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Nutritive value and antinutritional factors in different varieties of *Sesbania* seeds and their morphological fractions

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Abstract

Four different *Sesbania* seeds, *S. aculeata, S. rostrata, S. sesban* (accession 10865D) and *S. sesban* (accession 15019D), were analysed for their physicochemical properties, nutritive values and antinutritional factors (ANFs) in order to assess feasibility of use as an unconventional food resource. Seeds of both the accessions of *S. sesban* were smaller in size but their seed coat + endosperm fractions were higher (56–57%) than those of *S. aculeata* and *S. rostrata* (49–51%). *S. rostrata* had a lower hydration capacity, hydration index, swelling capacity and swelling index than other seeds. The crude protein content in different seeds ranged from 29.1 to 33.1%, crude lipid 4.7–6.0%, crude fibre 10.9–15.8%, total crude carbohydrates 44.6–47.4% and gross energy 19.2–20.0 kJ/g. Except for isoleucine, histidine and tryptophan, all other essential amino acids were deficient compared to the FAO/WHO reference pattern. The seed fractions showed similar amino acid pattern to that of whole seed with slightly higher values. Palmitic, stearic, oleic, linoleic and linolenic acids were the major fatty acids (FAs) and the total unsaturated and essential FAs ranged from 78.1 to 82.3% and 77.2 to 80.3%, respectively, with *S. aculeata* having the highest value. The ranges of starch fractions in different seeds were: total starch 17.5–20.4%, digestible starch 7.3–10.2% and resistant starch 10.2–11.2%. The soluble sugar contents ranged from 8.8 to 10.6%. The ranges of antinutritional factors in different seeds were: total phenols 2.96–5.95%, tannins 1.97–2.25%, condensed tannins 1.82–5.14%, phytic acid 1.89–2.37%, saponins 0.52–1.46%, trypsin inhibitors 5.25–14.0 (mg trypsin inhibited per gram of sample) and lectin activity 10.2–20.5 units. There was a wide variation in the ANFs contents of seed coat + endosperm, and cotyledons.

Keywords: *Sesbania*; Nutritive value; Antinutritional factors; Resistant starch; Morphological fractions

1. Introduction

Sesbania species, locally known as Dhaincha in Bangladesh and India, are not food plants except in some instances where leaves and flowers are consumed as vegetables (Evans & Rotar, 1987). Their major agricultural use has been as green manure to improve production of food crops. They are also grown for animal fodder and their wood is used for firewood, poles and light construction. They have been adopted for these applications because agriculturists have been impressed by their special qualities of vigorous growth, adaptation to varied soil environments, control of soil erosion and the enhancement of soil fertility where they are grown. Because of these characteristics *Sesbania* species constitute a valuable, promising, multipurpose plant resource.

Potential uses of *Sesbania* include improved animal nutrition. *Sesbania* leaves, flowers, pods and seeds are sources of animal feed and possibly also food for humans. The mature seeds of *S. bispinosa* are known to be cooked and eaten by the Indian tribal sects Katkharis and Ghonds (Siddhuraju, Vijaykumari & Janardhanan, 1995).

Sesbania leaves, flowers, pods and seeds are sources of animal feed. Hussain and Khan (1962) reported a *S. aculeata* seed yield of 1.5 t/ha. In a farm-scale economic analysis for Indian conditions, Chandra and Farooqi (1979) set 1 t/ha as a minimum yield. *Sesbania* seed

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contains 30–40% crude protein and at present the seeds are not being used for any other agricultural or industrial purpose. Presence of various antinutritional factors (ANFs), such as tannins, saponins, and trypsin inhibitors, in *Sesbania* seed is a major problem when used as animal feed, as reported by Evans and Rotar (1987). Removal or destruction of such ANFs by various processing techniques could pave the way for their efficient use as animal feed. However, detailed information on the nutritive value and ANF contents in *Sesbania* seeds is scanty. In view of this, in the present study: (1) the proximate composition, amino acid, fatty acid profile, and carbohydrate fractions; and (2) ANF content of four different *Sesbania* seeds, *S. aculeata, S. rostrata, S. sesban* (accession 10865D) and *S. sesban* (accession 15019D), are analysed.

2. Materials and methods

2.1. Samples

The mature and dry raw seeds of *S. aculeata* were collected from the field station of the Department of Plant Breeding and Genetics and *S. rostrata* from the Agronomy farm of Bangladesh Agricultural University, Mymensingh. The seeds were cleaned and dried once more in the sun. Seeds of two accessions of *S. sesban* (accession 10865D and accession 15019D) were collected from the International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. For convenience, hereafter, in this paper, the two accessions of *S. sesban* are called *S. seban* (1) (accession 10865D) and *S. sesban* (2) (accession 15019D). The seeds were stored at room temperature until used for analysis. It was not possible to separate the seed coat, endosperm and cotyledons mechanically. Seed coats, endosperm and cotyledons were separated manually by cutting each of the seed with a fine sharp blade after moistening the seed with water. A certain quantity of the seeds was placed in a Petri dish and moistened with a small amount of water and kept in a refrigerator (4°C) overnight so that the total water was absorbed by the seeds and the seeds became soft. Because of difficulty in removal of seed coat and endosperm separately, the seed coats and endosperms were kept together. The seed coats + endosperm and cotyledons were freeze-dried and ground into a particle size of less than 0.5 mm before chemical analysis.

