Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 2 (4): 433-439, 2007
ISSN 1816-4951

Biochemical and Nutritional Assessment of *Rhynchosia hirta* (Andr.) Meikle and Verdc (Papilionaceae)

[1]K. Sri Rama Murthy and [2]Vivek Babu Kandimalla
[1]Department of Botany, Center for Applied Biological Sciences,
Andhra Loyola College, Vijayawada-520008, India
[2]Department of Chemistry, Analytical Chemistry Division,
Nanjing University, Nanjing 210093, People's Republic of China

Abstract: Seeds of *Rhynchosia hirta* (Andr.) Meikle and Verdc. locally known as Adavi Kandulu in Telugu, used as food by local tribal in the Udayagiri forest of Nellore district 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 profiles, mineral and antinutritional factors. The investigated seed samples contained higher amount of crude protein, crude fat, ash and nitrogen free extractives constitute 19.05, 8.73, 4.48 and 48.78 g/100 seed powder, respectively. The calorific value of seed material was 1690.9 kJ/100 g 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, Phosphorous, calcium, zinc, manganese and iron. Anti-nutritional factors, such as total free phenols, (2.25%) tannins (4.75%), L-DOPA (0.65%), hydrogen cyanide (0.0654%) and phytic acid (1.32%) are, present in variable quantities. This paper presents the analytical data on the chemical composition and nutritional potential of *Rhynchosia hirta* seeds with regard to its suitability as a regular component in human diet.

Key words: Amino acids, fatty acids, protein fractions, Anti-nutritional factors, *Rhynchosia hirta*

INTRODUCTION

Knowledge of the chemical composition of foods is 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). Inadequate availability and consumption of protein foods in India due to both population exploration and urbanization, will remain if efforts are not made toward 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 (Murthy *et al*., 2003; Thangadurai *et al*., 2006). With increasing in 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 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

Corresponding Author: K. Sri Rama Murthy, Department of Botany, Center for Applied Biological Sciences, Andhra Loyola Collage, Vijayawada-520008, India
Tel: +91-866-2474902 Fax: 91-866-2486084

different tribal sects of India (Arora *et al*., 1980; Murthy and Pullaiah, 2005). 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 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 is tempted us to study the biochemical composition of the seeds to know its potential use in human nutrition.

MATERIALS AND METHODS

The seeds of *Rhynchosia hitra* were collected from the moist deciduous of Udayagiri forest of Nellore district, Andhra Pradesh, during April 2004 (early summer) 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 of the Department of Botany, Andhra Loyola College Herbarium. The moisture content was determined by drying 50 transversely cut seeds in an oven at 80°C for 24 h and is 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. 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 flowing 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 (1970) methods. 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).

The total true proteins were extracted by the method of Rajaram and Janardhanan (1990). The extracted protein were purified by precipitation with cold 20% 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 min the supernatant containing prolamine was air dried and dissolved in 0.1 N NaOH. The remaining pellet was extracted with 0.4 N NaOH (1:10 w/v) overnight and centrifuged at 20,000 x g for 20 min. 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°C for 24 h *in vaccuo*. After evaporation, the dried residue were dissolved in citrate buffer (pH 2.2), known aliquots were analyzer in 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 amino acids recovered are presented as mg/100 g proteins. The contents of different amino acids recovered were presented as mg g^{-1} protein. The essential amino acids were scored and compared with FAO, WHO, UNO (1985) reference pattern.

The total lipids from the seed fluor 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 Janardhanan, 1993) by gas chromatography (Shimadzu Model RIA) using an instrument equipped with a flame ionization detector and a glass column (2 m×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^{-1}. The column temperature was 190°C. Peaks were identified by comparison with authentic standards, quantified by peak area integration and relative weight percentage of pack fatty acid was determined from integrated peak areas.

The macrominerals 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°C for 12 h in a muffle furnace and dissolved in 3 N 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).

Anti-nutritional factors like total free Phenols, tannins L - DOPA (3,4-dihydrioxy 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, sample was neutralized and estimated with chloramines T and barbituric acid reagent (Cooke and Madugwu, 1978; Nambisan and Sunderasen, 1984). The colorimetric 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 1) shows that *Rhynchosia hitra* seed meal contained high amount crude protein of 19.05%, crude fat of 8.73% than in other commonly consumed legumes *Cicer arietinum* (Srivastava and Ali, 2004), *Vigna umbellata* (Rajaram and Janardhanan, 1990), *Canavalia virosa* (Thangadurai *et al*., 2001a) and *Tamarindus indica* (Pugalenthi *et al*., 2004). The remarkably high level of protein in the wild legume under study underscores their importance as source of this vital nutrient. The food energy value of the seed was 1690.9 kJ due to the protein, lipid and NFEs rich nature. Albumins and globulins (5.12 and 6.75 g/100 g, respectively) (Table 2) 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; Pugalenthi *et al*., 2004). The data on fatty acid composition of the seed lipids (Table 3) indicated that palmitic, linoleic, oleic and stearic acids are the predominant fatty acids. The occurrence of unsaturated fatty acids which account for more than 60% of the seed lipids were comparable with some other wild legumes (Vidivel and Janardhanan, 2004). The level of 43.76 and 21.75 g/100 g of palmitic and linoleic acids, respectively (Table 3), were more than the cultivated legumes of *Vigna* (Viswanathan *et al*., 2001).

