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Nutritional and anti nutritional assessment of under utilized legume *D. lablab* var. *vulgaris* L.

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Abstract

Two samples of seed materials of the under utilized tribal pulse, *D. lablab* var. *vulgaris* (dark brown and pale brown coloured seed coat) were collected from Anakodi, Krishnagiri district, Eastern Ghats, Tamil Nadu. The mature seed samples were analysed for proximate composition, mineral profiles, vitamins, seed protein fractions, fatty acid profiles, amino acid profiles and antinutritional factors. The investigated seed samples of *D. lablab* var. *vulgaris* contained higher amounts of crude protein and crude lipid when compared with most of the commonly consumed pulses. The investigated seeds were rich in minerals such as Na, K, P, Ca, Mg and Fe. Albumin and globulin fractions constituted the major bulk of seed protein. The essential amino acid profiles of total seed proteins were compared favourably with FAO/WHO (1991) requirement pattern. The fatty acid profiles of both the samples revealed that the seed lipids contained higher concentration of unsaturated fatty acids (66.78-69.08%) and had very high contents of linoleic acid (40.36-41.62%). Antinutrtional factors like total free phenolics, tannins, L-DOPA, phytic acid, hydrogen cyanide, trypsin inhibitor, oligosaccharides (raffinose, stachyose, verbascose) and phytohaemagglutinating activity were analyzed.

Keywords: *D. lablab* var. *vulgaris*; Vitamins; Protein fractions; Fatty acids profiles; Amino acids; Antinutritional factors

Introduction

Legumes with high protein content, energy values, vitamin and mineral content, have been recognized as "meat of poor people" (Bello-Perez *et. al.*, 2007). The increasing global protein consumption and high prices for meat and fish have led to the demand for new food protein sources, particularly from plants. 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). There are some 28 wild legumes commonly consumed by different tribal seeds of India (Arora *et. al.*, 1980; Arinathan *et. al.,* 2009). However, most of the underutilized legumes/tribal pulses remain uninvestigated biochemically and nutritionally. The tribal communities living in the forest of Eastern Ghats, Krishnagiri, Tamil Nadu, collected the seeds of underutilized legumes from the vicinity of the forests and consumed the seed after boiling. This was tempted to study the nutritional and antinutritional composition of the underutilized legumes for its potential use in human nutrition. In this context, in the present investigation, an attempt has been made to understand the chemical composition and antinutritional factors of the tribal pulses to suggests ways and means to remove the antinutritional /toxins and make the edible plants safe protein sources for mass consumption. Therefore, this study was designed to evaluate the nutritional and antinutritional properties of underutilized legume *Dolichos lablab* var. *vulgaris.*

Materials and methods

Collection of seed samples

The seeds of *Dolichos lablab* var. *vulgaris* (dark brown and pale brown coloured seed coat) were collected in wild conditions during the month of August 2011 from Anakodi, Krishnagiri district, Eastern Ghats, Tamil Nadu. With the help of local flora, the plants were botanically identified. The collected pods were thoroughly sun dried, the pods were thrashed to remove seeds. Broken seeds, foreign materials and immature seeds were removed, followed by thorough cleaning and storage in an air tight plastic jar at room temperature (25^{o}C).

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Proximate composition

Moisture and crude protein

The moisture content was determined by drying 50 transversely cut seed in an oven at 80ºC for 24 hr and is expressed on a percentage basis. The air-dried samples were powdered separately in a Wiley mill (Scientific Equipment, Delhi, India) to 60-mesh size and stored in screw capped bottles at room temperature for further analysis.

The nitrogen content was estimated by the micro-Kjeldahl method (Humphries, 1956) and the crude protein content was calculated (N x 6.25).

Crude lipid and ash

Crude lipid content was determined using Soxhlet apparatus (AOAC, 2005).The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr (AOAC, 2005).