2.1.1. Physicochemical properties

The physicochemical properties, such as density, hydration capacity, hydration index, swelling capacity and swelling index, of the seeds, were determined according to Chavan et al. (1999). The colour of the seeds was determined subjectively. The proportions of seed coat + endosperm and cotyledons in the different seeds were determined by taking the average measure of 1000 grain weight.

2.1.2. Proximate composition

Moisture contents of all *Sesbania* seeds were determined by oven drying to a constant mass at 105°C. The crude protein, crude lipid, crude fibre and ash contents were determined in accordance with the standard methods of AOAC (1990). Nitrogen-free extracts (NFE) were obtained by difference. Gross energy was determined using a bomb calorimeter (IKA C7000) using a benzoic acid standard.

2.1.3. Amino acid analysis

The amino acid composition of whole seed, seed coat + endosperm, and cotyledon samples of *Sesbania* were determined using an amino acid analyser (Bassler & Buchholz, 1993) after hydrolysing the samples with 6 M HCl at 100°C for 24 h. Sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. The tryptophan content was determined spectrophotometrically by the method of Mertz, Jambunathan and Misra (1975). The contents of different amino acids are presented as g/16 g of nitrogen and are compared with the FAO/WHO (1990) reference pattern. The essential amino acid (EAA) score was calculated as:

$$\text{EAA score} = \frac{\text{g of EEA in 16 g N of test sample}}{\text{g of EAA in 16 g N in FAO/WHO reference pattern}} \times 100$$

2.1.4. Fatty acid analysis

Total lipids were extracted from the whole seed flour by using hexane at room temperature under overnight stirring on a magnetic stirrer. Further, this lipid was purified according to the method of Folch, Lees and Solane-Stanley (1957), using a chloroform and methanol mixture in the ratio of 2:1 (v/v). Methyl esters were prepared from the total lipids by the method of Lepage and Roy (1986). The fatty acid methyl esters were analysed by gas chromatography (Perkin-Elmer, USA) using an instrument equipped with a flame ionisation detector (FID) and a SP2330 Supelco Capillary Columm (30 m × 0.32 mm ID). The column temperature gradient ranged from 70 to 225°C and the carrier gas was nitrogen at a flow rate of 2 ml/min. A standard fatty acid methyl ester mixture was run and retention times were used in identifying sample peaks. Fatty acid levels were estimated on the basis of peak areas of known concentrations of the standards.

2.1.5. Starch analysis

Total starch (TS) content was determined after dispersion of the seed samples in 2M KOH (50 mg sample,

6 ml KOH) at room temperature (30 min, constant shaking) and hydrolysis of the solubilized starch with 80 μl amyloglucosidase (EC 3.2.1.3; Cat. No. 102857, Boehringer-Mannheim, Germany) at 60°C during 45 min (Goni, Gania-Alinos, Manas, & Saura-Calixto, 1997). Glucose was quantified using the glucose oxidase/peroxidase reagent (Cat. No.510-A, Sigma Chemical Co., Deisenhofen, Germany). Total starch was calculated as glucose×0.9, after correction for the free glucose content. The glucose content of samples, both free glucose and the glucose moiety of sucrose, was determined as follows. Samples dispersed in 2M KOH were treated with invertase (EC 3.2.1.26) during 30 min at 37°C. After centrifugation, a 1 ml aliquot was precipitated with 2 ml of 96% ethanol, centrifuged again and glucose analysed in the supernatant using the glucose oxidase/peroxidase reagent.

Resistant starch (RS) was analysed as follows: Samples (100 mg) were treated with 200 mg pepsin (1 g pepsin/10 ml KCl-HCl buffer; Merck No. 7190,2000 FIT-U g^{-1}) to remove protein and then incubated for 16 h at 37°C with 1 ml pancreatic alpha amylase (solution containing 40 mg alpha amylase/ml Tris Maleate buffer; EC 3.2.1.1, A-3176, Sigma Chemical Co.) to remove digestible starch. After centrifugation (15 min, 3000×***g***) and removal of supernatants, the pellets were dispersed with 2M KOH, hydrolysed with amyloglucosidase and the liberated glucose quantified, as described above for total starch. RS was calculated as glucose×0.9 (Goni, Garia-Diz, Manas & Saura-Calixto, 1996). Digestible starch (DS) content was calculated as TS-RS.

2.1.6. Soluble sugar

For soluble sugars, the samples were extracted according to Bravo, Siddhuraju and Saura-Calixto (1999) and sugar contents were determined by the anthrone-sulphuric acid method (Hedge & Hofreiter, 1962).

2.1.7. Analysis of various antinutritional components

2.1.7.1. Phenolic substances. Total phenols, tannins and condensed tannins were determined by spectrophotometric methods described by Makkar, Blümmel, Borowy and Becker (1993). Total phenols were quantified by Folin-Ciocalteu reagent, and tannins as the difference between phenolics before and after tannin removal from the extract using insoluble polyvinylpyrolidone. Condensed tannins were determined by butanol-HCl-Fe_3^+ reagent (Porter, Hirstich & Chan, 1986). Total phenols and tannins were expressed as tannic acid equivalents and condensed tannins as leucocyanidin equivalents.

2.1.7.2. Trypsin inhibitor activity. Trypsin inhibitor activity was determined according to Smith, Van Megan, Twaalhoven and Hitchcook (1980). Ground samples (0.25 g each) were extracted in 12.5 ml of 0.01 M NaOH at pH 9.4 to 9.6 using and Ultra-Turrax at 20,000 rpm for 5 min (2×2.5 min) with intermittent cooling by keeping the tubes containing the samples in an ice bath. The contents were centrifuged at 3500 ***g*** for 15 min and the supernatants were collected; this supernatant was centrifuged a second time at 9500 ***g***, following which the supernatants were collected by slowly pipetting between the residue at the bottom and the fatty layer on top. These solutions were used for the assay after appropriate dilution (with distilled water), based on pre-assay trial results.