Table 1: Data on proximate composition of *Rhynchosia hirta**

Component	Percentage
Moisture	9.45
Total carbohydrates	57.75
Crude protein (Kjeldahl N x 6.25)	19.05
Crude fat	8.73
Crude fibre	9.89
Ash	4.48
Nitrogen Free Extractives (NFE)	48.78
kJ 100 g^{-1} Dm	1690.90

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

Table 2: Data on protein fractions of seeds of *Rhynchosia hirta**

Protein fractions	g/100 g seed flour
Total protein (True protein)	16.75
Albumins	5.12
Globulins	6.75
Prolamines	2.40
Glutelins	2.75

*Mean of triplicate determinations expressed on dry weight basis

Table 3: Data on fatty acid composition of seeds of *Rhynchosia hirta**

Fatty acids	Percentage
Palmitic acid (C_{16}: 0)	43.76
Stearic acid (C_{18}: 0)	12.89
Oleic acid (C_{16}: 1)	13.65
Linoleic acid (C_{16}: 2)	21.75
Linolenic acid (C_{16}: 3)	10.18

* Mean of triplicate determinations expressed on dry weight basis

Table 4: Amino acid composition of acid hydrolysed purified total seed proteins of seeds of *Rhynchosia hirta* (mg/100 g crude protein)

	g/100 g seed protein	FAO/WHO/UNO (1985) recommended amino acid requirements			
Amino acids	availability quantity	Infant	Pre-school child (2-5 years)	School child (10-12 years)	Adult
Glutamine	85				
Asparagine	53				
Serine	25				
Threonine	50	43	34	28	9
Proline	39				
Alanine	19				
Glycine	12				
Valine	30	55	35	25	13
Cysteine+Methionine	38+11	42	25	22	17
Leucine	91	93	66	44	19
Isoleucine	48	46	28	28	13
Tyrosine+Phenylalanine	43+82	72	63	22	19
Lysine	55	66	58	44	18
Histidine	26	26	19	19	16
Tryptophan	18	17	11	9	5
Arginine	93				

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, lysine, isoleucine, valine, threionine and histidine are present in higher concentrations (91, 55, 48, 30, 50 and 26 mg/100 g crude protein, respectively) when compared with FAO/WHO/UNO (1985) provisional pattern adequate for human maintenance and normal growth (Table 4). Minerals are rich (Table 5). 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 allowance value (NRC/NAS, 1989). Phosphorus content is more than in the legumes of *Abrus precatrius* and *Cassia obtusifolia* (Mohan and Janardhanan 1995), *Tamarindus indica*, *Erythrina indica* and *Sesbania bispinosa* (Pugalenthi *et al*., 2004).

The anti-nutritional factors of seed flour are present variably (Table 6). The seed contains relatively higher amount of 2.25 and 1.32 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 and Thorne, 1991). The contents of tannin and non-protein amino acids L-DOPA are found to be very low (4.75 and 0.65 g, respectively) when compared with other species in *Vigna* (Rajaram and Janardhanan, 1990; Siddhuraju *et al*., 1992). A part from these anti-nutritional factors, the presence of negligible amount of hydrogen cyanide (0.0654 g/100 g 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 wet or dry thermal treatments (Geervani and Theophilus, 1981). In an earlier

Table 5: Data on selected mineral composition of *Rhynchosia hirta* seed meal*

Mineral	Availability quantity * (mg/100 g seed flour)	FAO/WHO/UNO (1985) recommended dietary allowances in mg/100 g seed protein				
		Adult (Male)	Adult (Female)	Children (7-10 years)	Infant	Pregnant and lactating women
Sodium	33.58	500	500	400	120-200	500
Potassium	657.05	2000	2000	1600	500-700	2000
Calcium	1285.66	800	800	800	600	1200
Magnesium	414.27	350	280	170	60	355
Phosphorus	551.34	800	800	800	500	1200
Zinc	9.34	15	12	10	5	19
Manganese	3.55	2-5	2-5	2-3	0.3-1.0	2-5
Iron	15.96	10	15	10	10	13
Copper	1.31	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 *Rhynchosia hirta**

Components	g/100 g seed flour
Total free phenols	2.25
Tannins	4.75
L-DOPA	0.65
Hydrogen cyanide	0.0654
Phytic acid	1.32

* Mean of triplicate determinations expressed on dry weight basis

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 (Thangadurai *et al*., 2001b). Therefore, the presence of these antinutritional factors may not be a limiting factor in the utilization of these seeds for food and other purpose.

