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COMPARATIVE ASSESSMENT ON THE NUTRITIONAL AND ANTINUTRITIONAL ATTRIBUTES OF THE UNDERUTILIZED LEGUMES, *Canavalia gladiata* (JACQ.) DC, *Erythrina indica* LAM. AND *Abrus precatorius* L.

[EVALUACIÓN COMPARATIVA DE LOS ATRIBUTOS NUTRICIONALES Y ANTINUTRICIONALES DE LEGUMINOSAS SUBUTILIZADAS, *Canavalia gladiata* (JACQ.) DC, *Erythrina indica* LAM. Y *Abrus precatorius* L]

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SUMMARY

The seed samples of *Canavalia gladiata*, *Erythrina indica* and *Abrus precatorius* (red and black coloured seed coat and white coloured seed coat) were collected from the regions of Tamil Nadu and analyzed for their chemical composition with a view to evaluate their nutritional potential. The proximate composition revealed that, all the presently investigated seed samples were found to contain high content of crude protein and crude lipid. Mineral profiles were also analyzed in all the seed samples. All the investigated pulses appeared to be good sources of potassium, magnesium, sodium, calcium, phosphorus, iron, zinc, copper and manganese content were deficient when compared with Recommended Dietary Allowance (NRC/NAS, 1980, 1989). Vitamins (niacin and ascorbic acid) contents were found to be relatively high in the investigated pulses. The essential amino acid profiles of total seed proteins were compared favorably with FAO/WHO (1991) requirement pattern. Fatty acid profiles revealed that, all the investigated seed samples had rich unsaturated fatty acids (55.60-72.04%) and very high content of linoleic acid (24.16-34.14%). The IVPD of the investigated tribal pulses ranged from 63.31-71.36%. Antinutritional factors like total free phenolics, tannins, L-DOPA, phytic acid, hydrogen cyanide, trypsin inhibitor, oligosaccharides and phytohaemagglutinating activity were analyzed.

Key words: Legumes; nutritive value.

RESUMEN

Se colectaron muestras de semillas de of *Canavalia gladiata*, *Erythrina indica* y *Abrus precatorius* (roja y negra, semilla de cascara colorada y semilla de cascara blanca) de las regiones de Tamil Nadu y se analizó su composición química con la visión de evaluar su potencial nutricional. La composición proximal reveló que todas las muestras investigadas de semillas contenían altos niveles de proteína cruda y grasa. Los perfiles de minerales fueron también analizados en todas las muestras. Todas las muestras parecieron tener buenas fuentes de potasio, magnesio, sodio, calcio, fosforo, hierro, zinc, cobre, el contenido de manganeso fue deficiente cuando se comparó con los requerimentos diarios en la dieta (NRC/NAS, 1980, 1989). Los contenidos de vitaminas (niacina y ácido ascórbico) fueron relativamente altos en todas las muestras investigadas. Los perfiles de aminoácidos esenciales del total de proteína de la semilla se compararon favorablemente con los patrones de requerimientos de la FAO/WHO (1991). Los perfiles de ácidos grasos revelaron que todas las muestras de semilla fueron ricas en ácidos grasos insaturados (55.60-72.04) y muy altos niveles de ácido linoleico (24.16-34.14%). El IVPD de las muestras estuvo en un rango de 63.31-71.36%. Se analizaron los factores antinutricionales como fenoles totales, taninos, L-DOPA, ácido fítico, hidrogeno ciánico, inhibidor de tripsina, oligosacáridos y fitohematoaglutinación.

Palabras clave: Leguminosas; valor nutritivo.

INTRODUCTION

Legumes are widely grown throughout the world and their dietary and economic importance is globally appreciated and recognized. Legumes not only add variety to diet but also serve as an economical source of supplementary proteins for a large human population. In India, they provide the only high protein component of the average diet and over 10 million tones are consumed annually (Sood *et al*.,

2002). They have high protein content (20-26%) and can be considered as a natural supplement to cereals. Next to fish (dry) which provides 335g protein per kg, grain legumes provide 220-250g protein per kg. Hence legumes are considered as "poor man's meat" (Kakati *et al*., 2010). The availability and consumption of protein food in India will remain inadequate due to population explosion and urbanization leading to Protein Energy Malnutrition (PEM). The PEM problem can be alleviated by finding alternative cost effective sources of proteins (Waterlow, 1994).

With an increasing interest in new food resources, the seeds of wild plants including the tribal pulses receive more attention, because they are highly resistant to disease and pests and exhibit good nutritional qualities (Janardhanan *et al*., 2003a). The underutilized legumes/wild tribal pulses have tremendous potential for commercial exploitation but remain ignored. They offer good scope to meet the ever-increasing demands for vegetable protein. Although they have high protein content and good nutritional value, their utilization is limited by the presence of some antinutritional/antiphysiological/ toxic substances (Pugalenthi *et al*., 2004).

Hence, the present study deals with the nutritional and antinutritional aspects of three underexploited legumes/little known/underutilized tribal pulses viz, *Canavalia gladiata, Erythrina indica* and *Abrus precatorius*.

