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Biochemical and nutritional evaluation of *Neonotonia wightii* (Wight & Arn.) Lackey (Fabaceae)

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Abstract

Seeds of *Neonotonia wightii* (Wight & Arn.) Lackey (Fabaceae), used as food by Malayali tribals in the Kollihills of Namakkal District, Tamil Nadu in Peninsular India, locally known as Adutheenikai, were analyzed for proximate composition, total (true) seed proteins, amino acid composition, fatty acid composition, minerals and antinutritional factors. The information is reported here for the first time in science. Crude protein, crude fat, ash and nitrogen free extractives constitute 20.6, 12.8, 5.8%, and 52.1g/100g seed flour, respectively. The calorific value of 100-g dry matter of seed material is 1700 kJ. The essential amino acids, leucine, lysine, isoleucine, valine, therionine and histidine are present in higher concentrations as 79, 54, 44, 37, 34 and 32 mg/100g crude protein, respectively. The limiting essential amino acids were cystine, methionine and tryptophan. The unsaturated fatty acids constitute more than 60% of seed lipids. The seeds were found to be a potential source of minerals such as potassium, magnesium, manganese and copper. The concentration of these minerals was relatively more than that of NRC/NAC recommended dietary allowances. The anti-nutritional factors such as total free phenols, tannins, L-DOPA, hydrogen cyanide and phytic acid contents were also determined.

Keywords: Amino acids; Antinutritional factors; Fatty acids; Protein fractionation; *Neonotonia wightii*

1. Introduction

Some of the wild nuts and seeds are commonly used as proteinaceous foods in different parts of the world (Amubode & Fetuga, 1983). There are some 28 wild legumes commonly consumed by different tribal sects in India (Arora, Chandel, Joshi, & Pant, 1980; Jain, 1981; Gunjatkar & Vartak, 1982). However, most of the Indian legumes remain uninvestigated biochemically and nutritionally. The Malayali tribals of the Solakkadu, Semmedu, Keel Solakkadu, Valavanthinadu and Othakadai hamlets situated at the top of the Kollihills in the vegetation types of tropical moist deciduous and sholas of the tropical semi-evergreen forests above 1000-m MSL in the Kollihills of Namakkal District, Tamil Nadu in South India, are collecting the seeds of *Neonotonia wightii* (Wight & Arn.) Lackey (Fabaceae), a polymorphic species (Viswanathan & Lakshmanan, 1991), locally known as Adutheenikai, randomly in the vicinity of the forests, soak in water and consume the seed meal after boiling and decanting four times. No information is available on the chemical composition and nutritional potential of *Neonotonia wightii* (Wight & Arn.) Lackey (Fabaceae) grows in India. This tempted us to study the biochemical composition of the seeds to know its potential use in human nutrition.

2. Materials and methods

The seeds of *Neonotonia wightii* (Wight & Arn.) Lackey (Fabaceae), were collected from the moist deciduous and shola of semi-evergreen forests near the vicinity of tribal hamlets and were used for analysis. 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- and air-dried seeds were powdered separately in a Willy Mill (Scientific Equipment Works, Delhi, India), for 60 mm mesh size. The fine seed powder obtained was used for further analysis. The total carbohydrate content was estimated (Conrad & Palmer, 1976). The crude protein content was calculated by multiplying the factor of 6.25 of percent Kjeldahl nitrogen

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following Humphries method (1956). The crude fibre content was determined according to the method described by Eggum and Beame (1981). The contents of nitrogen free extractives (NFEs), crude fat and ash were estimated by AOAC methods (1970). The energy content was estimated by multiplying the percentages of crude protein, crude fat and nitrogen free extractives with the factors of four, nine and four, respectively (Osborne & Voogt, 1978). The total true proteins were extracted from air-dried seed powder by Basha, Chery, and Young's method (1976) with a slight modification (ethanol treatment was omitted to save prolamine fraction). The proteins were purified by cold 20% TCA precipitation. Albumin and globulin fractions of seed proteins were extracted by Basha and Beevers' method (1975). The left out pellet was treated with 75% ethanol (1:5 W/V) over night and the supernatant was collected, air-dried and dissolved in 0.1 N NaOH and the prolamine fraction was estimated. 0.4 N NaOH 1:10 (W/V) was added with the remaining pellet and left overnight and centrifuged at 20,000 $\times g$ for 20 min. The supernatant thus obtained was designated as glutelin. All the protein fractions so obtained were estimated by Lowry Rosebrough, Farr, and Randall's method (1951) after 20% TCA precipitation. The purified total seed proteins were acid hydrolyzed with 6 N HCl at 100 °C for 24 h in vacuo. After flash evaporation, the dried residue was dissolved in citrate buffer (pH 2.2), known aliquots were analyzed 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 was determined as methionine sulfone. The different amino acids recovered are presented as mg/100 g proteins.

