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Biochemical composition and nutritional assessment of selected under-utilized food legume of the genus *Rhynchosia*

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Abstract: Seeds of tribal pulses *Rhynchosia cana*, *R. filipes, R. rufescens*, and *R. suaveolens* were analyzed for proximate composition, mineral profiles, vitamins, fatty acid profiles, amino acid profiles of seeds, protein digestibility and anti-nutritional factors. The crude protein ranged from 14.28 – 19.40%, crude lipid 3.28 – 4.41%, total dietary fibre 6.39 – 8.44%, ash 2.80 – 3.50% and carbohydrates 60.29 – 72.51%. The fatty acid profiles revealed that, the seed lipids contained higher concentration of palmitic and linoleic acids. The essential amino acid profile of total seed proteins were compared favorably with FAO/WHO (1991) requirement pattern, except that, there were deficiencies of sulphur containing amino acids in all the *Rhynchosia* species. The concentration of anti-nutritients in *R. filipes* seeds recorded high values 1.98, 2.14, 433.14 mg/100g and 19.39 TIU/mg protein) of total free phenolics, L-Dopa, phytic acid, and trypsin inhibitor activity respectively. Based on the results of the present investigation, the lesser known pulses which is referred as under-utilized legumes, acts as a potential source of edible as well as a source of protein, minerals and energy supplements in livestock and human food particularly.

Key words: Tribal pulse, proximate composition, minerals, vitamins, amino acid profiles, L-Dopa, anti-nutrients

Introduction

Legumes seeds are important sources of nutrients and can serve as high quality dietary protein sources to meet nutrient requirements (Perumal *et al*., 2001; Escudero *et al*., 2006). Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high (Vijayakumari *et al.*, 1997). The under-utilized legumes, which have tremendous potential for commercial exploitation but remain ignored, offer a good scope in this context (Bhag Mal, 1992). Accounts of important under exploited pulses which await exploration for food, fodder, energy and industrial purposes have been given (Siddhuraju *et al.*, 2000; Kalidass and Mohan, 2011).

Some work has been carried out on the biochemical and nutritional composition of *Rhynchosia venulosa* in different areas of Zambia (Lovelace, 1977) and *R. hirta* in Udayagiri forest of Nellore district of Andhra Pradesh of southern Peninsular India (Murthy and Kandimalla, 2007), but no information is available on the nutrient and biochemical composition of other species of *Rhynchosia*. Therefore, in the present investigation, an attempt has been made to understand the biochemical and nutritional assessment of *Rhynchosia cana, R. filipes, R. rufescens* and *R. suaveolens* which is consumed by the tribes Kani / Kannikars of southern Western Ghats of Tamil Nadu, which is potential for human nutrition.

Materials and Methods

Collection of seed samples

The seed samples of three accessions of *Rhynchosia cana* were collected from three geographical regions South India via Siruvani, Coimbatore district, Srivilliputhur, Virudhunagar district and Petchiparai, Kanyakumari district, Tamil Nadu. The other species of *Rhynchosia filipes*, *R. rufescens* and *R. suaveolens* were collected from Thallaianai, Courtallam and Papanasam, Tirunelveli district, Tamil Nadu respectively. The immature and damaged seed were removed, and the mature seeds were sun-dried for two days and stored in plastic containers until further use.

Proximate composition

The proximate composition of *Rhynchosia* species seeds was determined according to the methods described by AOAC (2005). Energy value was calculated using the Bhat and Sridhar (2008).

Minerals and vitamins analysis

Sodium, potassium, calcium, magnesium,

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copper, manganese, zinc and iron were determined with an atomic absorption spectrophotometer – ECIL (Electronic Corporation of India Ltd., India) (Issac and Johnson, 1975). Total phosphorus was determined spectrophotometrically after incubation with triple acid solution (Dickman and Bray, 1940). Ascorbic acid and niacin contents were extracted and estimated as per the method given by Sadasivam and Manickam (1996).

