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SHORT NOTE [NOTA CORTA]

CHEMICAL COMPOSITION AND NUTRITIONAL EVALUATION OF *Paracalyx scariosus* (ROXB.) ALI A WILD RELATIVE OF *CAJANUS* FROM SOUTHERN PENINSULAR INDIA

[COMPOSICIÓN QUÍMICA Y VALOR NUTRICIONAL DE *Paracalyx scariosus* (ROXB.) ALI, ESPECIE SILVESTRE DE *CAJANUS* DEL SURESTE DE LA INDIA]

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SUMMARY

Seeds of *Paracalyx scariosus* (Roxb.) Ali locally known as "adavi tellakandulu" in Telugu, used as food by local tribes people in the Rollapenta forest, Kurnool district, Nallamalais of Andhra Pradesh of southern Peninsular India. The mature seed samples were analysed for proximate composition, total (true) seed protein fractions, amino acid composition, fatty acids profile, mineral and antinutritional factors. They contained higher amount of crude protein, crude fat, ash and nitrogen free extractives constitute 20.56, 5.19, 5.12 and 56.25 % respectively of the seed weight. The calorific value of 100g dry matter of seed material was 1680.92 kJ DM. The essential amino acids, isoleucine, 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 sodium, phosphorus, calcium, zinc, manganese and iron. Anti-nutritional factors such as total free phenols, (5.56%) tannins (2.78%), L -DOPA (0.63%), hydrogen cyanide (0.065%) and phytic acid (0.85%) are present in variable quantities. From the results these plant have a good potential as food crops in Andhra Pradesh. This paper presents the chemical composition and nutritional potential of *Paracalyx scariosus* seeds with regard to its suitability as a regular component in human diet.

Key words: Proximate composition, amino acids, fatty acids, protein fractions, anti-nutritional factors, hemagglutinating activity.

RESUMEN

Semillas de *Paracalyx scariosus* (Roxb.) Ali conocida de manera local en Telugu, India como "adavi tellakandulu" y empleada como alimento por la tribu native del bosque Rollapenta, Distrito Kurnool, Nallamalais Andhra Pradesh, región sureste de la India. Se determine la composición proximal de las semillas, fracciones totales de proteína (verdadera), amino ácidos, ácidos grasos, minerales y factores antinutricionales. Las semillas contenien un alto valor de proteína cruda, grasa, cenizas y extracto libre de nitrógeno (20.6, 5.2, 5.1 y 56.3 % respectivamente), así como 1680.9 kJ /100gMS. Los amino ácidos, isoleucina, tirosina y fenilalanina estuvieron presentes en cantidades relativamente altas. Las semillas contienen altas concentraciones de ácido palmítico y linoleico, son ricas en sodio, fósforo, calcio, zinc, manganeso y hierro. Los factores antinutricionales se encuentran en cantiddes variables tales como fenoles libres (5.56%) taninos (2.78%), L-DOPA (0.63%), cianuro de hidrógeno (0.065%) y ácido fítico (0.85%). Esta planta tiene un buen potencial para su cultivo comercial en Andhra Pradesh. El trabajo presenta la composición química y valor nutricional de la semilla de *Paracalyx scariosus* en relación a su potencial para la alimentación humana.

Palabras clave: Análisis proximal, amino ácidos, ácidos grasos, fracciones proteicas, factores antinutricionales, actividad hemoaglutinante.

INTRODUCTION

Legumes have high protein content and can an important source of cheap protein in many countries where animal protein is expensive. Seeds are second in important to cereals as sources of food but they are two to three times richer in protein than cereals (National Academy of Sciences, 1979). In spite of an

urgent need to meet the nutritional requirements of the ever increasing populations, the availability of cheap protein resources have remained relatively unexplored (Murthy *et al.*, 2003, Thangadurai *et al*., 2006). With the increasing search of new food sources, the seeds of wild plants, including the tribal pulses, are receiving more attention because they are well adapted to adverse environmental conditions, highly resistant to disease and pests, and exhibit good nutritional qualities (Maikhuri, *et al*., 1991).