Total dietary fibre

Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method (Li and Cardozo, 1994). To determine the TDF, duplicate 500 mg ground samples were taken in separate 250 mL beakers. To each beaker 25 mL water was added and gently stirred until the samples were thoroughly wetted, (*i.e*. no clumps were noticed). The beakers were covered with Al foil and allowed to stand 90 min without stirring in an incubator maintained at 37°C. After that, 100 mL 95% ethanol was added to each beaker and allowed to stand for 1 hr at room temperature (25±2°C). The residue was collected under vacuum in a pre-weighed crucible containing filter aid. The residue was washed successively with 20 mL of 78% ethanol, 10 mL of 95% ethanol and 10 mL acetone. The crucible containing the residue was dried for ≥ 2 hr at 105°C and then cooled for ≥ 2 hr in a desiccator and weighed. One crucible containing residue was used for ash determination at 525°C for 5 hr. The ash-containing crucible was cooled for ≥ 2hr in a desiccator and weighed. The residue from the remaining duplicate crucible was used for crude protein determination by the micro-Kjeldahl method as already mentioned. The TDF was calculated as follows.

$$TDF\% = 100 \times \frac{Wr - [(P+A)/100]\,Wr}{Ws}$$

Where *Wr* is the mg residue, *P* is the % of protein in the residue; *A* is the % ash in the residue, and *Ws* is the mg sample.

Nitrogen free extractives

The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980).

Calorific value

The energy value of the seed (kJ) was estimated by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, respectively (Siddhuraju *et. al.,* 1996).

Minerals and vitamins analysis

Five hundred milligrams of the ground legume seed was digested with a mixture of 10 mL concentrated nitric acid, 4 mL of 60% perchloric acid and 1 mL of concentrated sulphuric acid. After cooling, the digest was diluted with 50 mL of deionised distilled water, filtered with Whatman No. 42 filter paper and the filtrates were made up to 100 mL in a glass volumetric flask with deionised distilled water. All the minerals except phosphorus were analysed from a triple acid-digested sample by an atomic absorption spectrophotometer - ECIL (Electronic Corporation of India Ltd., India) (Issac and Johnson, 1975). The phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman and Bray, 1940).

Ascorbic acid and niacin contents were extracted and estimated as per the method given by (Sadasivam and Manickam, 1996). For the extraction of ascorbic acid, 3g air-dried powdered sample was ground with 25 mL of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10 mL of the filtrate until it turned orange-yellow to remove the enolic hydrogen atoms. The excess of bromine was expelled by blowing in air. This filtrate was made up to 25 mL with 4% oxalic acid and used for ascorbic acid estimation. Two millilitres of the extract was made up to 3 mL with distilled H_2O in a test tube. One millilitre of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. The contents of the test tube were mixed thoroughly. After 3 hr incubation at 37°C, 7 mL of 80% H_2SO_4 was added to dissolve the osazone crystals and the absorbance was measured at 540 nm against a reagent blank. For the extraction of niacin, 5g air-dried powdered sample was steamed with 30ml concentrated H_2SO_4 for 30 min. After cooling, this suspension was made up to 50ml with distilled H_2O and filtered. Five millilitres of 60% basic lead acetate was added to 25 mL of the filtrate. The pH was adjusted to 9.5 and centrifuged to collect the supernatant. Two millilitres of concentrated H_2SO_4 was added to the

supernatant. The mixture was allowed to stand for 1hr and centrifuged. The 5 mL of 40% $ZnSO_4$ was added to the supernatant. The pH was adjusted to 8.4 and centrifuged again. Then the pH of the collected supernatant was adjusted to 7 and used as the niacin extract. For estimation, 1 mLextract was made up to 6 mL with distilled water in a test tube, 3 mL cyanogen bromide was added and shaken well, followed by addition of 1 mL of 4% aniline. The yellow colour that developed after 5 min was measured at 420 nm against a reagent blank. The ascorbic acid and niacin contents present in the sample were calculated by referring to a standard graph and expressed as milligrams per 100 g of powdered samples.

