

Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna*

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Two samples of seed materials of the Indian tribal pulse, *Mucuna utilis*, were collected from different forests in Tamil Nadu, India. The seeds of another tribal pulse, *M. monosperma*, were collected from Aliyar forest in Tamil Nadu. The mature seed samples were analysed for proximate composition, seed protein fractions, amino acid composition, fatty acid profiles, minerals and antinutritional factors. The investigated seed samples of *M. utilis* and *M. monosperma* contained higher amounts of crude protein and crude lipid when compared with most of the commonly consumed pulses. Albumin and globulin fractions constituted the major bulk of seed proteins. Amino acid profiles of *M. utilis* (black seed coat sample) revealed that the seed proteins contained relatively higher levels of the sulpho-amino acid cystine. The seed proteins of both samples of *M. utilis* and *M. monosperma* also contained higher levels of the other essential amino acids, isoleucine, tyrosine and phenylalanine. The fatty acid profiles of both samples of *M. utilis* and *M. monosperma* revealed that the seed lipids contained higher concentrations of palmitic acid and linoleic acid. The antinutritional fatty acid, behenic acid, also was detected in the present study. The investigated seeds were rich in minerals such as Na, P, Mg, Zn and Fe. Antinutritional substances, total free phenols, tannins, L-DOPA and haemagglutinating activity, were also analysed/assayed.

INTRODUCTION

Insufficient protein of good quality is a serious problem in many developing countries due to the prohibitive cost of protein from animal sources. Alternative sources of proteins which could alleviate this problem include the proteins from different plants (Luz Fernandez & Berry, 1988). There is a world-wide interest in finding alternative sources of economically cheaper protein. Leguminous seeds are important sources of protein in the diet of millions of people. There has been a constant search for new legumes with high protein contents, and suggestions for utilization of unconventional legumes have been made from time to time (Pandey & Srivastava, 1990). In India, information on chemical composition of seeds of tribal pulses and wild progenitors of cultivated legumes is relatively meagre. In view of this, in the present study an attempt was made to understand the biochemical composition and assess the nutritional value of two samples of the tribal pulse *Mucuna utilis* wall ex Wight and one sample of *Mucuna monosperma* DC. ex Wight. The mature seeds of *Mucuna utilis* are known

to be eaten boiled by an Indian tribal sect called Kanikkars (Janardhanan & Lakshmanan, 1985). The boiled seeds of *Mucuna monosperma* are eaten by the tribes of North Eastern India and Oceanic groups of tribals like Onges, Great Andamanese and Sompens during extreme scarcity of food (Arora & Mehra, 1981; Gunjatkar & Vartak, 1982).

MATERIALS AND METHODS

Of the two samples of *Mucuna utilis*, the sample with white seed coats was collected from Mundanthurai Wildlife Sanctuary, a semi-evergreen forest in the Nellore District, during June 1991 (late summer). The other seed sample, with black seed coats, was collected from Myllaru, a semi-evergreen forest in the Kanyakumari District, also during June 1991. The seeds of another tribal pulse, *M. monosperma*, were collected from the evergreen forest of Aliyar in the Coimbatore District during April 1991 (early summer).

The moisture content was determined by drying 50 transversely cut seeds in an oven at 80°C for 24 h, and is expressed on a percentage basis. The seeds were powdered separately in a Willey Mill to 60 mesh size.

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Table 1. Proximate composition of the seeds of *Mucuna utilis* and *M. monosperma*^a along with some of the most common pulses

Pulse	Component g 100 g ⁻¹ seed flour					
	Moisture	Crude protein (Kjeldahl N × 6.25)	Crude lipid	Crude fibre	Ash	Nitrogen free extractive (NFE)
<i>Mucuna utilis</i> (Black seed coat)	11.40 ± 0.11	29.6 ± 0.57	8.47 ± 0.03	10.40 ± 0.02	4.12 ± 0.03	47.4
<i>M. utilis</i> (White seed coat)	11.60 ± 0.07	25.7 ± 0.21	10.75 ± 0.09	11.55 ± 0.13	3.56 ± 0.01	48.4
<i>M. monosperma</i>	6.90 ± 0.02	23.5 ± 0.28	14.39 ± 0.13	6.79 ± 0.01	3.21 ± 0.03	52.2
<i>Phaseolus mungo</i> ^b	—	23.3	1.25	4.04	—	67.8
<i>Phaseolus aureus</i> ^b	—	22.3	1.12	4.83	—	68.3
<i>Cicer arietinum</i> ^c	—	20.7	4.50	3.50	2.70	57.1
<i>Cajanus cajan</i> ^d	—	19.4	3.24	5.56	4.05	57.2
<i>Vigna unguiculata</i> ^e	—	22.5	1.60	5.33	3.81	56.9

^a All values are means of triplicate determinations expressed on a dry-weight basis. ± Denotes standard error.