2.1.7.3. Lectin or phytohaemagglutinating activity. Analysis for lectin activity was conducted by the haemagglutination assay described by Gordon and Marquardt (1974) in round-bottomed wells of Microtitre plates with 2% (v/v) trypsinised cattle blood erythrocytes suspension in phosphate-buffered saline (PBS), pH 7. Ground samples (0.5 g each) were extracted in 10 ml of the PBS using an Ultra-Taurrax at 20,000 rpm for 5 min (2×2.5 min) with intermittent 5 min cooling, by keeping the tube containing the sample in an ice bath. The contents were centrifuged and the supernatant was collected. The solution was mixed with an equal volume (0.05 ml) of 2% erythrocytes in microplates. The sedimentation patterns of the erythrocytes suspensions were read after 2 h at room temperature. A positive pattern, which indicated agglutination, was a uniform effacement of the bottom of the well by erythrocytes, while a negative pattern (indicating no agglutination) was a circular clump of erythrocytes surrounded by a concentric, clear zone of equal size to the blank (which contained PBS instead of sample). The formation of erythrocyte aggregates of at least four or five cells, that were not disrupted by gentle movement, was considered as evidence of agglutination. The haemagglutination activity was defined as the minimum amount of the seed material in mg per ml of the assay medium which produced agglutination. For better comparison, the results are presented as a reciprocal of the minimum amount of sample (mg) per ml of the assay medium which produced haemagglutination.

2.1.7.4. Saponins. Saponin (steroidal) content was determined using a spectrophotometric method described by Baccou, Lambert and Samvaire (1977). To 0.5 g defatted ground-meal samples, in a screw-capped centrifuge tube, was added 10 ml of 80% aqueous methanol. The tubes were tightly capped and the contents were stirred overnight using a magnetic stirrer. The tubes were centrifuged at 3000 ***g*** for 10 min and the supernatants were collected in 25 ml volumetric flasks. The residues were washed three times with 5 ml of 80% aqueous methanol, followed by centrifugation, and the supernatants were collected in volumetric flasks. The final volume was made to 25 ml with 80% aqueous

methanol. Aliquot samples from the flasks were used for saponin determination. The results are expressed as diosgenin equivalents from a standard curve of different concentrations of diosgenin in 80% aqueous methanol.

2.1.7.5. Thin-layer chromatographic (TLC) analysis of saponin. Saponin extracts were prepared from the seeds of *Sesbania* using 50 g of each of the seed samples extracted with 200 ml of 50% MeOH in a water bath sonicator for 20 min. The extract was centrifuged and methanol was evaporated by a rotary evaporator. The aqueous phase was fractionated 3 times with chloroform. Then the aqueous phase was again fractionated twice with butanol. The butanol was removed using a water bath at 50°C with nitrogen flush. The dried residue was dissolved in water, frozen and freeze-dried.

TLC was carried out according to Okenfull and Sidhu (1989), using Merck (#1.05721) TLC plates (20×20 cm, silica gel 60). Each lane was loaded with 3 μl of saponin extract (10 mg dissolved in 1 ml of 95% methanol). Separation was carried out using a mobile phase of chloroform:methanol:water (60:55:15, v/v/v) solution, air-dried and heated at 80°C for 10 min. Visualisation of the saponin on the developed plates was done by spraying with glacial acetic acid:H_2SO_4:para-anisaldehyde (97:2:1, v/v/v). A Quilaja saponin (Sigma-2149) was used as a reference TLC plate. A set of plates developed in the same way was also sprayed with 6% trypsinised cattle blood erythrocyte suspension in PBS, pH-7.0, to see the haemolytic nature of the saponin.

2.1.7.6. Phytic acid. Phytic acid content of the sample was determined by a colorimetric procedure described by Vaintraub and Lapteva (1988). Ground samples (0.5 g each) were stirred in 10 ml of 3.5% HCl for 1 h. The contents were centrifuged at 3500 rpm for 10 min to obtain supernatants. Suitable aliquots of the supernatants were diluted with distilled water to make 3 ml and then used for the assay. Results are expressed as percentage phytic acid by using standard (Sigma) phytic acid.

2.1.8. Statistical analysis

One-way analysis of variance (ANOVA) was carried out to compare between the mean values of different seeds and seed fractions, followed by Duncan's Multiple Range test. Standard errors (±S.E.) of means were calculated from the residual mean square in the analysis of variance.

3. Results and discussion

To compare the morphological characteristics of different seeds of *Sesbania*, their physicochemical properties were evaluated and the results are presented in Table 1. The colour of *S. aculeata* and *S. rostrata* were dark brown and brown and *S. sesban* (1) and *S. sesban* (2) greenish brown and greenish, respectively. The average seed weight of different varieties varied substantially (0.882–1.583 g/100 seeds). The weights of *S. aculeata* and *S. rostrata* were similar and the weights of *S.sesban* (1) and *S. sesban* (2) were much lower than that of *S. aculeata* and *S. rostrata*. The seed coat + endosperm and cotyledon proportions also varied between different *Sesbania* seeds. The seed coat + endosperm percentage ranged from 48.9 to 57.3%, with *S. aculeata* and *S. rostrata* showing the lower and *S. sesban* (1) and *S. sesban* (2) showing the higher values. A similar proportion of seed coat and endosperm to whole seed in *S. aculeata* was reported by Hussain and Khan (1962). The density (g/ml) of different seeds ranged from 0.83 to 0.88. The hydration capacity, hydration index and swelling capacity of *S. rostrata* seed were lower than other seeds. These characteristics may be a reflection of the fact that *S. rostrata* seeds have a very hard and impermeable coat and, as such, do not get hydrated easily. Therefore, *S. rostrata* seeds may require a longer time for germination, probably a reflection of their behaviour not to germinate after only a little rain. From all the observed physicochemical characteristics, *S. aculeata* seeds may be considered best among the seeds investigated.