Canavalia gladiate

The sword bean, *Canavalia gladiata* (Jacq.) DC (local name: Koliawarai) is a representative of the family Fabaceae and is distributed in North and Penninsular India. Fruits of sword bean are consumed by the Indian ethnic/tribal peoples of Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura and Meghalaya (Borthakur, 1996). The Kaddar, Mannan and Muthuvan tribal sects of Kerala state, South India, consume unripe fruits of sword bean (Radhakrishnan *et al*., 1996). Cooked young pods and seeds are known to be consumed by the Palliyar tribals living in Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, South-Eastern slopes of Western Ghats, Tamil Nadu, India (Arinathan *et al*., 2007). This species is also used as a cover crop and roasted seeds are ground to prepare a coffee-like drink in Guatemala (Bressani *et al*., 1987).

Erythrina indica

Erythrina indica Lam. is commonly known as Kalyana murungai, and grows throughout India, mostly in Assam, Bengal, Konkan, Tamil Nadu and Kerala. It is a deciduous tree with orange red flowers and grows to a height of 15m with rough bark. The young fruits and seeds are taken as vegetable by the tribes of Susale island, Pune district (Vartak and Suryanarayana, 1995). Despite, being edible, seeds of *E. indica* are also medicinally valuable. Seed paste is used to massage in case of paralysis and to kill worms (Pal and Jain, 1988).

Abrus precatorius

Abrus precatorius L., an underutilized food legume, is commonly known as Gundumani. It is indigenously found throughout India, even at altitudes upto 1200m on the outer Himalayas. It is now naturalized in all tropical countries (Dwivedi, 2004) and Andaman Islands, having good nutritional properties receive more attention as an alternative protein source. It is a twining herb, with pinkish white or pink coloured flowers. The seeds are brightly coloured, elliptic to sub globose, smooth, glossy, and shinning. The seeds of *A. precatorius* are very similar in weight. The seeds are much valued in making jewelry for their bright colouration. The cooked seeds have been consumed by Onges of Andaman and Katharis of Pune District, Maharashtra, India during extreme famine (Janardhanan *et al*., 2003b; Pugalenthi *et al*., 2007). The seeds of *A. precatorius* have medicinal values. The seeds are used to treat diabetes and chronic nephritiss. Dry seeds of *A. precatorius* are powdered and taken one teaspoonful once a day for two days to cure worm infection (Rain-tree, 2004).

MATERIALS AND METHODS

Collection of seed samples

The seeds of *C. gladiata* (Jacq.) DC were collected from Arumugamangalam, Tuticorin district, Tamil Nadu, during August 2010, *E. indica* Lam. seeds were collected from Spic Nagar, Tuticorin district, Tamil Nadu during July, 2010. The seeds of *A. precatorius* L. red and black coloured seed coat were collected from Sathankulam, Tuticorin district and white coloured seed coat of *A. precatorius* L. were collected from Vadavalli, Coimbatore district, Tamil Nadu during August 2010. The collected pods were thoroughly dried in the sun; the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds, foreign materials and immature seeds were stored in airtight plastic jars at room temperature (25^{o}C).

Proximate composition

The moisture content was determined by drying 50 transversely cut seed in an oven at 80°C 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 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 (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 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 ≥ 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 % protein in the residue; *A* is the % ash in the residue, and *Ws* is the mg sample.

The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). 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 mg of the ground legume seed was digested with a mixture of 10ml concentrated nitric acid, 4ml of 60% perchloric acid and 1ml of concentrated sulphuric acid. After cooling, the digest was diluted with 50ml of deionised distilled water, filtered with Whatman No. 42 filter paper and the filtrates were made up to 100ml 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 was determined colorimetrically (Dickman and Bray, 1940)

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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 25ml of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10ml 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 25ml with 4% oxalic acid and used for ascorbic acid estimation. Two millilitres of the extract was made up to 3ml 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 3hr incubation at 37°C, 7ml of 80% H_2SO_4 was added to dissolve the osazone crystals and the absorbance was measured at 540nm against a reagent blank.

For the extraction of niacin, 5g air-dried powdered sample was steamed with 30ml concentrated H_2SO_4 for 30min. After cooling, this suspension was made up to 50ml with distilled H_2O and filtered. Five ml of 60% basic lead acetate was added to 25ml of the filtrate. The pH was adjusted to 9.5 and centrifuged to collect the supernatant. Two ml of concentrated H_2SO_4 was added to the supernatant. The mixture was allowed to stand for 1hr and centrifuged. The 5ml 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, 1ml extract was made up to 6ml with distilled water in a test tube; 3ml cyanogen bromide was added and shaken well, followed by addition of 1ml of 4% aniline. The yellow colour that developed after 5min was measured at 420nm 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 mg per 100 g of powdered samples.

Extraction and estimation of total proteins and protein fraction

The total (true) protein was extracted by the method of Basha *et al* (1976) with slight modification (ethanol treatment was omitted to save prolamin

fraction). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA) and estimated by the method of Lowry *et al* (1951). The albumin and globulin fractions of the seed protein were extracted and separated according to the method of Murray (1979). The prolamin fraction was extracted from the residual pellet by treating the pellet with 80% ethanol (1:10w/v) overnight. After centrifugation (20,000g for 20 min at room temperature) the supernatant containing prolamins was air-dried and dissolved in 0.1N NaOH. The resulting pellet was extracted with 0.4N NaOH (1:10w/v) overnight and centrifuged as above. The supernatant was designated as glutelins. All four fractions so obtained were precipitated and washed with cold 10% TCA. All samples were redissolved in 0.2M NaOH and protein content was determined by the method of Lowry *et al* (1951).

