 1991). In Nigeria, powdered hairs on pods are administrated with honey for expelling intestinal worms (Gill and Nyawuame, 1994).

Despite the potential of this underutilized species as a source of protein/energy and active medicinal ingredients, to our knowledge, meager information is available on its germplasm collection from Western Ghats Tamil Nadu, India and its evaluation for chemical composition. In Tamil Nadu, the tropical forests of Western Ghats, one of the 18 biodiversity hot spots of the world (Gadgil, 1996) has a large group of underutilized food plants from Leguminosae, whose chemical potential hitherto remains untapped. In this context, chemical evaluation of five accessions of itching bean collected from five different agroclimate/ecological regions of Western Ghats, Tamil Nadu, India were investigated.

MATERIALS AND METHODS

Five accessions of itching bean, *Mucuna pruriens* (L.) DC. var. *pruriens* were colleted as pod from natural stands of five different agroclimatic regions of Western Ghats, Tamil Nadu, India during March and April, 2008 (Table 1). With the help of keys by Wilmot-Dear, 1987, the accessions were botanically identified. After thoroughly drying in the sun, the pods were thrashed to remove seeds. After through cleaning, removal of broken seeds, foreign materials and mature seeds were stored in airtight plastic jars at room temperature (25°C).

Table 1: Collection details of five accessions of itching bean.

Botanical name, & Accession No.	Place of Collection	Agro-climatic region
Mucuna pruriens (L.) DC var. *pruriens* VOC/Bot/VRM/0836	Ayyanarkovil Reserve Forest Virudhunagar Dist, Tamil Nadu.	Latitude: 09º 30" 52.68" N Longitude: 077º 26" 36.49" E Annual rain fall: 1595 – 1915 mm Habitat: Semi evergreen forest, slightly elevated in 1213 ft altitude, sandy soil.
VOC/Bot/VRM/0817	Sivagiri Reserve forest, Tirunelveli Dist, Tamil Nadu.	Latitude: 09º 22" 51.38" N Longitude: 077º 23" 17.13" E Annual rain fall: 1435 – 1895 mm Habitat: Semi evergreen forest, slightly elevated in 802 ft altitude, loamy soil.
VOC/Bot/VRM/0829	Aliyar Reserve Forest, Coimbatore Dist, Tamil Nadu.	Latitude: 10º 27"34.03" N Longitude: 076º 57" 55.04" E Annual rain fall: 1875 – 2015 mm Habitat: Semi-evergreen forest, slightly elevated in 1354 ft altitude, sandy soil.
VOC/Bot/VRM/0813	Anaikatti Reserve Forest, Coimbatore Dist, Tamil Nadu.	Latitude: 11º 05" 26.85" N Longitude: 076º 50" 14.20" E Annual rain Fall: 1654 – 1875 mm Habitat: Deciduous forest, slightly elevated in 1916 ft altitude, sandy soil.
VOC/Bot/VRM/0842	Seithur Reserve Forest, Virudhunagar Dist, Tamil Nadu.	Latitude: 09º 24" 21.63" N Longitude: 077º 26" 53.59" E Annual rain Fall: 1350 – 1570 mm Habitat: Semi-evergreen forest, slightly elevated in 645 ft altitudes, sandy soil.

Proximate composition

Seed moisture content, on a percent basis, was determined by drying 50 transversely cut seeds in an oven at 80°C for 24h. 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.

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). Ash was determined by heating 2g of the dried sample in a silica dish at 600°C for 6h (AOAC, 2005). Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method proposed by Li and Cardozo (1994). To determine the TDF, duplicate 500mg ground samples were taken in separate 250ml beakers. To each beaker 25ml water was added and gently stirred until samples were thoroughly wetted, (i.e. no clumps present); the beakers were then covered with Al foil and allowed to stand 90min without stirring in an incubator maintained at 37°C; after that, 100ml 95% ethanol were added to each beaker and allowed to stand for 1hr 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 20ml of 78% ethanol, 10ml of 95% ethanol and 10ml acetone. The crucible containing the residue was dried $\geq$2 h at 105°C and then cooled $\geq$ 2h in a desiccator and weighed. One crucible containing residue was used for ash determination at 525°C for 5h. The ash-containing crucible was cooled for $\geq$ 2h in a desiccator and weighed. The residue from the remaining duplicate crucible was used for crude protein determination by the micro-Kjeldahl method as already mentioned. The TDF was calculated as follows.

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

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).

Analysis of minerals and vitamins

Five hundred milligrams of the ground legume seed were digested with a mixture of 10ml concentrated nitric acid, 4ml of 60% perchloric acid and 1ml concentrated sulphuric acid. After cooling, the digest was diluted with 50ml of deionised H_2O, filtered through Whatman No. 42 filter paper and filtrates were made up to 100ml with deionised H_2O in a glass volumetric flask. All minerals, except phosphorus, were analyzed from a triple acid-digested sample by atomic absorption spectrophotometry, 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 were extracted and estimated after Sadasivam and Manickam (1996). For the extraction of ascorbic acid, 3g from the air-dried powdered sample were 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. A 2ml aliquot of the extract was made up to 3ml with distilled H_2O in a test tube; then, 1ml 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 were added to dissolve the osazone crystals and the absorbance was measured at 540nm against a reagent blank.

For the extraction of niacin, 5g from the 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. Next, 5ml of 60% basic lead acetate was added to 25ml of the filtrate, pH was adjusted to 9.5 and the filtrate was centrifuged (10,000g for 10 min at room temperature). The resulting supernatant was added with 2ml concentrated H_2SO_4 and the mixture was allowed to stand for 1hr and was then centrifuged (10,000g for 10 min at room temperature); five millilitres of 40% $ZnSO_4$ were added, pH was adjusted to 8.4 and the mixture was centrifuged (10,000g for 10 min at room temperature) again; pH of the supernatant was adjusted to pH 7.0 and used as the niacin extract. For estimation, 1ml extract was made up to 6ml with distilled water in a test tube; 3ml cyanogen bromides were added and the extract was then shaken vigorously, 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 in the sample were calculated by referring to a standard graph and expressed as milligrams per 100 grams of powdered samples.