Table 1
Physicochemical characteristics of different *Sesbania* seeds[a]

Parameters	*S. aculeata*	*S. rostrata*	*S. sesban* (1)	*S. sesban* (2)
Colour of the seed	Dark brown	Brown	Greenish brown	Greenish
Grain weight (g/100 seed)	1.583	1.581	0.951	0.882
Seed coat + endosperm (% whole seed)	48.9	51.12	57.31	56.06
Cotyledon (% whole seed)	51.1	48.88	42.69	43.94
Density (g/ml)	0.88	0.83	0.86	0.85
Hydration capacity (g/seed)	0.012	0.007	0.006	0.005
Hydration index	0.79	0.44	0.63	0.56
Swelling capacity	0.011	0.006	0.007	0.006
Swelling index	0.84	0.42	0.73	0.53

[a] Values are the average of duplicate determinations.

The proximate composition of all the studied *Sesbania* seeds is presented in Table 2. There were no wide variations between the crude protein contents (29.1–33.1%) among the different *Sesbania* seeds, except for *S. sesban* (2), which had a comparatively low crude protein (29.1%) value. Our findings of crude protein contents are higher than those reported for conventional legumes, such as chickpea (*Cicer arietinum*), mungbean (*Vigna radiata*) and cowpea (*Vigna unguiculata*; Khan, Jacobson & Eggum, 1979) and comparable to that of some of the under-utilised legumes such as *Mucuna utilis* (Ravindran & Ravindran, 1988) and *S. bispinosa* (Siddhuraju et al., 1995). The protein contents in seed coat + endosperm and cotyledons were 10.8 and 59.4% for *S. aculeata* and 10.9 and 54.5% for *S. rostrata*, respectively (Table 4). The nutritional differences of different *Sesbania* seeds could be related to varietal difference, agroclimatic conditions and fertility of the land. The crude lipid content of all the seeds is higher than other little-known *Vigna* species, such as *V. calcaratus, V. sublobata* and *V. glabrescens* (Rajaram & Janardhanan, 1992) and similar to *S. bispinosa* (Siddhuraju et al., 1995). The crude fibre contents in *S. sesban* (1) and *S. sesban* (2) are higher than those of *S. aculeata* and *S. rostrata*. The crude fibre content of all *Sesbania* seeds are much higher than those reported for commonly cultivated pulses, such as chick pea, horse gram, red gram and black gram (Premakumari, Fathima & Saraswati, 1984) and *S. bispinosa* (Siddhuraju et al., 1995). Presence of high crude fibre in food material is reported to decrease dry matter digestibility in animals. The high crude fibre content therefore provides a good indication of nutritive value of the feed material (Devendra, 1995). Because of the higher protein and lipid contents, total gross energy values in this study seem to be higher than those reported for *S. bispinosa* (Siddhuraju et al., 1995).

The amino acid compositions, along with EAA scores compared to the FAO/WHO (1990) reference pattern of

Table 2
Proximate composition of different *Sesbania* seeds (g 100 g^{-1} dry matter)[a]

Seeds	Dry matter	Crude protein	Crude lipid	Ash	Crude fibre	NFE[b]	Gross energy (kJ g^{-1})
S. aculeata	89.5	33.1	6.0	3.9	10.9	46.1	20.0
S. rostrata	90.4	32.0	4.7	4.0	11.8	47.4	19.2
S. sesban (1)	91.3	32.3	5.0	2.7	15.5	44.6	19.8
S. sesban (2)	92.2	29.1	6.0	3.3	15.8	45.8	20.0

[a] Values are means of triplicate determinations.
[b] Nitrogen-free extract calculated as 100−%(moisture + crude protein + lipid + ash + crude fibre).

Table 3
Amino acid composition and essential amino acid (EAA) scores in whole seeds of different *Sesbania* varieties

Amino acids	Amino acid composition g 16 g N^{-1}									
	S. aculeata	EAA score	*S. rostrata*	EAA score	*S. sesban* (1)	EAA score	*S. sesban* (2)	EAA score	FAO reference values[a]	Soybean[b]
Aspartic acid	7.27		7.25		5.96		6.48			11.3
Threonine	2.45	72.0	2.28	67.0	2.08	61.1	2.38	70.0	3.40	3.76
Serine	3.94		3.59		3.14		3.38			5.67
Glutamic acid	13.64		12.75		10.47		10.68			16.9
Glycine	4.76		4.75		3.63		4.06			4.01
Alanine	3.03		3.00		2.58		2.92			4.23
Valine	3.00	85.7	3.00	85.7	2.52	72.0	2.71	77.7	3.50	4.59
Cystine	0.70		0.66		0.68		0.75			1.70
Methionine	1.03	69.0	1.09	70.0	0.96	65.5	0.96	68.4	2.50[c]	1.22
Isoleucine	3.06	109.2	3.03	108.2	2.39	85.3	2.56	91.4	2.80	4.62
Leucine	5.36	81.2	5.03	76.2	4.32	65.5	4.56	69.1	6.60	7.72
Tyrosine	2.73		2.16		2.33		2.14			3.39
Phenylalanine	3.55	99.7	3.34	87.3	2.80	81.4	2.99	81.4	6.30[d]	4.84
Histidine	8.58	451.5	7.41	390.0	12.45	655.2	12.53	659.5	1.90	2.50
Lysine	4.55	78.4	4.31	74.3	3.86	63.1	4.20	72.4	5.80	6.08
Arginine	8.58		8.22		5.87		6.01			7.13
Proline	3.39		3.28		2.61		2.74			4.86
Tryptophan	1.36	123.6	1.28	116.4	1.58	143.6	1.63	148.2	1.10	1.24