Lipid extraction and fatty acid analysis

The total lipid from the seeds flours were extracted (Folch *et al*., 1957). Fatty acid methyl esters were prepared from the parental lipids (Metcalfe *et al*., 1966), and the fatty acid analysis was performed using a flame ionization detector in gas chromatography (ASHMACO, Japan; Model No: ABD20A).

Amino acid analysis

The purified seed proteins were acid hydrolyzed with 6N HCL at 110°C for 24 h in vacuo. After flesh evaporation, the dried residue was dissolved in citrate buffer (pH 2.2). Known aliquots were analyzed in an LKB-Biochrome Automated Amino acid Analyzer (model-4151), follwing the procedure of Siddhuraju *et al*. (1992). Tryptophan was determined according to the method of Spies and Chambers (1949) as modified by Rama Rao *et al*. (1974). The contents of different amino acids recovered were presented as mg/g proteins. The essential amino acid were scored and compared with FAO/WHO (1991) reference pattern.

Analysis of anti-nutritional compounds

The anti-nutritional 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 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Estimation of oligosaccharides

The oligosaccharides composition of raw seed samples was determined by taking 5 grams of the seed flours were extracted with 50 ml of 70% (v/v) aqueous ethanol. Separation of oligosaccharides was done by TLC. The separated spots were compared with standard (raffinose, stachyose and verbascose obtained from Sigma chemicals) and the sugar spots were scrapped, eluted in 2 ml of distilled water and filtered. 1 ml of the extract was treated with 1 ml of 0.2 M thiobarbituric acid and 1 ml of conc. HCL and kept on boiling water bath for 6 min. After cooling, the absorbency was measured at 432 nm and the oligosaccharide contents were quantified.

Determination of haemagglutinating activity

The haemagglutinating activity of selected samples was analyzed haemagglutinating assay in the presence of 10 mM Mn^{2+} in a round bottomed micro-titer plate using 2% (v/v) trypsinized human blood erythrocyte suspension in saline phosphate buffer (pH 7.0). One haemagglutinating unit (HU) is defined as the least amount of the extract per ml of the last dilution, which giving positive agglutination.

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 Chemicals. Calculated amounts of the control (casein) and sample were weighed out, hydrated in 10 ml 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.

Statistical analysis

All the data obtained are reported on dry weight basis and represents the mean of triplicates ± standard error. Differences between mean values were determined by ANOVA followed by comparisons using DMRT. Significance was accepted at $p < 0.05$.

Results and Discussion

The proximate composition of *Rhynchosia* species are presented in Table 1. The crude protein content was found to be high in *R. rufescens* (19.40%). This is comparable to some popular conventional source of protein such as *Centrosema pubescens* (18.97%), *Centrosema pascuorum* (18.15%), *Lablab purpureus* (17.28%) (Nworgu and Ajayi, 2005) and tribal food tubers of *Dioscorea pentaphylla var. pentaphylla* (9.18%), *Dioscorea oppositifolia var oppositifolia*

(7.00%), *Dioscorea spicata* (6.38%) and *Dioscorea tomentosa* (8.31%) (Mohan and Kalidass, 2010). The crude lipid content of Siruvani and Petchiparai accessions of *Rhynchosia cana*, *R. filipes* and *R. rufescens* seems to be higher than the previously studied common/tribal pulses such as *Mucuna flegellipes* (Ihedioha and Okoye, 2011) and tubers of *Dioscorea pentaphylla var. pentaphylla*, D*ioscorea oppositifolia var. oppositifolia*, *Dioscorea spicata* and *Dioscorea tomentosa* (Mohan and Kalidass, 2010). The total dietary fiber content of all the investigated *Rhynchosia* species is found to be more than the other tribal pulses *Luffa cylindrica* (Olaofe *et al*., 2008); *Canavalia ensiformis* (Doss *et al.*, 2011). The ash content of investigated Rhynchosia species (2.80 – 3.50%) would be important to the extent that it contains the nutritionally important mineral elements, which are depicted in Table 2. It appears that, the *Rhynchosia* species have a high range of carbohydrate (64.25 – 72.51%), because of their low fat content. All the investigated *Rhynchosia* species have a high energy range (1563.21 – 1593.37 kJ 100g^{-1} DM) than the commonly cultivated pulse crops like cowpea, green gram, horse gram, moth bean and peas (Narasinga Rao *et al.*, 1989), which are in the range of 1318 – 1394 kJ 100g^{-1} DM.