Lipid extraction and fatty acid analysis

Total lipids were extracted from the seeds according to the method by Folch *et al.*, (1957) using chloroform and methanol mixture in ratio of 2: 1 (v/v). Methyl esters were prepared from the total lipids (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 (2m x 3mm) 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 by Basha *et al.*, (1976). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). A protein sample of 30mg was hydrolysed by 6N HCL (5ml) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 hours. The sealed tube was broken and the acid removed completely by repeated flash evaporation after the addition of deionized H_2O. Dilution was effected by means of citrate buffer pH 2.2 to such an extent that the solution contained 0.5mg protein ml^{-1}. The solution was passed through a millipore filter (0.45µM) and derivatized 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 150mm). The flow rate was 1 ml min-1 with fluorescence detector. The cystine content of protein sample was obtained separately by the Liddell and Saville (1959) method. For the determination of tryptophan content of proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together

with 1 ml 5M NaOH. The ampoules were flame sealed and incubated at 110°C for 18 hours. The tryptophan content of the alkaline hydrolysates were determined colorimetrically using the method by Spies and Chambers (1949) as modified by 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 Kakade *et al.* (1974) by using benzoil-DL-arginin-*p*-nitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Extraction, TLC separation and estimation of Oligosaccharides

Extraction of oligosaccharides was done following the method of Somiari and Balogh (1993). Five grams raw seed flours of all the five accessions were extracted separately with 50ml of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 g for 13 hours and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25ml of 70% (v/v) ethanol. Filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in five ml of distilled water.

Separation of oligosaccharides was done by TLC. Thirty grams of cellulose-G powder (Sigma-Aldrich Brand) was 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. 5 µl aliquots of each sample were spotted three times separately. The plates were developed by using a solvent system of n-propanol, ethyl acetate and distilled H_2O (6:1:3), and dried (Tanaka *et al.*, 1975). The plates were sprayed with α –naphthol (1%, w/v). The separated spots were compared with standard sugar spots in a standard sugar mixture containing raffinose, stachyose and verbascose (procured from Sigma Chemical Co., St. Louis, MO, USA). Separated sugars included verbascose, stachyose and raffinose. The sugar spots were scrapped, eluted in 2ml of distilled H_2O, kept overnight and filtered through Whatman No. 1 filter paper. The final 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 0.2M 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 432nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was expressed on dry weight based.

Quantitative determination of phytoheamagglutinating (Lectin) activity

Lectin activity was determined by the method of Almedia *et al.* (1991). One gram of air-dried seed flour was stirred with 10ml of 0.15N sodium chloride solution for 2hr and the pH 4.0 was adjusted. The contents were centrifuged at 10,000 X g for 20min. and the supernatants were collected separately. Protein content was estimated after Lowry *et al.* (1951) method.

Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3min at low speed (3,000 g for 10 min at room temperature). Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24drops of phosphate – buffered saline. Human blood (blood groups A, B and O) was procured from the blood bank of Jothi Clinical Laboratory, Tuticorin.

The determination of lectin was done by the method of Tan *et al.* (1983). Clear supernatant (50µl) was poured into the depression (pit) on a microtitration plate and serially diluted 1:2 with normal saline. The human blood erythrocyte (A, B and O blood groups) suspensions (25µl) were added to each of the twenty depressions. The plates were incubated for 3hours at room temperature. After the incubation period, the titer values were recorded. One Haemagglutinating unit is defined as the least amount of heamagglutinin that will produce positive evidence of agglutination of 25µl of a

blood group erythrocyte after 3hr incubation at room temperature. The phytoheamagglutinating activity was expressed as heamagglutinating units (HU)/mg protein.

Determination of *in vitro* protein digestibility (IVPD)

The determination of *in vitro* protein digestibility 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 1hr. 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 with a multi-enzyme mixture [trypsin (porcine pancreatic trypsin–type IX with 14190 BAEE unites per mg protein), α-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.

Statistical analysis

Analysis of Variance (ANOVA) and turkey method (DMRT) were used for analysis (SPSS software for windows release 11.5; SPSS Inc., Chicago IL, USA) of any significant differences in chemical compositions among the five accessions collected from the five locations. Significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

The proximate composition of the five accession of itching bean (*M. pruriens* var. *pruriens*) is shown in Table 2. Crude proteins and carbohydrates (Nitrogen-Free Extract) are the major chemical constituents of the legume samples. Protein content ranged from 27.51 – 31.24%. The significant ($P<0.05$) difference in protein content was probably due to different growing conditions of the plants from which the seeds were collected (Vadivel and Janardhanan, 2002). The range in protein levels of itching bean compared well with earlier reports on *Mucuna monosperma* (Mohan and Janardhanan, 1995), *Mucuna pruriens* var. *utilis* (Mohan and Janardhanan, 1995; Vadivel and Janardhanan, 2000a), *Mucuna pruriens* var. *pruriens* (Vadivel and Janardhanan, 2000b), but it was higher than *Cicer arietinum* (Srivastava and Ali, 2004; Alajaji and El-Adawy, 2006). The remarkably high level of protein in the wild legume accessions under study underscores their importance as sources of this vital nutrient. In general, the Aliyar accession showed more crude fat than the other accessions. The Total dietary fiber ranged from 6.52 to 9.71%. The ash content range of this legume (4.31 – 6.00%) would be important to the extent that it contains the nutritionally important mineral elements shown in Table-3. From the data the itching bean has a higher range of carbohydrate (46.11 to 52.53%), than pea nut and soybeans (Rao *et al.*, 1989). The range in calorific values exceeds the energy values of *Vigna unguiculata, Vigna radiata, Macrotyloma uniflorum, Vigna aconitifolia* and *Pisum sativum* (Rao *et al.*, 1989), which are in the range of 1576.18 – 1620.43 kJ $100g^{-1}$ DM.