[a] Data from FAO/WHO (1990).
[b] Data from Bau, Villaume, Evard, Quemener, Nicolas and Mejean (1994).
[c] Methionine + cystine.
[d] Phenylalanine + tyrosine.

different *Sesbania* whole seed proteins are shown in Table 3. In general, the amino acid composition and EAA scores of *S. aculeata* seed protein are comparatively better than all other seed proteins. The histidine contents in all the seed proteins were extremely high [4–6 times higher than the FAO/WHO (1990) reference pattern]. Compared to the FAO/WHO (1990) reference pattern, only three EAAs, such as leucine, tryptophan and histidine in *S. aculeata* and *S. rostrata* and two EAAs, e.g. histidine and tryptophan in *S. sesban* (1) and *S. sesban* (2), were adequate or higher. The rest of the EAAs in all the seeds were limiting amino acids. Like all other legumes, the sulphur-containing amino acids methionine + cystine (EAA score 65.5–70.0) in the present study, were the most deficient followed by lysine (66.5–78.5). The relatively low content of methionine and cystine in legumes has been reported by other investigators (Apata & Ologhobo, 1994). The amino acid composition in different seed fractions of *S. aculeata* and *S. rostrata* is presented in Table 4. The amino acid composition of seed coat + endosperm and cotyledon showed a similar pattern to that of whole seed with slightly higher values. Although the *Sesbania* seeds are deficient in sulphur-containing EAAs, because of their comparatively high protein content, they could still be a potential source of dietary protein in monogastrics, including fish. This EAA deficiency could be overcome by adding synthetic EAA or using these seeds in combination with other feed ingredients rich in sulphur-containing amino acids.

The fatty acid compositions of lipids in different *Sesbania* seeds are given in Table 5. The fatty acid composition shows variation with respect to specific seeds, although the pattern is somewhat similar. Palmitic, stearic, oleic, linoleic and linolenic acid are the major fatty acids, as in soybean, black gram and green gram (Salunkhe, Sathe & Reddy, 1982). The total content of unsaturated fatty acids ranged from 78.1 to 81.3%, with *S. aculeata* having the highest. These values are higher than those of *S. bispinosa* (66.7%), cow pea (68.1%) and chick pea (67.1%; Salunkhe et al., 1982; Siddhuraju et al., 1995) and slightly lower than those of field pea (85.0%), beach pea (85.2%) and green pea (83.5%; Chavan, Shahidi, Bal & Mckenzie, 1999). Total essential fatty acid content ranged from 77.2 to 80.1%. The occurrence of high concentrations of linoleic acid (55.2–62.4%) in different *Sesbania* seed is comparable to that of field pea (56% Chavan et al., 1999). The composition of fatty acids of the seeds in this study is noteworthy in its contents of unsaturated fatty acids, particularly, the essential fatty acids, which are the needed components in the diet of man and animals.

Table 6 shows the total, digestible and resistant starch and soluble sugar contents in different *Sesbania* seeds. TS ranged from 17.5% to 20.4% with *S. aculeata* showing, significantly, the highest and *S. sesban* (1) the lowest value. TS values are much lower than the reported values of 29.7–49.6% by Ologhobo and Fetuga (1986) and 37–48% by Omueti and Singh (1987) for cow pea. Bravo et al. (1999) reported a TS content in the range of 31.8–39.9% for chickpeas and green grams. Marconi, Ruggeri and Carnovale (1997) reported that the low starch contents in *Vigna* spp seeds were related to their smaller size. The percentage contents of digestible

Table 4
Crude protein and amino acid composition (g 16 g N^{-1}) in different seed fractions of *S. aculeata* and *S. rostrata*

Amino acids	*S. aculeata*		*S. rostrata*		Soybean[a]
	Seed coat + endosperm	Cotyledon	Seed coat + endosperm	Cotyledon	
Crude protein	10.8	59.4	10.9	59.5	–
Aspartic acid	7.04	7.57	7.16	7.90	11.3
Threonine	2.69	2.46	2.57	2.54	3.76
Serine	5.56	3.84	5.14	3.94	5.67
Glutamic acid	12.4	14.4	12.0	14.95	16.9
Glycine	12.4	3.3	11.7	3.52	4.01
Alanine	3.61	3.02	3.49	3.18	4.23
Valine	3.06	3.04	3.03	3.27	4.59
Cystine	0.93	0.64	0.92	0.64	1.70
Methionine	1.20	1.08	1.19	1.15	1.22
Isoleucine	2.87	3.20	2.84	3.40	4.62
Leucine	4.91	5.63	4.86	5.72	7.72
Tyrosine	2.96	2.50	3.12	2.36	3.39
Phenylalanine	3.43	3.69	3.39	3.92	4.84
Histidine	6.76	9.16	6.61	10.34	2.50
Lysine	5.00	4.64	4.77	4.76	6.08
Arginine	6.02	9.60	5.87	9.85	7.13
Proline	3.24	3.56	3.03	3.50	4.86
Trytophan	1.38	1.24	1.19	1.16	1.24

