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Nutritional assessment and chemical composition of the lesser known tree legume, *Acacia leucophloea* (Roxb.) Willd.

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The seeds of *Acacia leucophloea* (Roxb.) Willd. were analysed for their proximate composition, minerals, protein fractions, seed protein amino acid profiles, fatty acid composition of lipids and antinutritional substances. Crude protein value was 26·5 g 100 g^{-1} DM. The other major nutrient contents (g 100 g^{-1}) were crude lipid, 5·13; crude fibre, 6·78; ash, 4·12 and total crude carbohydrates, 57·5. The seeds were a rich source of minerals such as Ca, Mg, P, Fe and Mn. The predominant seed protein fractions were globulins and albumins. The essential amino acids, cystine, methionine, tyrosine and phenylalanine, were low and threonine, valine, isoleucine and lysine were fairly high when compared with the FAO/WHO/UNO amino acid recommended pattern. The lipids contained high amounts of unsaturated fatty acids in which linoleic acid (51·1%) was the major fatty acid. Antinutritional substances, such as total free phenols, tannins, L-DOPA and haemagglutinating activity, were also analysed.

INTRODUCTION

Insufficient protein of good quality is a serious problem in many developing countries. Limitations of protein supplies make the hunt for new protein sources and their quality evaluation urgent. To meet the nutritional requirements of an ever-increasing population, there has been a constant search for new legumes of high protein content and suggestions for the utilization of unfamiliar ones (Pant & Tulsiram, 1969). With increasing interest in new food sources, the seeds of wild plants, including the tribal pulses, are now receiving more attention. Information available on the chemical analysis and nutritional evaluation of such tribal pulses is scant. Mature seeds of *Acacia leucophloea* are known to be eaten by the tribal people living in the hilly region of the Pune district of Maharashtra state, India (Gunjatkar & Vartak, 1982). In view of this, in the present study the proximate and mineral composition, seed protein fractionation, amino acid composition, fatty acid composition and some of the antinutritional factors of the aforesaid tribal pulses are analysed.

MATERIALS AND METHODS

Mature seeds of *Acacia leucophloea* (Roxb.) Willd. were collected from Bharathiar University Campus, Coimbatore, Tamil Nadu, India.

The moisture content of the seeds was estimated by taking 50 transversely cut mature and dry seeds, weighing the sample before and after drying in an oven at 80°C for 24 h and cooling in a desiccator. The nitrogen content was estimated by the micro-Kjeldahl method (Humphries, 1956) and the crude protein content was calculated by multiplying the Kjeldahl N by 6·25. The contents of crude lipid, crude fibre and ash were determined by AOAC (1970) methods. The nitrogen-free extractives (NFE) were calculated by difference (100 − crude protein + crude lipid + crude fibre + ash in percentage) (Muller & Tobin, 1980). The energy content of the seed was determined by multiplying the crude protein, crude lipid and total crude carbohydrates by the factors 4, 9 and 4, respectively (Osborne & Voogt, 1978).

Seed samples were triple acid-digested. By using an atomic absorption spectrophotometer (Perkin Elmer, Model-5000) sodium, potassium, calcium, magnesium, iron, copper, zinc and manganese were analysed (Issac & Johnson, 1975). Phosphorus was estimated colorimetrically (Dickman & Bray, 1940).

The total proteins were extracted from the air-dried seed flour following the method of Rajaram & Janardhanan (1990) and purified by cold 200 g $litre^{-1}$ TCA precipitation. Seed protein fractions, albumins and globulins, were extracted following the method of Murray (1979). The residual pellet was extracted with 80% (v/v) ethanol 1 : 10 (w/v) overnight and centrifuged at 20 000 × *g* for 20 min. The supernatant thus obtained was designated as prolamin. From the above

residual pellet the glutelin fraction was extracted with 0·2 N NaOH 1 : 15 (w/v) overnight and centrifuged at 20 000 × *g* for 20 min. The supernatant containing glutelin was saved. The fractions so obtained were precipitated with cold 200 g litre^{-1} trichloroacetic acid and centrifuged at 20 000 × *g* for 20 min at 4°C. The purified protein pellets were dissolved separately in 0·25 N NaOH and protein content was estimated by the Lowry *et al.* (1951) method.