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 (5ml) 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 (0.45µM) 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 C_{18} 5 micron column (4.6X 150mm). The flow rate was 1 ml min^{-1} 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 were expressed as g100g-1 proteins and were compared with FAO/WHO (1991) reference pattern. The essential amino acid score (EAAS) was calculated as follows:

$$EAAS = \frac{\text{Essential a.a. g /100g total protein}}{\text{Essential a.a. g/100g FAO/WHO (1991) reference pattern}} \times 100$$

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 (2mX3mm) 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 30ml/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.

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) 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 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Extraction, TLC separation and 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 was dissolved in 45 ml of double distilled water and shaken well to get homogeneous slurry. 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 was 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

Quantitative determination of phyto-haemagglutinating (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 0.15N sodium chloride solution for 2hours and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20min. 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 3min 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 (50µl) 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 (25µl) 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 25µl 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 10ml of distilled water and refrigerated at 5°C for 1h. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37°C. The IVPD was determined by the sequential digestion of the samples containing protein with a multi-enzyme mixture (trypsin, ∝-chymotrypsin and peptidase) at 37°C followed by protease at 55°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.

RESULTS AND DISCUSSION

Crude protein

Proteins are required for maintenance (replacement of wear and tear of tissues) in adults for the growth of infants and children, for foetal development in pregnancy and secretion of milk during lactation. The requirement of proteins for the later groups is higher than the adults (Rao *et al*., 1989). Legume seeds are valuable source of protein, oil, carbohydrates, minerals and vitamins. They are playing an important role in human nutrition mainly in developing countries (Yanez *et al*., 1995). In the present study, *C. gladiata* has high content of crude protein than the other pulses investigated (Table 1). The crude protein content of *C. gladiata, E. indica* and *A. precatorius* (both samples) was higher than the other tribal pulses like *Dolichos trilobus, Entada rheedi, Rhynchosia cana, R. suaveolens, R. filipes, Vigna radiata* var. *sublobata, V. unguiculata* subsp. *cylindrica, Atylosia scarabaeoides, Neonotonia wightii* var. *coimbatorensis* and *V. unguiculata* subsp. *unguiculata* (Arinathan *et al*., 2003; 2009). To meet the protein demands in developing countries where

animal protein is grossly inadequate, considerable attention is being paid to less consumed protein sources, especially in legumes (Balogun and Fetuga, 1986) which are considered as protein tablets (Salunkhe, 1982). The crude protein levels of the studied samples suggest its usefulness as alternative source of protein.

Crude lipid

Fat is an important component of diet and serves a number of functions in the human body. Fat is a concentrated source of energy and supplies per unit weight more than twice the energy furnished by either proteins or carbohydrates. *Abrus precatorius* (both samples) contained a high level of crude lipid content (Table 1). This value is found to be more or less equal to that of earlier reports in the same species (Mohan and Janardhanan, 1995a). Crude lipid content of *Canavalia gladiata* is found to be higher than that of *Canavalia ensiformis* (Doss *et al*., 2011). Crude lipid content of *Erythrina indica* is found to be higher than that of same species (Pugalenthi *et al*., 2004) and the tribal pulses *Vigna aconitifolia* and *V. unguiculata* subsp. *unguiculata* (Tresina and Mohan, 2011a).

Total Dietary Fibre and ash content

The presence of fibre in the diet is necessary for digestion and for elimination of wastes. The contraction of muscular walls of the digestive tract is stimulated by fibre, thus counteracting constipation (Rao *et al*., 1989). The World Health Organization (WHO) has recommended an intake of 22-23 kg of fibre for every 1000 Kcal. of diet (Kanwar *et al*., 1997). *Abrus precatorius* (red and black coloured seed coat) contained the highest percentage of TDF (Table 1) among the legumes of this study. However, the TDF level of presently investigated tribal pulses seems to be higher compared to certain legumes like cowpea and kidney bean (Singh *et al*., 2000); different varieties of *Vigna mungo* (Tresina *et al*., 2010); Co_9, Co_{11} and Co_{12} varieties of *Lablab purpureus* (Kala *et al*., 2010a).

The ash content of the presently investigated tribal pulses (4.11-5.21%) (Table 1) would be important to the extent that it contains the nutritionally important mineral elements, which are presented in Table 2. In the present study, all the investigated pulses exhibited the highest level of ash when compared with an earlier report in different varieties of *Vigna mungo* (Tresina *et al*., 2010).

Nitrogen Free Extractives and Calorific value

Among the presently investigated tribal pulses, *A. precatorius* (white coloured seed coat) exhibited higher level of Nitrogen Free Extractives (NFE) than *C. gladiata*, *E. indica* and *A. precatorius* (red and black coloured seed coat) (Table 1). These values are found to be higher than that of some of the earlier investigated tribal pulses like, three accessions of *Mucuna pruriens* var. *pruriens* (Kala and Mohan, 2010a; Fathima *et al*., 2010); *M. pruriens* var. *utilis* and *M. deeringiana* (Kala and Mohan, 2010b); five accessions of *M. atropupurea* (Kala *et al*., 2010b); five accessios of *M. pruriens* var. *pruriens* (Kalidass and Mohan, 2011) and *V. aconitifolia* (Tresina and Mohan, 2011a). The high NFE content of these legumes act as a good source of calories which would be antimarasmus, especially infant nutrition (Vadivel and Janardhanan, 2000). The range in calorific values exceeds the energy values of different varieties of *Lablab purpureus* (Kala *et al*., 2010a) which are in the range of 1524-1589 kJ $100g^{-1}$DM respectively.

