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Nutritional and Antinutritional Properties of the Underexploited Wild Legume *Rhynchosia bracteata* Benth

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Abstract

The nutritional composition and antinutritional factors of *Rhynchosia bracteata* Benth. underexploited tribal pulses locally known as Advi Chekkudu in Telugu, used as food by local tribals in the Rollapenta forest, Kurnool district, Nallamalais of Andhra Pradesh. The mature seed samples were analysed for proximate composition, total (true) seed protein fractions, amino acid composition, fatty acids profile, mineral and antinutritional factors. The investigated seed samples contained higher amount of crude protein, crude fat, ash and nitrogen free extractives constitute 20.18, 6.16, 6.12 and 61.31 % respectively. The calorific value of the seed weight 100 g dry seed material was 366.86 K.cal. The essential amino acids, leucine, tyrosine and phenylalanine, were present in relatively large quantities. The fatty acid profiles revealed that the seed lipids contained higher concentrations of palmitic and linoleic acids. The seeds are rich in magnesium, iron potassium and phosphorous. Antinutritional factors such as total free phenols, (3.76 %) tannins (0.29%), L -DOPA (0.51%), hydrogen cyanide (0.066%) and phytic acid (0.18%) are present in variable quantities. This paper presents the chemical composition and nutritional potential of *Rhynchosia bracteata* seeds with regard to its suitability as a regular component in human diet.

Key words. *Rhynchosia bracteata*, Proximate composition. Amino acids, Antinutritional factors, Fatty acids, Protein fractions. Hemagglutinating activity

Introduction

The role of seed legumes in the diets of animal and man in developing countries is well documented (Oke *et al.*, 1995). Knowledge of the chemical composition of foods is the key recommendations of foods for providing a balanced nutritional diet. A table of food composition is used at the macro-level in planning food demand and supply, and at the micro level in developing prescribed diet as well as in determining and correcting the nutritional values of a given diet (Southgate, 1974). There is serious problem of inadequate availability and consumption of protein foods in India due to both population explosion and urbanization. There is urgent need for putting forth efforts towards finding alternate and cheaper sources of proteins. In spite of an urgent need to meet the nutritional requirements of the ever increasing populations, the available cheap protein resources have remained relatively unexplored (Murhty *et al.*, 2003). With increasing in new food sources, the seeds of wild plants, including the tribal pulses, are receiving more attention because they are well adopted to adverse environmental conditions, highly resistant to disease and pests, and exhibit good nutritional qualities (Maikhuri, *et al.,* 1991).

Some of the wild nuts and seeds are commonly used as proteinaceous foods in different parts of the world (Amubode and Fetuga, 1983). There are some 28 wild legumes commonly consumed by different tribal sects of India. (Arora, *et al.*, 1980). However, most of the Indian legumes remain uninvestigated biochemically and nutritionally. The tribal communities living in the forests of Eastern Ghats in the vegetation of tropical moist deciduous and semi-evergreen forests, collect the seeds of wild legumes randomly in the vicinity of the forests. The seeds were soaked in water and consume the seed meal after boiling and decanting for four to twelve times. With this back ground the present study was under taken.

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Material and Methods

The seeds of *Rhynchosia bracteata* Benth. were collected from the dry deciduous forests near Rollapenta, Nallamalias of Andhra Pradesh, near the vicinity of tribal hamlets and were used for analysis. The accessions were botanically identified by using botanical keys of Pullaiah and Murthy, (2001) and were deposited in the herbarium, Department of Biotechnology, Montessori Mahila Kalasala, Vijayawada.

The moisture content was determined by drying 100 transversely cut seeds in an oven at 80^{o} C for 24 hours and was expressed on a percentage basis. The oven dried and air-dried seeds were powdered separately in a Kemi Mill, for 60-mesh size.

The fine powder obtained was used for further analysis. The total carbohydrate content was estimated by Conrad and Palmer (1976). The crude protein content was calculated by multiplying the factor of 6.25 time's percent Kjeldahl nitrogen following Humphries (1956) method. The crude fibre content was determined according to the methods described by Eggum and Beame (1983). The contents of nitrogen free extractives (NFEs), crude fat and ash were estimated by AOAC methods (1970). The energy content was determined by multiplying the percentage of crude protein, crude fat and nitrogen free extractives by factors of 4, 9, and 4 respectively (Osborne and Voogt, 1978). Data represents the means of 3 replicates.

The total true proteins were extracted by the method of Rajaram and Janardhanan (1990). The extracted protein was purified by precipitation with 20% cold TCA and determined by the method of Lowry *et al.*, (1951). The seed protein fractions, albumins and globulins were extracted following the method of Murray (1979), from the remaining pellet; the prolamine protein fraction was extracted by treating it with 80% ethanol (1:5 w/v) overnight. After centrifugation at 20, 000 x g for 20 minutes the supernatant containing prolamine was air dried and dissolved in 0.1 N Na OH. The remaining pellet was extracted with 0.4 N Na OH (1:10 w/v) overnight and centrifuged at 20, 000 x g for 20 minutes. The supernatant thus obtained was assumed to be the glutelin protein.