The purified total seed proteins were acid-hydrolysed with 6 N HCl at 110°C for 24 h *in vacuo*. After removing the acid by flash evaporation, the dried residue was dissolved in citrate buffer (pH 2·2) and analysed in a LKB-Biochrome Automated Amino Acid Analyser, Model-4151-Alpha plus. The essential amino acid score was calculated (Bhanu *et al.*, 1991) and tabulated.

The total lipids were extracted from the seed flour according to the method of Folch *et al.* (1957) using chloroform and methanol 2 : 1 (v/v). Fatty acid methyl esters were prepared from the total lipids by the Metcalfe *et al.* (1966) method. Fatty acid analysis was performed with a gas chromatograph (Shimadzu, Model-RIA) equipped with a flame ionization detector and glass column (2 m × 3 mm) packed with 1% diethylene glycol succinate on chromosorb W (silanized 80/100 mesh). The carrier gas was nitrogen, at a flow rate of 32 ml min^{-1}. The column temperature was 190°C. Peaks were identified by comparison with authentic standards and the relative percent of each fatty acid was determined from integrated peak areas.

The antinutritional factors, such as total free phenols (Bray & Thorne, 1954), tannins (Burns, 1971) and non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine) (Brain 1976), were analysed. The haemagglutinating activity of the albumin and globulin fractions of seed proteins (Liener, 1976) was also assayed.

RESULTS AND DISCUSSION

The proximate composition of *Acacia leucophloea* is given in Table 1. The contents of crude protein and crude lipids of *A. leucophloea* are found to be higher than the commonly cultivated legumes such as black gram, green gram (Kadwe *et al.*, 1974; Gupta & Wagle, 1978); chickpea (Jambunathan & Singh, 1980), pigeonpea (Nwokolo, 1987) and some *Acacia* species (Kapoor *et al.*, 1973; Rajaram & Janardhanan, 1991). Due to their lipid-rich nature, the seeds of *A. leucophloea* had a higher energy value than the commonly cultivated pulse crops (Kuzayli *et al.*, 1966).

Mineral analysis (Table 1) reveals that the contents of calcium, phosphorus and iron seem to be higher than those of the cultivated legumes (Kuzayli *et al.*, 1966) and different ricebean varieties (Singh *et al.*, 1980). Magnesium, iron and manganese are present in more than adequate levels when compared with the Recommended Dietary Allowances (NRC/NAS, 1980). Potassium is the predominant mineral in *A. leucophloea*.

Table 1. Proximate and mineral composition of *Acacia leucophloea* seeds[a]

Component	Proximate composition (g 100 g^{-1} seed flour)	Mineral	Mineral composition (mg 100 g^{-1} seed flour)
Moisture	5·92 ± 0·31	Na	32 ± 0·1
Crude protein (Kjeldahl N × 6·25)	26·5 ± 2·77	K	1 020 ± 1·2
Crude lipid	5·13 ± 0·17	Ca	314 ± 1·0
Crude fibre	6·78 ± 0·49	Mg	261 ± 0·6
Ash	4·12 ± 0·35	P	474 ± 1·2
Nitrogen-free extractives or total crude carbohydrates	57·47	Fe	22 ± 0·3
Calorific value		Cu	2 ± 0·1
kcal kg^{-1} DM	382	Zn	6 ± 0·1
		Mn	4 ± 0·1

[a] All values are means of triplicate determinations expressed on a dry weight basis.
± Denotes the standard error.

Seed protein fractionation of *A. leucophloea* (Table 2) shows that the globulins and albumins constitute the major bulk of the seed proteins, as in many legumes reported earlier (Boulter & Derbyshire, 1976; Murray, 1979; Janardhanan & Lakshmanan, 1985; Rajaram & Janardhanan, 1991), and the percentage distributions of both the aforesaid protein fractions are comparable to that of *Vigna sesquipedalis* (Rajaram & Janardhanan, 1990).

