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The biochemical composition and nutritional potential of the tribal pulse, *Alysicarpus rugosus* (Willd.) DC

P. Siddhuraju, K. Vijayakumari & K. Janardhanan

Seed Physiology Laboratory, Department of Botany, Bharathiar University, Coimbatore—641 046, India

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Seeds of the tribal pulse, *Alysicarpus rugosus* (Willd.) DC, were analysed for proximate composition, seed protein fractions, amino acid composition, fatty acid profiles, minerals and antinutritional factors. The seed materials 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. Though the level of the essential amino acid, threonine, was deficient, all the other essential amino acids, especially sulpho-amino acids, together, of the seed proteins, were relatively abundant when compared with those of soybean seed proteins. The seed lipids contained a large proportion of unsaturated fatty acids with linoleic acid as the predominant fatty acid. The seeds were rich in minerals such as Ca, Mg, Zn, Mn, Fe and Cu. Antinutritional substances, such as total free phenols, tannins, L-DOPA and haemagglutinating activity, were also analysed/assayed.

INTRODUCTION

Plant foods such as cereals and legumes have consistently been listed as the major potential sources of dietary protein for feeding the world of tomorrow and research efforts are being directed to this area to identify and evaluate underexploited food sources (Egbe & Akinyele, 1990). Wild plants may contain useful amounts of nutrients, as was indicated by an American study of seeds from a large number of species not usually cultivated for food. They are found to contain about 27·9% crude protein and 27% oil (Van Etten *et al.*, 1967). Recently certain Indian tribal pulses have been investigated to decipher their biochemical composition and to evaluate their nutritional potential (Janardhanan & Lakshmanan, 1985; Prakash & Misra, 1987, 1988; Rajaram & Janardhanan, 1991*a,b,c*; Janardhanan & Nalini, 1991; Mary Josephine & Janardhanan, 1992; Arulmozhi & Janardhanan, in press). In view of this, in the present study an attempt was made to understand the biochemical composition and assess the nutritional value of the tribal pulse, *Alysicarpus rugosus* (Willd.) DC. The seeds are eaten by the tribal people living in the hilly region of the Pune district, Maharashtra, India (Gunjatkar & Vartak, 1982).

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MATERIALS AND METHODS

Only the mature seeds were collected from *Alysicarpus rugosus* plants at the same time in the same place (Mallur Village of Salem district, Tamil Nadu, India). The seeds were dried in the open (sun only) after collection for 2 days. The moisture content was determined by drying 50 transversely cut seeds in an oven at 80°C for 24 h and it is expressed on a percentage basis. The seeds were powdered separately in a Wiley Mill to 60 mesh size. 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 the factor 6·25. The contents of crude lipid, crude fibre and ash were estimated by 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 the factors 4, 9 and 4, respectively (Osborne & Voogt,

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 cold 20% TCA. The albumin and globulin fractions of seed proteins were extracted following the method of Basha & Beevers (1975). From the left out pellet the prolamin fraction was extracted by treating it with 70% ethanol 1:5 (w/v) overnight; after centrifugation, the supernatant containing 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 20000 *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 citrate buffer (pH 2·2). Known aliquots were analysed in a LKB-Biochrome Automated Amino Acid Analyser, Model 4151-Alpha Plus. The total lipids were extracted from the seed flour with a mixture of chloroform and methanol in the 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.

RESULTS AND DISCUSSION

The crude protein and crude lipid contents in the present investigation for the seeds of *Alysicarpus rugosus* (Table 1) are higher than the commonly consumed pulse crops in India such as black gram, green gram, pigeonpea, chickpea and cowpea reported earlier (Gupta & Wagle, 1978; Jambunathan & Singh, 1980, 1981; Nwokolo & Oji, 1985; Nwokolo, 1987). The seed protein fractionation of *A. rugosus* (Table 2) shows that the albumins and globulins constitute the major bulk of the seed proteins as in most of the legumes reported earlier (Boulter & Derbyshire, 1976; Murray, 1979; Janardhanan & Lakshmanan, 1985; Rajaram & Janardhanan, 1991*a*; Mary Josephine & Janardhanan, 1992).