The purified total seed proteins were acid hydrolyzed with 6 N HCl at 100^{o}C for 24 h in vacuo. After evaporation, the dried residue was dissolved in citrate buffer (pH 2.2), known aliquots were analyzed in LKB-Biochrome Automated Amino acid Analyzer. The essential amino acids were scored and compared with FAO, WHO, UNO (1985) reference pattern.

The total lipids from the seed flours were extracted (Folch, *et al.*, 1957) using chloroform and methanol mixtures in the ratio of 2:1 (V/V). Methyl esters were prepared from the total lipids by the method of Metcalfe *et al.,* (1966). Fatty acid analysis was prepared (Mohan and Janandhanan, 1993) by gas chromatography using an instrument equipped with a flame ionization detector and a glass column (2 m x 3 mm) packed with 1 % diethylene glycol succinate on chromosorb W (Silanised 80 / 100 mesh). The carrier gas was nitrogen, at flow rate of 32 ml / mim. The column temperature was 190^{o} C. Peaks were identified by comparison with authentic standards, quantified by peak area integration and relative weight percentage of each fatty acid was determined from integrated peak areas. The macro minerals and trace elements were estimated (Issac and Johanson 1975, Meines *et al.,* 1976) in Atomic Absorption Spectrophotometer.

Ant-nutritional factors like total free Phenols, tannins, L-DOPA (3,4,- dihydrixy phenylalanine), hydrogen cyanide and phytic acid were qualitative. The concentration of total free phenols was determined using the method of Mole and Waterman (1987). Tannins were captured and determined in a polyamide chromatography column following the method described by Burns (1971). L - DOPA content was determined by Brain method (1976). Hydrogen Cyanide was estimated by extraction with 0.1. M orthophosphoric acid. After extraction, sample was neutralized and estimated with chloramines T and barbituric acid reagent (Cooke and Madugwu, 1978; Nambisan and Sundaresan, 1984). The calorimetric technique of Wheeler and Ferrel (1971) as modified by Reddy *et al.,* (1978) was used to estimate phytic acid.

Results and Discussion

The proximate composition (Table I) shows that *Rhynchosia breacteata* seed meal contained high amount crude protein of 21.18 %, crude fat of 6.16 % than in other commonly consumed legumes *Cicer arietinum* (Luz Fernandez and Berry, 1988), *Vigna umbellata* (Rajaram and Janardhanan, 1990) and *Canavalia virosa* (Thangadurai, *et al.*, 2001). The food energy value of the seed was 366.86 K. cal due to the protein, lipid and NFEs rich ture. The seed protein fractionations content of *Rhynchosia breacteata* is given in Table II. Albumins and globulins (4.92 and 8.96 % respectively)

Table I: Proximate composition of *Rhynchosia bracteata

Component	Percentage
Moisture	12.36
Total carbohydrates	58.92
Crude protein (Kjeldahl N x 6.25)	21.18
Crude fat	6.16
Crude fibre	6.12
Ash	7.29
Nitrogen Free Extractives (NFE)	61.31
Energy K.Cal	366.86

* Mean of triplicate determinations expressed on dry weight basis (except moisture)

constitute the major bulk of the seed proteins as in many other legumes, and percentage distribution of both proteins are more or less equal to that of *Vigna sesquipedalis* (Rajaram and Janardhanan, 1990), *Vigna trilobata* (Sidduraju *et al*., 1992) *Phaseolus lunatus* (Vijayakumari, *et al.,* 1993) and *Abrus precatorius* (Mohan and Janardhanan 1995). The data

Table II. Protein fractions of seeds of *Rhynchosia bracteata* *

Protein fractions	% Seed flour
Total Protein (True protein)	17.10
Albumins	4.92
Globulins	8.96
Prolamines	1.87
Glutelins	1.45

* Mean of triplicate determinations expressed on dry weight basis

on fatty acid composition of the seed lipids (Table III) indicated that palmitic, linoleic, and stearic acid are the predominant fatty acids. The occurence of unsaturated fatty acids,

Table III: Fatty acid composition of seeds lipid of *Rhynchosia bracteata**

Fatty acids	Percentage
Palmitic acid (C_{16} : 0)	34.10
Stearic acid (C_{18} : 0)	14.40
Oleic acid C_{16} : 1)	16.16
Linoleic acid (C_{16} : 2)	25.86
Linolenic acid (C_{16} : 3)	9.88

* Mean of triplicate determinations expressed on dry weight basis.

which account for more than 60% of the seed lipids were comparable with some other wild legumes (Mohan and Janardhanan 1995). The level of 34.10 and 25.86 % of palmitic and linoleic acids, respectively (Table III), were more than the cultivated legumes of *Vigna* (Salunkhe *et al.*, 1982).