Some of the wild seeds are commonly used as pertinacious 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, Murthy and Pullaiah, 2005). However, most of the Indian legumes need biochemical and nutritional investigation. 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 genetic resource, randomly in the vicinity of the forests, soak in water and consume the seed meal after boiling and decanting for four to twelve times. This has stimulated us to study the biochemical composition of the seeds to understand its potential use in human nutrition.

MATERIAL AND METHODS

Preparation of seeds

The 3.5 kg seeds of *Paracalyx scariosus* (Roxb.) Ali were collected during February 2005, from the dry deciduous forests near Rollapenta, Nallamalais of Andhra Pradesh, near the vicinity of tribal hamlets and were used for analysis. The accessions were botanically identified by using the botanical keys of (Pullaiah and Murthy, 2001) and deposited in the Herbarium, Department of Biotechnology, Montessori Mahila Kalasala.

The moisture content was determined by drying 50 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 (Scientific equipment works, Kerala), for 60-mesh size.

Chemical analysis

The fine powder obtained was used for further analysis. The total carbohydrate content was estimated (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 mean of 3 replicates.

Protein fractionation

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 × 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 × 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^{0} C for 24 h in vaccuo. After evaporation, the dried residue was dissolved in citrate buffer (pH 2.2), known aliquots were analyzed using LKB-Biochrome Automated Amino acid Analyzer Model 4151 - Alpha Plus. For the determination of cystine, samples were oxidized with formic acid and hydrogen peroxide. Methionine was determined as methionone sulfone. The different amino acids recovered were presented as mg/100g proteins. The contents of different amino acids recovered were presented as mg/g protein. The essential amino acids were scored and compared with FAO, WHO, UNO (1985) reference pattern.

Fatty acids

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 (Shimadzu, Model - RIA) using an instrument equipped with a flame ionization detector and a glass column (2m×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/min. The column temperature was 190^{0} 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.

Minerals

The macro minerals and trace elements were estimated (Issac and Johanson 1975, Meines et al., 1976) in Perkin Elmer Model 5000 Atomic Absorption Spectrophotometer. Dry ashing procedures were used for the preparation of mineral solutions. The samples were ignited at 450^0 C for 12 h in a muffle furnace and dissolved in 3N HNO_3. For correction of error for the determination of calcium and magnesium, a 1 % lanthanum solution was added to the sample. Phosphorus was measured by calorimetric means (Virmani and Narula, 1995).

Antinutritional factors

Antinutritional factors like total free phenols, tannins, L- DOPA (3, 4, - dihydroxy 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

Legumes seeds are valuable source of protein, carbohydrates, minerals, vitamins etc. They are also playing an important role in human nutrition mainly in developing countries (Yanez et al., 1995). In the present study (Table 1) *Paracalyx scariosus* seed meal contained high amount crude protein of 20.56 %, crude fat of 5.19 % than in other commonly consumed legumes *Cicer arietinum* (Srivastava and Ali 2004), *Vigna umbellata* (Rajaram and Janardhanan, 1990) and *Canavalia virosa* (Thangadurai, *et al*., 2001). The food energy value of the seed was 1680.92 kJ. due to the protein, lipid and NFEs rich nature. The seed protein fractionations content of *Paracalyx scariosus* is given in Table 2. Albumins and globulins (4.92 and 8.73g/100g, respectively) 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).

Table 1. Data on proximate composition of *Paracalyx scariosus.**

Component	Percentage
Moisture	14.38
Total carbohydrates	65.05
Crude protein (Kjeldahl N x 6.25)	20.56
Crude fat	5.19
Crude fibre	6.10
Ash	5.12
Nitrogen Free Extractives (NFE)	56.25
kJ 100 g^{-1} Dm	1680.9

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

Table 2. Data on protein fractions of seeds of *Paracalyx scariosus**.