Lipid extraction and fatty acid analysis

The total lipid was extracted from the seeds according to the method of (Folch *et. al*., 1957) using chloroform and methanol mixture in 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 performed by gas chromatography (ASHMACO, Japan; Model No: ABD20A) using an instrument equipped with a flame ionization detector and a glass column (2m x 3mm) packed with 1% diethylene glycol succinate on chromosorb W. The temperature conditions for GC were injector 200°C and detector 210°C. The temperature of the oven was programmed from 180°C and the carrier gas was nitrogen at a flow rate of 30 ml/min. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters; the relative weight percentage of each fatty acid was determined from integrated peak areas.

Amino acid analysis

The total seed protein was extracted by a modified method of (Basha *et. al.*, 1976). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). A protein sample of 30mg was hydrolysed by 6N HCL (5 mL) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 hr. The sealed tube was broken and the acid removed completely by repeated flash evaporation after the addition of de-ionized water. Dilution was effected by means of citrate buffer pH 2.2 to such an extent that the solution contained 0.5 mg protein ml^{-1}. The solution was passed through a millipore filter (0.45 µM) and derivitized with O-phthaldialdehyde by using an automated pre-column (OPA). Aminoacids were analysed by a reverse - phase HPLC (Method L 7400, HITACHI, Japan) fitted with a denali C18 5 micron column (4.6 x 150 mm). The flow rate was 1 mL per min with fluorescence detector. The cystine content of protein sample was obtained separately by the (Liddelle and Saville, 1959) method. For the determination of tryptophan content of proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together with 1 mL 5M NaOH. The ampoules were flame sealed and incubated at 110°C for 18 hr. The tryptophan contents of the alkaline hydrolysates were determined colorimetrically using the method of (Spies and Chambers, 1949) as modified by (Rama Rao *et. al.*, 1974). The contents of the different amino acids (AA) were expressed as g/100g proteins and were compared with FAO/WHO (1991) reference pattern. The essential amino acid score was calculated as follows:

Essential AA score =

$$\frac{\text{Essential AA in g/100g of total protein}}{\text{Essential AA in g/100g of FAO/WHO (1991) reference pattern}} \times 100$$

Analysis of antinutritional compounds

The antinutritional compounds, total free phenolics (Bray and Thorne, 1954), tannins (Burns, 1971), the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) (Brain, 1976), phytic acid (Wheeler and Ferrel, 1971) and hydrogen cyanide (Jackson, 1967) were quantified. Trypsin inhibitor activity was determined by the enzyme assay of (Kakade *et. al.,* 1974) by using benzoil-DL-arginin-p-nitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410 nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Estimation of oligosaccharides

Extraction of oligosaccharides was done following the method of (Somiari and Balogh, 1993). Five grams each of all the samples of seed flours were extracted with 50 mL of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 hr and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25 mL of 70% (v/v) ethanol. The filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in five mL of double-distilled water. Separation of oligosaccharides was done by TLC. Thirty g of cellulose-G powder were dissolved in 45 ml of double distilled water and shaken well until the slurry was homogeneous. TLC plates were coated with the slurry and air-dried. Spotting of the

sugar samples was done by using micropipettes. Five µL aliquots of each sample were spotted thrice separately. The plates were developed by using a solvent system of n-propanol, ethyl acetate and distilled water (6:1:3), and dried (Tanaka *et. al*., 1975). The plates were sprayed with α -naphthol reagent (1%, w/v). Plates were dried in a hot-air oven. The separated spots were compared with standard sugar spots. A standard sugar mixture containing raffinose, stachyose and verbascose (procured from sigma chemical co., St. Louis, USA). Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped, eluted in 2 mL of distilled water kept overnight and filtered through Whatman No. 1 filter paper. The filtrates were subjected to quantitative estimation. The eluted individual oligosaccharides were estimated by the method of (Tanaka *et. al*., 1975). One mL of the eluted and filtered sugar solution was treated with one ml of 0.2 M thiobarbituric acid and one ml of concentrated HCl. The tubes were boiled in a water bath for exactly 6 min. After cooling, the oligosaccharide contents were quantified in an Elico UV-Spectrophotometer model SL 150 at 432 nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was expressed on dry weight basis.