In comparison with earlier studies (Vadivel and Janardhanan, 2000b) the content in sodium, potassium, calcium, magnesium and phosphorus were found to be higher in all the accessions of itching bean investigated in the present study (Table-3). Based on the above analysis, all the itching bean accessions can be considered as potential sources of minerals because any diet that contains 2/3 of the RDA (Recommended Dietary Allowances) values is considered to be adequate (Robinson, 1987). Generally, the statistically significant difference presented in Table 3 may have been related to the soil types in which the legume plant was found growing and/or to the efficiency of the uptake from the soil by the plants (Vadivel and Janardhanan, 2002). The itching bean is rich source of niacin and ascorbic acid (Table 4). The niacin content ranged from 33.27 – 64.76 $mg100g^{-1}$, and ascorbic acid also ranged from 25.15 – 48.62 mg100g-1. In the present study, all the accessions showed a higher level of niacin compared to an earlier report in *Cajanus cajan, Dolichos lablab, Dolichos biflorus, M. pruriens, P. mungo, Vigna catjang* and *Vigna* species (Rajyalakshmi and Geervani, 1994). Itching bean accessions showed a higher content in ascorbic acid than *Cicer arietinum* (Fernandez and Berry, 1988); *Atylosia scarbaeoides* and *Teramnus labilis* (Arinathan *et al.*, 2003; 2009).

The fatty acid profiles (Table 5) reveal that all the accessions of *M. pruriens* var. *pruriens* seeds contain high levels of linoleic acid, as in the case of some edible legumes such as *Vigna radiata, V. mungo*, (Salunkhe *et al.*, 1982), *Phaseolus vulgaris* and *Vigna unguiculata* (Omogbai, 1990). Palmitic acid is present in large quantity in the seeds of *M. pruriens* var. *pruriens* (all the accessions) as in *Cajanus cajan, Vigna radiata, V. mungo* (Salunkhe *et al.*, 1982) *Phaseolus vulgaris* and *Vigna unguiculata* (Omogbai, 1990). The antinutritional fatty acid, behenic acid, is detected in all the accessions of *M. pruriens* var. *pruriens*. Earlier reports indicate the presence of behenic acid in *Arachis hypogea* (Kritchevsky *et al.*, 1973), *Psophocarpus tetragonolobus* (Bean *et al.*,

1984; Fernando and Bean, 1986); *Parkia roxburghii* and *Entada phaseoloides* (Mohan and Janardhanan, 1993) and *Mucuna* species (Mohan and Janardhanan, 1995). The presence of behenic acid has been associated with atherogenic properties (Kritchevsky *et al.*, 1973).

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

Components	Ayyanarkoil	Sivagiri	Aliyar	Anaikatti	Seithur
Moisture	11.17±1.06^{a}	10.18±0.75^{a}	10.69±0.31^{a}	11.54±0.10^{a}	11.56±0.66^{a}
Crude protein (Kjeldhal N × 6.25)	27.51±1.08^{c}	28.26±0.41^{c}	30.85±0.32^{ab}	31.24±0.90^{a}	29.67±0.30^{b}
Crude lipid	7.84±1.87^{ab}	6.82±0.17^{b}	8.80±0.17^{a}	7.41±0.91^{ab}	6.57±0.50^{b}
TDF (Total Dietary Fibre)	9.47±1.05^{a}	8.75±0.71^{a}	8.53±0.05^{a}	9.71±0.32^{a}	6.52±0.49^{b}
Ash	6.00±0.49^{a}	5.05±0.07^{b}	5.71±0.10^{a}	4.34±0.23^{c}	4.71±0.31^{bc}
Nitrogen Free Extractives (NFE)	49.17±0.49^{ab}	51.12±0.31^{b}	46.11±0.12^{b}	47.29±0.39^{b}	52.53±0.16^{a}
Calorific value (kJ100g^{-1} DM)	1576.18±0.57^{abc}	1582.70±0.25^{bc}	1616.99±0.10^{a}	1590.99±0.33^{ab}	1620.43±0.15^{c}

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

Table 3: Mineral composition of five accessions of *M. pruriens* var. *pruriens*. (mg 100 g^{-1} seed flour)* "

Components	Ayyanarkoil	Sivagiri	Aliyar	Anaikatti	Seithur
Sodium	65.61±0.50^{c}	78.07±0.07^{b}	98.51±0.50^{a}	57.36±0.55^{d}	34.53±0.41^{e}
Potassium	1561.38±0.62^{c}	1569.72±0.69^{b}	1600.93±1.20^{a}	1498.26±1.06^{d}	1395.41±0.75^{d}
Calcium	740.72±0.63^{a}	678.09±0.15^{b}	548.15±1.05^{d}	674.03±4.39^{c}	569.39±1.46^{d}
Magnesium	537.37±4.06^{b}	440.78±9.04^{c}	511.41±0.71^{c}	594.60±1.55^{a}	487.40±0.83^{d}
Phosphorus	438.15±0.25^{c}	531.14±0.88^{b}	583.07±8.51^{a}	410.37±1.04^{d}	440.16±0.99^{c}
Iron	5.47±0.52^{c}	5.38±0.10^{c}	6.40±0.01^{b}	7.56±0.10^{a}	4.45±0.07^{d}
Zinc	2.23±0.36^{b}	1.83±0.09^{c}	1.42±0.01^{c}	2.18±0.08^{ab}	2.63±0.47^{a}
Copper	0.45±0.06^{b}	0.55±0.07^{a}	0.35±0.05^{c}	0.48±0.01^{ab}	0.57±0.02^{a}
Manganese	7.46±0.06^{b}	8.36±0.30^{a}	5.55±0.01^{d}	7.95±0.09^{ab}	6.76±0.61^{c}

