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COMPARATIVE ANALYSIS AND NUTRITIONAL ASSESSMENT OF RAW SEEDS OF CROTALARIA SPECIES

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ABSTRACT : Five different species of *Crotalaria* were analysed for their proximate and mineral composition, protein fractions, amino acid profile of total seed proteins, fatty acid profile of seed lipids, in vitro protein digestibility vitamins (niacin and ascorbic acid) and certain anti-nutritional factors. The major findings of the study were as follows: crude protein content ranged from 12.50 - 27.12%, crude lipid 0.77 - 1.63%, crude fibre 0.92 - 6.26%, ash 3.20 -3.90%, carbohydrates 66.21 - 82.16% and calorific value 1546.25 - 1611.74 $kJ100^{-1}g$ DM. The investigated seed samples contained minerals such as Na, K, Ca, Mg and P were in abundance. Albumins and globulins seem to be the principle protein of the investigated species of *Crotalaria*. The essential amino acid profile of total seed proteins was found to be high when compared to the FAO/WHO (1991) recommended pattern. The fatty acid profiles of all the five species revealed that the seed lipids contained linoleic and linolenic and palmitic acid in high concentration. The IVPD of the studied samples ranged from 71.84 - 74.30%. The anti-nutritional factors ranged from: total free phenolics 0.14 - 2.25%, tannins 7.61 - 17.50%, L-DOPA 2.31 - 9.41%, phytic acid 0.04 - 0.09 %, trypsin inhibitor activity 21.70 - 28.60 TIUmg-1 protein. Low levels of phytohaemagglutinating activity for human erythrocytes of 'O' blood group than 'A' and 'B' blood groups were found in all the varieties.

Key Words: Vitamins, amino acid profiles, in vitro protein digestibility, anti-nutritional factors.

INTRODUCTION

Legumes/pulses are considered to be a very important group of plant food stuffs, particularly in the developing world, as a cheap source of protein when animal protein is scarce. A significant part of human population relies on legumes as staple food for subsistence, particularly in combination with cereals. They are unique foods because of their rich nutrient content including starch, protein dietary fibre, oligosaccharides, phyto-chemicals and minerals [1]. Their nutritional contents contribute to many health benefits to the humans beings [2, 3].

Most of the research on dry beans has been related to varietal selection. The criteria for selection have always been resistance to diseases or yields but nutritional quality [4]. A study of the composition and nutritive quality of dry beans would therefore be of great interest, because the knowledge provided would help to orient the work of investigators involved in varietal selection and also reduce or eliminate anti-nutritional factors to make edible and non edible legume seeds more acceptable as an inexpensive source of protein. The data on nutritional /anti-nutritional content of grain legumes in one region may help to select the specific variety or type of legume to grow on large scale. These kinds of studies would also help to increase the availability of food by processing underutilized varieties into edible forms through research and development [5].

The potential and use of forage legumes in farming systems and particularly in livestock nutrition has been well summarized by Tothill [6]. However research on indigenous wild legumes has been very limited compared to improved legumes. Tropical legumes which are used for forage have been cultivated during the last 50 years [7] yet the volume of unexplored genetic resource remains vast. The aim of the present study was to determine the variation in some of the nutrient and anti-nutrients, which are interesting from the nutritional point of view for five different species of *Crotalaria* grown in Nilgiri Biosphere Reserve (NBR) of Tamil Nadu.

MATERIALS AND METHODS

Collection of seed samples

The five different species of *Crotalaria* were procured from different agro-climatic and ecological regions of the Nilgiri Biosphere Reserve (NBR) in Nilgiris and Coimbatore districts, Tamil Nadu State, India. 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˚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 Willy 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 [8] and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus [9].The ash content was determined by heating 2g of the dried sample in a silica dish at 600°C for 6hr [9]. Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method [10]. 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 ≥ 2 hr at 105°C and then cooled ≥ 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 [11]. 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 [12].

Minerals analysis

Five hundred milligrams 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) [13]. The phosphorus content in the triple acid digested extract was determined colorimetrically [14].

Extraction and estimation of total proteins and protein fraction

The total (true) protein was extracted by the method of Basha *et al* [15] 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* [16].

The albumin and globulin fractions of the seed protein were extracted and separated according to the method of Murray [17]. 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 Lowry *et al* method [16].