[a] Data from Bau et al. (1994).

starch in different *Sesbania* seeds were quite low (39.7–49.9%). In fact, the RS was very high (40–50% of total starch) whereas, in general, the RS content in raw legume seed is 21–44% of total starch (Lintas, Cappelloni & Ruggeri, 1992). However, Marconi et al. (1997) found high resistant starch contents (64–72% of total starch) in wild *Vigna* spp. Siddhuraju, Bravo and Sauro-Calixto (1998) reported RS contents of 12.2, 26.4 and 19.7% (% dry matter) in raw moth bean, horse bean and black gram, respectively. The lower digestible starch in *Sesbania* seeds may be due to the higher lectin and tannin contents (Deshpande & Salunkhe, 1982; Thampson & Gabon, 1987). Fish and Thompson (1991) demonstrated that the reduction of amylase activity by lectins and tannins is a result of the interaction of the lectins or polyphenols with either the enzyme itself or with its substrate. In addition, the relatively high amylose and amylase inhibitors may further decrease in vitro digestibility of starch (Dreher & Dreher, 1984). The soluble sugar contents in different *Sesbania* seeds

Table 5
Fatty acid composition of different whole *Sesbania* seed (% of total lipid)[a]

Fatty acids (%)	*S. aculeata*	*S. rostrata*	*S. sesban* (1)	*S. sesban* (2)
C12:0 (Lauric)	0.09	0.12	ND[b]	ND
C14:0 (Mystiric)	0.13	0.18	0.14	0.13
C16:0 (Palmitic)	11.1	15.3	14.1	13.3
C16:1 (Palmitoleic)	0.29	0.14	0.18	0.24
C17:1 (*cis*-10-Hepadecenic)	ND	0.16	0.33	0.45
C18:0 (Stearic)	5.79	4.88	5.52	5.30
C18:1n9c (Oleic)	19.1	16.8	13.2	10.3
C18:2n6t (Linolelaidic)	0.08	0.08	ND	ND
C18:2n6c (Linoleic)	56.0	55.2	58.9	62.4
C18:3n3 (Linolenic)	4.99	5.22	5.64	5.04
C20:0 (Arachidic)	0.73	0.89	0.18	0.21
C20:1n9 (*cis*-11-Eicosenoic)	0.24	0.14	0.22	0.62
C21:0 (Henicosanoic)	0.02	0.07	ND	ND
C20:2 (*cis*-11,14-Eicosadienoic)	0.12	ND	ND	ND
C20:3n6 (cis-8,11,14-Eicosatrienoic)	0.35	0.09	0.23	0.83
C20:4n6 (Arachidonic)	ND	ND	ND	0.36
C22:0 (Behenic)	0.37	0.47	0.89	ND
C22:1n9 (Erucic)	ND	ND	0.15	ND
C22:5n3 (*cis*-7,10,13,16,19-Docosapentaenoic)	0.07	ND	ND	ND
C22:6n3 (*cis*-4,7,10,13,16,19 (Docosapentaenoic)	ND	0.07	0.13	ND
C23:0 (Tricosanoic)	0.08	ND	ND	ND
C24:0 (Lignoceric)	0.34	0.13	0.20	0.30
C24:1 (Nervonic)	0.02	0.05	0.09	0.52
C26:0 (Cerotic)	0.07	0.03	ND	ND
Total unsaturated fatty acid	81.3	78.1	79.0	80.7
Total saturated fatty acid	18.7	21.9	21.0	19.3
Total essential fatty acids	80.1	77.2	77.7	77.7

[a] Average value of duplicate determinations.
[b] ND, not detected.

Table 6
Composition of the starch fractions and soluble sugars in different *Sesbania* seeds (g 100 g^{-1}, dry matter)[a]

Seeds	Total starch	Digestible starch[b] (% of Total starch)	Resistant starch	Soluble sugar
S. aculeata	20.4a	10.2a (49.90)	10.2b	10.6a
S. rostrata	19.7b	8.6b (43.90)	11.0a	8.83b
S. sesban (1)	17.5d	7.2c (41.32)	10.3b	8.85b
S. sesban (2)	18.6c	7.4c (39.68)	11.2a	8.93b
±S.E[c]	0.045	0.112	0.025	0.051

[a] Figures in the same column with same letters are not significantly ($P > 0.05$) different.
[b] Calculated by difference as total starch−resistance starch.
[c] Standard error of means calculated from residual mean square in the analysis of variance.

varied from 8.8 to 10.6%, with *S. aculeata* having the highest value. These values are slightly higher than the values of 5.1–6.6% for yam bean, pigeon pea and cornpea (Ene-Obong & Carnovale, 1992) and 6.0–8.3% for common legumes, such as moth bean, black gram, green gram and chick pea (Bravo et al., 1999).