Mineral composition

The determination of minerals and trace elements in food stuffs is an important part of nutritional and toxicological analyses. Copper, chromium, iron and zinc are essential micronutrients for human health. In addition, these elements play an important role in human metabolism, and interest in these elements is increasing together with reports of relationships between trace elements status and oxidative diseases. Legumes supply adequate protein while being a good source of vitamins and minerals (Fennema, 2000). In the present study, all the investigated tribal pulses exhibited lower level of sodium when compared to Recommended Dietary Allowances (RDA) of NRC/NAS (1980).

In the present study, all the investigated tribal pulses registered a higher level of potassium (Table 2) when compared to earlier reports in chick pea (Alajaji and Ed-Adawy, 2006); *L. purpureus* varieties (Kala *et al*., 2010a) and *V. mungo* varieties (Tresina *et al*., 2010) and Recommended Dietary Allowance value (RDA) of infants and children (< 1550mg) (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).

Table: 1 Proximate composition of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius* (g $100g^{-1}$) [a]

Components	*Canavalia gladiata*	*Erythrina indica*	*Abrus precatorius* (red and black coloured seed coat)	*Abrus precatorius* (white coloured seed coat)
Moisture	5.65 ± 0.14	7.04 ± 0.12	7.38 ± 0.21	6.76 ± 0.34
Crude protein (Kjeldahl N x 6.25)	26.35 ± 0.56	22.56 ± 0.48	20.40 ± 0.78	19.34 ± 1.10
Crude lipid	6.31 ± 0.11	6.58 ± 0.18	8.34 ± 0.31	8.56 ± 0.26
Total Dietary Fibre (TDF)	7.24 ± 0.09	7.40 ± 0.27	7.86 ± 0.14	6.24 ± 0.11
Ash	5.21 ± 0.07	4.14 ± 0.11	4.30 ± 0.08	4.11 ± 0.07
Nitrogen Free Extractive (NFE)	54.89	59.32	59.10	61.31
Calorific value kJ $100g^{-1}$DM	1594.595	1615.46	1642.67	1669.57

[a] All values are means of triplicate determination expressed on dry weight basis; ± denotes standard error.

All the presently investigated tribal pulses contained more calcium content when compared to *Cicer arietinum* (Alajaji and Ed-Adawy, 2006); *Phaseolus vulgaris, Cajanus cajan* (Sangronis and Machado, 2007); *Rhynchosia cana* and *R. suaveolens* (Arinathan *et al*., 2009). But, all the presently investigated legumes were deficient in calcium content compared to RDA's of infants (NRC/NAS, 1980).

Among the presently examined tribal pulses, *Erythrina indica* registered the highest level of magnesium content (Table 2) when compared to some legumes like, *P. vulgaris* (black and white beans), *C. cajan* (Sangronis and Machado, 2007); *Cicer arietinum* (Alajaji and Ed-Adawy, 2006); *Dolichos trilobus, R. cana, R. suaveolens, Vigna radiata* var. *sublobata, V. unguiculata* subsp. *cylindrica* (Arinathan *et al*., 2009); *V. mungo* varieties (Tresina *et al*., 2010) and *Vigna* species (Tresina and Mohan, 2011a). *Erythrina indica* is found to contain more than adequate level of magnesium compared to RDA's of NRC/NAS (1980).

Among the presently studied tribal pulses, *C. gladiata, E. indica* and *A. precatorius* (white coloured seed coat) registered the highest level of phosphorus content than that of earlier reports in *C. arietinum* (Alajaji and Ed-Adawy, 2006); *Atylosia scarabaeoides, Neonotonia wightii* var. *coimbatorensis, R. cana, R. suaveolens, V. radiata* var. *sublobata, V. unguiculata* subsp. *cylindrica, D. trilobus* (Arinathan *et al*., 2003; 2009) and *Vigna* species (Tresina and Mohan, 2011a). But the phosphorus content of presently studied species is deficient according to RDA's (NRC/NAS, 1980).

Among the presently investigated tribal pulses, *E. indica* registered high level of iron (Table 2) and this value seems to be lower than that of an earlier report in the same species (Pugalenthi *et al*., 2004). Among the presently investigated tribal pulses, *E. indica* exhibited the highest level of zinc and manganese; and the copper level is low in all the presently studied pulses. But all the presently investigated pulses were deficient in Fe, Cu, Zn and Mn content when compared to children RDA's of (NRC/NAS, 1989).

The ratios of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are also shown in Table 2. 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 samples would 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 lost in the urine, decreasing the calcium level in bones. Food is considered "good" if the ratio is above one and "poor" if the ratio is less than 0.5 (Nieman *et al*., 1992). The Ca/P ratio in the present study ranged between 0.74 to 1.19 indicating they would serve as good sources of minerals for bone formation.