Table 1. Proximate and mineral composition of *Alysicarpus rugosus* seed[a]

	Proximate composition (g 100 g^{-1} seed flour)		Mineral composition (mg 100 g^{-1} seed flour)
Moisture	6·27 ± 0·7	Na	53·3 ± 0·02
Crude protein (Kjeldahl $N \times 6{\cdot}25$)	27·1 ± 1·8	K	960 ± 0·08
Crude lipid	14·0 ± 1·1	Ca	341 ± 0·12
Crude fibre	4·25 ± 0·6	Mg	307 ± 0·04
Ash	3·61 ± 0·4	P	491 ± 0·28
Nitrogen free extractives (NFE)	51·1	Fe	22·0 ± 0·04
Calorific value (kcal 100 g^{-1} DM)	439	Cu	3·68 ± 0·07
		Zn	7·82 ± 0·08
		Mn	4·27 ± 0·26

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

The amino acid profiles of the purified seed proteins are given in Table 3. The most noteworthy feature of this profile is that the sulpho-amino acids, cystine and methionine, together seem to be higher than most of the legumes including soyabean reported earlier (Boulter & Derbyshire, 1976; Prakash & Misra, 1988; Laurena *et al.*, 1991; Rajaram & Janardhanan, 1991*a*,*b*,*c*; Mary Josephine & Janardhanan, 1992). The seed proteins are markedly deficient only in threonine. The concentrations of the other essential amino acids, valine, isoleucine, leucine, tyrosine, phenylalanine and lysine, are found to be relatively high when compared with the WHO requirement pattern (FAO/WHO, 1973) and comparable to those of soyabean (Boulter & Derbyshire, 1976). The fatty acid profile (Table 4) reveals that *A. rugosus* seeds contain a large proportion of unsaturated fatty acids as in the case of some edible legumes such as *Phaseolus vulgaris* and *Vigna unguiculata* (Omogbai, 1990), *Tylosema esculentum* (Bower *et al.*, 1988) and *Psophocarpus tetragonolobus* (Rao & Belavady, 1979). Linoleic acid is found to be the predominant fatty acid, accounting for 45·6% of the total fatty acids. The high

Table 2. Data on seed total (true) proteins and protein fractionation of *Alysicarpus rugosus*

Name of the fraction	Seed flour[a] (g 100 g^{-1})	Seed protein (g 100 g^{-1})
Total protein (true protein)	21·3 ± 0·07	100
Albumins	4·79 ± 0·05	22·5
Globulins	13·4 ± 0·13	62·7
Prolamins	1·21 ± 0·09	5·68
Glutelins	1·95 ± 0·07	9·15

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

Table 3. Amino acid profiles of acid-hydrolysed, purified total seed proteins of *Alysicarpus rugosus*

Amino acid	Protein[a] (g 100 g^{-1})	FAO/WHO (1973) requirement pattern (g 100 g^{-1} protein)	Soyabean[b] (g 100 g^{-1} protein)
Glutamic acid	11·7 ± 0·08	—	—
Aspartic acid	9·52 ± 0·16	—	—
Serine	4·12 ± 0·09	—	—
Threonine	2·20 ± 0·13	4·0	3·9
Proline	3·31 ± 0·15	—	—
Alanine	6·17 ± 0·21	—	—
Glycine	7·59 ± 0·16	—	—
Valine	5·54 ± 0·13	5·0	4·8
Cystine	2·53 ± 0·07	3·5	1·3
Methionine	0·91 ± 0·04		1·3
Isoleucine	5·12 ± 0·10	4·0	4·5
Leucine	8·27 ± 0·07	7·0	7·8
Tyrosine	2·65 ± 0·08	6·0	3·1
Phenylalanine	5·53 ± 0·14		4·9
Lysine	6·13 ± 0·11	5·5	6·4
Histidine	5·86 ± 0·09	—	—
Tryptophan	ND	1·0	1·3
Arginine	3·55 ± 0·13	—	—

[a] All values are means of triplicate analyses.
[b] Boulter & Derbyshire (1976).
± denotes standard error.
ND, not detected.

concentration of linoleic acid in *A. rugosus* is comparable with that of other commonly consumed legumes like soyabean, chickpea and horse gram (Salunkhe *et al.*, 1982).

The data on the mineral analysis reveal that the seeds are a rich source of Ca, Mg, Cu, Fe and Zn when compared with the domesticated pulses (Meiners *et al.*, 1976). The contents of Mn, Fe and Cu seem to be higher than the RDA (Recommended Dietary Allowances) values (NRC/NAS, 1980).