Determination of phytohaemagglutinating (Lectin) activity

Lectin activity was determined by the method of (Almedia *et. al.,* 1991). One g of air-dried seed flour was stirred with 10ml of 0.15 N sodium chloride solution for 2 hours and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 x g for 20 min. and the supernatants were collected separately. The protein content was estimated by the (Lowry *et. al.*, 1951) method. Human blood (blood groups A, B and O) was procured from the blood bank of Jothi Clinical Laboratory, Tuticorin.

Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3 min at low speed. Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24 drops of phosphate - buffered saline.

The determination of lectin was done by the method of Tan *et. al.,* (1983). Clear supernatant (50 µL) was poured into the depression (pit) on a micro-titration plate and serially diluted 1:2 with normal saline. The human blood erythrocyte (A, B and O blood groups) suspensions (25 µL) were added to each of the twenty depressions. The plates were incubated for 3 hours at room temperature. After the incubation period, the titre values were recorded. One haemagglutinating unit (HU) is defined as the least amount of haemagglutinin that will produce positive evidence of agglutination of 25 µL of a blood group erythrocyte after 3hr incubation at room temperature. The phytohaemagglutinating activity was expressed as haemagglutinating units (HU) / mg protein.

Determination of in vitro protein digestibility (IVPD)

This was determined using the multi-enzyme technique (Hsu *et. al*., 1977). The enzymes used for IVPD were purchased from Sigma Chemical Co., St. Louis, MO, USA. Calculated amounts of the control (casein) and sample were weighed out, hydrated in 10 mL of distilled water and refrigerated at 5^{o}C for 1h. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37^{o}C. The IVPD was determined by the sequential digestion of the samples containing protein with a multi-enzyme mixture (trypsin, oo-chymotrypsin and peptidase) at 37^{o}C followed by protease at 55^{o}C. The pH drop of the samples from pH 8.0 was recorded after 20min of incubation. The IVPD was calculated according to the regression equation Y= 234.84 - 22.56 X, where Y is the % digestibility and X the pH drop.

Statistical analysis

Proximate composition of minerals, vitamins (niacin and ascorbic acid), antinutritional factors like total free phenolics, tannins, L-DOPA, phytic acid, hydrogen cyanide and oligosaccharides were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

Results and discussion

The proximate composition of *Dolichos lablab* var. *vulgaris* are shown in the Table I. The crude protein content of *Dolichos lablab* var. *vulgaris* is higher than the commonly consumed legumes *Cicer arietinum* (Srivastava and Ali, 2004; Khatoon and Prakash, 2006); tribal pulses like *Dolichos trilobus, Rhynchosia cana, R. shaveolens, Vigna radiata var. sublobata, V. unguiculata subsp. cylindrical* (12.8 - 18.5 g/100gm) (Arinathan *et. al*., 2009) and *Lablab purpuries* var. CO_{12} (Kala *et. al*., 2010). The remarkably high level of protein in the wild legume under study underscores their importance as source of this vital nutrient. Similarly both the seed samples of *Dolichos lablab* var. *vulgaris* contained higher lipids than those in other tribal pulses, *Vigna aconitifolia* and *V. unguiculata* subsp. *unguiculata* (Maroon coloured seed coat) (Tresina and Mohan, 2011).

The high NFE content of *Dolichos lablab* var. *vulgaris* (both the samples) enable this legume to act as a good source of calories which would be antimarasmus, especially infant nutrition (Vadivel and Janardhanan, 2000). The range in calorific values (1606.08-1609.21 KJ $100g^{-1}$ DW) exceeds energy value of cowpea, green gram, horse gram, moth bean and peas (Rao *et. al.*, 1989) which are in the range of 1318-1394 KJ $100g^{-1}$ DW.