Robinson (1987) reported that, a diet that meets two-thirds of the RDA (Recommended Dietary Allowances) values is considered to be adequate for an individual. Food legumes are a good source of minerals such as calcium, iron, copper, zinc, potassium and magnesium (Salunkhe *et al*., 1985). Table 2 furnishes the mineral composition of the investigated samples. The seeds of all the investigated Rhynchosia species contained higher levels of sodium, potassium and calcium, when compared with other legumes, P*haseolus vulgaris, Phaseolus limensis, Vigna unguiculata, Cicer arietinum, Pisum sativum* and *Lens culinaris* (Meiners *et al.*, 1976). In the present investigation, all the *Rhynchosia* species registered a higher level of potassium when compared with recommended dietary allowance value (RDA) of infants and children (<1550 mg) (NRC/NAS 1980). The high content of potassium can be utilized beneficially in the diet of people who take diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid (Siddhuraju *et al*., 2001). The iron content of *Rhynchosia filipes*, manganese content of Siruvani and Petchiparai accessions of *Rhynchosia cana*, *Rhynchosia rufescens* and *Rhynchosia suaveolens* are found to be higher than that of the recommended dietary allowance of iron and manganese by the ICMR (1992).

Table 1. Proximate composition of *Rhynchosia* species / accessions. (g 100 g^{-1} seed flour)*$

Components	*R. cana* Siruvani	*R. cana* Srivilliputhur	*R. cana* Petchiparai	*R. filipes* Thallaianai	*R.rufescens* Courtallam	*R.suaveolens* Papanasam
Moisture	7.50±0.01^{c}	8.40±0.01^{b}	10.50±0.02^{a}	7.46±0.03^{d}	5.10±0.01^{f}	6.78±0.02^{e}
Crude protein (Kjeldahl N × 6.25)	16.40±0.01^{c}	14.28±0.10^{f}	15.30±0.01^{e}	17.38±0.01^{b}	19.40±0.01^{a}	16.08±0.01^{d}
Crude lipid	4.38±0.01^{b}	3.40±0.02^{e}	3.91±0.02^{d}	4.20±0.01^{c}	4.41±0.01^{a}	3.28±0.01^{f}
TDF (Total Dietary Fibre)	7.80±0.01^{ab}	6.48±0.02^{b}	7.41±0.01^{ab}	6.39±0.01^{b}	8.44±0.03^{a}	7.29±0.04^{ab}
Ash	3.20±0.01^{d}	3.33±0.02^{c}	2.80±0.01^{f}	3.48±0.04^{b}	3.50±0.02^{a}	3.06±0.12^{e}
Nitrogen Free Extractives (NFE)	68.22	72.51	70.58	68.55	64.25	60.29
Calorific value (kJ100g^{-1} DM)	1578.28	1577.57	1581.60	1593.37	1563.21	1566.04

*All values are means of triplicate determinations expressed on a dry weight basis ± denotes standard error.
$Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

Table 2. Mineral composition of *Rhynchosia* species / accessions (mg 100 g^{-1} seed flour)*$