The total lipid was extracted from the seeds according to the method of Folch, Lees, and Solane-Stanley (1957) using chloroform and methanol mixture in the ratio of 2:1 (V/V). Methyl esters were prepared from the total lipids by the method of Metcalfe, Schemitz, and Pelka (1966). Fatty acid analysis was performed (Mohan & 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 a flow rate of 32 ml/min. The column temperature was 190 °C. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters and relative weight percentage of each fatty acid was determined from integrated peak areas.

Minerals of sodium, potassium, calcium, magnesium, zinc, manganese, iron and copper were estimated (Issac & Johnson, 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 3N HNO_3. For correction of error for the determination of calcium and magnesium, a 1% lanthanum solution was added to the samples. Phosphorus content in the triple acid digested extract was determined colorimetrically (Virmani & Narula, 1995).

Table 1
Data on proximate composition of *Neonotonia wightii* seeds[a]

Component	Percentage
Moisture	8.3
Crude protein (Kjeldahl N×6.25)	20.6
Crude fat	12.8
Crude fiber	8.7
Ash	5.8
Nitrogen Free Extracts	52.1
kJ 100 g^{-1} Dm	1700.0

[a] Mean of triplicate determinations expressed on dry weight basis.

Anti-nutritional factors such as total free phenols, tannins, L-DOPA (3,4-dihydroxy phenyl alanine), hydrogen cyanide and phytic acid were quantified. The concentration of total free phenols was obtained by using the Folins–Denis method as applied by Mole and Waterman (1987). Tannins were captured in a polyamide chromatography column following the method described by Burns (1971). L-DOPA content was estimated by Brain method (1976). Hydrogen cyanide was estimated by extraction with 0.1 M orthophosphoric acid. After extraction, sample was neutralized and estimated with chloramine T and barbituric acid reagent (Cooke & Madugwu, 1978; Nambisan & Sunderesan, 1984). The colorimetric technique of Wheeler and Ferrel (1971) as modified by Reddy, Pubols, and McGinnis (1978) was used to estimate phytic acid.

3. Results and discussion

The proximate composition (Table 1) shows that *Neonotonia wightii* seed meal contained high amount of crude protein of 20.6%, crude fat of 12.8% than in other commonly consumed legumes such as *Cassia occidentalis* (Niranjan & Gupta, 1973), *Cicer arietinum* (Luz Fernandez & Berry, 1988) and *Vigna umbellata* (Rajaram & Janardhanan, 1990). The food energy value of the seed was 1700 kJ due to the protein, lipid and NFEs rich nature. The seed protein fractionations content of *Neonotonia wightii* is given in Table 2. Albumins and globulins (33.6 and 51.5g/100g, respectively) constitute the major bulk of the seed proteins as in many other legumes, and percentage distributions of both proteins are more or less equal to that of *Vigna sesquipedalis* (Rajaram & Janardhanan, 1990), *Vigna trilobata* (Siddhuraju, Vijayakumari, & Janardhahan, 1992), *Phaseolus lunatus* (Vijayakumari, Siddhuraju, &

Table 2
Data on protein fractionation of *Neonotonia wightii* seeds[a]

Protein fraction	Seed flour (g/100 g)	Seed protein (g/100 g)
Total protein (True protein)	15.2	100.0
Albumins	5.1	33.6
Globulins	7.8	51.5
Prolamines	1.1	7.0
Glutelins	1.2	7.9

[a] Mean of triplicate determinations expressed on dry weight basis.

Table 3
Data on fatty acid composition of *Neonotonia wightii* seeds[a]

Fatty acid	Percentage
Palmitic acid (C_{16}:0)	36.1
Stearic acid (C_{18}:0)	15.2
Oleic acid (C_{18}:1)	17.2
Linoleic acid (C_{18}:2)	23.6
Linolenic acid (C_{18}:3)	7.9
Others (unidentified)	–

[a] Mean of triplicate determinations expressed on dry weight basis.

Janardhanan, 1993) and *Abrus precatorius* (Mohan & Janardhanan, 1995). The data on fatty acid composition of the seed lipids (Table 4) 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 (Mohan & Janardhanan, 1995). The level of 36.1 and 23.6g/100g of palmitic and linoleic acids, respectively (Table 3), were more than the cultivated legumes of *Vigna* (Salunkhe, Sathe, & Reddy, 1982).