Food legumes are a good source of minerals such as calcium, iron, copper, Zinc, potassium and magnesium (Salunkhe *et. al.*, 1985). Table II showed the mineral composition of the seed samples of *Dolichos lablab* var. *vulgaris* registered a higher level of potassium when compared with recommended dietary allowance value of infants and children (< 1550 mg) (NRC/NAS 1980). The high content of potassium can be utilized beneficially in the diets of people who take diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid (Siddhuraju *et. al*., 2001). Both the investigated samples of *Dolichos lablab* var. *vulgaris* contained high levels of sodium, calcium, phosphorus, magnesium and iron when compared with

Table I. Proximate composition of *Dolichos lablab* var. *vulgaris* (g/100g seed flour)[a]

Component	*Dolichos lablab* var. *vulgaris* (Dark brown coloured seed coat)	*Dolichos lablab* var. *vulgaris* (Pale brown coloured seed coat)
Moisture	7.80±0.11	6.98±0.07
Crude Protein (Kjeldahl N× 6.25)	21.34±0.21	22.30±0.26
Crude lipid	5.64±0.09	5.32±0.03
Total dietary fibre (TDF)	6.66±0.06	6.23±0.03
Ash	4.26±0.03	4.01±0.01
Nitrogen free Extractives (NFE)	62.10	62.05
Calorific value (KJ $100g^{-1}$ DM)	1606.08	1609.21

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

± denotes standard error.

Robinson (1987) reported that a diet that meets two-thrids of the RDA (Recommended Dietary Allowances) values is considered to be adequate for an individual. Food legumes have been recognized as important sources of several mineral in India diets (Gopalan *et. al.,* 1978).

Table II. Mineral composition and Vitamins (niacin and ascorbic acid) of *Dolichos lablab* var. *vulgaris* (mg/100g seed flour)[a]

Component	*Dolichos lablab* var. *vulgaris* (Dark brown coloured seed coat)	*Dolichos lablab* var. *vulgaris* (Pale brown coloured seed coat)
Sodium	36.80±0.09	28.24±0.19
Potassium	1888.01±1.38	1936.16±2.46
Calcium	268.56±0.31	236.12±0.28
Magnesium	108.04±0.17	98.20±0.11
Phosphorus	238.12±0.24	256.07±0.36
Iron	17.20±0.06	14.21±0.07
Copper	1.21±0.04	0.098±0.01
Zinc	2.24±0.03	1.96±0.03
Manganese	1.36±0.01	1.56±0.02
Na/K	0.019	0.015
Ca/P	1.13	0.92
Niacin	26.21±0.14	29.30±0.16
Ascorbic acid	34.01±0.17	31.26±0.13

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

± denotes standard error.

Phaseolus vulgaris, P. limensis, Vigna unguiculata, Pisum sativum, Lens culinaris and *Cicer arietinum* (Meiners *et. al*., 1976). The zinc, copper and manganese content were found to be higher than the *Vigna aconitifolia* (Tresina and Mohan, 2011). The ratios of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are also shown in Table II. Na/K ratio in the body is of great concern for prevention of high blood pressure Na/K ratio less than one is recommended. Hence, in the present study, all the seed probably reduce high blood pressure disease because they had Na/K less than one. Modern diets which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine (Shills and Young, 1988). This had led to the concept of the Ca/P ratio. If the Ca/P ratio is low (low calcium, high phosphorus intake), more than the normal amount of calcium may be loss in the urine, decreasing the calcium level in bones. Food is considered "good" if the ratio is above 1 and "poor" if the ratio is less than 0.5 (Nieman *et. al.,* 1992). The Ca/P ratio in the present study ranged between 0.92 to 1.13 indicating they would serve as good sources of minerals for bone formation.