Though food legumes are important sources of dietary protein in the developing countries, their acceptability and utilization has been limited due to the presence of relatively high concentrations of certain antinutritional factors (Nowacki, 1980). Protease inhibitors, lectins, tannins, cyanogens, goitrogens, antivitamins and amylase inhibitors form the heat-labile antinutritional factors (Liener, 1980) and toxic amino acids, alkaloids and cyanogenic glucosides constitute the heat-stable antinutritional factors (Nowacki, 1980). The data on antinutritional factors are presented in Table 5. The content of total free phenols present in the seeds of *A. rugosus* appears to be low when compared with *Phaseolus lunatus* (Egbe & Akinyele, 1990), *Cajanus cajan* (Singh, 1988) and *Cyamopsis tetragonaloba* (Kaushal & Bhatia, 1982). Tannins interact strongly with proteins to form insoluble complexes (Bressani & Elias, 1979) and they also inhibit the activities of digestive enzymes (Jambunathan & Singh, 1980). The level of tannins present in the seeds of *A. rugosus* is insignificant when compared with the commonly consumed legume seeds such as green gram, cowpea, pigeonpea and black gram (Khan *et al.*, 1979; Rao & Deosthale, 1982). The concentration of the non-protein amino acid, L-DOPA, in *A. rugosus* has been found to be relatively very low when compared with the other tribal pulses reported earlier from our laboratory (Janardhanan & Lakshmanan, 1985; Janardhanan & Nalini, 1991; Rajaram & Janardhanan, 1991*a*; Arulmozhi & Janardhanan, in press).

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 and B blood groups specifically. Similarly, the albumin fraction agglutinates erythrocytes specifically from human B and O blood groups in *Entada scandens*

Table 4. Fatty acid composition (%) of *Alysicarpus rugosus* seed lipids[a]

Fatty acid	
C16:0 (Palmitic acid)	15·47 ± 0·17
C18:0 (Stearic acid)	4·81 ± 0·13
C18:1 (Oleic acid)	23·73 ± 0·23
C18:2 (Linoleic acid)	45·61 ± 0·26
C18:3 (Linolenic acid)	5·72 ± 0·18
Others (unidentified)	4·66 ± 0·21

[a] All values are means of triplicate analyses.
± denotes standard error.

Table 5. Data on antinutritional factors of *Alysicarpus rugosus* seeds

Component	Seed flour (g 100 g^{-1})
Total free phenols	0·48 ± 0·02[a]
Tannins	1·17 ± 0·05[a]
L-DOPA	0·65 ± 0·01[a]
Phytohaemagglutinating activity[b]	

Name of the protein fraction	Erythrocytes from the human blood group	Haemagglutinating activity
Albumins	A	+
Albumins	B	+
Albumins	O	−
Globulins	A	++
Globulins	B	++
Globulins	O	++

[a] Denotes mean of triplicate determinations expressed in percentage on dry weight basis.
[b] Values of two independent experiments.
+, some clumping, pellet disperses partially.
−, no clumping, pellet disperses easily.
++, no dispersion of pellet.
± denotes the standard error.

(Janardhanan & Nalini, 1991) and O blood group only in *Mucuna pruriens* (Lucknow germplasm) (Mary Josephine & Janardhanan, 1992).

The observations made in the present study show that the seeds of *Alysicarpus rugosus* are rich in crude protein, crude lipid, most of the essential amino acids, unsaturated fatty acids and some minerals. The anti-nutritional factors (total free phenols, tannins and lectins) except L-DOPA are heat-labile and can be eliminated by a cooking process. The tribal people in the Pune district consume a diet consisting of cultivated cereals along with some wild legumes such as *Mucuna monosperma*, *Abrus precatorious*, *Vigna vexillata*, *Bauhinia vahlii* and *Alysicarpus rugosus*. All the above mentioned legumes except *Alysicarpus rugosus* are either roasted or boiled before consumption, whereas the seeds of *Alysicarpus rugosus* are cooked and eaten. In view of this, it is suggested that the consumption of seeds of *A. rugosus*, in preference to the other legumes mentioned above, is advocated, since the seed proteins of this tribal pulse seem to be rich in most of the essential amino acids including sulphur-containing amino acids.

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