Amino acid analysis

The total seed protein was extracted by a modified method of Basha *et al* [15]. 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 C18 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 [18] 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 [19] as modified by Rama Rao *et al* [20]. The contents of the different amino acids were expressed as $g100g^{-1}$ proteins and were compared with FAO/WHO (1991) reference pattern [21]. The essential amino acid score was calculated as follows:

$$\text{Essential amino acid score} = \frac{\text{grams essential amino acid in 100g of total protein}}{\text{grams of essential amino acid in 100g of FAO/WHO (1991) reference pattern}} \times 100$$

Lipid extraction and fatty acid analysis

The total lipid was extracted from the seeds according to the method of Folch *et al* [22] 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* [23]. 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 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.

Determination of in vitro protein digestibility (IVPD)

This was determined using the multi-enzyme technique [24]. 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, α-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.

Vitamins analysis

Ascorbic acid and niacin contents were extracted and estimated as per the method given by Sadasivam and Manickam [25]. 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 millilitres 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 millilitres 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 milligrams per 100 grams of powdered samples.

Analysis of anti-nutritional compounds

The anti-nutritional compounds, total free phenolics [26], tannins [27], the non-protein amino acid, LDOPA (3, 4-dihydroxyphenylalanine) [28] and phytic acid [29] were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al* [30] 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 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Quantitative determination of phytohaemagglutinating (lectin) activity

Lectin activity was determined by the method of Almedia *et al* [31]. 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* [15] method. Human blood (blood groups A, B and O) was procured from the blood bank of Micro Clinical Laboratory, Coimbatore. 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* [32]. 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.

RESULTS AND DISCUSSION

The proximate composition of seeds of five different species of *Crotalaria* was shown in Table 1. Crude protein content ranged from 12.50 - 27.12%.

Among the five species, *C. clarkei* was found to be higher (27.12%) amount of crude protein when compared to certain legumes such as *Phaseolus vulgaris* (22.4%) and *Cajanus cajan* (22.7%) as reported by Apata and Ologhobo, [33]. The crude lipid content of *C. fysonii* was higher when compared with *Vigna radiata* and *Vigna mungo* [34]. The crude fibre content of five different species ranged from 0.92 – 6.26%. The ash content range of these legumes (3.20- 3.90%) would be important to the extent that it contains the nutritionally important mineral elements shown in Table 2. From the data the different species of *Crotalaria* had a higher range of carbohydrate (NFE) (66.21 – 82.16%), than peanut and soybeans [36]. The high NFE contents of the *Crotalaria* enable these legumes to act as a good source of calories which would be anti-marasmus, especially infant nutrition [36]. The range in calorific values exceeds energy values of cowpea, green gram, horse gram, moth beans and peas [36] which are in the range of 1546.25 – 1611.74 KJ 100 g^{-1} DM.

Table.1. Data on proximate composition of *Crotalaria species* [a] germplasms.

Components	Crotalaria semperflorens	Crotalaria clarkei	Crotalaria paniculata	Crotalaria fysoni	Crotalaria walkeri
	g 100 g–1 seed flour				
Moisture	8.80 ± 0.08b	7.60 ± 0.08b	8.50 ± 0.07b	7.50 ± 0.07a	8.70 ± 0.08b
Crude protein (Kjeldahl N x 6.25)	20.94 ± 0.36bc	27.12 ± 0.09b	24.25 ± 0.14c	17.19 ± 0.16b	12.50 ± 0.15a
Crude lipid	1.03 ± 0.02b	0.77 ± 0.00a	0.99 ± 0.03b	1.63 ± 0.04c	0.82 ± 0.01a
Crude fibre	2.88 ± 0.08c	1.32 ± 0.14b	4.65 ± 0.10d	6.26 ± 0.03e	0.92 ± 0.03a
Ash	3.70 ± 0.03b	3.30 ± 0.08a	3.90 ± 0.02c	3.20 ± 0.03a	3.60 ± 0.06b
Nitrogen Free Extractives (NFE)	71.45	67.49	66.21	71.72	82.16
Calorific value (kJ 100g-1DM)	1581.74	1609.02	1548.01	1546.25	1611.74

a - Mean values in the rows sharing a common letter are not statistically significant according to Duncan's Multiple Range Test (DMRT)

± - Standard error

Food legumes are good source of minerals such as calcium, iron, copper, zinc, potassium and magnesium [37]. In the present investigation, all the species of *Crotalaria* registered a higher level of potassium (Table - 2) when compared with recommended dietary value (RDA) for infants and children (<1550mg) [38]. 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 [39]. The seeds of five different species of *Crotalaria* contained higher level sodium, potassium, calcium and phosphorus when compared with *Phaseolus limensis, Cicer arietinum, Pisum sativum and Lens culinaris* [40]. The manganese content of all the species of *Crotalaria* was found to be higher than that of Estimated Safe and Adequate Daily Dietary Intake of minerals (ESADDI) [41].