Ascorbic acid that is vitamin C, is an essential nutrient for man as he lacks the capacity to synthesis it like many other animal species. Ascorbic acid is a strong reducing agent. It is involved in collagen synthesis, bone and teeth calcification and many other reactions in the body as a reducing agent. Nicotinic acid (also called niacin) is a vitamin intimately connected with several metabolic reactions. It takes part as a component of coenzyme in oxidative reactions and is concerned with metabolism of carbohydrate, fats and proteins. The presently investigated legumes *Dolichos lablab* var. *vulgaris* exhibited the highest level of niacin content (Table II) which was found to be higher than that of an earlier report in *Cajanus cajan, Dolichos lablab, D. biflorus, Mucuna pruriens, Phaseolus mungo, Vigna catjang* and *Vigna* species (Rajalakshmi and Geervani, 1994); *Vigna unguiculata* subsp *cylindrica* (Arinathan *et. al.,* 2009) and *Vigna* species (Tresina and Mohan, 2011). The investigated tribal pulses also registered higher level of ascorbic acid content than *Cicer arietinum* (Fernandez and Berry, 1988); *Teramnus labialis* (Arinathan *et. al.*, 2009); *Vigna mungo* varieties (Tresina *et. al.*, 2010) and *Vigna* species (Tresina and Mohan, 2011).

In the samples investigated, albumin and globulins (5.83-6.58% and 11.63-12.19% respectively) constitute the major bulk of the proteins (Table III) as in most legume reported earlier (Tresina *et. al.,* 2010; Murthy, 2011; Murthy and Emmanuel, 2011). The amino acid profiles of the purified seed proteins and the essential amino acid score are presented in Table IV. The content of essential amino acid except tryptophan in the presently investigated *Dolichos lablab* var. *vulgaris* (both the samples) was found to be higher compared to the FAO/WHO (1991) requirement pattern. In the presently investigated seed samples registered higher amounts of *in vitro* protein digestibility than that of earlier investigations in the seeds of *Vigna mungo* varieties (Tresina *et. al.*, 2010) and *Lablab purpuries* varieties CO_1 and CO_{12} (Kala *et. al.,* 2010).

Table III. Data on total (free) proteins and protein fractionation of seed flour of *Dolichos lablab* var. *vulgarisa*[a]

Fraction	*Dolichos lablab* var. *vulgaris* (Dark brown coloured seed coat)		*Dolichos lablab* var. *vulgaris* (Pale brown coloured seed coat)	
	g /100g seed flour	g /100g Protein	g /100 g seed flour	g /100g Protein
Total protein (free protein)	18.48±0.14	100	19.96±0.24	100
Albumins	5.83± 0.06	31.55	6.58±0.09	32.96
Globulins	11.63±0.03	62.93	12. 19±0.11	61.07
Prolamins	0.54±0.01	2.92	0.61±0.01	3.06
Glutelins	0.48±0.01	2.60	0.58±0.03	2.91

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

± denotes standard error.

The fatty acid composition of the total seed lipids of *Dolichos lablab* var. *vulgaris* (both the samples) were given in Table V. The data revealed that, both the seed lipids were rich in unsaturated fatty acids (66.78-69.08%) and had very high contents of linoleic acid (40.36-41.62%). These values are nutritionally desirable and also higher than certain legumes *Vigna radiata* (Salunkhe *et. al*., 1982); *Phaseolus vulgaris* (Omogbai, 1990); *Vigna mungo* varieties (Tresina *et. al*., 2010) *Vigna aconitifolia* and *Vigna unguiculata* subsp unguiculata (Tresina and Mohan, 2011). The palmitic acid content of *Dolichos lablab* var. *vulgaris* (both the samples)

Table IV. Amino acid profiles of acid-hydrolysed, purified total seed proteins of *Dolichos lablab* var. *vulgarisa*[a]

Amino acid	*Dolichos lablab* var. *vulgaris* (Dark brown coloured seed coat)	Essential amino acid score	*Dolichos lablab* var. *vulgaris* (Pale brown coloured seed coat)	Essential amino acid score	FAO/WHO (1991) requirement pattern
Glutamic acid	15.63		15.12		
Aspartic acid	13.68		14.36		
Serine	5.21		5.06		
Threonine	5.01	147.35	5.31	156.18	3.4
Prolien	4.32		3.98		
Alanine	4.12		4.24		
Glycine	4.36		4.84		
Valine	4.98	142.29	5.16	147.43	3.5
Cystine	0.38	63.60	0.46	63.20	2.5
Methionine	1.21		1.12		
Isoleucine	4.56	162.86	4.84	172.86	2.8
Leucine	7.36	11.52	7.86	119.09	6.6
Tyrosine	4.01	147.46	4.36	157.14	6.3
Phenylalamine	5.28		5.54		
Lysine	7.65	131.90	7.02	121.03	5.8
Histidine	3.54	186.32	3.74	196.84	1.9
Tryptophan	0.94	85.45	0.88	80.00	1.1
Arginine	5.62		5.39		