Components	*R. cana* Siruvani	*R. cana* Srivilliputhur	*R. cana* Petchiparai	*R. filipes* Thallaianai	*R. rufescens* Courtallam	*R. suaveolens* Papanasam
Sodium	31.80±1.01^{c}	24.34±0.01^{g}	28.30±0.99^{d}	21.48±0.19^{f}	34.14±0.03^{b}	36.40±0.01^{a}
Potassium	1662.20±10.01^{f}	1584.00±2.00^{g}	1712.12±0.02^{d}	2048.30±0.01^{a}	1849.30±0.13^{b}	1783.26±0.01^{c}
Calcium	154.31±0.90^{g}	148.30±0.99^{g}	171.10±0.05^{d}	178.14±0.07^{c}	194.10±0.98^{b}	222.10±0.04^{a}
Magnesium	174.34±2.00^{b}	164.39±0.01^{d}	169.14±0.0^{c}	144.10±0.05^{g}	178.36±0.01^{a}	148.30±0.15^{g}
Phosphorus	194.20±1.00^{g}	202.10±1.00^{d}	212.40±0.18^{c}	165.16±0.01^{f}	288.00±9.97^{b}	348.21±0.01^{a}
Iron	7.20±0.99^{c}	9.48±0.01^{b}	7.78±0.01^{c}	20.14±0.02^{a}	9.08±0.01^{b}	6.30±0.03^{d}
Zinc	5.10±1.01^{a}	4.78±0.01^{ab}	4.21±0.01^{b}	5.12±0.02^{a}	4.14±0.02^{b}	4.80±0.01^{ab}
Copper	1.40±0.01^{g}	1.58±0.04^{c}	1.64±0.01^{b}	1.14±0.01^{f}	1.78±0.01^{a}	1.48±0.01^{d}
Manganese	8.80±0.10^{a}	5.36±0.03^{g}	6.78±0.01^{b}	4.38±0.01^{f}	5.76±0.01^{d}	6.47±0.01^{c}
Na/K	0.02	0.02	0.02	0.01	0.02	0.02
Ca/P	0.79	0.73	0.81	1.08	0.67	0.64

*All values are of means of triplicate determination expressed on dry weight basis ± denotes Standard error.
$Mean values in the following row sharing a common letter are not statistically significant according to DMRT

In the present investigation, *Rhynchosia* species exhibited higher levels of niacin content (Table 3) which is found to be higher than that of an earlier report in *Cajanus cajan, Dolichos lablab, Dolichos biflorus, Mucuna pruriens, Phaseolus mungo, Vigna catjang* and *Vigna* species (Rajyalakshmi and Geervani, 1994) and compared to that of legumes, green leafy vegetables and nuts have been reported to be high in niacin (Chatterjea and Shinde, 2007; Akubugwo *et al*., 2007). The ascorbic acid content of *Rhynchosia* species was high when compared to

Table 2. Mineral composition of *Rhynchosia* species / accessions (mg 100 g^{-1} seed flour)*$

Species / Accessions	Niacin	Ascorbic acid
R. cana - Siruvani	36.34±0.99^{c}	64.90±1.01^{d}
R. cana - Srivilliputhur	21.40±1.01^{f}	79.24±1.00^{a}
R. cana - Petchiparai	28.64±0.02^{d}	69.32±0.04^{e}
R. filipes - Thallaianai	24.10±0.02^{e}	69.74±0.02^{e}
R. rufescens - Courtallam	42.63±0.02^{b}	74.22±1.99^{b}
R. suaveolens - Papanasam	44.10±0.01^{a}	56.44±0.03^{g}

*All values are of means of triplicate determination expressed on dry weight basis ± denotes standard error.
Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

Table 3. Vitamins (niacin and ascorbic acid) content of *Rhynchosia* species / accessions (mg 100g^{-1} seed flour)*$

Fatty acid (%)	*R. cana* Siruvani	*R. cana* Srivilliputhur	*R. cana* Petchiparai	*R. filipes* Thallaianai	*R. rufescens* Courtallam	*R. suaveolens* Papanasam
Palmitic acid [C16:0]	35.28	33.10	36.36	30.13	36.04	29.38
Stearic acid [C18:0]	12.36	14.40	10.26	14.10	11.15	15.30
Oleic acid [C18:1]	14.14	16.12	16.70	12.43	13.25	18.10
Linoleic acid [C18:2]	25.04	23.43	22.30	24.15	25.10	23.14
Linolenic acid [C18:3]	12.10	10.08	13.03	13.00	14.46	13.01
Others [unidentified]	1.08	2.87	1.35	1.19	-	1.07

*All values are of two determination.

Cicer arietinum (Fernandez and Berry, 1988); Indian goose berry, guava fruit and *Amaranthus hybridus* (Vasudevan and Sreekumari, 2007; Akubugwo *et al*., 2007).