Sources: ^b Gupta & Wagle (1978); ^c Jambunathan & Singh (1980); ^d Nwokolo (1987); ^e Nwokolo & Oji (1985).

The fine seed powder so obtained was used for further analysis. The crude protein content was calculated by multiplying the percentage Kjeldahl nitrogen (Humphries, 1956) by a factor of 6.25. The contents of crude lipid, crude fibre and ash were estimated by Association of Official Analytical Chemists (AOAC, 1970) methods. The nitrogen-free extractives (NFE) or total crude carbohydrates were calculated by difference (Muller & Tobin, 1980). The energy content of the seeds was determined by multiplying the crude protein, crude fat and nitrogen-free extractives (total crude carbohydrates) contents by factors of 4, 9 and 4, respectively (Osborne & Voegt, 1978). The total true proteins were extracted by the method of Basha *et al.* (1976) with slight modification (ethanol treatment was omitted in order to save the prolamin fraction). The extracted proteins were purified by precipitation with 20% TCA. The albumin and globulin fractions of seed proteins were extracted following the method of Murray (1979). From the residual pellet the prolamin fraction was extracted by treatment with 70% ethanol 1:5 (w/v) overnight; after centrifugation, the supernatant containing the prolamins was air-dried and dissolved in 0.1 N NaOH. The resulting pellet was extracted with 0.4 N NaOH 1:10 (w/v) overnight and centrifuged at 20 000 g for 20 min. The supernatant thus obtained was designated as glutelins. The proteins in the fractions so obtained were estimated by the Lowry *et al.* (1951) method after 20% cold TCA precipitation. The purified total seed proteins were acid-hydrolysed with 6 N HCl at 110°C for 24 h *in vacuo*. After flash evaporation, the dried residue was dissolved in a citrate buffer (pH 2.2). Known aliquots were analysed in a LKB-Biochrome Automated Amino Acid Analyser, Model 4151-Alpha plus. The essential amino acids were scored following the method of the Food and Agriculture Organisation and the World Health Organisation (FAO/WHO, 1990). True protein digestibilities of the samples were determined following the method of Eggum (1973). Protein digestibility-corrected amino acid scores of the

samples were calculated by multiplying the lowest amino acid ratio by the true protein digestibility (FAO/WHO, 1990). The total lipids were extracted from the seed flour with a mixture of chloroform and methanol in a ratio of 2:1 respectively, following the method of Folch *et al.* (1957). Fatty acid methyl esters were prepared according to the procedure outlined by Metcalfe *et al.* (1966). Fatty acid methyl esters were analysed using gas chromatography (Shimadzu Model-R1A). All the minerals except phosphorus were analysed by Atomic Absorption Spectrophotometer (Perkin-Elmer, Model-5000) (Issac & Johnson, 1975). The phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman & Bray, 1940). The antinutritional factors such as tannins, (Burns, 1971), total free phenols (Bray & Thorne, 1954) and the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) (Brain, 1976) were analysed. The haemagglutinating activity of the seed proteins (Liener, 1976) was also assayed.

Table 2. Calorific value of the seeds of *Mucuna utilis* and *M. monosperma* along with other *Mucuna* species / germplasms^a

Name of the pulse	Calorific value (kCal 100 g ⁻¹ DM)
<i>Mucuna utilis</i> (Black seed coat)	384
<i>M. utilis</i> (White seed coat)	393
<i>M. monosperma</i>	432
<i>M. gigantea</i> ^b	375
<i>M. pruriens</i> ^c (Begur)	394
<i>M. pruriens</i> ^c (Silent Valley)	385
<i>M. pruriens</i> ^c (Lucknow)	419

^a All values are means of triplicate determinations expressed on a dry-weight basis.
Sources: ^b Rajaram & Janardhanan (1991a); ^c Mary Josephine & Janardhanan (1992).

