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Phytochemical composition and antioxidant potential of itching beans (*Mucuna pruriens* var. *pruriens* (L.) DC): A less-known food and medicinal legume

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Abstract
Five accessions of itching beans (*Mucuna pruriens* var. *pruriens* (L.) DC) were collected from five different locations in Western Ghats and Eastern Ghats in South India. They were analysed for their proximate and mineral composition, amino acid profiles of total seed protein, *in vitro* protein digestibility (IVPD) and certain antioxidants. Crude protein ranged from 25 to 34.5%, crude lipid 5.7-8.6%, crude fibre 5.5-7.8 %, ash 3-4.8% and carbohydrates 48.9-58.9%. Mineral profiles, viz., sodium, potassium, calcium, magnesium, phosphorus, iron, zinc and manganese ranged from 31-107.7, 723.8-1599, 304.5-780, 68.5-639.6, 119.6-640.2, 10.7-20, 1.2-3.9 and 1-4.3 mg $100g^{-1}$ seed flour, respectively. The essential amino acid profile of total seed proteins compared favourably with FAO/WHO requirements, except that there were deficiencies of threonine, lysine and sulphur containing amino acids. The IVPD of the accessions ranged from 65.64 to 74.66%. Antioxidant substances like phenols, tannins and L-DOPA (3, 4-dihydroxyphenylalanine) were also investigated.

Keywords: itching beans, *Mucuna pruriens* var. *pruriens*, chemical composition, amino acid, antioxidants

1. Introduction
Pulses are good source of nutritional components, they are low in fat and rich in complex carbohydrates, vegetable protein and minerals [1]. Researchers have found a positive correlation between the anticarcinogenic effects and various components present in pulses including dietary fiber [2] and folate [3]. The major polyphenolic compounds of pulses are tannins, flavonoids and phenolic acids. These compounds are the products of secondary metabolism of plants; they have a good antioxidant activity [4].
Itching bean [*Mucuna pruriens* var. *pruriens* (L.) DC], with the vernacular name, Naikuruna, is found wild in the forests of South India. It is a hardy, herbaceous, vigorous and twining annual, which forms a thick soil covering and smothering the growth of weeds. It can be grown on almost every type of soil in tropical and subtropical plains [5]. Mature seeds, seeds from unripe pods and young pods of itching beans are soaked and boiled / roasted and eaten as such or mixed with salt by the tribes of North-East India: Khasi, Naga, Kuki, Jaintia, Chakma and Mizo [6]; tribes of North-Western part of Madhya Pradesh: Abujh-Maria, Maria, Muria, Gond and Halba [7]; tribes of South India: Mundari, Dravidian, 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 [9]. Janardhanan and Lakshmanan [10] have attributed the presence of a non-protein amino acid L-DOPA (3, 4-dihydroxyphenylalanine), which is pharmacological active, and is known to cure Parkinson's disease.
The seed powder of *M. pruriens* has a faster hypothermic and anti-parkinsonian activity than synthetic L-DOPA [11]. The seed powder increases the sexual activity of male albino rats considerably and L-DOPA also is reported to arouse sexual desire in the patients suffering from Parkinson's disease [12]. Vigorex-SF capsule, an Ayurvedic herbomineral formulation, comprising *M. pruriens* is found to have adaptogenic effect to improve one's libido, disturbed due to psychological fear and emotional imbalance and other allied ailments [13]. Alcohol extracts of leaf and fruit trichomes of *M. pruriens* are found to increase the pain threshold and decrease body temperature. The extracts also showed anti-inflammatory activity, as they are able to inhibit carrageenin-induced edema [14]. Seeds of this plant species are widely used for treating male sexual dysfunction in Unani medicine [15]. The blocking effect of King cobra venom at the neuromuscular junction is removed by the aqueous extract of *M. pruriens* seeds [16]. Rhinax, an herbal formulation comprising *M. pruriens* possesses anti-hepatotoxic activity [17]. The tribe, Garos of Meghalaya, India consume the seeds for increasing potency, and the hairs of the pod are used as vermifuge [18]. In Nigeria, powdered hairs on pods are administered with honey for expelling intestinal worms [19]. The roots are used as tonic, stimulant, diuretic, purgative and emmenagogue. An ointment prepared from the root is applied for elephantiasis. The leaves of the plant are applied to ulcers [20]. Besides, the potential use of itching bean flour in high protein biscuits is highlighted [21].
Despite the potential of this species as a source of less-known food and medicine, to our knowledge, meagre information is available on the germplasm collection from South India and their evaluation for chemical composition. In this context, five accessions of itching beans collected from five locations of South India have been investigated phytochemically and the results are discussed with earlier findings on cultivated food legumes.