Table 2. Mineral composition of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius* (mg $100g^{-1}$)[a]

Components	*Canavalia gladiata*	*Erythrina indica*	*Abrus precatorius* (red and black coloured seed coat)	*Abrus precatorius* (white coloured seed coat)
Sodium	78.04 ± 0.17	44.08±0.78	33.16 ± 0.54	25.08 ± 0.16
Potassium	1938.51±2.34	1838.12±1.24	1970.30 ± 1.56	1864.14 ± 2.52
Calcium	341.14 ± 0.76	294.04±0.61	246.10 ± 0.38	213.08 ± 0.24
Magnesium	106.06 ± 0.36	324.68 ± 0.54	94.26 ± 0.14	114.16 ± 0.11
Phosphorus	313.68 ± 0.53	396.02 ± 0.36	206.13 ± 0.32	256.53 ± 0.46
Iron	4.74 ± 0.11	5.37 ± 0.11	3.48 ± 0.15	4.11 ± 0.13
Zinc	1.56 ± 0.03	0.67 ± 0.03	0.74 ± 0.03	0.82 ± 0.02
Copper	0.76 ± 0.01	6.34 ± 0.17	2.44 ± 0.07	2.58 ± 0.01
Manganese	0.59 ± 0.02	0.56 ± 0.01	0.31 ± 0.01	0.338 ± 0.01
Na/K	0.04	0.02	0.02	0.01
Ca/P	1.09	0.74	1.19	0.83

[a]All values are means of triplicate determination expressed on dry weight basis; ± denotes standard error.

Table 3 Vitamins (niacin and ascorbic acid) content of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius* (mg $100g^{1}$)[a]

Components	*Canavalia gladiata*	*Erythrina indica*	*Abrus precatorius* (red and black coloured seed coat)	*Abrus precatorius* (white coloured seed coat)
Niacin	33.20 ± 0.38	11.36 ± 0.08	9.42 ± 0.11	10.12 ± 0.07
Ascorbic acid	27.37 ± 0.11	14.36 ± 0.13	17.38 ± 0.21	14.07 ± 0.32

[a]All values are means of triplicate determination expressed on dry weight basis. ± denotes standard error.

Vitamins

Legumes constitute an important part of the human diet in many parts of the world and are sources of vitamins (Shills *et al*., 1999). Niacin or nicotinic acid is a true anti-pellagra vitamin. It is an essential vitamin needed for our health. The presently investigated tribal pulse exhibited highest level of niacin content (Table 3) which was found to be higher than that of an earlier report in *Cajanus cajan, D. lablab, D. biflorus, M. pruriens,, P. mungo, Vigna catjang* and *Vigna* species (Rajyalakshmi and Geervani, 1994); *V. unguiculata* subsp. *unguiculata* (Arinathan *et al*., 2003); *C. arietinum* (Alajaji and Ed-Adawy, 2006) and *V. mungo* varieties (Tresina *et al*., 2010).

Ascorbic acid is an essential nutrient for humans, but they lack the capacity to synthesize it. It is involved in collagen synthesis, bone and teeth calcification. The presently investigated tribal pulses registered higher level of ascorbic acid content (Table 3) than *C. arietinum, P. aureus, D. biflorus* (Khatoon and Prakash, 2006); *P. vulgaris* (white and black beans); *C. cajan* (Sangronis and Machado, 2007), *V. radiata*, *V. mungo* (Kakati *et al*., 2010) and *V. mungo* varieties (Tresina *et al*., 2010).

Total protein and protein fractionation

Proteins are indispensable for normal growth and metabolism of human. The storage or reserve proteins

constitute the major portion of the proteins in grains. Among the studied tribal pulses, *C. gladiata* exhibited highest level of total proteins than the other pulses (Table 4). *Canavalia gladiata* is found to contain more total protein than that of *L. purpureus* (Co_2, Co $_9$, Co_{11}, Co_{12} varieties) (Kala *et al*., 2010a) and *V. mungo* (TMV-1 and Vamban-1 varieties) (Tresina *et al*., 2010).

In general, the globulin constitutes the major seed storage protein in legumes. In all the presently investigated tribal pulses, globulins constitute the major bulk of the proteins (Table 4). This is in consonance with some earlier reports in *Lablab purpureus* (Kala *et al*., 2010a) and *V. mungo* varieties (Tresina *et al*., 2010).

Fatty acid composition

Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance. The fatty acid composition of the total seed lipids of presently investigated tribal pulses were given in Table 6. The current data revealed that, all the seed lipids were rich in unsaturated fatty acids (55.60-72.04%) and had very high contents of linoleic acid (24.16-34.14%). These values are nutritionally desirable.

The palmitic acid content of *C. gladiata* was higher than the other legumes such as *L. purpureus* (Kala *et al*., 2010a), *M. pruriens* var. *pruriens* (Fathima *et al*., 2010; Kala and Mohan, 2010a; Kalidass and Mohan, 2011) and *Vigna* species (Tresina and Mohan, 2011a). Oleic acid was found to be higher than the pulse crop commonly consumed in India such as *C. cajan* (Salunkhe, 1982); tribal pulses *M. atropurpurea* (Kala *et al*., 2010b) and *Vigna* species (Tresina and Mohan, 2011a). Similarly, the level of stearic acid detected in the samples investigated in the present study seems to be higher than the samples of *L. purpureus* (Kala *et al.*, 2010a); *V. mungo* and *V. aconitifolia* (Tresina *et al*., 2010; Tresina and Mohan, 2011a).

The detected level of antinutritional fatty acid, behenic acid in *E. indica* (1.04%) is in agreement with earlier reports in Shenbagathoopu accession of *M. pruriens* var. *pruriens* (Kala and Mohan, 2010a) and Seithur accession of *M. pruriens* var. *pruriens* (Kalidass and Mohan, 2011). The presence of behenic acid has been implicated with antherogenic properties (Kritchevsky *et al*., 1973).