Protein fractions	g/100g seed flour
Total Protein (True protein)	19.82
Albumins	4.92
Globulins	8.73
Prolamines	3.76
Glutelins	2.45

* Mean of triplicate determinations expressed on dry weight basis

The data on fatty acid composition of the seed lipids (Table 3) indicated that palmitic, linoleic, oleic, stearic and linolenic acid are the predominant fatty acids. The occurrences of unsaturated fatty acids, which account for more than 60% of the seed lipid were comparable with some other wild legumes (Mohan and Janardhanan, 1995). The level of 33.75 and 24.10 g/100g of palmitic and linoleic acids, respectively (Table 3), 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, cystine, methionine and tryptophan are the conspicuous limiting amino acids. Whereas, the other essential amino acids leucine, isoleucine, valine,lysine, threionine and histidine are present in higher concentrations (95, 74, 56, 41, 32 and 29 mg/100g crude protein, respectively) when compared with FAO/WHO/UNO (1985) provisional pattern adequate for human maintenance and normal growth (table 4). The seeds were found to be a potential source of minerals with are compared with the recommended dietary allowances of NRC/NAS (1989) (Table 5) 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 precatorius* and *Cassia obtusifolia* (Mohan and Janardhanan 1995).

Table 3 Data on fatty acid composition of seeds of *Paracalyx scariosus**.

Fatty acids	Percentage
Palmitic acid (C_{16} : 0)	33.75
Stearic acid (C_{18} : 0)	16.62
Oleic acid (C_{16} : 1)	20.78
Linoleic acid (C_{16} : 2)	24.10
Linolenic acid (C_{16} : 3)	4.75

* Mean of triplicate determinations expressed on dry weight basis.

The anti-nutritional factors of seed flour are present variably (Table 6). The seed contains relatively higher amount of 5.56 and 0.85 g 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 (2.78 and 0.63 g 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 (0.065g/100g seed flour) was also noticeable. The phytohaemagglutinating activity of albumins and globulins are similar showing without 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 (Geervani and Theophilus, 1981). In an earlier study, it has been 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 (Viswanthan *et al*., 2001; Vadivel and Janardhanan 2004.). Therefore, the presence of these antinutritional factors may not be a limiting factor in the utilization of these seeds for food and other purpose.

Table 4. Amino acid composition of acid hydrolysed purified total seed proteins of *Paracalyx scariosus* (mg/100g crude protein).

Amino acids	g/100g seed protein availability	FAO/WHO/UNO recommended amino acid requirements (1985)			
		Infant	Pre-school child (2-5 years)	School child (10-12 years)	Adult
Glutamine	56				
Asparagine	52				
Serine	23				
Threonine	56	43	34	28	9
Proline	31				
Alanine	18				
Glycine	12				
Valine	32	55	35	25	13
Cysteine + Methionine	32+21	42	25	22	17
Leucine	95	93	66	44	19
Isoleucine	74	46	28	28	13
Tyrosine + Phenylalanine	19+32	72	63	22	19
Lysine	41	66	58	44	18
Histidine	29	26	19	19	16
Tryptophan	11	17	11	9	5
Arginine	68				

Table 5. Data on selected mineral composition of *Paracalyx scariosus* seed meal.

Mineral	Availability (mg/100g seed flour)*	FAO/WHO/UNO recommended dietary allowances in mg / 100 g seed protein (1989)				
		Adult (Male)	Adult (Female)	Children (7-10 years)	Infant	Pregnant and Lactating women
Sodium	38.10	500	500	400	120-200	500
Potassium	1260.80	2000	2000	1600	500-700	2000
Calcium	589.79	800	800	800	600	1200
Magnesium	482.10	350	280	170	60	355
Phosphorus	526.70	800	800	800	500	1200
Zinc	8.90	15	12	10	5	19
Manganese	2.80	2-5	2-5	2-3	0.3-1.0	2-5
Iron	6.70	10	15	10	10	13
Copper	2.30	1.5-3	1.5-3	1-2	0.6-0.7	1.5-3

* Mean of triplicate determinations expressed on dry weight basis

Table 6. Data on antinutritional factors present in the seed flour of *Paracalyx scariosus.*

Components	g/100g seed flour*
Total free phenols	5.56
Tannins	2.78
L - DOPA	0.63
Hydrogen cyanide	0.065
Phytic acid	0.85

* Mean of triplicate determinations expressed on dry weight basis.

CONCLUSION

In view of the above facts, *Paracalyx scariosus* 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. From the results of this study, it can be concluded that the carbohydrate, crude fat, crude protein and potassium contents of the flour of *Paracalyx scariosus* varied significantly. More agronomic studies should be done on this legume with a view of cultivating it. They are a cheap source of nutrients and could be useful in formulating balanced diets. 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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