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 five species of *Crotalaria* 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 [42]. 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 a above one and "poor" if the ratio is less than 0.5 [43]. This indicating they would serve as good sources of minerals for bone formation.

In the samples investigated, albumins and globulins (4.27 – 5.34% and 12.12 – 13.62% respectively) constitute the major bulk of the proteins (Table 3) as in most of the legume species reported earlier [49, 50, 35]. The amino acid profiles of the purified seed proteins and the essential amino acid score were presented in Table 4. The content of essential amino acids in all the five different species of *Crotalaria* were found to be higher compared to the FAO / WHO [21] requirement pattern.

Table.2. Data on mineral composition of *Crotalaria species* [a] germplasms

Components	Crotalaria semperflorens	Crotalaria clarkei	Crotalaria paniculata	Crotalaria fysonii	Crotalaria walkeri
	mg 100 g–1 seed flour				
Sodium	192.99 ± 0.55a	448.99 ± 0.51c	364.00 ± 0.51b	643.00± 0.31d	10.70 ± 0.76e
Potassium	369.34 ± 0.84a	779.00 ± 0.30d	650.00 ± 0.79b	904.00 ± 0.51e	741.00 ± 0.18c
Calcium	329.03 ± 0.27a	384.24 ± 0.05c	426.52 ± 0.02d	345.10 ± 0.17b	479.47 ± 0.38e
Magnesium	197.51 ± 0.31d	187.42 ± 0.48b	144.01 ± 0.65a	145.12 ± 0.18a	191.75 ± 0.32c
Phosphorus	528.33 ± 0.18d	407.30 ± 0.39c	192.58 ± 0.57c	129.37 ± 0.47a	1191.60 ± 0.94b
Iron	3.86 ± 0.04b	3.42 ± 0.02a	4.50 ± 0.07c	5.87 ± 0.16d	3.99 ± 0.06b
Copper	7.85 ± 0.17c	9.95 ± 0.11d	2.63 ± 0.09a	4.38 ± 0.06b	2.55 ± 0.06a
Manganese	6.21 ± 0.43c	7.95 ± 0.19d	1.22 ± 0.07a	2.07 ± 0.08b	1.02 ± 0.00a

a - Mean values in the rows sharing a common letter are not statistically significant according to Duncan's Multiple Range Test (DMRT)

± - Standard error

Table.3. Data on total protein and protein fractions of seed flour of *Crotalaria species* [a] germplasms

Protein fraction	Crotalaria semperflorens		Crotalaria clarkei		Crotalaria paniculata		Crotalaria fysonii		Crotalaria walkeri	
	g 100 g–1 seed flour	g 100 g–1 seed protein	g 100 g–1 seed flour	g 100 g–1 seed protein	g 100 g–1 seed flour	g 100 g–1 seed protein	g 100 g–1 seed flour	g 100 g–1 seed protein	g 100 g–1 seed flour	g 100 g–1 seed protein
Total protein	20.66 ±0.37b	100	20.44 ± 0.46ab	100	20.90 ± .58b	100	20.11 ± 0.38ab	100	19.39 ± 0.04a	100
Albumins	4.62 ± 0.22b	22.36	5.20 ± 0.06c	25.44	5.34 ± 0.14c	25.55	4.88 ± 0.10c	24.27	4.27 ± 0.10a	22.02
Globulins	12.75 ±0.10c	61.71	11.36 ± 0.22a	55.58	13.62 ± .06d	65.17	12.12 ± 0.06b	60.27	13.46 ± 0.13d	69.42
Prolamins	1.07 ± 0.02b	5.18	1.89 ± 0.03d	9.25	0.96 ± 0.05b	4.59	1.35 ± 0.05c	6.71	0.64 ± 0.05a	3.30
Glutelins	2.22 ± 0.09d	10.75	1.99 ± 0.03c	9.73	0.98 ± 0.02a	4.69	1.76 ± 0.02b	8.75	1.02 ± 0.03a	5.26

a - Mean values in the rows sharing a common letter are not statistically significant according to Duncan's Multiple Range Test (DMRT) ± - Standard error