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

Table 4: Vitamins (niacin and ascorbic acid) content of five accessions of *M. pruriens* var. *pruriens*. (mg 100g^{-1})* "

Components	Ayyanarkoil	Sivagiri	Aliyar	Anaikatti	Seithur
Niacin	33.27±0.15^{a}	42.10±0.01^{a}	38.29±0.05^{b}	49.62±0.04^{a}	64.76±0.14^{b}
Ascorbic acid	25.15±0.16^{b}	31.55±0.11^{a}	33.45±0.06^{a}	29.30±0.05^{a}	48.62±0.09^{a}

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

Table 5: Fatty acid profile of lipid of five accessions of *M. pruriens* var. *pruriens*[a].

Fatty acid (%)	Ayyanarkoil	Sivagiri	Aliyar	Anaikatti	Seithur
Palmitic acid [C16:0]	26.14	24.68	28.15	27.46	25.10
Stearic acid [C18:0]	16.41	15.10	14.30	17.13	14.30
Oleic acid [C18:1]	13.42	16.28	14.46	15.39	17.32
Linoleic acid [C18:2]	30.10	32.17	29.50	28.40	33.14
Linolneic acid [C18:3]	11.59	9.21	10.85	9.48	8.86
Behenic acid [C22:0]	2.34	2.56	2.74	2.14	1.28

[a] All values are of two determination.

The amino acid profile of the purified seed proteins and the essential amino acid score are presented in Table 6. The contents of sulphur containing amino acids, cystine, methionine, valine and leucine in Ayyanarkoil accession, tryptophan in Sivagiri, Aliyar and Seithur accessions, leucine in Anaikatti and

Seithur accessions and lysine in all the investigated accessions seem to be deficient; whereas threonine and histidine in all the five accessions, isoleucine in all accessions except Aliyar and tryptophan in Ayyanarkoil and Anaikatti accessions of present investigation are found to be higher compared to the FAO/WHO (1991) requirement pattern. Among the five accessions of itching bean, the Aliyar accession registered higher amounts of IVPD than the other four accessions (Table 7) and their protein digestibility was found to be higher than that of *Cajanus cajan* (Singh and Eggum, 1984); *Phaseolus lunatus*, *Dolichos lablab* (Alector and Aladetimi, 1989), *Cicer arietinum* (Attia *et al*., 1994) and different cultivars of *Vicia faba* (Markar *et al*., 1997).

The problem of plant protein digestibility has been attributed to the interplay of several factors, including protease inhibitors, phytates, oxalates, lectins, goitrogens and other antinutritional factors. Naturally, in societies where legumes are consumed, rather than much more expensive animal foods, there is great concern over the level of antinutrients present in the diet. For this reason, a preliminary evaluation of some of these factors in raw itching bean was made (Table 7). Total free phenolics occurred within the range of 3.40 – 4.78% and tannins ranged from 0.14 – 0.23%. These levels seem to be higher than those reported in earlier studies (3.16 – 3.18% and 0.03 – 0.06% total free phenolics and tannins respectively) in the *Mucuna utilis* (Mohan and Janardhanan, 1995). Tannins are suspected to affect adversely protein digestibility (Sathe and Salunkhe, 1984). In *Mucuna* beans Mary Josephine and Janardhanan (1992) reported that most of the tannins are located in the seed coat with only traces in the cotyledons. 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. The concentration of the non-protein amino acid L-DOPA in *Mucuna pruriens* var. *pruriens* has been found to be slightly low when compared with the values reported earlier in the same species (Mary Josephine and Janardhanan, 1992). 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 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 compounds (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 levels of phenolics, tannins and L-DOPA is significantly eliminated by dry-heat treatment (Siddhuraju *et al.*, 1996) and cooking and autoclaving (Vijayakumari *et al.*, 1996) and their reduction improves the protein digestibility.

Phytic acid has an antinutritional property because of its ability to lower the bioavilability of essential minerals, and to form a complex with proteins, thereby inhibiting the enzymatic digestion of protein (Nolan and Duffin, 1987). Phytic acid content of five accessions of *M. pruriens* var. *pruriens* is comparable with that of some of the commonly consumed legumes like *Vigna mungo* (Kataria *et al.*, 1988) and *V. radiata* (Kataria *et al.*, 1989). The level of hydrogen cyanide in *M. pruriens* var. *pruriens* (all the accessions) seems to be negligible when compared with the lethal level of HCN (36 mg/100g) (Oke, 1969). Though the trypsin inhibitor activity has been studied in a number of pulses, the results obtained in the present investigation cannot be compared because the expression of trypsin inhibitor activity, nature and concentration of the substrate, etc. are different (Vadivel and Janardhanan, 2004). However, based on investigations that have been reported and where the same experimental conditions were used, the trypsin inhibitor activity in cultivated legumes likes pigeon pea (67.1 – 71.3 TIU mg-1 protein) (Singh and Eggum, 1984) is higher than that of itching bean. Trypsin inhibitor activity has the greatest impact on the IVPD of the legumes where the former is known to be heat labile. The oligosaccharide content of the presently investigated seeds of *M. pruriens* var. *pruriens* is comparable with that of five accessions of other species of *M. pruriens* var. *utilis* (Janardhanan *et al.*, 2003). Verbascose is found to be the major oligosaccharide in all the accessions of *M. pruriens* var. *pruriens*, as has been reported earlier in *M. pruriens* var. *utilis* (Janardhanan *et al.*, 2003). 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). Phytohaemagglutinating activity of all five accessions of *M. pruriens var. pruriens* registers higher activity with respect to, A' blood group of human erythrocytes. All the accessions had low levels of phytohaemagglutinating activity with respect to erythrocytes of, O' blood group. This is in good agreement with earlier reports in the other *Mucuna* species (Vijayakumari *et al.*, 1996). However, dry-heat and autoclaving are known to inactivate completely the trypsin inhibitors and phytohaemagglutins in *Mucuna* beans (Siddhuraju *et al.*, 1996).