The data on amino acids profile of the purified seed proteins revealed that the essential amino acids, cysteine, methionine and tryptophan are the conspicuous limiting amino acids. Whereas, the other essential amino acids leucine, lysine, isoleucine, valine, threionine and histidine are present in higher concentrations (89, 72, 63, 25, 49 and 22 % crude protein, respectively) when compared with FAO/WHO/ UNO (1985) provisional pattern adequate for human maintenance and normal growth (Table IV). Minerals are rich (Table V). The seeds were found to be a potential source of minerals such as calcium, potassium, magnesium, manganese and copper than in the legumes of *Phaseolus lunatus, Leucaena leucocephala* and *Lathyrus sativus* (Duke, 1981) and in comparison with recommended dietary content is more than in the legumes of *Abrus precatrius* and *Cassia obtusifolia* (Mohan and Janardhanan 1995).

The anti-nutritional factors of seed flour are present variably (Table VI). The seed contains relatively higher amount of 5.56 % and 0.85 % of total free phenols and phytic acids, respectively, than the commonly cultivated legumes as observed earlier. (Bressani *et al*., 1983; Khan *et al.,* 1979; Rajaram and Janardhanan, 1992; Rodrigues andThorne, 1991) The contents of tannin and non-protein amino acids $_{L}$ -DOPA are found to be very low (0.29 % and 0.5 1% respectively) when compared with other species in Vigna (Rajaram and Janardhanan, 1990; Siddhuraju *et al*., 1992). Apart from these anti-nutritional factors, the presence of negligible amount of hydrogen cyanide (0.066g % seed flour) was also noticeable. The phytohaemagglutinating activity of albumins and globulins are similar showing with out any specificity against human ABO system as observed earlier (Siddhuraju *et al.*, 1992)

The conventional method of repeated soaking and boiling of seeds in water followed by decanting five to six times before consumption is being practiced by the local tribes to eliminate most of the antinutritional factors. All the antinutritional factors reported except $_{L}$-DOPA are heat labile. Hence they can be removed by wet or dry thermal treatments (Geervavi and Theophilus, 1981). In an earlier study, it has been

Table IV: Amino acid composition of acid hydrolysed purified total seed proteins of *Rhynchosia bracteata* (mg % crude protein)

Amino acids	mg% seed protein Availability quantity	FAO / WHO / UNO recommended amino acid requirements (mg/Kg body Wt/day) (1985)			
		Infant	Pre-school child (2-5 years)	School child (10-12 years)	Adult
Glutamine	57				
Asparagine	44				
Serine	22				
Threonine	49	43	34	28	9
Proline	38				
Alanine	26				
Glycine	11				
Valine	25	55	35	25	13
Cysteine + Methionine	29+18	42	25	22	17
Leucine	89	93	66	44	19
Isoleucine	63	46	28	28	13
Tyrosine + Phenylalanine	25+81	72	63	22	19
Lysine	72	66	58	44	18
Histidine	22	26	19	19	16
Tryptophan	11	17	11	9	5
Arginine	62				

demonstrated that the $_{L-}$ DOPA contents can also be significantly reduced by repeated soaking and boiling of the seeds in water, under optimum heat conditions to realize the maximum nutritional advantages (Thangadurai *et al.*, 2001). Therefore, the presence of these antinutritional factors may not be a limiting factor in the utilization of these seeds for food and other purpose.

The study indicates that these seeds have potential for human feeding judging from their proximate composition, gross energy, amino acid profile and mineral content which, in several aspects, compared favorably with those reported for many conventional edible legumes. Also it is clear from this study that the seeds contained some anti-nutrients, which

Table V: Selected mineral composition of *Rhynchosia bracteata* seed meal *

Mineral	Availability quantity * (mg % seed flour)	FAO/WHO/UNO recommended dietary allowances in mg % seed (1989)				
		Adult (Male)	Adult (Female)	Children (7-10 years)	Infant	Pregnant and Lactating women
Sodium	24.63	500	500	400	120-200	500
Potassium	1870.16	2000	2000	1600	500-700	2000
Calcium	346.12	800	800	800	600	1200
Magnesium	612.73	350	280	170	60	355
Phosphorus	781.28	800	800	800	500	1200
Zinc	5.26	15	12	10	5	19
Manganese	2.17	2-5	2-5	2-3	0.3-1.0	2-5
Iron	13.70	10	15	10	10	13
Copper	1.92	1.5-3	1.5-3	1-2	0.6-0.7	1.5-3

* Mean of triplicate determinations expressed on dry weight basis

could be reduced or eliminate by some of the processing methods adopted. However, the raw and processed seed flours appeared to be unsuitable as sole sources of directory protein especially in human diets. Their incorporation in diets along with other protein resources is therefore suggested as a way of enhancing the utilization of these differently processed legume seed flours.

Table VI: Antinutritional factors present in the seed flour of *Rhynchosia bracteata

Components	% seed flour
Total free phenols	3.76
Tannins	0.29
L - DOPA	0.51
Hydrogen cyanide	0.066
Phytic acid	0.18

* Mean of triplicate determinations expressed on dry weight basis.

Conclusion

In view of the above facts, *Rhynchosia bracteata* may further be exploited in breeding programmes and popularized for mass cultivation and consumption in third world countries such as India to alleviate hunger and poverty. As its domestication for commercial exploitation is considered in a number of biogeographical regions, such nutritional information is also very crucial to overcome the food crisis of ever expanding world's population.

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