Table.4. Amino acid profiles of acid hydrolyzed purified total seed proteins of *Crotalaria species* germplasms

Amino acid	Crotalaria semperflorens		Crotalaria clarkei		Crotalaria paniculata		Crotalaria fysonii		Crotalaria walkeri		FAO / WHO (1991) requirement pattern
	g 100 g–1 protein	EAA	g 100 g–1 protein	EAA	g 100 g–1 protein	EAA	g 100 g–1 protein	EAA	g 100 g–1 protein	EAA	
Glutamic acid	19.8		19.9		15.8		16.7		15.2		
Aspartic acid	10.6		11.9		13.7		9.9		10.6		
Serine	6.9		3.7		4.9		4.2		4.0		
Threonine	3.4	100.00	3.9	114.70	4.8	141.18	3.2	94.12	3.5	102.94	3.4
Proline	4.8		3.4		3.6		3.5		3.2		
Alanine	6.5		3.5		4.1		4.2		3.5		
Glycine	8.1		3.7		4.5		3.6		2.9		
Valine	4.5	128.57	5.3	151.43	4.9	140.00	4.5	128.57	6.4	182.86	3.5
Cystine	Trace	20.00	Trace	48.00	Trace	28.00	Trace	20.00	Trace	28.00	2.5
Methionine	0.5		0.8		0.7		0.5		0.7		
Isoleucine	4.6	164.29	5.2	185.71	4.5	160.71	3.9	139.29	6.0	214.29	2.8
Leucine	6.6	100.00	7.6	115.15	8.3	125.76	7.4	112.12	7.4	112.12	6.6
Tyrosine	1.3	61.90	2.8	130.16	3.6	133.33	3.2	146.03	3.1	150.79	6.3
Phenyl alanine	2.6		5.4		4.8		6.0		6.4		
Lysine	3.4	58.62	7.5	129.31	7.0	120.69	6.4	110.34	8.9	153.45	5.8
Histidine	2.8	147.36	2.3	121.05	3.4	178.95	3.0	157.89	2.5	131.58	1.9
Tryptophan	N.D.		N.D.		N.D.		N.D.		N.D.		1.1
Argnine	7.9		5.1		5.9		7.2		5.3		

N. D - Not Detected

Fatty acid composition of the total seed lipids of five different species of *Crotalaria* were given in Table 5. The data revealed that all the seed lipids were rich in unsaturated fatty acids and had very high contents of linolenic acid (12.23- 46.35%) and low level of saturated fatty acids. The fatty acid composition and high amounts of unsaturated fatty acids make *Crotalaria* a special legume. Linoleic and Linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance [52]. The presence of high levels of unsaturated fatty acids, in all the presently studied varieties, is nutritionally desirable.

Table.5. Fatty acid composition of the seed lipids of *Crotalaria species* germplasms

Fatty acid	Crotalaria semperflorens	Crotalaria clarkei	Crotalaria paniculata	Crotalaria fysonii	*Crotalaria walkeri*
	Percentage				
Myristic acid (C 14:0)	-	-	-	3.56	-
Palmitic acid (C 16:0)	17.65	19.23	27.72	13.61	14.52
Pamitolic acid (C 16:1)	-	-	-	9.08	
Stearic acid (C 18:0)	5.19	4.04	1.01	7.42	4.37
Oleic acid (C 18:1)	19.21	17.36	14.26	17.93	19.58
Linoleic acid (C 18:2)	11.32	10.60	43.40	20.62	17.51
Linolenic acid (C18:3)	45.38	46.35	12.23	19.21	35.08

Among the five species of *Crotalaria,* the seed materials of *C. walkeri* registered highest level of *in vitro* protein digestibility (74.30%) and higher than that of an earlier investigation in the seeds of *Phaseolus mungo* [51]. The presently investigated five species of *Crotalaria* exhibits the highest level of niacin content (Table 6) which was found to be higher than that of earlier report in *Cajanus cajan, Phaseolus mungo* and *Vigna catajang* [44]; *Phaseolus aureus* and *Cicer arietinum* [45] and *Vigna unguiculata* subsp. *cylindrica* [46]. The presently investigated *V. mungo* varieties also registered higher level of ascorbic acid content than *Cicer arietinum* [47]; *Vigna radiata* and *Vigna mungo* [48].