***In vitro* protein digestibility (IVPD)**

Among the presently investigated tribal pulses, *Canavalia gladiata* registered the highest level of IVPD (71.36%) than the other pulses (Table 7), and their protein digestibility was found to be higher than that of *V. mungo* varieties (Tresina *et al*., 2010) and comparable with some edible legumes (Table 7).

Table 4 Data on total (true) protein and protein fractions of seed flour of *Canavalia gladiata*, *Erythrina indica* and *Abrus precatorius* [a]

Components	*Canavalia gladiata*		*Erythrina indica*		*Abrus precatorius* (red and black coloured seed coat)		*Abrus precatorius* (white coloured seed coat)	
	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein
Total protein	22.34±1.26	100	17.48±0.74	100	16.30±0.94	100	15.26±0.41	100
Albumins	5.98 ± 0.31	26.77	5.21±0.34	29.81	5.16±0.21	29.81	4.86±0.08	31.85
Globulins	13.61±0.65	60.92	10.28±0.56	58.81	8.74±0.76	58.81	8.77±0.16	57.47
Prolamins	0.78±0.06	3.49	0.84±0.03	4.81	0.94±0.01	4.81	0.62±0.03	4.06
Glutelins	1.97±0.04	8.82	1.15±0.06	6.57	1.46±0.03	6.57	1.01±0.05	6.62

[a]All values are means of triplicate determination expressed on dry weight basis; ± denotes standard error.

Table 5. Amino acid profiles of acid- hydrolysed, purified seed proteins of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius* (g $100g^{-1}$)

Amino acid	*Canavalia gladiata*	EAAS	*Erythrina indica*	EAAS	*Abrus precatorius* (red and black coloured seed coat)	EAAS	*Abrus precatorius* (white coloured seed coat)	EAAS	FAO WHO 1991
Glutamic acid	14.38		14.28		14.82		13.84		
Aspartic acid	13.36		11.02		11.14		12.56		
Serine	3.78		4.34		2.41		2.74		
Threonine	3.53	103.82	3.30	97.06	2.86	84.12	2.31	67.94	3.4
Proline	4.08		2.94		3.84		3.72		
Alanine	4.16		4.14		5.46		5.84		
Glycine	4.26		4.38		5.08		5.20		
Valine	4.06	116	4.58	130.86	6.18	176.57	5.98	170.86	3.5
Cystine	1.21		0.38		0.42		0.56		2.5
Methionine	0.48	67.60	1.04	56.80	1.68	84	1.34	76	
Isoleucine	7.12	254.29	2.78	99.29	4.34	155	4.68	167.14	2.8
Leucine	6.28	95.15	6.96	105.45	7.14	108.18	6.96	105.45	6.6
Tryosine	2.78		3.41		2.81		3.04		6.3
Phenylalanine	3.38	97.78	5.04	134.13	4.30	112.86	4.61	121.43	
Lysine	5.64	97.24	6.28	108.28	5.86	101.03	5.92	102.07	5.8
Histidine	3.14	165.26	2.14	112.63	1.34	70.53	1.76	92.63	1.9
Tryptophan	0.78	70.91	0.64	58.18	0.76	69.09	0.84	76.36	1.1
Arginine	4.36		6.18		5.43		5.88		

EAAS-Essential amino acid score

Table 6. Fatty acid profile of lipids of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius* [a]

Components	*Canavalia gladiata*	*Erythrina indica*	*Abrus precatorius* (red and black coloured seed coat)	*Abrus precatorius* (white coloured seed coat)
Myristic acid (C14:0)		2.14	-	-
Palmitic acid (C16:0)	32.14	12.68	20.48	21.43
Stearic acid (C18:0)	12.26	11.30	8.41	34.41
Oleic acid (C18:1)	21.48	27.46	32.08	7.08
Linoleic acid (C18:2)	24.16	34.14	29.40	26.61
Linolenic acid (C18:3)	9.36	9.40	8.14	8.88
Behenic acid (C22:0)	-	1.04	-	-
Others		1.84	1.49	1.59

[a]Average values of two determinations.

Antinutritional factors

The problem of protein digestibility has been attributed to the interplay of several factors, including protease inhibitors, phytates, oxalates, goitrogens and other antinutritional factors. Naturally, in societies where legumes are consumed, rather than much more expensive animal foods, there is great concern over the level of antinutrients present in the diet. For this reason, a preliminary evaluation of some of the antinutritional factors in raw seeds of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius* (both samples) was examined (Table 8).

Total free phenolics and tannins

Phenolic compounds inhibit the activity of digestive as well as hydrolytic enzymes such as amylase, trypsin, chymotrypsin and lipase (Salunkhe *et al*, 1992) and decrease the digestibility of proteins, carbohydrates and availability of vitamins and minerals (Rao and Deosthale, 1982). However, recent researchers report that the phenolic compounds are the main human dietary antioxidant and have a decreased incidence of chronic diseases. A number of polyphenolic compounds are present, which contribute towards the defense mechanism of plants. Although these are considered earlier as antinutritional compounds, under the present nomenclature, phenols fall under the category of nutraceuticals, offering many nutritional advantages to man (Shanthakumari *et al*., 2008). The content of total free phenolics of currently investigated tribal pulses appeared to be higher than the earlier reports in *L. purpureus* (Kala *et al*., 2010a), *V. mungo* (Tresina *et al*., 2010) and lower than those of other tribal pulses such as *Dolichos lablab* (Ramakrishnan *et al*., 2006), *D. trilobus, Entada rheedi, M. atropurpurea, Rhynchosia cana, R. suaveolens, Tamarindus indica, Teramnus labialis, V. radiata* var. *sublobata, V. unguiculata* subsp. *cylindrica* (Arinathan *et al*., 2009) and *V. aconitifolia* (Tresina and Mohan, 2011a). The tannin content of the investigated pulses were relatively lower than red gram, bengal gram, lentil (Salunkhe *et al*., 2006), *C. arietinum* (Alajaji and Ed-Adawy, 2006), *P. vulgaris* (white and black beans), *C. cajan* (Sangronis and Machado, 2007), *Pisum sativum* (Nikolopoulou *et al*., 2007); *V. radiata, V. mungo* (Kakati *et al*., 2010) and *V. aconitifolia* (Tresina and Mohan, 2011a).