Table 6: Amino acid profiles of acid-hydrolysed, purified total seed proteins of five accessions of *Mucuna pruriens* var. *pruriens.*

Amino acid	Ayyanarkoil	EAAS	Sivagiri	EAAS	Aliyar	EAAS	Anaikatti	EAAS	Seithur	EAAS	FAO/WHO (1991) requirement pattern
Glutamic acid	11.14		9.48		8.78		13.10		9.56		
Aspartic acid	12.70		16.40		14.20		13.48		15.37		
Serine	4.44		4.93		3.34		4.50		3.78		
Threonine	4.08	120.00	4.84	142.35	5.28	155.29	5.10	150.00	4.50	132.35	3.4
Proline	3.10		2.50		3.14		4.54		3.74		
Alanine	4.68		5.70		5.84		4.15		3.60		
Glycine	4.35		4.17		4.40		4.53		5.12		
Valine	3.33	95.14	4.31	123.14	5.48	156.57	6.30	180.00	5.78	165.14	3.5
Cystine	1.62	}86.40	1.34	}97.60	2.20	}118.40	1.12	}86.40	1.69	}105.20	2.5
Methionine	0.54		1.10		0.76		1.04		0.94		
Isoleucine	3.34	119.29	3.56	127.14	2.54	90.71	5.30	189.29	5.14	183.57	2.8
Leucine	5.74	86.97	6.48	98.18	6.50	98.48	5.17	78.33	5.80	87.88	6.6
Tyrosine	4.32	}118.25	2.43	}114.76	5.11	}139.84	3.40	}124.44	3.78	}134.29	6.3
Phenylalanine	3.13		4.80		3.70		4.44		4.68		
Lysine	5.12	89.83	5.40	93.10	4.85	83.62	5.17	89.14	5.08	87.59	5.8
Histidine	2.23	117.37	3.28	172.63	4.31	226.84	4.12	216.84	3.40	178.95	1.9
Tryptophan	1.14	103.64	1.04	94.55	0.78	70.91	1.21	110.00	0.92	83.64	1.1
Arginine	5.38		3.50		4.34		4.02		2.52		

EAAS: Essential amino acid score.

Table 7: Data an IVPD and antinutritional factors of five accessions of *Mucuna pruriens* var. *pruriens.*

Components	Ayyanarkoil			Sivagiri			Aliyar			Anaikatti			Seithur		
in vitro protein digestibility (%)[b]	72.22±0.74[c]			71.51±0.06[d]			76.52±0.10[a]			70.12±0.09[e]			74.12±0.02[b]		
Total free phenolics[a] g $100g^{-1}$	4.78±0.11[a]			3.40±0.10[e]			3.79±0.02[d]			4.40±0.02[b]			3.85±0.10[c]		
Tannins[a] g $100g^{-1}$	0.23±0.03[a]			0.15±0.02[d]			0.19±0.01[c]			0.22±0.03[b]			0.14±0.01[e]		
L-DOPA[a] g $100g^{-1}$	5.90±0.02[d]			6.98±0.05[a]			6.34±0.04[c]			5.38±0.06[e]			6.54±0.02[b]		
Phytic acid [a] mg $100g^{-1}$	478.00±2.08[e]			568.00±0.06[c]			608.07±0.03[b]			648.10±0.01[a]			510.16±0.03[d]		
Hydrogen cyanide [a] mg $100g^{-1}$	0.38±3.21[a]			0.33±0.01[c]			0.24±0.15[e]			0.35±0.02[b]			0.28±0.21[d]		
Trypsin inhibitor activity [b] (TIU mg^{-1} protein)	45.32			47.12			40.40			48.24			42.20		
Oligosaccharides [a] g $100g^{-1}$	Raff	Stac	Verb	Raff	Stac	Verb	Raff	Stac	Verb	Raff	Stac	Verb	Raff	Stac	Verb
	1.02± 0.02[c]	1.21± 0.01[b]	4.30± 0.02[c]	0.98± 0.01[d]	1.05± 0.02[e]	3.78± 0.08[d]	1.12± 0.03[a]	1.28± 0.01[a]	4.38± 0.68[b]	1.08± 0.01[b]	1.20± 0.01[c]	3.75± 0.02[e]	0.85± 0.15[e]	1.18± 0.01[d]	4.76± 0.02[a]
Phytohaemagglutinating activity Hu mg^{-1} protein[b]	A group 140	B group 60	O group 14	A group 162	B group 74	O group 12	A group 158	B group 76	O group 10	A group 166	B group 86	O group 12	A group 148	B group 78	O group 9

Raff: Raffinose; Stac: Stachyose; Verb: Verbascose;

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

[b] All values of two independent experiments.

CONCLUSION

The observations made in the present study reveal that the nutritional profiles of *M. pruriens* var. *pruriens* seems to be similar to higher than other *Mucuna* species / accessions reported earlier and can also be explored as an alternate protein source to protein-energy-malnutrition among the economically weaker sections of people in developing countries. The presence of antinutritional factors identified in the current report should not pose a problem for humans if the beans are properly processed.

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REFERENCES

Afolabi, O.A., Osshuntogun, B.A., Adewusi, S.R., Fapojuwo, O.O., Ayorinde, F.O., Grissom, F.E., Oke, O.L. 1985. Preliminary nutritional and chemical evaluation of raw seeds from *Mucuna solanel*; an underutilized food source. Journal of Agricultural and Food Chemistry. 33: 122 -124.