Though legumes are important sources of dietary protein for both humans and animals, their acceptability and utilisation has been limited due to the presence of relatively high concentrations of some ANFs (Nowacki, 1980). Some of the ANFs, such as protease inhibitors, lectins, goitriogens, cyanogens, anti-vitamin factors and amylase inhibitors, constitute the heat-labile ANFs, whereas toxic amino acids, cyanogenic glucosides, saponins, flavones, iso-flavones, phenolics, tannins, and alkaloids form the heat-resistant ANFs (Liener, 1994). The data on ANFs of different *Sesbania* seeds and seed fractions are presented in Table 7. The total phenol contents in different *Sesbania* seeds ranged from 2.96 to 5.95% and both *S. sesban* (1) and *S. sesban* (2) showed significantly higher values. These values are higher than those reported for common legumes (Singh, 1988). Levels of tannins in different *Sesbania* seeds varied from 1.97 to 2.25% with *S. aculeata* showing the highest value. These values are relatively higher than those of commonly consumed legume seeds, such as green gram, chick pea, black gram and pigeon pea (Rao & Deosthale, 1982). The condensed tannin contents in different *Sesbania* seeds ranged from 1.89 to 5.14% and both the accessions of *S. sesban* seeds showed significantly higher values (Table 7). Tannins, in legumes, are located in the seed coat and, thus, its removal may be expected to reduce the tannin content after dehulling (Ma & Bliss, 1978). In this study the amounts of tannic acid and condensed tannins in the cotyledons were very low. Ravindran and Ravindran (1988) also reported that only a trace amount of tannin was present in the cotyledon of mucuna bean.

Tannins are known to inhibit the activities of digestive enzymes (Jambunathan & Singh, 1981) and nutritional effects of tannins are mainly related to their interaction with protein (Laurena, Van & Mendoza, 1984). Tannin-protein complexes are insoluble and the protein digestibility is decreased (Carnovale, Lugano & Marconi, 1991). However, in legumes, soaking and cooking processes are known to eliminate phenols and tannins significantly (Singh, 1988).

Data on phytic acid contents in whole seed and seed fractions of different *Sesbania* seed are presented in Table 7. The phytic acid content ranged from 1.89 to 2.37%, 0.16 to 0.36%, and 3.02 to 4.20% in whole seed, seed coat + endosperm, and cotyledon, respectively. *S. rostrata* seed had the lowest (1.89%) amount of phytic acid. The seed coat + endosperm fractions had very low

Table 7
Antinutritional factor contents in whole seed and seed fractions in different *Sesbania* seeds (g $100g^{-1}$ dry matter unless otherwise stated)[a]

Seeds	Total phenol	Tannins[b]	Condensed tannins[c]	Phytic acid	Saponin[d]	Trypsin inhibitor[e] (mg g^{-1})	Lectin[f] activity
Whole seed							
S. aculeata	3.08c	2.25a	1.89c	2.16b	0.52c	5.25b	10.2b
S. rostrata	2.96c	1.99b	2.52b	1.89c	0.50c	5.64b	20.5a
S. sesban (1)	4.85b	1.97b	5.05a	2.35a	1.46a	14.01a	20.5a
S. sesban (2)	5.95a	2.02b	5.14a	2.37a	1.26b	13.70a	20.5a
±S.E.[g]	0.020	0.015	0.059	0.008	0.020	0.141	0.000
Seed coat + endosperm							
S. aculeata	2.89c	2.49c	3.02b	0.28b	0.44b	2.24c	5.12a
S. rostrata	2.87c	3.85a	2.33c	0.16c	0.41b	2.80b	5.12a
S. sesban (1)	6.45b	2.62b	6.87a	0.30ab	1.52a	3.77a	2.56b
S. sesban (2)	6.87a	2.68b	6.98a	0.36a	1.48a	3.96a	5.12a
±S.E.	0.030	0.020	0.060	0.006	0.019	0.070	0.000
Cotyledon							
S. aculeata	1.75b	0.23b	0.11b	3.22b	0.47c	6.44c	20.5b
S. rostrata	1.90a	0.31a	0.31a	3.02c	0.48c	6.86c	41.0a
S. sesban (1)	1.36c	0.14c	0.12b	4.17a	1.28a	16.22a	41.0a
S. sesban (2)	1.45c	0.13c	0.14b	4.20a	0.96b	15.39b	41.0a
±S.E.	0.007	0.014	0.107	0.015	0.160	0.131	0.000

[a] Figures in the same column under whole seeds, seed coat + endosperm, and cotyledon, respectively, with same letters are not significantly ($P > 0.05$) different.
[b] As tannic acid equivalents.
[c] As leucocyandinin equivalents.
[d] As diosgenin equivalents.
[e] mg pure trypsin inhibited per g of sample.
[f] 1/Minimum quantity (mg) of sample per ml of the assay medium which produced haemagglutination.
[g] Standard error of means calculated from residual mean square in the analysis of variance.

contents (0.16–0.36%) of phytic acid. The accessions of *S. sesban* had significantly more phytic acid, in both whole seed and cotyledons. The phytic acid contents in whole seeds (1.89–2.37%) of *Sesbania* are within/or higher than the range of reported values for several other legumes (Reddy, Balakrishnan & Salunkhe, 1978). In many cases, phytic acid content may vary depending upon the variety and/or cultivar, climatic conditions, location, irrigation conditions, type of soil, and year during which they are grown (Miller, Youngs & Oplinger, 1980). The high levels of phytic acid are of nutritional significance as phytic acid might decrease bioavailability of minerals. The insoluble complexes, soformed, resist breakdown in the digestive tract, resulting in reduced availability of these minerals for non-ruminants. The phytic acid could, however, be substantially eliminated by processing methods, such as soaking and cooking (Reddy, Sathe & Salunkhe, 1982).