The data on amino acid 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, therionine and histidine are present in higher concentrations (79, 54, 44, 37, 34 and 32mg/100g crude protein, respectively) when compared with FAO/WHO/UNO (1985) provisional pattern adequate for human maintenance and normal growth (Table 4). The essential amino acid, tryptophan is totally absent. Minerals are rich (Table 5). The seeds were found to be a potential source of minerals such as 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 values (NRC/NAS, 1989). Phosphorus content is more than in the legumes of *Abrus precatorius* and *Cassia obtusifolia* (Mohan & Janardhanan, 1995).

The anti-nutritional factors of seed flour are present variably (Table 6). The seed contains relatively higher amounts of 2.6 and 1.2g of total free phenols and phytic acid, respectively, than the commonly cultivated legumes as observed earlier (Bressani, Brenes, Garcia, & Elias, 1983; Khan, Jacobson, & Eggum, 1979; Rajaram & Janardhanan, 1992; Rodrigues & Thorne, 1991). The contents of tannin and non-protein amino acid, L-DOPA are found to be very low (1.0 and 0.9g, respectively) when

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

Amino acid	Available quantity	FAO/WHO/UNO recommended amino acid requirements (1985) for adults
Glutamate	137	
Aspartate	82	
Serine	44	
Therionine	34	9
Proline	86	
Alanine	37	
Glycine	39	
Valine	37	13
Cystine + Methionine	12	17
Leucine	79	19
Isoleucine	44	13
Tyrosine + Phenylalanine	77	19
Lysine	54	18
Histidine	32	16
Tryptophan	–	5
Arginine	48	

Table 5
Data on mineral composition of *Neonotonia wightii* seeds

Mineral	Available quantity[a] (mg/100g seed protein)	Recommended dietary allowance in mg/100g seed protein (1989)	
		Adult (Males)	Adult (Females)
Sodium	112	500	500
Potassium	2,899	2000	2000
Calcium	613	800	800
Magnesium	571	350	280
Phosphorus	392	800	800
Zinc	3.2	15	15
Manganese	2.9	2–5	2–5
Iron	5.9	10	10
Copper	1.8	1.5–3	1.5–3

[a] Mean of triplicate determinations expressed on dry weight basis.

Table 6
Data on anti-nutritional factors present in the seed flour[a]

Component	Seed flour (g/100 g)
Total free phenols	2.6
Tannins	1.0
L-DOPA	0.2
Hydrogen cyanide	0.01
Phytic acid	1.2

[a] Mean of triplicate determinations expressed on dry weight basis.

compared with other species in *Vigna* (Rajaram & Janardhanan, 1990; Siddhuraju et al., 1992). Apart from these anti-nutritional factors, the presence of negligible amount of hydrogen cyanide (0.01g/100 g 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 four times before consumption is being practiced by the Malayalis to eliminate most of the anti-nutritional factors. All the anti-nutritional factors reported except L-DOPA are heat labile. Hence, they can be removed by wet or dry thermal treatments (Geervani & Theophilus, 1981). In an earlier study, it has been demonstrated that the L-DOPA content 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, Viswanathan, & Ramesh, 2001; Viswanathan, Thangadurai, Tamil Vendan, & Ramesh 1999). Therefore, the presence of these anti-nutritional factors may not be a limiting factor in the utilization of these seeds for food and other purposes.

Thus, the present study reveals the chemical composition and nutritional quality of lesser known legumes, *Neonotonia wightii* seeds. The presence of relatively higher amounts of crude protein, crude fat and NFEs were found to be responsible for *Neonotonia wightii* seeds as a good source of energy, which merit consideration as alternative source for human diet. The seeds are also a good source of essential amino acids such as leucine, lysine, isoleucine, valine, therionine and histidine, and minerals such as potassium, magnesium, manganese and copper, when compared with other commonly consumed legumes. Significantly, the anti-nutritional factors can be inactivated by wet or dry thermal cooking process. Therefore, it is possible to reduce the activity of most of the anti-nutritional factors. There are well-recognized shortcomings in consuming animal proteins due to unhygienic processing, storage and consequent microbial contamination. In addition, legumes have been reported to reduce the levels of plasma cholesterol and blood glucose in experimental animals (Singh & Singh, 1992). If this effect also occurs in human, it may have considerable physiological significance in reducing the risk of heart disease in people having pulses in their diet. That the low cholesterol and high unsaturated fatty acid contents reduces the risk of coronary heart diseases and arteriosclerosis has been shown elsewhere (Soni, George, & Singh, 1982). It is very interesting to note the fewer incidences of heart diseases and malaria fever among the Malayali tribals may be due to the consumption of this legume seeds, having high unsaturated fatty acid content and negligible amount of hydrogen cyanide warrants further study.

In view of the above facts, *Neonotonia wightii* 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. As its domestication for commercial exploitation is to be 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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