The fatty acid composition of the total seed lipids of *Rhynchosia* species are represented in Table 4. Fatty acid profiles of all *Rhynchosia* species revealed that, the lipids are a good source of the nutritionally essential linoleic and oleic acids. Linolenic acid was the dominating unsaturated fatty acid, followed by oleic acid. The nutritional value of linoleic acid is due to its metabolism at tissue levels which produce the hormone-like prostaglandins. The activity of these prostaglandins includes lowering of blood pressure and construction of smooth muscle (Aurand *et al.*, 1987). Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance (Pugalenthi *et al*., 2004). All the presently investigated seed lipids are rich in unsaturated fatty acids. These values are nutritionally desirable and also comparable to those of certain common legume seeds (Salunkhe *et al*., 1985). The seed samples also contain more palmitic acid (29.30 – 36.36%) and stearic acid (10.26 – 15.30%) than the other legume seeds like Vigna radiata, and Vigna mungo (Salunkhe *et al.*, 1982), Glycine max (Ologhobo and Fetuga, 1984) and Vigna unguiculata, and Phaseolus vulgaris (Omogbai, 1990).

The amino acid compositions and essential amino acid scores of the total seed proteins of all the *Rhynchosia* species are shown in Table 5 along with those for FAO/WHO (1991) reference pattern. The contents of theronine, isoleucine, thyrosine, phenylalanine and histidine in all the investigated *Rhynchosia* species, valine in Srivilliputhur and Petchiparai accessions of *Rhynchosia cana, Rhynchosia filipes, Rhynchosia rufescens* and *Rhynchosia suaveolens,* leucine in Srivilliputhur accessions of *Rhynchosia cana* and *Rhynchosia rufescens* and tryptophan in *Rhynchosia filipes* and *Rhynchosia rufescens* are found to be higher than those of the FAO/WHO (1991) recommended pattern. However, except sulphur containing amino acids, levels of all other essential amino acids are more or less comparable to soyabean protein (Bau *et al*., 1994).

The in vitro protein digestibilities (IVPD) of raw seeds of *Rhynchosia* species are present in Table 6. IVPD of the Rhynchosia seeds were higher than that of cooked pearl millet flour (Ali *et al*., 2009). Among the investigated *Rhynchosia* species, *Rhynchosia filipes* registers the lowest level of in vitro protein digestibility (69.54%). The improvement in digestibility may be attributed to denaturation of protein, destruction of the trypsin inhibitor or reduction of tannins and phytic acid.

The consequence of legumes is decreased by toxic or antinutritional compounds associated with the larger protein content in their seeds (Nowacki, 1980). Table 6 summarizes the data on anti-nutritional factors. The total free phenolics occurred within the range of 1.10 – 1.98% and tannins ranged from 0.43 – 0.78%. The content of total free phenolics of investigated seed samples of *Rhynchosia* species is found to be equal when compared with *Vigna unguiculata*

Table 5. Amino acid profiles of acid-hydrolysed, purified total seed proteins of *Rhynchosia* species / accessions (g100g^{-1} proteins)[a]