Saponins (steroid or triterpenoid) are a diverse group of compounds containing an aglycone moiety linked to one or more sugar or oliogosaccharide residues. Some of the major biological effects of saponin in animal include erythrocyte haemolysis, effects on growth, effects on feed intake, effects on nutrient absorption and effects on cholesterol and bile acid metabolism (Cheke, 1996). The saponin (steroidal) contents in different *Sesbania* seeds ranged from 0.50 to 1.46% (as diosgenin equivalents) with both *S. sesban* (1) and *S. sesban* (2) showing significantly higher values of 1.26 and 1.46%, respectively. Fenwick, Price, Tsukamoto and Okubo (1991) reported saponin values of 0.05–0.23% for mung beans and chickpeas, respectively. Earlier, Fenwick and Oakenfull (1983) reported much higher values of 0.57 and 5.6% for mung beans and chick peas, respectively. Dandanell Daveby, Aman, Betz and Obermeyer (1995) reported a soya saponin 1 content of 0.08–0.25% in dehulled seed from peas. In the present study, there was not much variation among the saponin contents in seed coat + endosperm and cotyledons.

To see the separation pattern and to verify the haemolytic nature of the saponins, the TLC analysis of saponins in different *Sesbania* seeds was carried out according to Okenfull and Sidhu (1989). Images of the plates with spray solution showed similarities between saponins from 4 different seeds with Rfs 0.55–0.96. The butanol extract was separated into seven spots. The TLC pattern of a reference Quilaja saponin (Sigma-2149) indicated presence of only three spots with Rfs 0.70–0.77. Similar Rf values of 0.60–0.85 have been reported by Khalil and El-Adawy (1994) in peas, beans and soybeans. Visualisation of separate TLC plates by spraying with diluted cattle blood (6% trypsinised erythrocytes suspension in PBS), showed two distinct haemolytic spots on each of the *Sesbania* seed extracts and the spots were larger than those of the Quilaja saponins. This clearly indicated that the saponin present in the *Sesbania* seed under investigation is haemolytic in nature.

The trypsin inhibitor activity (TIA) of different *Sesbania* seeds varied from 5.25 to 14.01 (mg of trypsin inhibited/g of DM). Both *S. sesban* (1) and *S. sesban* (2) showed about 2.5 times more TIA than *S. aculeata* and *S. rostrata*. The TIA of *S. aculeata* and *S. rostrata* were significantly lower and similar. The seed coat + endosperm had a very low TIA compared to that of cotyledons. It is very difficult to compare the enzyme inhibitory activities of legumes, as reported by different investigators, primarily because of differences in methods and units used. Smith, Van Megan, Twaalhoven and Hitchcook (1980) reported trypsin inhibitor values (using the same method as used in the present investigation) of 16.6–30 mg of trypsin inhibited/g of raw soybean meal and consumption of raw soybean meals is known to produce adverse effects in animals. A TIA range of 4.6–13.9 mg/g seed in *Vigna unguiculata* has been reported by Kochhar, Walker and Pike (1988). The TIA of *Sesbania* seeds in the present study are lower than the value reported for *S. bispinosa* (Sidduraju et al., 1995). In peas, TIA is located in cotyledons whose TIA is about 13 times higher than hulls (Valdebouze, Bergeron, Garborit & Delort-Lavel, 1980) but, in the present study, TIA is about 3–4 times higher in cotyledon. As regards the significant correlation between tannins and TIA, several authors have reported that TIA activity is due to two factors: the heat-labile protein factor, mainly present in the cotyledons, and the heat-resistant factor, located mainly in the seed coat and associated with tannins (De Lumen & Salamat, 1980; Elias, De Fernandez & Bressani, 1979).

Lectins, otherwise referred to as phytohaemagglutinins, are glycoprotein compounds which in vitro agglutinate red blood cells and in vivo can bind to receptors of epithelial cells of the intestinal mucosa and disturb the digestive process (Gatel, 1992). The lectin activity of different *Sesbania* seed ranged from 10.2 to 20.4 units (one unit is the reciprocal of minimum amount of mg sample/ml of assay medium which produced haemagglutination). Among the seeds *S. aculeata* showed significantly the lowest lectin activity. Presence of lectin activity in legume seeds has been reported by many authors (D'Mello, 1995; Gatel, 1992). The seed coat + endosperm showed lower and cotyledons higher lectin activity. In field beans, Marquardt, Mckirdy, Ward and Campbell (1975) reported that lectins were located in the cotyledons. In contrast to most dietary proteins, lectins resist proteolytic breakdown in vivo and thus substantial quantities of ingested lectins may be found intact in the digestive tract and faeces of man and animals fed jack beans (Nakata & Kimura, 1985) and *Canavalia brasiliensis* seeds (Oliveira et al., 1994). However, the lectin activity can easily be eliminated by moist heat treatments (Liener, 1994).

Most tropical legumes contain varieties of antinutritional factors with the capacity to cause profoundly adverse effects in man and animals. Nevertheless, as a whole, these antinutrients elicit relatively minor effects on food and feeding values of tropical pulses and legumes (D'Mello, 1995). Acute shortage of conventional foodstuffs for feeding of livestock in developing countries has forced planners and nutritionists to look for unconventional food resources wherein there is no competetion with humans. These unconventional food resources are rich in antinutritional factors and sometimes have low biological value. In the present study, the unconventional food resource, *Sesbania* seed, is a good source of protein (29–33%) and essential fatty acids, and may be considered as a potential dietary protein source for monogastrics, including fish. However, the main constraint to extend their use in animal feeding is the deficiency of essential amino acids and the presence of various antinutritional factors. Therefore, efforts should be made to use such unconventional foodstuffs efficiently by removal or inactivation of antinutritional factors and increasing their biological value by chemical or biotechnological means.

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