[a] All values are of single determinations.

was higher than the other legumes such as *Vigna radiata* (Salunkhe *et. al.,* 1982); *V. unguiculata, Phaseolus vulgaris* (Omogbai, 1990); *Glycine max* (Ologhobo and Fetuge, 1984) and *Vigna* species (Tresina and Mohan, 2011).

Legumes commonly consist of toxic factors, which decrease the digestibility and prevent bioavailability of nutrients. For this reason, preliminary evaluations of some of these factors in raw pulses were made (Table VI). The content of total free phenolics of currently investigated pulses appears to be lower than the earlier reports in *Canavalia gladiata, Neonotonia wightii var.* coimbatorensis*, Vigna trilobata, Vigna unguiculata* subsp *unguiculata, Vigna radiata* var. *sublobata, Dolichos trilobus, Rhynchosia cana, R. shaveolens, Teramnus labialis, V. unguiculata* subsp *cylindrica* (Arinathan *et. al.,* 2003; 2009); *V.mungo* varieties (Tresina *et. al.*, 2010) *Vigna* species (Tresina and Mohan, 2011) and *Cajanus albicans* (Murthy, 2011). The tannin content of the investigated samples were relatively lower than the *Phaseolus vulgaris, Cajanus cajan* (Sangronis and Machado, 2007); *Vigna radiata, V. mungo* (Kakati *et. al.*, 2010); *Phaseolus aureus, Cajanus cajan, Lens esculenta* and

Table V. Fatty acid profile of the seed lipids of *Dolichos lablab* var. *vulgaris* (g/100g)[a]

Fatty acid	*Dolichos lablab* var. *vulgaris* (Dark brown coloured seed coat)	*Dolichos lablab* var. *vulgaris* (Pale brown coloured seed coat)
Palmitic acid (C16:0)	28.32	27.30
Stearic acid (C18:0)	3.10	2.84
Oleic acid (C18:1)	14.28	14.56
Linoleic acid (C18:2)	40.36	41.62
Linolenic acid (C18:3)	12.14	12.90
Others	1.80	0.78

[a] All values are of two determinations.

Table VI. Data on *in vitro* protein digestibility (IVPD) and antinutritional factors o *Dolichos lablab* var. *vulgaris*[a]

Components	*Dolichos lablab* var. *vulgaris* (Dark brown coloured seed coat)	*Dolichos lablab* var. *vulgaris* (Pale brown coloured seed coat)
In vitro protein digestibility (%)[a]	66.34±1.21	67.60±0.76
Total free phenolics[b] g /100 g	0.61±0.03	0.52±0.04
Tannins[b] g /100g	0.17±0.02	0.19±0.01
L-DOPA[b]g /100g	0.91±0.11	0.87±0.12
Phytic acid[b] g /100g	413.26±2.46	388.21±2.14
Hydrogen cyanide[b] mg /100g	0.31±0.01	0.24±0.02
Trypsin inhibitora(TIU mg^{-1} protein)	29.28±0.07	31.24±0.14
Oligosaccharides[b]g /100g		
Raffinose	0.58±0.06	0.41±0.03
Stachyose	1.76±0.03	1.66±0.09
Verbascose	1.20±0.04	1.24±0.03
Phytohaemagglutinating activity[a](Hu mg^{-1} protein)		
A group	34	42
B group	164	174
O group	29	33

[a] All values of two independent experiments

[b] All values are of means of triplicate determination expressed on dry weight basis