2. Materials and methods

2.1. Sources of seed

Five accessions (each 5kg) of itching beans were gathered as mature pods from natural stands in five locations. Locality, district and state of collection are given in Table 1. The accessions were botanically identified by using the botanical key of Wilmot-Dear [22]. The mature pods were collected from tropical rain forests of Western Ghats (three accessions) and deciduous forests in Eastern Ghats (two accessions). After thoroughly drying in the Sun, the pods were thrashed to remove seeds. After thorough clearing and removal of broken seeds and foreign materials, seeds were stored in airtight plastic containers at room temperature (25°C±2°C). The air-dried seeds (nearly 50g from each accession) were powdered in a Wiley Mill to pass a 60-mesh screen and stored in screw-capped bottles at room temperature for further analysis.

Table 1: Information on location of collection of five accessions of itching bean seeds

Locality	District	State
Aliyar (Western Ghats)	Coimbatore	Tamil Nadu
Arunooli (Western Ghats)	Trichur	Kerala
Elagiri hills (Eastern Ghats)	Vellore	Tamil Nadu
Karwar (Western Ghats)	Uttarkannada	Karnataka
Salem (Eastern Ghats)	Salem	Tamil Nadu

2.2. Proximate composition

The moisture content of the seed was estimated by taking 50 transversely cut seeds at a time and the weight was taken before and after incubation in a hot-air-oven at 80°C for 24h, followed by cooling in a desiccator. Nitrogen content in the powdered seed samples was estimated by the micro-Kjeldahl method [23] and crude protein was calculated (*N* x 6.25). The recommended methods of Association of Official Analytical Chemists [24] were used for the determination of ash, crude lipid and crude fibre. Ash content was determined by incineration of 2g of sample in a muffle furnace kept at 550°C for 6h. Crude lipid was determined by exhaustively extracting 2g of sample with petroleum ether, using a Soxhlet apparatus. Crude fibre was determined by acid and alkaline digestion methods. The carbohydrate content was calculated by subtracting the total of the percentages of crude protein, crude lipid, crude fibre and ash on moisture-free basis from 100. The energy value of the seed was estimated (in kJ) by multiplying the percentages of crude protein, crude lipid and carbohydrates by the factors 16.7, 37.7 and 16.7, respectively. All these constituents were analysed in triplicate. All the results were expressed on a dry weight basis.

2.3. Mineral 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 de-ionised distilled water, filtered with Whatman no. 42 filter paper and filtrates made up to 100ml in a glass volumetric flask with de-ionised distilled water. All the minerals except phosphorus were analysed from triple acid digested samples by using an atomic absorption spectrophotometer [25]. Phosphorus content in the triple acid digested extract was colorimetrically analysed [26] at 650nm using a spectrophotometer.

2.4. Amino acid analysis

The total seed protein was extracted by a modified method of Basha *et al.* [27] The ethanol treatment was omitted to retain the prolamin fraction. The extracted proteins were purified by precipitation with cold 20% trichloroactic acid (TCA). A protein sample of 30mg was hydrolysed by 6*N* HCl (5ml) in an evacuated sealed tube, which was kept in air oven maintained at 110°C for 24h. The sealed tube was broken and the acid removed completely by repeated flash evaporation after the addition of de-ionised distilled water. 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). Amino acids were analysed by a reversed-phase HPLC (Model 23250, ISCO, Lincoln, NE, USA) fitted with a spherisorp C_{18} column (4.6 x 250mm) and ISCO-dual pump. The flow rate was 1.5ml min^{-1} and a fluorescence detector (excitation 305-395nm; emission 430-470nm) was used. The cystine content of protein samples was obtained separately by the Liddell and Saville (1959) [28] method. For the determination of tryptophan content of proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together with 1ml 5*M* NaOH. The ampoules were flame sealed and incubated at 110ºC for 18h. The tryptophan contents of the hydrolysates were determined colorimetrically using the method of Rama Rao *et al*. [29]. The contents of the different amino acids were expressed as g $100g^{-1}$ proteins.

2.5. Determination of *in vitro* protein digestibility (IVPD)

Protein digestibility was assayed by the *in vitro* method described by Hsu *et al*. [30]. 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.

2.6. Quantification of antioxidants

The antioxidant compounds, phenols [31], tannins [32] and the non-protein amino acid L-DOPA (3, 4-dihydroxyphenylalanine) [33] were quantified.

2.7. Statistical analysis

Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used for analysis [MSTAT–'C' software (version 1.4.1 Michigan State University, MI, USA)] of any significant difference in chemical compositions among the five accessions collected from five

locations. Significance was accepted at $p \leq 0.05$.