The amino acid profiles of the purified seed proteins are given in Table 3. The potential food value of the seed proteins (as a source of amino acids) can be justified by comparison with the FAO reference pattern (FAO/WHO/UNO, 1985). The sulphur-containing amino acids, cystine and methionine, of seed proteins are the most conspicuous limiting amino acids, as in some *Acacia* species (Prakash & Misra, 1987), *Acacia catechu* (Rajaram & Janardhanan, 1991) and *Cassia* species (Ramachandra & Monteiro, 1990; Bhanu *et al.*, 1991). Phenylalanine and tyrosine contents are at lower, whereas the other essential amino acids such as threonine, valine, isoleucine and lysine, are at higher than adequate concentrations when compared with recommended amino acid pattern (FAO/WHO/UNO, 1985).

Table 2. Seed protein fractionation of *Acacia leucophloea*[a]

Protein fraction	g 100 g^{-1} seed flour	g 100 g^{-1} seed protein
Total protein (true protein)	17·3 ± 0·81	100
Albumins	4·34 ± 0·04	25·0
Globulins	10·1 ± 0·58	58·3
Prolamins	1·10 ± 0·02	6·35
Glutelins	1·80 ± 0·01	10·4

[a] All values are averages of three independent experiments expressed on a dry weight basis.
± Denotes the standard error.

Table 3. Amino acid profile of acid-hydrolysed, purified total seed proteins of *Acacia leucophloea*

Amino acid	g 100 g^{-1} protein	Recommended FAO/WHO/UNO (1985) pattern for 2–5 years old (g 100 g^{-1} protein)	Essential amino acid score
Glutamic acid	14·6		
Aspartic acid	10·2		
Serine	5·81		
Threonine	4·32	3·6	120
Proline	5·60		
Alanine	6·51		
Glycine	8·11		
Valine	4·84	3·7	131
Cystine + Methionine	1·12 + 0·47	2·6	61
Isoleucine	3·59	3·0	120
Leucine	6·96	7·0	99
Tyrosine + Phenylalanine	1·71 + 4·43	6·6	93
Lysine	6·46	6·2	104
Histidine	2·20		
Tryptophan	ND		
Arginine	5·43		

ND—not detected.

The data on fatty acid composition of the total lipids of *A. leucophloea* (Table 4) indicate that linoleic acid is the predominant fatty acid accounting for about 51·1% of the total fatty acids. The unsaturated fatty acids (76·0%) constitute the bulk of fatty acids as in the case of certain edible legumes such as *Phaseolus vulgaris* and *Vigna unguiculata* (Omogbai, 1990) and *Psophocarpus tetragonolobus* (Rao & Belavady, 1979). The occurrence of a high concentration of linoleic acid in *A. leucophloea* is comparable to that of other commonly consumed legumes such as soybean, chickpea and horse gram (Salunkhe *et al.*, 1982) and *Cajanus indicus* and *Pisum sativum* (Choudhury & Rahman, 1973).

Although the protein content of legume grains is high (20–25%) its digestibility is somewhat low. This has been attributed to the presence of antinutritional substances such as trypsin inhibitors, haemagglutinins, cyanogenic glucosides, saponins, flatulence factors and phytates (Liener, 1980). The content of total free phenols is found to be lower when compared with some tribal pulses such as *Mucuna utilis* (Janardhanan & Lakshmanan, 1985), *Acacia catechu* (Rajaram & Janardhanan, 1991) and *Mucuna pruriens* (Mary Josephine & Janardhanan, 1992). The level of tannins in *A. leucophloea* seed is insignificant when compared with the cultivated legume crops such as green gram, cowpea, pigeonpea and black gram (Khan *et al.*, 1979; Rao & Deosthale, 1982). The albumin fraction of *A. leucophloea* exhibits weak agglutination with the erythrocytes of 'O' blood group specifically as in *Mucuna pruriens* (Lucknow germplasm) (Mary Josephine & Janardhanan, 1992). While the globulin fraction weakly agglutinates erythrocytes from A and B blood groups, it exhibits strong agglutination with 'O' blood group erythrocytes.