Aguiji, J.C., Uguru, M.O., Johnson, P.B., Obi, C.I., Adogo, G.I. 1997. Effects of *Mucuna pruriens* seed extract on smooth and skeletal muscle preparations. Fitoterapia, 68: 366 – 370.

Alajaji, S.A., El-Adawy, T.A. 2006. Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. Journal of Food Composition and Analysis. doi: 10.1016/j.jfca.2006.03.015.

Aletor, V.A., Aladetimi, O.O. 1989. Compositional evaluation of some cowpea varieties and some under-utilized edible legumes in Nigeria. Natrung, 33: 999 – 1007.

Almedia, N.G., Calderon de la Barca, A.M., Valencia, M.E. 1991. Effect of different heat treatments on the anti-nutritional activity of *Phaseolus vulgaris* (variety ojode Carbra) lution. Journal of Agricultural and Food Chemistry, 39: 1627 – 1630.

Amin, K.M.Y., Khan, M.N., Zillur-Rehman, S., Khan, N.A. 1996. Sexual function improving effect of *Mucuna pruriens* in sexually normal rats. Fitoterapia, 67: 58 – 68.

AOAC, (Association of Official Agricultural Chemists), 2005) In: *Official methods of analysis*, 18th Eds. Washington DC.

Arinathan, V., Mohan, V.R., John de Britto, A. 2003. Chemical composition of certain tribal pulses in South India. International Journal of Food Science and Nutrition. 54: 209 – 217.

Arinathan, V., Mohan, V.R., Maruthupandian, A., Athiperumalsami, T. 2009. Chemical evaluation of raw seeds of certain tribal pulses in Tamil Nadu, India. Tropical and Subtropical Agroecosystem. 10: 287 – 294.

Arinthan, V., Mohan, V.R., John de Britto, A. Murugan, C. 2007. Wild edibles used by Palliyars of the Western Ghats, Tamil Nadu. Indian Journal of Traditional Knowledge. 6: 163 – 168.

Arora, R.K. 1991. Native food plants of the northeastern India. In: *Contributions to Ethnobotany of India* (Eds. SK Jain), Scientific Publishers, Jodhpur, India. pp. 137 – 152.

Attia, R.S., El-Tabey Shehata, A.M., Aman, M.E., Hamza, M.A. 1994. Effect of cooking and decortication on the physical proerties, the chemical composition and the nutritive value of chick pea (*Cicer arietinum* L.). Food Chemistry. 50: 125 – 131.

Basha, S.M.M., Cherry, J.P., Young, C.T. 1976. Changes in free amino acids, Carbohydrates and proteins of maturity seeds from various peas (*Arachis hypogaea*) cultivars. Cereal Chemistry. 53: 583 – 597.

Bean, G., Fernando, T., Holden, H., Patterson, G. 1984. Total plant analysis of sterols and fatty acids of the winged bean, *Psophocarpus tetragonolobus*. Journal of Food Science. 49: 964 – 965.

Brain, K.R. 1976. Accumulation of L-DOPA in cultures from *Mucuna pruriens.* Plant Science Letters. 7: 157-161.

Bray, H.G., Thorne, W.V. 1954. Analysis of phenolic compounds methods. Biochemical. Analyst. 1: 27-52.

Burns RR. 1971. Methods of estimation of tannin in grain Sorghum. Agronomy Journal, 63: 511-512.

Conway, G., Toenniessen, G. 1999. Feeding the world in the twenty – first century. Nature. 402 (Suppl.), C55-C68.

Dhuley, J.N., Naik, S.R. 1997. Protective effect of Rhinax, a herbal formulation against CCl4-induced liver injury and survival in rats. Journal of Ethnopharmacology**,** 56: 159 – 164.

Dickman, S.R., Bray, R.H. 1940. Colorimetric determination of phosphate. Industrial and Engineering Chemistry Analytical Education. 12: 665-668.

Duke, J.A. 1981. Handbook of Legumes of World Economic Importance, Duke, JA, Ed. Plenum Press: New York, pp: 170 -173.

FAO/WHO. 1991. Protein quality evaluation, (p 66). Rome, Italy: Food and Agricultural Organization of the United Nations.

Fernandez, M.L., Berry, J.W. 1988. Nutritional evaluation of chickpea and germinated chickpea flours. Plant Foods for Human Nutrition. 38: 127-134.

Fernando, T., Bean, G. 1986. The reduction of the antinutritional behenic acid content among the cultivars of winged bean, *Psophocarpus tetragonolobus* (L.) DC. seeds. Plant Foods for Human Nutrition. 36: 93 – 96.

Folch, J., Lees, M., Solane-Stanly, G.M. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226: 497 – 506.

Gadgil, M. 1996. Documenting diversity: An experiment, Current Science. 70: 36 – 44.

Gill, L.S., Nyawuame, H.G.K. 1994. Leguminosae in ethnomedicinal practices of Nigeria. Ethnobotany. 6: 51 – 64.

Haq, N. 1983. New food legume crop for the tropics. In: *Better crops for food* Eds. N. Nugent and M.O. Conor . Pitman Books, London (Cuba Foundation Symposium 97). pp. 144-160.

Hsu, H.W., Vavak, D.L., Satterlee, L.D., Miller, G.A. 1977. A multi-enzyme technique for estimating protein. Digestibility. Journal of Food Science. 42: 1269 -1271.

Humphries, E.C. 1956. Mineral composition and ash analysis, in *Modern Methods of Plant Analysis* (Vol. 1), Peach, K Tracey, M.V., (Eds) Springer-Verlag, Berlin. pp. 468-502.

Hussain, G., Manyam, B.V. 1997. *Mucuna pruriens* proves more effective than L-DOPA in Parkinson's disease animal model. Phytotherapy Research. 11: 419 – 423.