In view of the above facts, *Rhynchosia hirta* may further be exploited in breeding programs and popularized for mass cultivation and consumption in third world countries such as India to alleviate hunger and poverty. More efforts are needed to encourage the increased production and consumption of processed indigenous legumes, especially the *Rhynchosia hirta* as they seem to be economical sources of vegetable protein. 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.

ACKNOWLEDGMENTS

KSM is grateful to the Department of Science and Technology, New Delhi for the award of SERC-Young Scientist research grant No. SR/FT/L-16/2003.

REFERENCES

Amubode, F.A. and B.L. Fetuga, 1983. Proximate composition and chemical assay of methionine lysine, tryptophan in some Nigerian forest trees. Food Chem., 12: 67-72.

AOAC, 1970. Official Methods of Analysis. 11th Edn., Washington, DC: Association of Official Analytical Chemists, pp: 211-214.

Arora, R.K., K.P.S. Chandel, B.S. Joshi and K.C. Pant, 1980. Rice bean: Tribal pulse of Eastern India. Econ. Bot., 34: 260-263.

Brain, K.R., 1976. Accumulation of L-DOPA in cultures from *Mununa pruriens*. Plant Sci. Lett., 7: 157-161.

Bressani, R., R.G. Brenes, A. Garcia and L.G. Elias, 1983. Chemical composition, amino acid content and protein quality of *Canavalia* sp. seeds. J. Sci. Food Agric., 40: 17-23.

Burns, R.R., 1971. Methods for estimation of tannins in grain, Sorghum. Agron. J., 63: 511-512.

Conrad, E.C. and J. Palmer, 1976. Rapid analysis of carbohydrates by high-pressure liquid chromatography. Food Technol., 30: 84-93.

Cooke, R.D. and E.N. Madugwu, 1978. The effects of simple processing on the cyanie content of *Cassaca chips*. J. Food Technol., 13: 299-306.

Duke, J.A., 1981. Handbook of Legumes of World Economic Importance. New York, Plenum Press.

Eggum, B.O. and R.M. Beame, 1983. The Nutritive Value of Seed Proteins. In: Seed Proteins Biochemistry, Genetics and Nutritive Values. Gotteschalk, W. and P.H. Muller (Eds.). The Hague: Junk J.N. Publishers .

FAO/WHO/UNO, 1985. Energy and Protein Requirements. WHO Tech, Rep. Ser. No. 724, Generva, Switzerland.

Folch, J., M. Less and G.H. Solane-Stantely, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-506.

Geervani, P. and F. Theophilus, 1981. Effect of home processing on the protein quality of selected legumes. J. Food Sci., 32: 71-78.

Humphries, E.C., 1956. Mineral Components and Ash Analysis. In: Modern Methods of Plant Analysis, Vol. 1. Paech, K. and M.V. Tracey (Eds.), Berlin: Springer Verlag, pp: 468-502.

Issac, R.A. and W.C. Johanson, 1975. Collaborative study of wet and dry ashing techniques for the elemental analysis of plant tissue by Atomic Absorption Spectrophotometer. J. Assoc. Official Anal. Chem., 58: 436-440.

Khan, K.M., L. Jacobson and O.B. Eggum, 1979. Nutritive value of some improved varieties of legumes. J. Sci. Food Agric., 30: 394-400.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Maikhuri, R.K., M.C. Nautiyal and M.P. Khali, 1991. Lesser known crops of foods value in Garhwal Himalaya and a strategy to conserve them. FAO/IBPGR Plant Genrt. Res. Newslett., 86: 33-36.

Meines, C.R., N.L. Derise, H.C. Lau, M.G. Grews, J. Ritchey and E.W. Merphy, 1976. The Content of nine mineral elements raw and cooked mature dry legumes. J. Agric. Food Chem., 24: 1126-1130.

Metcalfe, L.D., A.A. Schemitz and J.R. Pelka, 1966. Rapid preparation of fatty acid esters from lipids for as chromatographic analysis. Anal. Chem., 38: 514-515.

Mohan, V.R. and K. Janardhanan, 1993. Chemical and nutritional evolution of raw seeds of the tribal pulses. *Parkia roxburghii* G. Don and *Entada phaseoloides* (L.) Merr. Int. J. Food Sci. Nutr., 44: 47-53.

Mohan, V.R. and K. Janardhanan, 1995. Chemical determinations of nutritional and antinutritional properties in tribal pulses. J. Food Sci. Technol., 32: 459-469.

Mole, S. and P.G. Waterman, 1987. A critical analysis of techniques for measuring tannins in ecological studies. A. Techniques for anemically detecting tannins. Oecologia, 72: 137-147.