L-DOPA

L-DOPA (3,4- dihydroxyphenylalanine) is a non-protein amino acid which causes skin eruptions and increases body temperature in the consuming people when present in high concentrations (Jebedhas, 1980). L-DOPA, a compound chiefly used in the treatment of Parkinson's disease, has been reported to cause hallucinations, in addition to causing gastrointestinal disturbance such as nausea, vomiting and anorexia (Reynolds, 1989). All the presently investigated tribal pulses contained low level of L-DOPA (Table 8) when compared with *M. atropurpurea* (Fathima and Mohan, 2009; Kala *et al*., 2010a); *M. pruriens* var. *pruriens* (Kala and Mohan, 2010a; Kalidass and Mohan, 2011). Among the investigated tribal pulses, *C. gladiata* exhibited the highest percentage of L-DOPA. The level of L-DOPA is significantly reduced by repeated soaking and boiling of seeds (Jebedhas, 1980). It is also observed that, drying effects substantial lose in content of L-DOPA (Longo *et al*., 1974; Larher *et al*., 1984). Repeated boiling of seeds in water and decanting the water for seven times resulted in significant reduction in the level of L-DOPA (Janardhanan, 1982). Dry heat treatment also has been found to be more effective in reducing the L-DOPA content (Siddhuraju *et al*., 1996).

Table 7. IVPD of seeds of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius* (two samples) compared with some pulses

Pulses	(IVPD %)	Sources
Canavalia gladiata	71.36	
Erythrina indica	69.73	
Abrus precatorius (red and black coloured seed coat)	64.34	
Abrus precatorius (white coloured seed coat)	63.31	
Dolichos diflorus	71.00	Rajyalakshmi and Geervani (1990)
Vigna radiata	80.05-85.33	Reddy and Gowramma (1987)
Vicia faba	57.20-72.07	Moneam (1990)
Vigna umbellata	73.48-74.30	Laurena *et al*., (1991)
Lablab purpureus	64.36-70.30	Kala *et al*., (2010a)
Vigna species	69.32-74.21	Tresina and Mohan, (2011a)

Table 8 Data on antinutritional factors of *Canavalia gladiata, Erythrina indica* and *Abrus precatorius*

Components	*Canavalia gladiata*	*Erythrina indica*	*Abrus precatorius* (red and black coloured seed coat)	*Abrus precatorius* (white coloured seed coat)
Total free phenolics[a] g 100 g^{-1}	1.21±0.03	0.94±0.07	0.76±0.11	0.84±0.11
Tannins[a] g $100g^{-1}$	0.41±0.01	0.36±0.03	0.54±0.02	0.61±0.07
L-DOPA[a]g $100g^{-1}$	2.50±0.21	2.48 ±0.16	1.12±0.07	0.98±0.03
Phytic acid[b] mg $100g^{-1}$	354.30±1.76	386.24±1.04	348.14±2.21	366.32±1.34
Hydrogen cyanide[a] mg $100g^{-1}$	0.31±0.01	0.09±0.01	0.12±0.01	0.09±0.01
Trypsin inhibitor (TIU mg^{-1} protein)	25.54±0.48	36.24±0.21	34.16±0.14	35.30±0.02
Oligosaccharide[a] $g100g^{-1}$	0.64±0.06	1.24±0.04	1.38±0.05	1.21±0.04
Raffinose				
Stachyose	1.64±0.11	0.98±0.06	1.12±0.03	1.08±0.03
Verbascose	2.11±0.10	5.86±0.74	3.34±0.56	3.96±0.24
Phytohaemagglutinating activity[b] (Hu mg^{-1} protein)	138	138	58	64
A group				
B group	94	68	178	168
O group	36	16	28	32

[a] All values are of means of triplicate determination expressed on dry weight basis; [b] All values of two independent experiments; ± Standard error

Phytic acid

Phytic acid, a major phosphorus storage form in plants and its salts are known as phytates, which regulates the various cellular functions such as DNA repair, chromatin remodelling, endocytosis, nuclear messenger and potential hormone signalling which is important for plants and seed development (Zhou and Erdman, 1995), as well as animal and human nutrition (Vucenik and Shamsuddin, 2006). It is often regarded as an antinutrient because of strong mineral, protein and starch binding properties, thereby decreasing their bioavailability (Weaver and Kannan, 2002). Phytic acid content of investigated seed samples was found to be low when compared to that of some commonly consumed legumes,TMV-1 variety of *V. mungo* (Tresina *et al*., 2010); *V. radiata, V. mungo* (Kakati *et al*., 2010) tribal *pulses M. pruriens* var. *pruriens* (Fathima *et al*, 2010; Kala and Mohan, 2010a); *M. pruriens* var. *utilis* and *M. deeringiana* (Kala and Mohan, 2010b) and *V. aconitifolia* (Tresina and Mohan, 2011a). It is worthwhile to note that, the phytate content in *Mucuna* beans could be substantially eliminated by processing methods such as soaking and cooking (Vijayakumari *et al*., 1996).