± Standard error

Cicer arietinum (Khandelwal *et. al.*, 2010). Phenolics and tannins are known to inhibit activities of digestive enzymes and hence, the presence of even low levels of tannins and phenolics is not desirable from a nutritional point of view. However, in legumes, the soaking and cooking process is known to reduce phenolics and tannins significantly (Vadival and Pugalenthi, 2008). Recently phenolics have antioxidative, antiallergic, antidiabetic, anticarcinogenic, antimicrobial, antimutagenic and anti-inflammatory activities. (Arts and Hollman, 2005; Scalbert *et. al.*, 2005). In the currently investigated samples, the content of the non-protein amino acid, L-DOPA is low compared with those of the tribal pulses such as *Entada rheedi, Vigna radiata* var. sublobata and *Vigna unguiculata* subsp *unguiculata* (Arinathan *et. al*., 2003; 2009) *Paracalyx scariosus* (Murthy and Rao, 2009) and *Rhynchosia bracteata* (Murthy and Emmanuel, 2011).

Phytic acid has an antinutritional property because of its ability to lower the bioavailability of essential minerals and to form a complex with protein, thereby inhibiting the enzymatic digestion of investigated protein (Nolan and Duffin, 1987). Phytic acid content of investigated seed samples was found to be low when compared with that of some commonly consumed legumes, *Lablab purpureus* (Osman, 2007); *Vigna radiata* and *V. mungo* (Kakati *et. al.*, 2010) and more or less equal to the *Vigna mungo* varities Tmv1 and Vamban1 (Tresina *et. al.*, 2010). Hydrogen cyanide is known to cause acute or chronic toxicity. The content of HCN level in the presently investigated legume was far below the level *i.e*. 36mg/100g (Oke, 1969) and comparable with those of *Dolichos trilobus, Rhynchosia cana R.suaveolens, Teramnus labialis, Vigna radiata* var. *sublobata, Vigna unguiculata* subsp *cylindrica* (Arinathan *et. al*., 2009); *Vigna* aconitifolia and *Vigna unguiculata* subsp *unguiculata* (Tresina and Mohan, 2011). The trypsin inhibitor activities of the presently studied samples were higher than that of *Vigna radiata, Phaseolus vulgaris* (Mohamed *et. al.,* 2011) and *Vigna* species (Tresina and Mohan, 2011). Stachyose seems to be the principle oligosaccharide of investigated *Dolichos lablab* var. *vulgaris* (both the samples). It was in conformity with the earlier reports in *Cicer arietinum* (Alajaji and El-Adawy, 2006); *Sphenostylis stenocarpa, Voandzeia subterranean, Phaseolus vulgaris, P. lunatus, Cajanus cajan, Canavalia ensiformis* (Apata, 2008); *Lablab purpures* varieties (Kala *et. al.*, 2010); *Vigna aconitifolia* and *Vigna unguiculata* subsp *unguiculata* (Tresina and Mohan, 2011). The lectin (Phytoheamagglutinating activity) of seed samples exhibited

a high level of agglutination activity specifically in 'B' group compared to other two blood groups 'A' and 'O'. This is in good agreement with earlier reports in the *Vigna aconitifolia* and *Vigna unguiculata* subsp *unguiculata* (Tresina and Mohan, 2011).

Investigations on the nutritional and antinutritional qualities of seeds of *Dolichos lablab* var. *vulgaris* are scanty. On the basis of the above findings, it is concluded that the tribal pulse investigated seem to be a good source of protein, essential amino acids, essential fatty acids, minerals and vitamins. All the antinutritional factors reported except L-DOPA are heat liable. Hence they can be removed by wet or dry thermal treatment. It has been demonstrated that, the level of L-DOPA is significantly eliminated by soaking and autoclaving (Vadivel and Pugalenthi, 2008). The presently studied tribal pulses exhibit high level of nutrients, besides *in vitro* protein digestibility and low level of antinutritional factors. From these chemical investigations it is concluded that, the presently investigated tribal pulses can be used as protein source to curtail with problem of protein deficiency in most of the developing countries which may result in many child killer diseases. After conducting toxicology/animal feeding experiments, these little known tribal pulses may be recommended for large scale consumption as an alternative potential source of protein.

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