Although legumes provide 20% of all plant protein in human diets and are even more important in the diets of livestock, their usefulness is decreased by anti-nutritional or toxic compounds associated with the large content of protein in their seeds [53]. For this reason, in the present investigation an attempt has been made to detect the presence of certain anti-nutritional factors in five different species of *Crotalaria.* The content of total free phenolics and tanninin the samples investigated appeared to be lower when compared with earlier reports *Vigna mungo* [51]. The tannin content of the investigated samples were relatively lower than the other domesticated legumes like black gram, chick pea, cow pea and green gram [54; 55].

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 [56]. Recently plant phenolics are increasingly gaining importance in relation to human health as wellness since they exhibit anti-carcinogenic, anti-oxidant, anti-viral, anti-microbial, anti-inflammatory and hypotensive properties [57]. The content of non-protein amino acid, L-DOPA is relatively low when compared with other pulses reported earlier from our laboratory [58; 46].

The phytate molecule is negatively charged at physiological pH and is reported to bind with essential, nutritionally important divalent cations such a Fe, Zn, Mg and Ca etc., and forms insoluble complexes, thereby making minerals unavailable for absorption [59]. It also formed complexes with proteins and starch and inhibits their digestion [60]. The phytic acid content of investigated seed samples were found to be low when compared with that of some commonly consumed legumes such as *Dolichos lablab* var. *vulgaris* [61]; tribal pulses *Mucuna pruriens* var, *utilis* [62] and *Mucuna atropurpurea* [63].

Table.6. Data on IVPD, Vitamins and anti nutritional factors of *Crotalaria* species [a] germplasms

Name of the plant	Crotalaria semperflorens	Crotalaria clarkei	Crotalaria paniculata	Crotalaria fysonii	Crotalaria walkeri
	g 100 g–1 seed flour				
IVPD	72.70 ± 0.12b	73.69 ± 0.23c	74.03 ± 0.12c	71.84 ± 0.10a	74.30 ± 0.15c
Ascorbic acid	3.00 ± 0.08a	4.50 ± 0.07b	3.10 ± 0.06a	8.00 ± 0.08d	7.10 ± 0.18c
Niacin	1.40 ± 0.06b	2.90 ± 0.12c	4.20 ± 0.05d	3.10 ± 0.06c	0.68 ± 0.03a
Total free phenolics	0.54 ± 0.01b	0.14 ± 0.00 a	0.78 ± 0.01c	1.79± 0.01d	2.25± 0.02e
Tannins	7.61 ± 0.10a	12.80 ± 0.17c	13.60 ± 0.22d	10.40 ± 0.23b	17.50 ± 0.15e
Phytic acid (Phytol)	0.04 ± 0.00a	0.08 ± 0.00c	0.04 ± 0.00b	0.04 ± 0.00a	0.09 ± 0.00d
L-DOPA	3.40 ± 0.06b	2.31 ± 0.17a	4.90 ± 0.04c	6.83 ± 0.04d	9.41 ± 0.06e
Tripsin inhibitor (TIU mg^{-1} protein)	26.67 ± 0.44^{bc}	21.70 ± 0.29^{a}	28.60 ± 0.51^{d}	25.30 ± 0.33^{b}	27.40 ± 0.27^{cd}
Phytohaemagglutinating activity [b]					

Name of the protein fraction	Erythrocytes from human blood group	Haemagglutinating activity (HU/mg protein)				
Albumins	A	9	19	9	7	15
Albumins	B	0	8	4	6	3
Albumins	O	5	4	10	0	16
Globulins	A	22	39	59	53	46
Globulins	B	31	15	64	12	35
Globulins	O	12	48	32	0	24

a - Mean values in the rows sharing a common letter are not statistically significant according to Duncan's Multiple Range Test (DMRT)

b - Values of two independent experiments ± - Standard error

TIU - Tripsin inhibitor unit HU - Haemagglutinating units

The lectin (phytohaemagglutinating activity) of seed samples exhibit a high level of agglutination activity specifically in 'B' group compared other two blood group 'A' and 'O' (Table 6). This is in good agreement with earlier reports in the tribal pulse *Dolichos lablab* var. *vulgaris* [61]. On the basis of the above findings, it is concluded that *the five different species of Crotalaria* investigated seem to be a good source of protein, essential amino acids, essential fatty acids, minerals and vitamins like niacin and ascorbic acid. The adverse effect of most of the anti-nutritional factors detected in the present study can be eliminated by moist heat treatment or a cooking process since they are heat liable.

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