3. Results and discussion

The proximate compositions of five accessions of itching bean are shown in table 2. The crude protein content of the itching bean accessions ranged from 25.0 to 34.5%. This range is higher than those reported for and black gram, horse gram and pigeon peas [9]. These legumes are used extensively in typical Indian diets and are expected to play a significant role in improving protein nutrition in India and Asia. The significant ($p \leq 0.01$) difference in protein content was probably due to different growing conditions for the plants from which the seeds were collected [34]. The fat content range is higher than that of black gram and green gram [35]. The range in ash content of this wild legume (Table 2) would be important to the extent that it contains the nutritionally important mineral elements shown in Table 3. It appears that itching bean has a high range of carbohydrate probably because of their low lipid content. The range in calorific values exceeds the energetic values of cowpea, green gram, horse gram, moth bean and peas [36], which are in the range of 1318-1394 kJ $100g^{-1}$ DM.

Table 2: Proximate composition of five accessions of itching bean (g $100g^{-1}$ seed flour) †

Component	Location					CD (5%)
	Aliyar	Arunooli	Elagiri hills	Karwar	Salem	
Moisture	3.2 ± 0.2^{d}	8.4 ± 0.2^{a}	6.2 ± 0.2^{c}	7.4 ± 0.2^{b}	7.9 ± 0.1^{ab}	0.6042^{**}
Crude protein	25.0 ± 0.1^{d}	32.4 ± 0.5^{b}	28.0 ± 0.1^{c}	34.5 ± 1.3^{a}	33.0 ± 0.1^{ab}	1.9500^{**}
Crude lipid	5.9 ± 0.1^{d}	5.7 ± 0.2^{d}	7.3 ± 0.2^{b}	6.4 ± 0.1^{c}	8.6 ± 0.1^{a}	0.3854^{**}
Crude fibre	6.5 ± 0.1^{b}	7.8 ± 0.3^{a}	6.0 ± 0.1^{bc}	5.5 ± 0.2^{c}	5.6 ± 0.2^{c}	0.6341^{**}
Ash	3.7 ± 0.1^{b}	3.5 ± 0.2^{b}	4.8 ± 0.2^{a}	4.6 ± 0.2^{a}	3.0 ± 0.2^{c}	0.5388^{**}
CHO	58.9 ± 0.1^{a}	50.6 ± 0.6^{c}	54.0 ± 0.1^{b}	48.9 ± 1.0^{c}	50.0 ± 0.6^{c}	1.8720^{**}
Energy (kJ $100g^{-1}$ DM)	1623.3 ± 0.1^{b}	1601.7 ± 11.4^{c}	1643.4 ± 7.0^{b}	1635.3 ± 2.7^{b}	1706.7 ± 5.0^{a}	20.4304^{**}

Mean values in the row sharing common letters are not statistically significant according to Duncan's Multiple Range Test (DMRT). ** Significant at 1% level

† Mean of three replications expressed on dry weight basis (± S E); CHO- Carbohydrate

The mineral elements analysed and presented in Table 3, are important nutritionally. Potassium, as in most legumes, is the predominant macro-mineral and sodium levels are low. The low amounts of sodium in the legume seeds is good for health because of the relationship that low sodium diet has to hypertension in humans [37]. Among the micro-minerals, iron concentration ranged between 10.7 and 20mg 100g-1. In general, all the five accessions are found to contain higher levels of sodium compared to chick peas, kidney beans, peas and cowpeas [38]; higher potassium and magnesium contents compared to cowpeas [39] and higher calcium and iron contents compared to chickpea, pigeonpea, black gram and green gram [40]. The variability in the content of minerals for the same species may be related to genetic origin, geographical source, level of soil fertility and the efficiency of uptake from the soil [34].

Table 3: Mineral composition of five accessions of itching beans (mg 100g-1 seed flour) †

Component	Location					CD (5%)
	Aliyar	Arunooli	Elagiri hills	Karwar	Salem	
Sodium	104.5 ± 3.4^{a}	47.8 ± 6.8^{b}	42.6 ± 1.8^{bc}	107.7 ± 1.4^{a}	31.0 ± 3.2^{c}	12.0848^{**}
Potassium	1588.0 ± 2.4^{a}	835.1 ± 5.7^{c}	1599.0 ± 2.4^{a}	723.8 ± 3.4^{d}	1326.0 ± 4.3^{b}	12.2045^{**}
Calcium	780.0 ± 3.8^{a}	304.5 ± 7.4^{d}	568.5 ± 1.1^{c}	316.0 ± 4.7^{d}	695.2 ± 8.8^{b}	18.3780^{**}
Magnesium	68.5 ± 4.7^{e}	209.0 ± 4.4^{d}	639.6 ± 3.5^{a}	241.3 ± 4.8^{c}	417.1 ± 6.5^{b}	15.3502^{**}
Phosphorus	$633.\ 8 \pm 3.3^{a}$	119.6 ± 3.8^{d}	408.6 ± 7.8^{c}	423.7 ± 3.4^{b}	640.2 ± 2.0^{a}	14.1959^{**}
Iron	17.3 ± 1.4^{ab}	16.4 ± 0.4^{b}	10.7 ± 0.8^{c}	20.0 ± 0.9^{a}	10.8 ± 0.6^{c}	2.7271^{**}
Zinc	1.8 ± 0.5^{b}	2.3 ± 0.5^{ab}	3.9 ± 0.3^{a}	2.6 ± 0.8^{ab}	1.2 ± 0.5^{b}	1.6437^{*}
Manganese	1.0 ± 0.6^{b}	1.8 ± 0.4^{b}	4.3 ± 0.3^{a}	1.0 ± 0.5^{b}	1.1 ± 0.2^{b}	1.3130^{**}