RESULTS AND DISCUSSION

The contents of crude protein and crude lipid detected in the samples of the present investigation (Table 1) were found to be higher than the pulse crops commonly consumed in India, such as black gram, green gram, pigeonpea, chickpea and cowpea, which have been reported earlier (Gupta & Wagle, 1978; Jambunathan & Singh, 1980; Nwokolo & Oji, 1985; Nwokolo, 1987). Nonetheless, the occurrence of higher contents of crude lipid, compared to the values of the present samples, in *Bauhinia vahlii* (Rajaram & Janardhanan, 1991b), *Mucuna hirsuta* (Rajaram & Janardhanan, 1992), and *Parkia roxburghii* (Mohan & Janardhanan, 1993) from our laboratory has already been detected and reported.

The seeds of *M. monosperma* exhibit the highest calorific value, a value greater than the other *Mucuna* species/germplasm, investigated earlier in our laboratory (Table 2) (Rajaram & Janardhanan, 1991a; Mary Josephine & Janardhanan, 1992).

The data on seed protein fractionation of the samples of *M. utilis* and *M. monosperma* (Table 3) show that the albumins and globulins constitute the major bulk of the seed proteins, as in most legumes and the other *Mucuna* species reported earlier (Boulter & Derbyshire, 1976; Duranti & Cerletti, 1979; Rajaram & Janardhanan, 1992; Siddhuraju *et al.*, 1992). The amino acid profiles of the purified seed proteins, the essential amino acid score and the protein digestibility-corrected amino acid score are presented in Table 4. The data

Table 3. Data on total (true) proteins and protein fractionation of *Mucuna utilis* and *M. monosperma*.^a

Fraction	<i>M. utilis</i> (Black seed coat)		<i>M. utilis</i> (White seed coat)		<i>M. monosperma</i>	
	g 100 g ⁻¹ seed flour	g 100 g ⁻¹ protein	g 100 g ⁻¹ seed flour	g 100 g ⁻¹ protein	g 100 g ⁻¹ seed flour	g 100 g ⁻¹ protein
Total protein (true protein)	23.4 ± 0.14	100	22.73 ± 0.11	100	20.4 ± 0.23	100
Albumins	8.32 ± 0.04	35.5	8.39 ± 0.07	36.9	4.22 ± 0.01	20.7
Globulins	12.5 ± 0.07	53.2	11.7 ± 0.13	51.3	14.0 ± 0.11	68.6
Prolamins	0.89 ± 0.01	3.80	0.76 ± 0.01	3.34	0.68 ± 0.02	3.33
Glutelins	1.75 ± 0.03	7.47	1.92 ± 0.06	8.45	1.51 ± 0.03	7.39

^a All values are means of triplicate determinations expressed on a dry-weight basis. ± Denotes standard error.

Table 4. Amino acid profiles of acid-hydrolysed, purified total seed proteins of *Mucuna utilis* and *M. monosperma*

Amino acid	<i>M. utilis</i> (Black seed coat) (mg g ⁻¹ protein)	Essential amino acid score	<i>M. utilis</i> (White seed coat) (mg g ⁻¹ protein)	Essential amino acid score	<i>M. monosperma</i> (mg g ⁻¹ protein)	Essential amino acid score	FAO/WHO (1990) requirement pattern (mg g ⁻¹ protein)
Glutamic acid	126		140		149		
Aspartic acid	147		142		113		
Serine	45.0		44.0		41.2		
Threonine	49.1	1.44	45.9	1.35	33.6	0.99	34
Proline	29.8		35.3		51.2		
Alanine	46.0		41.6		60.4		
Glycine	51.2		66.6		43.6		
Valine	33.9	0.97	42.5	1.21	44.9	1.28	35
Cystine	20.2		16.2		Trace		
Methionine	06.8	1.08	03.2	0.78	Trace		25
Isoleucine	72.0	2.57	80.2	2.86	63.1	2.25	28
Leucine	64.8	0.98	64.1	0.97	51.9	0.79	66
Tyrosine	43.0		52.1		33.9		
Phenylalanine	46.3	1.42	40.6	1.47	59.8	1.49	63
Lysine	56.1	0.97	59.8	1.03	52.4	0.97	58
Histidine	29.3		30.1		26.8		
Tryptophan	ND ^a		ND		ND		11
Arginine	71.4		63.0		55.8		
True protein digestibility	0.70 (70%)		0.71 (71%)		0.64 (64%)		
Protein digestibility- corrected amino acid score	0.68		0.55		0.51		

^aND, not detected.