Hydrogen cyanide (HCN)

Hydrogen cyanide is known to cause acute or chronic toxicity. A lot of HCN (known to inhibit the respiratory chain at the cytochrome oxidase level) is lost during soaking and cooking (Kay *et al*., 1977). The content of HCN level in the presently investigated tribal pulses was far below the lethal level i.e., 36mg/100g (Oke, 1969) and comparable with those of *V. mungo* (Tresina *et al*., 2010), *L. purpureus* (Kala *et al*., 2010a) and certain tribal pulses investigated in our laboratory (Arinathan, 2003; 2009; Tresina and Mohan, 2011b).

Trypsin inhibitor activity

The presence of protease inhibitors such as trypsin and chymotrypsin inhibitors in the diet leads to the formation of irreversible trypsin enzyme-trypsin inhibitor complexes, causing a decrease in trypsin in the intestine and decrease in the digestibility of dietary protein, thus leading to slower animal growth.

As a result, the secretary activity of the pancreas increases, which could cause pancreatic hypertrophy and hyperplasia (Liener, 1994). The trypsin inhibitor activities of all the studied samples were higher than that of *P. vulgaris* Roba variety (4.59mg/g) (Shimelis and Rakshit, 2007); *C. arietinum* (11.90mg/g) (Alajaji and Ed-Adawy, 2006); *P. vulgaris* (white and black beans), *C. cajan* (4.13-4.75mg/g) (Sangronis and Machado, 2007) and they seem to be lower than that of Co_5 and TMV-1 varieties of *V. mungo* (38.74-41.36mg/g) (Tresina *et al*., 2010) *M. pruriens* var. *pruriens* (40.40-48.24%) (Kala and Mohan, 2010a; Kalidass and Mohan, 2011). Trypsin inhibitor activity has greater impact on the IVPD of the legumes where the trypsin inhibitor activity was known to be heat labile.

Oligosaccharides

Ingestion of large quantities of beans is known to cause flatulence in humans and animals. The raffinose family sugars (raffinose, stachyose and verbascose) are important contributors of flatus. These are not digested by man due to lack of α-galactosidase enzyme (Gitzelmann and Aurricctuo, 1965). The microflora in the lower intestine metabolizes these oligosaccharides and produces flatus gases.

Among the presently investigated tribal pulses, the seed sample of *E. indica* is found to contain the highest level of total oligosaccharides (Table 8). In the present study, all the investigated samples contained verbascose as the major oligosaccharide. This is in agreement with earlier reports in *C. cajan* (Mulimani and Devendra, 2010); *M. pruriens* var. *utilis* (Janardhanan *et al*., 2003a; Kala and Mohan, 2010b), *M. pruriens* var. *pruriens* (Kala and Mohan, 2010a; Kalidass and Mohan, 2011).

Haemagglutinins (lectins)

Lectins are toxic glycoproteins that have the ability to bind with carbohydrate moieties on the surface of the human red blood cells (RBC) and cause them to agglutinate. Lectins can combine with intestinal mucosal cells and cause interference with the absorption of available nutrients (Liener, 1994).

Regarding phytohaemagglutinating activity, among the presently investigated tribal pulses, *C. gladiata* and *E. indica* registered higher haemagglutinating activity with respect to the 'A' blood group of human erythrocytes; whereas, the seed samples of *A. precatorius* registered higher haemagglutinating activity with respect to the 'B' blood group of human erythrocytes. All the presently investigated seed samples showed low levels of phytohaemagglutinating activity with respect to the 'O' blood group. Haemagglutinating activity of *C. gladiata* and *E. indica* is in agreement with earlier reports in *Mucuna* (Kala and Mohan, 2010a; Fathima *et al*., 2010; Kalidass and Mohan, 2011). Similarly haemagglutinating activity of *A. precatorius* (both samples) is in good agreement with earlier reports in the tribal pulse *Vigna* species (Tresina and Mohan, 2011a).

Lectins are highly sensitive to heat treatment (Singh *et al*., 1988). Haemagglutinating activity decreases during germination in *Glycine max, P. vulgaris, Vicia faba* and *V. radiata* (Valdebouze *et al*., 1980). A significant reduction in lectin activity has been noticed when the seeds of certain pulses were subjected to dry heat treatment and autoclaving (Siddhuraju *et al*., 1996).

CONCLUSION

On the basis of the comparative assessment, *C. gladiata, E. indica* and *A. precatorius* (red and black coloured seed coat and white coloured seed coat) which are referred as underutilized legumes are a valuable source of nutrition due to high proteins and carbohydrates with an adequate quantity of minerals, vitamins, essential amino acids and unsaturated fatty acids. The currently investigated tribal pulses possess a variety of antinutritional factors that cause adverse effects on consumers. The presence of antinutritional factors identified in the current report should not pose a problem for humans, if the beans are not properly processed. The overall interpretation of this present investigation may offer a scientific basis for increased and versatile utilization of these protein-rich underutilized legumes as a food and protein supplement.

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