Mean values in the row sharing common letters are not statistically significant according to Duncan's Multiple Range Test (DMRT). * Significant at 5% level, ** Significant at 1% level

† Mean of three replications expressed on dry weight basis (± S E)

The essential amino acid profile of total seed proteins compared favourably with FAO/WHO [41] requirements, except that there were deficiencies of threonine, lysine and sulphur containing amino acids (Table 4).

Table 4: Amino acid profiles of five accessions of itching bean (g $100g^{-1}$ protein)

Amino acid	Aliyar	Arunooli	Elagiri hills	Karwar	Salem	FAO/WHO [41] requirement pattern
Aspartic acid	9.54	10.53	8.96	10.24	13.21	
Glutamic acid	10.18	9.84	10.51	12.47	15.10	
Alanine	2.00	2.55	2.91	3.83	4.72	
Valine	9.74	8.57	5.32	6.75	7.86	3.5
Glycine	8.83	10.73	6.53	7.54	5.12	
Arginine	3.91	7.14	5.76	4.87	6.12	
Serine	4.08	5.75	4.58	3.83	4.14	
Cystine	0.85	0.65	0.98	0.84	0.93	2.5
Methionine	0.91	1.27	0.51	0.83	0.74	

Threonine	2.40	2.58	2.02	2.78	2.21	3.4
Phenylalanine	3.97	3.38	3.89	4.83	4.83	6.3
Tyrosine	6.01	5.27	5.03	5.54	5.60	
Isoleucine	3.66	3.96	3.08	3.14	3.98	2.8
Leucine	8.86	7.28	7.40	7.57	8.40	6.6
Histidine	2.57	4.27	2.30	2.85	2.80	1.9
Lysine	3.51	5.06	4.51	4.85	3.30	5.8
Tryptophan	1.34	1.72	1.22	1.52	1.32	1.1
Proline	N.D	N.D	N.D	N.D	N.D	

N.D- Not Detected

The *in vitro* protein digestibility (IVPD) range of itching bean (Table 5) was higher than that of black gram [42] and green gram [43]. Data on antioxidants such as phenols, tannins and L-DOPA were shown in Table 5. Among the five accessions, the Salem accession had the highest percentage of phenolics and tannins. In the present study, L-DOPA range (Table 5) seems to be higher as compared to earlier reports in *M. utilis* [44]. The high range of L-DOPA is encouraging from the point of view of pharmacological industries. Cultivar differences and accession variations are known to exist in the L-DOPA content of *Mucuna* beans [44].

Table 5: *In vitro* protein digestibility (IVPD) and antioxidant contents of itching beans

Component	Location					CD (5%)
	Aliyar	Arunooli	Elagiri hills	Karwar	Salem	
IVPD (%)‡	74.66	72.41	65.64	70.78	70.16	--
Phenols (%)†	5.29 ± 0.13^{b}	5.96 ± 0.11^{a}	5.34 ± 0.22^{b}	3.89 ± 0.06^{c}	6.39 ± 0.29^{a}	0.5702^{**}
Tannins (%)†	0.14 ± 0.01^{b}	0.05 ± 0.01^{c}	0.06 ± 0.01^{c}	0.08 ± 0.01^{c}	0.47 ± 0.03^{a}	0.0521^{**}
L-DOPA (%)†	7.62 ± 0.13^{a}	8.37 ± 0.39^{a}	7.78 ± 0.68^{a}	7.54 ± 0.52^{a}	7.82 ± 0.14^{a}	n.s

Mean values in the row sharing common letters are not statistically significant according to Duncan's Multiple Range Test (DMRT).

** Significant at 1% level and n.s. means not significant

† Mean of three replications expressed on dry weight basis (± SE)

‡ Mean of two independent determinations.

4. Conclusion

To conclude, it is suggested that the less-known food and medicinal legume, itching beans, might be useful as a source of crude protein, crude fat, carbohydrate, certain essential amino acids and some minerals and antioxidants such as phenolis, tannins and L-DOPA. *In vitro* protein digestibility of itching bean is also found to be higher than that of certain common legumes.

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