Table 5. Fatty acid composition of lipids of *Mucuna utilis* and *M. monosperma* seeds^a compared with common legume seed lipids

Pulse	Fatty acid (%)										
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	Others
<i>Mucuna utilis</i> (Black seed coat)	—	27.1	—	15.0	20.3	32.0	3.38	—	—	2.26	—
<i>M. utilis</i> (White seed coat)	—	39.4	—	11.7	14.6	21.4	8.99	—	—	3.99	—
<i>M. monosperma</i>	—	24.6	—	11.7	30.8	24.7	4.74	—	—	3.52	—
<i>Cajanus cajan</i> ^b	—	20.5	—	6.90	10.5	56.3	5.00	0.80	—	—	—
<i>Vigna radiata</i> ^b	—	14.1	—	4.30	20.8	16.3	35.7	—	—	9.30	—
<i>V. mungo</i> ^b	—	17.8	—	5.90	17.3	11.6	47.5	—	—	—	—
<i>V. unguiculata</i> ^c	2.00	14.6	9.30	7.30	18.9	20.7	18.2	—	7.90	—	1.10
<i>Phaseolus vulgaris</i> ^c	—	15.1	6.30	9.10	19.8	20.6	17.3	—	3.80	—	8.00
<i>Glycine max</i> ^d	0.27	10.6	0.53	2.94	25.5	48.7	5.03	2.03	—	—	—

^a All values are of two determinations.

Sources: ^b Salunkhe *et al.*, (1982); ^c Omogbai (1990); ^d Ologhobo & Fetuga (1984).

reveal that the limiting amino acids are as follows: in *M. utilis* (black seed coat sample), both valine and lysine; in *M. utilis* (white seed coat sample), sulphur-containing amino acids; and in *M. monosperma*, leucine. The seed proteins of *M. utilis* (both samples) and *M. monosperma* also contain higher levels of the other essential amino acids, isoleucine, tyrosine and phenylalanine, when compared with the WHO requirement pattern (FAO/WHO, 1990). The threonine content of *M. utilis* (both samples) is comparable with that of soybean (Boulter & Derbyshire, 1976). The fatty acid profiles (Table 5) reveal that both the samples of *Mucuna utilis* and *Mucuna monosperma* 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), *V. unguiculata* and *Phaseolus vulgaris* (Omogbai, 1990). Oleic acid is present in large quantity in the seeds of *Mucuna monosperma*, as in *Cajanus cajan*, *Vigna radiata*, *V. mungo*, (Salunkhe *et al.*, 1982), *Phaseolus vulgaris* and *Vigna unguiculata* (Omogbai, 1990) and *Glycine max* (Ologhobo & Fetuga, 1984). Nonetheless, the palmitic acid level of an earlier investigation in *Bauhinia racemosa* (Mohan & Janardhanan, in press) is higher than the present samples. However, its level is comparable with that of other investigations (El Refai *et al.*, 1987; Vasanthan & Hoover, 1992). Similarly, the level of stearic acid detected in the samples investigated in the present study seems to be lower than that of samples of *Bauhinia racemosa* of an earlier investigation (Mohan & Janardhanan, in press) and more or less comparable with that of another tribal pulse, *Parkia roxburghii* (Mohan & Janardhanan, 1993). The antinutritional fatty acid, behenic acid, is detected in *Mucuna utilis* (both samples) and *M. monosperma*. Earlier reports indicate the detection of behenic acid in groundnut (Kritchevsky *et al.*, 1973), winged bean (Bean *et al.*, 1984; Fernando & Bean, 1985, 1986), *Parkia roxburghii* and *Entada phaseoloides* (Mohan & Janardhanan, 1993).

The mineral analysis (Table 6) reveals that the samples of *Mucuna utilis* and *Mucuna monosperma* appear

Table 6. Mineral composition of the seeds of *Mucuna utilis* and *M. monosperma*^a

Mineral (mg 100 g ⁻¹ seed flour)	<i>M. utilis</i> (Black seed coat)	<i>M. utilis</i> (White seed coat)	<i>M. monosperma</i>
Sodium	71.3 ± 0.11	72.0 ± 0.02	62.0 ± 0.11
Potassium	1660 ± 1.21	1244 ± 0.84	1761 ± 0.99
Calcium	759 ± 0.51	688 ± 0.64	172 ± 0.31
Magnesium	271 ± 0.09	261 ± 0.33	72.2 ± 0.23
Phosphorus	327 ± 0.57	260 ± 0.14	129 ± 0.13
Iron	5.87 ± 0.03	6.33 ± 0.06	5.16 ± 0.07
Copper	0.45 ± 0.05	0.45 ± 0.03	0.43 ± 0.02
Zinc	2.03 ± 0.03	1.81 ± 0.03	1.07 ± 0.01
Manganese	9.26 ± 0.06	8.82 ± 0.08	8.16 ± 0.02

^a All values are mean of triplicate determinations expressed on a dry-weight basis. ± Denotes standard error.

to be rich sources of Na, Mg, P, Zn, Fe and Mn, when compared with the other *Mucuna* species (Rajaram & Janardhanan, 1992).

