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Evaluation of the proximate and phytochemical compositions of an underexploited legume *Mucuna pruriens* var. *utilis* (Wall ex Wight) L.H.Bailey

*Kalidass, C. and Mahapatra, A. K.

Taxonomy and Conservation, Regional Plant Resource Centre, Nayapalli, Bhubaneswar – 751015, Odisha, India

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Abstract

The present study aimed to compare the nutritional and biochemical composition of Indian legume seed (white and black seeds of Velvet beans) flour used by tribals of South India. The crude protein content was higher in white seed compared to black seed; but the nutritive values of black seeds were better compared to the white seeds. The mineral constituents of both seed flour was found to be high in K, Ca, Mg, P and Fe. The proximate and mineral elements suggest that both seed varieties to be a cheap source of protein and macro minerals, therefore an useful food supplement for tribals. The concentration of unsaturated fatty acids was relatively higher in the black velvet beans, but both seed flours contained palmitic, omega-3 and omega-6 fatty acids. Total free phenolics are abundant in white seed sample (3.13 g/100 g), whereas there are less tannins in black velvet beans. The black seed flour contains L-dopa, phytic acid, hydrogen cyanide, showed higher trypsin inhibitor activity. These wild legumes constitute not only a rich source of protein, but also is high in fiber content than other commonly consumed pulses, and therefore can be considered in breeding for better nutritional qualities.

Introduction

From time immemorial, Legumes continue to be the world most important sources of staple food (John, 2005). Velvet beans [*Mucuna pruriens* var. *utilis* (Wall. ex Wight) L.H.Bailey] are an important constituent in the Ayurvedic system of medicine, and forward to be having aphrodisiac effect on against, parkinsonism, lymphoedema and it is a good source of L-DOPA (Vaidya *et al.*, 1978). With reference to the nutritional value, this wild legume can supply significant amount of energy, vitamins and minerals in addition to protein (24-31%). More importantly other parts of this plant are also used for medicinal purposes, e.g. trichomes of pods are used for de-worming, decoction of root to contain delirium, root powder as a diuretic and anti-inflammatory agent. Similarly, the paste of fresh root is used in the treatment of lymphoedema.

With ever-increasing population pressure and fast depletion of natural resources, it has become extremely important to bring in diversity to agriculture, as we are becoming dependent on limited cultivated crops (Janardhanan *et al.*, 2003). The increasing interest in search for alternative/ additional food and livestock feed is of paramount importance mainly for two reasons, firstly for low production of oil seeds food grains and secondly on account of competition between man and the livestock industry for food and feed materials (Siddhuraju and Beckar, 2003a, b). The continuing food scarcity, malnutrition and poverty combined with population growth in developing countries have promoted scientists to seek more esoteric plant species. In spite of an urgent need to meet the nutritional requirements of the ever increasing populations, the availability of cheap protein resources has remained relatively unexplored (Thangadurai, 2005). With the increasing search for new food sources, the seeds of wild plants are receiving more attention because they are well adapted to adverse environmental conditions, resistant to disease, pests, and possess desired nutritional qualities (Maikhuri *et al*., 1991). With this backdrop in the present study we characterized the biochemical profile of the Velvet bean from Southern Western Ghats, India.

Materials and Methods

Collection of seeds samples

White and black mature seeds of velvet bean [*Mucuna pruriens* var. *utilis* (Wall. ex Wight) L.H.Bailey] were collected/ harvested from Petchiparai Reserve Forest, Southern Western Ghats, India. With the help of keys by Wilmot-Dear, 1987, the taxon was botanically identified. After thoroughly

*Corresponding author.
Email: *kalidassindia@gmail.com*

drying in the sun, the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds and foreign materials 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 to 60-mesh size and stored in screw capped bottles at room temperature for further analysis. The nitrogen content was estimated by the micro-Kjeldahl method (Humphries, 1956) and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus (AOAC, 2005). The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr (AOAC, 2005). Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method proposed by Li and Cardozo (1994). The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). The energy value of the seed (kJ) was estimated by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, respectively (Siddhuraju *et al.*, 1996).

Minerals analysis

500 mg of the ground legume seed was digested with a mixture of 10 ml concentrated nitric acid, 4ml of 60% perchloric acid and 1 ml of concentrated sulphuric acid. After cooling, the digest was diluted with 50 ml of deionised distilled water, filtered with Whatman No. 42 filter paper and the filtrates were made up to 100 ml in a glass volumetric flask with deionised distilled water. All the minerals except phosphorus were analysed from a triple acid-digested sample by an Atomic Absorption Spectrophotometer – ECIL (Electronic Corporation of India Ltd., India) (Issac and Johnson, 1975). The phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman and Bray, 1940).