Amino acid	*R. cana* Siruvani	EAAS	*R. cana* Srivillip uthur	EAAS	*R. cana* Petchipar ai	EAAS	*R. filipes* Thallai anai	EAAS	*R. rufescens* Courtall am	EAAS	*R. suaveolens* Papanas am	EAAS	FAO/WHO (1991) requirement pattern
Glutamic acid	9.81		8.21		10.10		11.12		12.31		9.41		
Aspartic acid	10.11		9.42		10.30		9.81		8.21		9.52		
Serine	3.12		2.60		3.40		3.53		3.32		2.91		
Threonine	4.30	126.47	4.80	141.17	4.30	126.47	3.70	108.82	3.50	102.94	4.40	129.41	3.4
Proline	3.30		3.70		3.90		2.80		2.11		2.70		
Alanine	2.20		2.72		3.10		1.91		3.42		4.10		
Glycine	1.80		2.51		2.20		2.32		2.61		3.11		
Valine	3.40	97.14	4.20	120.00	3.80	108.57	3.60	102.85	4.40	125.71	3.50	100.00	3.5
Cystine	1.11	}57.20	0.78	}71.60	1.20	}83.60	1.20	}95.20	1.30	}89.20	0.94	}70.80	}2.5
Methionine	0.33		1.01		0.89		1.10		0.93		0.83		
Isoleucine	5.50	196.43	6.60	235.71	5.80	207.14	5.30	189.28	3.40	121.42	4.50	160.71	2.8
Leucine	6.11	92.42	7.20	109.09	6.30	95.45	5.80	87.87	7.80	118.18	5.90	89.39	6.6
Tyrosine	4.20	}136.51	3.90	}153.97	4.60	}166.66	3.60	}182.53	5.10	}166.66	4.20	}150.79	}6.3
Phenylalanine	4.40		5.81		5.90		4.30		5.40		5.30		
Lysine	5.20	89.65	5.60	96.55	6.10	105.17	5.90	101.72	6.20	106.89	5.40	93.10	5.8
Histidine	2.80	147.37	3.11	163.16	2.60	136.84	2.41	126.31	2.10	110.53	3.50	184.21	1.9
Tryptophan	1.10	100.00	0.78	70.90	0.98	99.09	1.20	109.09	1.40	127.27	0.84	76.36	1.1
Arginine	5.90		6.30		5.41		4.80		3.41		4.31		

Table 6. Data on *in vitro* protein digestibility (IVPD) and anti-nutritional factors of five *Rhynchosia* species / accessions[$]

Components		*R. cana* Siruvani	*R. cana* Srivilliputhur	*R. cana* Petchiparai	*R. filipes* Thallaianai	*R. rufescens* Courtallam	*R.suaveolens* Papanasam
IVPD (%)[g]		70.48	71.30	72.41	69.54	70.70	71.48
Total free phenolics[h] g 100g^{-1}		1.48±0.02^{e}	1.10±0.01^{a}	1.32±0.01^{d}	1.98±0.03^{c}	1.88±0.02^{b}	1.88±0.02^{f}
Tannins[h] g 100g^{-1}		0.78±0.01^{a}	0.54±0.02^{d}	0.43±0.02^{f}	0.66±0.03^{b}	0.56±0.08^{c}	0.48±0.01^{e}
L-DOPA[h] g 100g^{-1}		1.01±0.01^{d}	0.98±0.04^{e}	1.26±0.02^{b}	2.14±0.02^{a}	0.88±0.02^{f}	1.10±0.01^{c}
Phytic acid[h] mg 100 g^{-1}		392.00±0.99^{d}	431.21±0.99^{b}	348.60±0.05^{f}	433.14±0.01^{a}	378.30±0.15^{e}	410.40±0.11
Hydrogen cyanide[h] mg 100g^{-1}		0.22±0.01^{d}	0.26±0.01^{c}	0.27±0.01^{ab}	0.25±0.01^{c}	0.30±0.01^{a}	0.29±0.02^{ab}
Trypsin inhibitor activity[g] (TIU mg^{-1} protein)		12.34	15.36	14.78	19.39	15.48	16.26
Oligosaccharides[h] g100 g^{-1}	**Raff**	0.46±0.01^{d}	0.42±0.01^{e}	0.68±0.02^{b}	0.58±0.01^{c}	0.31±0.01^{f}	0.78±0.01^{a}
	Stac	1.58±0.06^{b}	1.68±0.01^{a}	1.38±0.01^{d}	1.46±0.01^{c}	1.21±0.01^{e}	1.36±0.03^{d}
	Verb	1.26±0.03^{a}	1.01±0.01^{d}	1.21±0.01^{b}	1.12±0.01^{c}	0.98±0.01^{e}	0.94±0.01^{f}
Phytohaemagglutinating activity Hu mg^{-1} protein[g]	**A**	49	39	44	56	59	41
	B	134	156	148	136	112	133
	O	39	33	31	44	68	37

Raff: Raffinose; **Stac**: Stachyose; **Verb**: Verbascose; [g]All values of two independent experiments, [h]All values are of means of triplicate determination expressed on dry weight basis ± standard error, [$]Mean values in the following row sharing a common letter are not statistically significant according to DMRT.