Issac, R.A., Johnson, W.C. 1975. Collaborative study of wet and dry techniques for the elemental analysis of plant tissue by Atomic Absorption Spectrophotometer. J. Assoc. Off. Analytical Chemistry. 58: 436-440.

Jackson, M.L. 1967. Cyanide in Plant tissue. In: *Soil Chemical Analysis*. Asia Publishing House New Delhi India. pp. 337.

Jain, S.K. 1981 (Ed.). *Glimpses of Indian Ethnobotany*, Oxford & IBH publishing Co., New Delhi, India.

Janardhanan, K., Gurumoorthi, P., Pugalenthi, M. 2003. Nutritional potential of five accessions of a south Indian tribal pulse, *Mucuna pruriens* var. *utilis* I. The effect of processing methods on the content of L-DOPA, phytic acid and oligosaccharides. Tropical and Subtropical Agroecosystem. 1: 141 – 152.

Jebadhas, A.W. 1980. Ethnobotanical studies on some hill tribes of South India. Ph.D Thesis, Madras University, Madras, Tamil Nadu, India.

Josephine, M. R., Janardhanan, K. 1992. Studies on chemical composition and anti-nutritioal factors in three germplasm seed materials of the tribal pulses, *Mucuna pruriens* (L.) DC. Food Chemistry. 43: 13 – 18.

Kakade, M.L., Rackis, J.J., McGhce, J.E., Puski, G. 1974. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. Cereal Chemistry. 51: 376 -382.

Kataria, A., Chauhan, B.M., Gandhi, S. 1988. Effect of domestic processing and cooking on the antinutrients of black gram. Food Chemistry. 30: 149 – 156.

Kataria, A., Chauhan, B.M., Punia, D. 1989. Antinutrients and protein digestibility (*in*

vitro) of mungo bean as affected by domestic processing and cooking. Food Chemistry. 32: 9 – 17.

Kritchevsky, D., Tepper, S.A., Verrelinovitch, D., Wirrler, R.W. 1973. Cholesterol vehicle in experimental atherosclerosis, Randomised peanut oils. Athrosclerosis. 17: 225 – 237.

Kumar, K.V.A., Srinivasan, K.K., Shanbhag, T., Rao, S.G. 1994. Aphrodisiac activity of the seeds of *Mucuna pruriens*. Indian Drugs. 31: 321 – 327.

Li, B.W., Cardozo, M.S. 1994. Determination of total dietary fiber in foods and products with little or no starch, nonenzymatic-gravimetric method: collaborative study. Journal of Association of Official Analytical Chemists International. 77: 687-689.

Liddell, H.F., Saville, B. 1959. Colorimetric determination of cysteine. Analyst. 84: 133 - 137.

Liener, I.E. 1994. Antinutritional factors related to proteins and amino acids. In Foodborne disease handbook; Hui, Y.H, Gorham JR, Murrell, KD, Cliver DO, Eds, Marcel Dekkar: New York. 3: 261 -309.

Lowry, O.H., Rorebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with folin phenol reagent. Journal of Biological Chemistry. 193: 265 – 275.

Makker, H.D.S., Becker, K., Abel, H., Pawelzik, E. 1997. Nutrient contents, rumen protein digestibility and antinutritional fctors in some colour – and white flowering cultivars of *Vicia faba* beans. Journal of Science Food and Agriculture, 75: 511 – 520.

Mal, B. 1992. Biodiversity utilization and conservation in underutilized plants: Indian perspective. Indian Journal of Plant Genetic Resource. 5: 1 - 22.

Metcalfe, L.D., Schemitz, A.A., Pelka, J.R. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical Chemistry. 38: 514 – 515.

Mohan, V.R., Janardhanan, K. 1993. Chemical and nutritional evaluation of raw seeds of the tribal pulses *Parkia roxburghii*. G. Don and *Entada phaseoloides* (L.) Merr. International Journal of Food Science and Nutrition. 44: 47-53.

Mohan, V.R., Janardhanan, K. 1995. Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna*, Food Chemistry. 52: 275 – 280.

Muller, H.G., Tobin, G. 1980. Nutrition and food processing: Croom Helm Ltd., London.

Nechama, S.I., Edward, R.K. 1967. Does 3,4-dihydroxyphenylalanine play a part in favism? Nature. 215: 285 – 286.

Nolan, K.B., Duffin, P.A. 1987. Effect of phytate on mineral bioavailability. In vitro studies of MG2+, Ca2+, Fe2+, Cu2+ and Zn2+ solubilities in the presence of phytate, Journal of Science Food and Agriculture. 40: 79 -83.

Oke, O.L. 1969. The role of hydrocyanic acid in nutrition. World Review Nutrition & Diabetics. 11: 118 - 174.

Omogbai, F.E. 1990. Lipid composition of tropical seeds used in the Nigerian diet. Journal of Science Food and Agriculture. 50: 253 – 255.

Pieris, N., Jansz, E.R., Dharmadasa, H.M. 1980. Studies on *Mucuna* specis of Sri Lanka, I. The L-DOPA content of seeds. Journal of Natural Science. Found. Sri Lanka, 8: 35 – 40.

Pugalenthi, M., Vadivel, V., Siddhuraju, P. 2005. Alternative food/feed perspectives of an underutilized legume *Mucuna pruriens* var. *utilis* – A review. Plant Foods for Human Nutrition. 60: 201 – 218.

Radhakrishnan, K., Pandurangan, A.C., Pushpangadan, D. 1996. Ethnobotany of the wild edible plants of Kerala, India. In: *Ethnobotany in Human Welfare* (Eds. S.K, Jain), Deep Publications, New Delhi, India, pp. 48 – 51.

Rajendran, V., Joseph, T., David, J. 1996. Reappraisal of dopamineric aspects *Mucuna pruriens* and comparative profile with L-DOPA on cardiovascular and central nervous system in animals. Indian drugs. 33: 465 – 472.