Lipid extraction and fatty acid analysis

The total lipid was extracted from the seeds according to the method of Folch *et al.* (1957) using chloroform and methanol mixture in ratio of 2: 1 (v/v). Methyl esters were prepared from the total lipids by the method of Metcalfe *et al.* (1966). Fatty acid analysis was performed by gas chromatography (ASHMACO, Japan; Model No: ABD20A) using an instrument equipped with a flame ionization detector and a glass column (2 m x 3 mm) packed with 1% diethylene glycol succinate on chromosorb W. The temperature conditions for GC were injector 200°C and detector 210°C. The temperature of the oven was programmed from 180°C and the carrier gas was nitrogen at a flow rate of 30 ml/min. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters; the relative weight percentage of each fatty acid was determined from integrated peak areas.

Amino acid analysis

The total seed protein was extracted by a modified method of Basha *et al.* (1976). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). A protein sample of 30 mg was hydrolysed by 6N HCL (5 ml) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 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 (0.45 μM) and derivitized with O-phthaldialdehyde by using an automated pre-column (OPA). Aminoacids were analysed by a reverse – phase HPLC (Method L 7400, HITACHI, Japan) fitted with a denali C18 5 micron column (4.6 x 150 mm). 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 hr. The tryptophan contents of the alkaline hydrolysates were determined colorimetrically using the method of Spies and Chamber (1949) as modified by Rama Rao *et al.* (1974). The contents of the different amino acids were expressed as g100 g^{-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 anti-nutritional compounds

The anti-nutritional compounds, total free phenolics (Bray and Thorne, 1954), tannins (Burns, 1971), the non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine) (Brain, 1976), phytic acid (Wheeler and Ferrel, 1971) and hydrogen cyanide

(Jackson, 1967) were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al.* (1974) by using benzoil-DL-arginin-p-nitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410 nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Statistical analysis

The results of present study were expressed as the mean values of three separate determinations, except for the fatty acids, amino acids profiles and trypsin inhibitor activity. Data were statistically analyzed using analysis of variance and Duncan Multiple Range Test using SPSS 11.5; SPSS Inc., Chicago IL, USA). Significance differences were determined at the $p < 0.05$ level.

Results and Discussion

The proximate compositions of the white and black seeds of Velvet bean are shown in Table 1. Proximate analysis results showed that the white seed has the higher crude protein content (28.82 g/100 g) whereas the protein content of black seed was 26.26 g/100 g. These selected samples seeds make the legumes a good source of nutrition. Higher fiber content was observed in white seed. It is observed that, white and black seeds of selected samples have moderate range of carbohydrates. The black seeds recorded high energy (1602.14 kJ 100g^{-1} DM) content. The proximate composition of Velvet bean seeds are shown in Table 1, moisture content is 7.86 g/100 g and 6.87 g/100 g of white seed accession and black seed respectively. Crude proteins and carbohydrates are the major chemical constituents of the legume samples. The crude protein content was significant at 28.82 g/100 g, and higher than commonly consumed legumes in India such as *Vigna unguiculata* (Ayssiwede *et al.*, 2011); wild legumes as *Rhychosia cana*, *R. fillips*, *R. rufescens* and *R. suaveolens* and species of *Vigna* (Kalidass and Mohan, 2012a, b). The remarkably high level of protein in the wild legume accessions under study underscores their importance as sources of a vital nutrient. The currently investigated underexploited legume, such as *Mucuna pruriens* var. utilis (both the seed) are found to have higher crude lipid content than the common pulses such as *Mucuna flegellipes* (Ihedioha and Okoye, 2011) and other pulses i.e., *Rhychosia cana*, *R. fillips*, *R. rufescens* and *R. suaveolens* and species of *Vigna* (Kalidass and Mohan, 2012a, b). The total dietary fibre content is found to be higher in

Table 1. Proximate composition of *Mucuna pruriens* var. *utilis* (g 100 g^{-1} seeds)

Components	White seed	Black seed
Moisture	7.86 ± 0.06^{a}	6.87 ± 0.05^{b}
Crude protein (Kjeldhal N × 6.25)	28.82 ± 0.14^{a}	26.26 ± 0.07^{a}
Crude lipid	6.45 ± 0.02^{b}	7.19 ± 0.02^{a}
TDF (Total Dietary Fibre)	9.73 ± 0.02^{a}	8.46 ± 0.01^{b}
Ash	4.52 ± 0.02^{a}	4.68 ± 0.01^{a}
Nitrogen Free Extractives (NFE)	50.62	53.37
Calorific value (kJ100 g^{-1} DM)	1567.93	1602.14