Murray, D.R., 1979. The seed proteins of kowhai *Sophora microphylla* AIT Zeitschrift fur Pflanzenphysiologie, 93: 423-428.

Murthy, K.S.R., S. Sandhya Rani and T. Pullaiah, 2003. Wild edible plants of Andhra Pradesh, India. J. Econ. Taxon. Bot., 27: 613-630.

Murthy, K.S.R. and T. Pullaiah, 2005. Wild Relatives and Related Species of Cultivated Crop Plants of Eastern Ghats, India. Recent Trends in Plant Sciences. Pullaiah *et al*. (Eds.), Regency Publications, New Delhi, India, pp: 96-103.

Nambisan, B. and S. Sunderasen, 1984. Spectrophotometric determination of cyanoglucosides in Cassia. J. Assoc. Official Anal. Chem., 67: 641-643.

NRC/NAS, 1989. Recommended Dietary Allowances. Washington: National Academy Press.

Osborne, D.R. and P. Voogt, 1978. Calculation of Calorific Value. In the Analysis of Nutrients in Food. New York, Academic Press, pp: 239-240.

Pugalenthi, M., V. Vadivel, P. Gurumoorthi and K. Janardhanan, 2004. Comparative nutritional evolution of little known legumes, *Tamarindus indica*, *Erythrina indica*, *Sesbania bispinosa*. Trop. Subtrop. Agroecosyst., 4: 107-123.

Pullaiah, T. and K.S.R. Murthy, 2001. Flora of Eastern Ghats, India. Vol. II Leguminosae (Fabaceae) Regency Publications, New Delhi, India.

Rajaram, N. and K. Janardhanan, 1990. Checmcial composition and nutritional evaluation of certain under-explored *Vigna* sp. Food Sci. Nutr., 42: 213-221.

Rajaram, N. and K. Janardhanan, 1992. Nutritional and chemical evaluation of raw seeds of *Canavalia gladiata* (Jacq.) DC and *C. ensiformis* DC: The under utilized food and fodder crops in India. Plant Foods Hum. Nutr., 42: 329-336.

Reddy, S.J., M.H. Punols and Mc J. Ginnis, 1978. Effect of gamma irradiation on nutritional value of dry filed beans (*Phaseolus vulgrais*) for chick. J. Nutr., 109: 1307-1312.

Rodrigues, B.F. and S.G. Thorne, 1991. A chemical study of seeds in three *Canavalia species*. Trop. Sci., 31: 101-103.

Sidduraju, P., K. Vijayakumari and K. Janardhanan, 1992. Nutritional and chemical evalution of raw seeds of the tribal pulses *Vigna trilobata* (L.) Verdc. Int. J. Food Sci. Nutr., 43: 97-103.

Southgate, D.A., 1974. Guidelines for the Preparation of tables of Food Composition, Karger, Basel, pp: 7-21.

Srivastava, R.P. and M. Ali, 2004. Nutritional quality of common pulses: Indian Institute of pulses Research, Kanpur, pp: 14-22.

Thangadurai, D., M.B. Viswanathan and N. Ramesh, 2001a. The chemical composition and nutritional evaluation of *Canavalia virosa*: A wild perennial bean from Eastern Ghats of Peninsular India. Eur. Food Res. Technol., 213: 456-459.

Thangadurai, D., M.B. Viswanathan and N. Ramesh, 2001b. Nutritional potential of biochemical composition in *Galactia longifolia* Benth. (Fabaceae) Nahrung Food, 45: 97-100.

Thangadurai, D., K.S.R. Murthy and T. Pullaiah, 2006. Characterization, Conservation and Utilization of Plant Genetic Resources for Future Food, Agriculture and Medicine. In: Biodiversity Assessment and Conservation. In: Trivedi, P.C. (Ed.), pp 247-263.

Vadivel, V. and K. Janardhanan, 2004. The nutritional and Antinutritional attributes of sword bean [*Canavalia glabiata* (Jacq.) DC.]: An under-utilised tribal pulse from South India. Int. J. Food Sci. Technol., 39: 917-926.

Vijayakumari, K., P. Sidduraju and K. Janardhanan, 1993. Nutritional and Antinutritional properties of certain under exploited legume seeds. Int. J. Food Sci. Nutr., 44: 181-189.

Virmani, O.P. and A.K. Narula, 1995. Applied Chemistry Theory and Practice. London; New Internal Publishers, pp: 74-89.

Viswanathan, M.B., D. Thangadurai and N. Ramesh, 2001. Biochemical and Nutritional evolution of *Neonotonia wightii* (Wight and Arn). Lackey (Fabaceae). Food Chem., 75: 275-279.

Wheeler, E.L. and R.E. Ferrel, 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem., 48: 312-320.