subsp. *cylindrica* (Thangadurai, 2005). The contents of tannins in different species of *Rhynchosia* are lower than those in domesticated legumes such as varieties of cowpea (Ajeigbe *et al*., 2008). Phenolics and tannins are known to inhibit the activities of digestive enzymes and hence, the presence of ever low levels of tannins and phenolics is not desirable from nutritional point of view. However, in legumes, the soaking and cooking process is known to reduce phenolics and tannins significantly (Vijayakumari *et al.,* 1996). Recently, phenolics have been suggested to exhibit health related functional properties such as anticarcinogenic, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity (Shetty, 1997). The levels of L-Dopa content in all the investigated species of *Rhynchosia* species has been found to be low when compared with the values reported earlier in *Mucuna pruriens var. utilis* (Vadivel and Janardhanan, 2000). However, the pharmacologically active factor, L-Dopa (Pieris *et al.*, 1980) is potentially toxic (Duke, 1981; Afolabi *et al*., 1985) if ingested in large amounts. It has been demonstrated that, in Mucuna pruriens, the level of L-Dopa is significantly eliminated by dry heat treatment (Siddhuraju *et al.,* 1996); cooking and autoclaving (Vijayakumari *et al.*, 1996).

Phytic acid has an anti-nutritional property because of its ability to lower the bioavailability of essential minerals and to form a complex with proteins, thereby inhibiting the enzymatic digestion

of ingested protein (Nolan and Duffin, 1987). Phytic acid content in the seeds of Rhynchosia species is low when compared with those of *Dolichos lablab var. vulgaris* (Vijayakumari *et al.*, 1993); Mucuna pruriens (Vijayakumari *et al*., 1996); *Mucuna pruriens var. utilis* (Janardhanan *et al*., 2003). It is worth-while 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). Negligible amount of hydrogen cyanide was also present. The range of trypsin inhibitor activity (12.34 – 19.39 TIU mg^{-1} protein) is found to be low when compared to Glycine max (Salunke *et al*., 2006). Slight variation in the levels of oligosaccharides was detected in raffinose ranging from 0.31 – 0.78%, stachyose ranging from 1.21 – 1.58% and verbascose ranging from 0.94 – 1.26% of seed flour. Stachyose seems to be the principle oligosaccharide in different species of *Rhynchosia*. It is in conformity with the earlier reports in jack bean, lima and sword bean (Ravilleza *et al*., 1990). Lectins combine with the cells that line the intestinal mucosa and cause a nonspecific interference with the absorption of available nutrients, and also reduce feed intake (Liener, 1994). The seeds of all the *Rhynchosia* species exhibit comparable to *Myrsine africana* (Bashir *et al.*, 2011) and high levels of haemagglutinating activity were compared to different varieties of *Phaseolus vulgaris* (Bender and Reaidi, 1982) and *Phaseolus lunatus* (Vega and Sotelo, 1986). The lectins of *Rhynchosia* exhibited a high level of agglutinating activity specifically in 'B' group compared to the other blood groups 'A' and 'O'.

The processing methods used by the Palliyar tribals mostly eliminate the anti-nutritional factors such as total free phenolics, tannins, lectins, phytic acid, HCN, oligosaccharides and trypsin inhibitors; thus potentially increasing the in vitro protein digestibility of the seed meal. However, the L-Dopa could be eliminated after repeated boiling and decanting the seeds in water.

From these chemical investigations, it is concluded that, the presently investigated tribal pulses can be used as protein sources to curtail the problem of protein deficiency in most of the developing countries, which may result in many child killer diseases. In this document, only foods reported in the reference list pertaining underexploited legumes of food biodiversity contributes to the "Nutritional indicators of Biodiversity". The results obtained could be served as useful source of low-cost protein for human and animal food. It also provided a significant data, which may be utilized in breeding for better nutritional qualities. A more attractive food product will make seed proteins more acceptable for consumption. It is, therefore, necessary to improve nutritional quality and balance essential amino acids and remove toxins and anti-nutritional factors.

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