Table 2. Mineral composition of *Mucuna pruriens* var. *utilis*. (mg 100 g^{-1} seeds)

Components	White seed	Black seed
Sodium	128.12 ± 0.01^{a}	98.39 ± 0.02^{b}
Potassium	1628.36 ± 0.08^{b}	1846.23 ± 0.09^{a}
Calcium	689.45 ± 0.02^{b}	746.15 ± 0.06^{a}
Magnesium	341.44 ± 0.09^{a}	298.41 ± 0.01^{b}
Phosphorus	456.35 ± 0.11^{a}	327.47 ± 0.02^{b}
Iron	14.74 ± 0.04^{a}	12.41 ± 0.04^{b}
Zinc	6.12 ± 0.02^{b}	8.25 ± 0.01^{a}
Copper	2.11 ± 0.02^{a}	1.96 ± 0.03^{b}
Manganese	4.49 ± 0.01^{a}	3.78 ± 0.02^{b}
Na/K	0.08	0.05
Ca/P	1.51	2.28

the seeds of both the accessions of *Mucuna pruriens* var. *utilis* compared to other commonly cultivated pulses like chick pea, peas, horse gram, red gram and black gram (Premakumari *et al.*, 1984). The values of total dietary fibre content of above said samples are in consonance with the values reported for *Canavalia ensiformis* (Doss *et al.*, 2011). The ash content of investigated *Mucuna beans* (above 4%) would be important to the extent that it contains the nutritionally important mineral elements. Similar values were reported in *Cassia obtusifolia* (Vijayakumari *et al.*, 1993); species of *Vigna* (Kalidass and Mohan, 2012b). The food energy value was calculated to be 1602.14 kJ 100 g^{-1} DM based on crude protein, crude lipid and NFE. The high caloric values were due to its high fat content. This is comparable to previous studies, in the seeds of *Canavalia gladiata* (Vadivel and Janardhanan, 2004). Both seeds of Velvet beans investigated have higher energy than the reported value for pulses *Rhychosia cana*, *R. fillips*, *R. rufescens* and *R. suaveolens* (Kalidass and Mohan, 2012a) and *Canavalia ensiformis* (Doss *et al.*, 2011).

Analysis showed that both had significantly different ($p < 0.05$) levels of minerals content (Table 2). Higher concentration of sodium (128.12 mg/100 g), magnesium (341.44 mg/100 g), phosphorus (456.35 mg/100 g), iron (14.74 mg/100 g), copper (2.11 mg/100 g) and manganese (4.49 mg/100 g) are present in white seed, whereas black seed possessed higher quantum of potassium, calcium and zinc. The mineral composition of both seed expressed in mg per 100 g dry matter (DM) showed that it is rich sodium, potassium, calcium and phosphorus and fairly rich in iron, zinc and manganese. The ratio of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are shown in Table 2. The Na/K ratio in the beans can be useful for prevention of high blood pressure. Food is considered 'good' if the Ca/P ratio is above 1 and

Table 3. Fatty acid profile of *Mucuna pruriens* var. *utilis* a seeds

Fatty acid (%)	White seed	Black seed
Palmitic acid [C16:0]	25.12	28.02
Stearic acid [C18:0]	15.46	13.36
Total Saturated	**40.58**	**41.38**
Oleic acid [C18:1]	16.36	17.14
Linoleic acid [C18:2]	29.41	28.31
Linolneic acid [C18:3]	11.20	11.14
Total Unsaturated	**56.97**	**56.59**
Behenic acid [C22:0]	2.45	2.03

Table 4. Amino acid profiles of *Mucuna pruriens* var. *utilis* (g 100 g^{-1} proteins)

Amino acid	White seed	EAAS	Black seed	EAAS	FAO/WHO(1991) requirement pattern
Glutamic acid	17.42		15.26		
Aspartic acid	11.36		12.40		
Serine	4.32		4.00		
Threonine	3.52	103.53	3.40	100.00	3.4
Proline	2.94		3.26		
Alanine	5.10		4.36		
Glycine	5.45		4.94		
Valine	3.64	104.00	4.60	131.43	3.5
Cystine	0.38	} 53.60	0.78	} 64.80	} 2.5
Methionine	0.96		0.84		
Isoleucine	5.26	187.86	6.01	214.64	2.8
Leucine	7.01	106.21	5.36	81.21	6.6
Tyrosine	4.36	} 129.20	5.12	} 135.55	} 6.3
Phenylalanine	3.78		3.42		
Lysine	5.94	102.41	5.36	92.41	5.8
Histidine	3.44	181.05	2.20	115.79	1.9
Tryptophan	1.10	100.00	0.96	87.27	1.1
Arginine	5.74		5.26		

EAAS: Essential Amino Acids

'poor' if it is 1. In the present study, the Ca/P ratio 1.51 for white seeds and 2.28 for black seeds. The Ca/P ratio of white and black the seeds of *M. pruriens* var *utilis* were higher than the recommended value of 1.00 (Adeyeye and Fagbohun, 2005). The Ca/P ratio in the present study ranged between 1.51 to 2.28 indicated they would serve as good sources of minerals for bone formation.