Rajyalakshmi, P., Geervani, P. 1994. Nutritive value of the foods cultivated and consumed by the tribals of South India. Plant Food Human Nutrition. 46: 53-61.

Rao, B.S.N., Deosthale, Y.G., Pant, K.C. 1989. Nutritive value of Indian foods. pp. 47 – 91. Hyderabad, India: National Institute of

Nutrition, Indian Council of Medical Research.

Rao, M.V.R., Tara, M.R., Krishnan, C.K. 1974. Colorimetric estimation of tryptophan content of pulses. Journal of Food Science and Technology. (Mysore), 11: 213 – 216.

Reynolds, J.E.F. 1989. Martindale; *The Extra pharmacopoeia*. The Pharmaceutical press: London. pp 1015 – 1020.

Robinson, D.R. 1987. *Food biochemistry and nutritional value*. Longman Scientific and Technol, Burnmell, Haslow, England, pp 327-328.

Sadasivam, S., Manickam, A. 1996. Biochemical methods, New Age International (P) Limited Publishers New Delhi India. pp 1-250.

Sahu, T.R. 1996. Life support promising food plants among aboriginal of Baster (M.P), India. In: *Ethnobiology in Human Welfare* (Eds. SK Jain), Deep Publications, New Delhi, India, pp. 26 – 30.

Salunkhe, D.K., Sathe, S.K., Reddy, N.R. 1982. Legume lipids. In: S.K. Arora. (Eds.) Chemistry and Biochemistry of Legumes Oxford and IBH Publishing Co., New Delhi, India. pp.51-107.

Sathe, S.K., Salunkhe, D.K. 1984. Technology of removal of unwanted components of dry bean. CRC Critical Review Food Science and Nutrition, 21: 263 – 286.

Shaw, B.P., Bera, C.H. 1993. A preliminary clinical study to evaluate the effect of Vigorex-SF in sexual debility patients. Indian Journal of International Medicine, 3: 165 – 169.

Siddhuraju, P., Becker, K., Makker, P.S. 2000. Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilised tropical legume, *Mucuna pruriens* var. *utilis*. Journal of Agricultural and Food Chemistry. 48: 6048 – 6050.

Siddhuraju, P., Vijayakumari, K., Janardhanan, K. 1993. Genetic resources of tribal pulses. *FAO/IPGRI* Plant Genetic Resource Newsletter. 96: 47 – 49.

Siddhuraju, P., Vijayakumari, K., Janardhanan, K. 1996. Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC), Journal of Agricultural and Food Chemistry. 44: 2636-2641.

Singh, V., Eggum, B.O. 1984. Factors affecting the protein quality of pigeon pea (*Cajanus cajan* L.), Plant Foods for Human Nutrition. 34: 273 – 283.

Somiari, R.T., Balogh, E. 1993. Effect of soaking, cooking and alpha- galactoside treatment on the oligosaccharide content of cowpea flours. Journal of Science Food and Agriculture. 61: 339 – 343.

Spies, J.R., Chamber, D.C. 1949. Chemical determination of tryptophan in proleins. Analytical Chemistry. 21: 1249 – 1266.

Srivastava, R.R., Ali, M. 2004. Nutritional quality of common pulses: Indian Institute of Pulses Research, Kanpur, pp.14 -22.

Takasaki, S., Kawakishi, S. 1997. Formation of protein-bound 3,4-dihydroxyphenylalanine and 5-S-Cysteinyl-3,4-dihydroxyphenyalanine as new cross-limkers in gluten. Journal of Agricultural and Food Chemistry. 45: 3472 – 3475.

Tan, N.H., Rahim, Z.H.A., Khor, H.T., Wong, K.C. 1983. Winged bean (*Psophocarpus tetragonolobus*). Tannin level, phytate content and Haemagglutinating activity. Journal of Agricultural and Food Chemistry. 31: 916 – 917.

Tanaka, M., Thanankul, D., Lee, T.C., Chichester, L.O. 1975. A simplified method for the quantitative determination of sucrose, raffinose and stachyose in legume seeds. Journal of Food Science. 40: 1087 – 1088.

Vadivel V, Janardhanan K. 2002. Agrobotanical traits and chemical composition of *Cassia obtusifolia* L. a lesser-known legume of the Western Ghatsregion of South India. Plant Foods for Human Nutrition, 57: 151 – 1643.

Vadivel, V., Janardhanan, K. 2000a. Nutritional and anti-nutritional composition of velvet bean: an underutilized food legume in South India. International Journal of Food Science and Nutrition. 51: 279-287.

Vadivel, V., Janardhanan, K. 2000b. Preliminary agrobotanical traits and chemical evaluation of *Mucuna pruriens* (Itching bean): a less-known food and medicinal legume. Journal of

Medicinal Aromatic Plant Science. 22: 191 - 199.

Vadivel, V., Janardhanan, K. 2004. The nutritional and antinutritional attributes of swored bean (Canavalia gladiata (Jacq.)DC): an under-utilised tribal pulse from South India. International Journal of Food Science and Technology. 39: 917 – 926.

Vasudeva Rao, M.K., Shanpru, R. 1991. Some plants in the life of the Garos of Meghalaya. In: *Contributions to Ethnobotany of India* (Eds. SK Jain), Scientific Publishers, Jodhpur, India, pp. 183 – 190.

Vijayakumari, K., Siddhuraju, P., Janardhanan, K. 1996. Effects of different post-harvest treatments on anti-nutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) DC. International Journal of Food Science and Nutrition. 47: 263-272.

Wheeler, E.L., Ferrel, R.E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chemistry. 48: 312 – 320.

Wilmot-Dear, C.M. 1987. A revision of *Mucuna* (Leguminosae-Phaseoleae) in the Indian sub-continent and Burma.

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