Table 4. Fatty acid composition of *Acacia leucophloea* seed flour lipids

Fatty acid	Area (%)
Palmitic acid (C16:0)	17·0
Stearic acid (C18:0)	5·80
Oleic acid (C18:1)	22·7
Linoleic acid (C18:2)	51·1
Linolenic acid (C18:3)	2·13
Others (unidentified)	1·25

Table 5. Antinutritional factors of *Acacia leucophloea* seeds

Component	g 100 g^{-1} seed flour
Total free phenols[a]	0·90 ± 0·03
Tannins[a]	0·68 ± 0·02
L-DOPA[a]	NP

	Phytohaemagglutinating activity[b]	
Name of protein fraction	Erythrocytes from human blood group	Haemagglutinating activity
Albumin	A	—
Albumin	B	—
Albumin	O	+
Globulin	A	+
Globulin	B	+
Globulin	O	++

[a] Values are mean of triplicate determinations expressed on a dry weight basis.
(NP = not present.)
[b] Values of two independent experiments.
—, No clumping, pellet disperses easily.
+, Some clumping, pellet partially disperses.
++, No dispersion of pellet.
± Denotes standard error.

The observations made in the present study reveal that the seeds of *A. leucophloea* are rich in crude protein, crude lipid, some minerals and most of the essential amino acids and unsaturated fatty acids. Since the antinutritional factors (Table 5) detected in the present study are heat-labile, they can be eliminated easily by the cooking process. Taking into account the overall nutritional qualities of *A. leucophloea*, it may be adopted as an alternative cheap source of protein for the economically weaker sections of the population in the developing third world.

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REFERENCES

AOAC (1970). *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington DC.

Bhanu, N. V., Ramachandra, G. & Monteiro, P. V. (1991). Evaluation of protein isolate from *Cassia uniflora* as a source of plant protein. *J. Sci. Food Agric.*, **54**, 659–62.

Boulter, D. & Derbyshire, E. (1976). The general properties, classification and distribution of plant proteins. In *Plant Proteins*, ed. G. Norton. Butterworths, London, UKL, pp. 3–24.

Brain, K. R. (1976). Accumulation of L-DOPA in cultures from *Mucuna pruriens*. *Plant. Sci. Lett.*, **7**, 157–61.

Bray, H. G. & Thorne, W. V. (1954). Analysis of phenolic compounds. *Meth. Biochem. Anal.*, **1**, 27–52.

Burns, R. R. (1971). Methods for estimation of tannin in grain. *Sorghum. Agron. J.*, **63**, 511–12.

Choudhury, K. & Rahman, M. M. (1973). Fatty acids in diffrent pulses produced and consumed in Bangladesh. *J. Sci. Food. Agric.*, **24**, 471–3.

Dickman, S. R. & Bray, R. H. (1940). Colorimetric determination of phosphate. *Ind. Eng. Chem. Anal. Ed.*, **12**, 665–8.

FAO/WHO/UNO (1985). Protein and energy requirements. Tech. Rep. Ser. No. 724, United Nations Organisation, Rome, Italy.

Folch, J., Lees, M. & Solane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–506.

Gunjatkar, N. & Vartak, V. D. (1982). Enumeration of wild edible legumes from Pune district, Maharashtra state. *J. Econ. Tax. Bot.*, **3**, 1–9.

Gupta, K. & Wagle, D. S. (1978). Proximate composition and nutritive value of *Phaseolus mungoreous*. A cross between *Phaseolus mungo* and *Phaseolus aureus*. *J. Food. Sci. Technol.*, **15**, 34–5.

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

Issac, R. A. & Johnson, W. C. (1975). Collaborative study of wet and dry techniques for the elemental analysis of plant tissue by Atomic Absorption Spectrophotometer. *J. AOAC*, **58**, 436.

Jambunathan, R. & Singh, U. (1980). Studies on Desi and Kabuli chickpea (*Cicer arietinum*) cultivars. 1. Chemical composition. In *Proc. Int. Workshop on Chickpea Improvement*, 28 Feb.–2 March 1979. ICRISAT, Hyderabad, India.

Janardhanan, K. & Lakshmanan, K. K. (1985). Studies on the tribal pulse, *Mucuna utilis*: Chemical composition and antinutritional factors. *J. Food Sci. Technol.*, **22**, 369–71.

Kadwe, R. S., Thakare, K. K. & Badhe, N. N. (1974). A note on the protein content and mineral composition of twenty five varieties of pulses. *Ind. J. Nutr. Dieter.*, **11**, 83–5.