From the present investigation it is established that white and black velvet bean is composed of more than 56% unsaturated fatty acids, such as mono unsaturated fatty acid present in Oleic acid [C18:1], poly unsaturated fatty acids present in Linoleic acid [C18:2] and Linolneic acid [C18:3] (Table 3). These are considered essential fatty acids. The fatty acid composition indicates that linoleic acids were 29.41% of white seed and 28.31% of black seed and palmitic acids were higher than *M. pruriens* var pruriens (Kalidass and Mohan, 2010) *Rhychosia cana*, *R. fillips*, *R. rufescens* and *R. suaveolens* (Kalidass and Mohan, 2012a). The amino acid profile of white and black seeds of velvet beans (Table 4) indicated a deficiency of sulphur amino acids. Cystine + methionine and tryptophan are found also low when compared to the FAO/WHO standard. Based on the concentration of essential amino acids white seeds ranked higher than that of black seeds. The potential of seed protein as a source of amino acids can be assessed by comparison with other legume seeds.

Table 5. Second metabolites and anti-nutritional compositions of *Mucuna pruriens* var. *utilis* seeds

Components	White seed	Black seed
Total free phenolics[h] g100g^{-1}	3.13 ± 0.01[c]	2.84 ± 0.01[b]
Tannins[h] g 100g^{-1}	0.34 ± 0.01[d]	0.26 ± 0.02[b]
L-DOPA[h] g 100g^{-1}	3.24 ± 0.01[b]	3.64 ± 0.01[c]
Phytic acid [h] mg100g^{-1}	423.00 ± 0.57[b]	444.14 ± 0.58[c]
Hydrogen cyanide [h] mg100g^{-1}	0.21 ± 0.01[a]	0.31 ± 0.01[c]
Trypsin inhibitor activity[g] (TIU mg^{-1} protein)	43.50	44.10

The amino acid profile of *M. pruriens* var *utilis* was found to be higher compared to FAO/WHO (1991) requirement pattern, and also reported for *Senna obtusifolia* (Ingweye *et al*., 2010) *Rhychosia cana*, *R. fillips*, *R. rufescens* and *R. suaveolens* (Kalidass and Mohan, 2012a). Valine, cystine + methionine, threonine and histidine were found to be rich in the essential amino acids. The other amino acids are present in moderate amounts. This value was comparable to *Phaseolus aureus* (Mubarak, 2005); and less than species of *Vigna* (Kalidass and Mohan, 2012b).

The secondary metabolites of white and black seeds of velvet beans are shown in Table 5. Total free phenolics, tannins, L-Dopa, phytic acid and hydrogen cyanide were significantly $p < 0.05$ level. Phytic acid content in white seed is low compared black seed. Trypsin inhibitor activity investigated in both seed samples showed no distinct difference. Some of the secondary metabolites such as protease inhibitors, lectins, tannins, goitrogens, cyanogens, amylase inhibitors are heat-labile; whereas toxic amino acids, alkaloids, cynogenic glycosides, saponins, flavones and isoflavones and pyrimidine glucosides are heat-stable. The acceptability and utilization of legumes in general, and *M. pruriens* var *utilis* in particular, have been limited because of the presence of certain anti-nutritional factors. The concentration of the non-protein amino acid L-DOPA in *M. pruriens* var. utilis is found to be slightly lower compared to other *Mucuna* species. Hydrogen cyanide is known to cause acute or chronic toxicity. The content of HCN level in the presently investigated samples is far below toxic level i.e. 36 mg/100 g (Oke, 1969) and comparable to those of *Vigna sinensis* and *Pisum sativum* (Montgomery, 1980), *Dolichos lablab* var. *vulgaris* and *Bauhinia purpurea* (Vijayakumari *et al.*, 1995, 1997).

From the study it is evident that *Mucuna pruriens* var. *utilis* is a potential source of protein supplement for livestock as well as in human food. The result supports argument in favour of prospecting wild legumes in the Southern Western Ghats, Tamil Nadu, India whose nutritional and economic values could be determined and put to use human and animal feed. The wild pulses if domesticated can improve

crop diversity, would be drought hard and a cheaper option of protein rich food.

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