The usefulness of legumes is decreased by toxic or antinutritional compounds associated with the large protein content in their seeds (Nowacki, 1980). Some of the antinutritional factors such as protease inhibitors, lectins, tannins, goitrogens, cyanogens, amylase inhibitors and antivitamin factors are heat-labile (Liener, 1980); Whereas others such as toxic amino acids, alkaloids, cyanogenic glucosides, saponins, flavones and isoflavones, and pyrimidine glucosides are heat-stable (Nowacki, 1980). The data on antinutritional factors are presented in Table 7. The contents of tannins present in the seeds of *Mucuna utilis* (both samples) and *M. monosperma* appear to be low when compared with the other *Mucuna* species reported earlier (Rajaram & Janardhanan, 1991a; Mary Josephine & Janardhanan, 1992) and the commonly consumed legume seeds such as green gram, cowpea, pigeonpea and black gram (Khan *et al.*, 1979; Rao & Deosthale, 1982). The content of total free phenols of investigated seed samples of *Mucuna utilis* and *M. monosperma* is found to be

Table 7. Data on antinutritional factors of *Mucuna utilis* and *M. monosperma* seeds^a

Component	<i>M. utilis</i> (Black seed coat)	<i>M. utilis</i> (White seed coat)	<i>M. monosperma</i>	
Total free phenols (%)	3.18 ± 0.07	3.16 ± 0.11	0.85 ± 0.05	
Tannins (%)	0.06 ± 0.02	0.03 ± 0.001	0.06 ± 0.01	
L-DOPA (%)	6.86 ± 0.23	6.08 ± 0.51	4.24 ± 0.21	
Phytohaemagglutinating activity ^b				
Protein fraction	Erythrocytes (human blood groups)	Haemagglutinating activity ^c		
Albumins	A	+	+	+
Albumins	B	+	+	+
Albumins	O	+	+	+
Globulins	A	++	++	+
Globulins	B	++	++	+
Globulins	O	++	++	+

^a Values are means of triplicate determinations expressed on a dry-weight basis. ± denotes standard error.

^b Values of two independent experiments.

^c + some clumping, pellet disperses partially;

++ no dispersion of pellet.

low when compared with *Mucuna pruriens* (Mary Josephine & Janardhanan, 1992). The concentration of the non-protein amino acid L-DOPA in *Mucuna utilis* (both samples) and *Mucuna monosperma* has been found to be slightly low when compared with the values reported earlier in *M. pruriens* (Mary Josephine & Janardhanan, 1992). The globulin proteins strongly agglutinate all types of trypsinized human erythrocytes (A, B and O) without any specificity, as in the case of *Entada scandens* (Janardhanan & Nalini, 1991), whereas albumin proteins weakly agglutinate erythrocytes from the human A, B and O blood groups. This observation accords with the earlier investigations in *Dolichos lablab* (Kaushik, 1984), *Psophocarpus tetragonolobus* (Kotaru *et al.*, 1987) and *Psophocarpus scandens* (Kortt, 1988). In soyabean seeds, haemagglutinins (lectins) play a minor role in nutritional value (Liener, 1980). Nonetheless, Jaffe (1960) (cited by Liener (1980)) postulated that the action of haemagglutinin was to combine with cells lining the intestinal mucosa, causing non-specific interference with the absorption of nutrients.

The observations made in the present study show that both seed samples of *Mucuna utilis* are rich in crude protein, most of the essential amino acids, fatty acids such as palmitic acid and linoleic acid, and some minerals. The seeds of *Mucuna monosperma* are rich in crude protein, crude lipid and exhibit a higher calorific value. The seed proteins register higher levels of the essential amino acids, isoleucine, tyrosine and phenylalanine. The seed lipids seem to be rich in fatty acids such as palmitic acid, oleic acid and linoleic acid. They are also rich in some minerals such as K and Mn. The antinutritional factors (total free phenols, tannins and lectins), except L-DOPA, are heat-labile and can be eliminated by ordinary cooking processes.

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