Kapoor, V. P., Raina, R. M., Khan, P. S. H. & Farooqi, M. I. H. (1973). Chemical analysis of seeds—Part II. From another 60 leguminous species. *Sci. & Cult.* **39**, 444–6.

Khan, A. M., Jacobsen, I. & Eggum, O. B. (1979). Nutritive value of some improved varieties of legumes. *J. Sci. Food. Agric.*, **30**, 395–400.

Kuzayli, M. V., Cowan, J. W. & Sabry, Z. I. (1966). Nutritive value of middle eastern food stuffs. II. Composition of pulses, seeds, nuts and cereal products of Lebanon. *J. Sci. Food. Agric.*, **17**, 82–4.

Liener, I. E. (1976). Phytohaemagglutinins (phytolectins). *Ann. Rev. Plant. Physiol.*, **27**, 291–319.

Liener, I. E. (1980). *Toxic Constituents of Plant Foodstuffs*. Academic Press, New York, USA.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

Mary Josephine, R. & Janardhanan, K. (1992). Studies on chemical composition and antinutritional factors in three germplasm seed materials of the tribal pulse, *Mucuna pruriens* (L.) DC. *Food. Chem.*, **24**, 1126–30.

Metcalfe, L. D., Schemitz, A. A. & Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.*, **38**, 514–15.

Muller, H. G. & Tobin, G. (1980). *Nutrition and Food Processing*. Groom Helm Ltd, London, UK.

Murray, D. R. (1979). The seed proteins of Kowhai, *Sophora microphylla* AIT. *Z. Pflanzenphysiol.*, **93**, 423–8.

NRC/NAS (1980). *National Research Council Committee on Dietary Allowances. Recommended Dietary Allowances* (9th edn). National Academy of Sciences Press, Washington DC.

Nwokolo, E. (1987). Nutritional evaluation of pigeonpea meal. *Plant. Foods. Hum. Nutr.*, **37**, 283–90.

Omogbai, F. E. (1990). Lipid composition of tropical seeds used in the Nigerian diet. *J. Sci. Food. Agric.*, **50**, 253–5.

Osborne, D. R. & Voogt, P. (eds) (1978). Calculation of calorific value. In *The Analysis of Nutrients in Foods*, Academic Press, New York, USA, pp. 239–40.

Pant, R. & Tulsiram,. D. R. P. (1969). Solubility, amino acid composition and biological evaluation of protein isolated from leguminous seeds. *J. Agric. Food. Chem.*, **17**, 361–6.

Prakash, D. & Misra, P. S. (1987). Protein and amino acid composition of some wild leguminous seeds. *Plant Foods. Hum. Nutr.*, **38**, 61–5.

Rajaram, N. & Janardhanan, K. (1990). Chemical analysis and nutritional evaluation of certain under exploited *Vigna* spp. *Food Sci. Nutr,*, **42**, 213–21.

Rajaram, N. & Janardhanan, K. (1991). Studies on the under exploited tree pulses, *Acacia catechu* Willd., *Parkinsonia aculeata* L. and *Prosopis chilensis* (Molina) Stunz. Chemical composition and antinutritional factors. *Food Chem.*, **42**, 265–73.

Ramachandra, G. & Monteiro, P. V. (1990). Preliminary studies on the nutrient composition of *Cassia sericea* Sw—An unexploited legume seed. *J. Food Comp. Anal.*, **3**, 81–7.

Rao, P. U. & Belavady, B. (1979). Chemical composition and biological evaluation of Goa beans (*Psophocarpus tetragonolobus*) and their tubers. *J. Plant Foods*, **3**, 169–74.

Rao, P. U. & Deosthale, Y. G. (1982). Tannin content of pulses varietal differences and effects of germination and cooking. *J. Sci. Food. Agric.*, **33**, 1013–16.

Salunkhe, D. K., Sathe, S. K. & Reddy, N. R. (1982). Legume lipids. In: *Chemistry and Biochemistry of Legumes*, ed. S. K. Arora. Oxford & IBH, New Delhi, India, pp. 51–109.

Singh, S. P., Misra, B. K., Chandel, K. P. S. & Pant, K. C. (1980). Major food constituents of ricebean (*Vigna umbellata*). *J. Food Sci. Technol.*, **17**, 238–40.