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SHORT NOTE [NOTA CORTA]

CHEMICAL EVALUATION OF RAW SEEDS OF CERTAIN TRIBAL PULSES IN TAMIL NADU, INDIA

[EVALUACIÓN QUÍMICA DE SEMILLAS CRUDAS DE FRIJOLES DE TAMIL NADU, INDIA]

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SUMMARY

Raw seeds of tribal pulses *Dolichos trilobus, Entada rheedi, Mucuna atropurpurea, Rhynchosia cana, R. suaveolens, Tamarindus indica, Teramnus labialis, Vigna radiata* var. *sublobata* and *V. unguiculata* subsp. *cylindrica* were investigated for their proximate composition, minerals, vitamins (niacin and ascorbic acid) and certain anti-nutritional substances (total free phenolics, tannins, L-DOPA and hydrogen cyanide were also analysed). The seeds of *Mucuna atropurpurea*, *Tamarindus indica* and *Teramnus labialis* had a higher content of crude protein than commonly consumed Indian pulses. The seeds were found to be a rich source of minerals like potassium when compared with Recommended Dietary Allowances (RDA) values (NRC/NAS, 1980).

Key words: Tribal pulses; proximate composition; vitamins; anti-nutrients; western ghats.

RESUMEN

Se analizó la composición química, mineral, vitaminas (niacina y ácido ascorbico) y compuestos antinutricionales de semillas crudas de *Dolichos trilobus*, *Entada rheedi, Mucuna atropurpurea, Rhynchosia cana, R. suaveolens, Tamarindus indica, Teramnus labialis, Vigna radiata* var. *sublobata* y *V. unguiculata* subsp. *cylindrica*. Las semillas de *Mucuna atropurpurea*, *Tamarindus indica* y *Teramnus labialis* tuvieron una mayor concentración de proteína cruda en relación a otros especies hindús. Se encontró que las semillas fueron una buena fuente de minerales (como potasio) en relación a los valores diarios recomendados por NRC/NAS, 1980.

Palabras clave: composición próximal; vitaminas; factores antinutricionales; India.

INTRODUCTION

The search for novel high quality but cheap sources of protein and energy has continued to be a major concern in many parts of the developing world (Balogun and Fetuga, 1986). In India, information on chemical composition of seeds of tribal pulses and wild progenitors of cultivated legume is relatively meager. While searching for new food sources, nutritionally improved plants within the domesticated lines and wild plants are now receiving more attention. In this context, in the present investigation, an attempt has been made to understand the chemical composition and anti-nutritional factors of the tribal pulses to suggest ways and means to remove the anti-nutritional/ toxins and make the edible plants safe protein sources for mass consumption. With this view the tribal pulses taken up for study are *Dolichos trilobus* L., *Entada rheedi* Spreng, *Mucuna atropurpurea* DC, *Rhynchosia cana* DC, *R. suavelolens* (L.F.) DC, *Tamarindus indica* L., *Teramnus labialis* (L.F.) Spreng, *Vigna radiata* (L.) Wilczek var. *subolobata* (Roxb.) Verdc., *V. unguiculata* (L.) Walp. subsp. *cylindrica* (L.) Eselt.

In India the cooked seeds of these tribal pulses are known to be consumed by the *Palliyar* tribals living Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, South-Eastern Slopes of Western Ghats, Tamil Nadu, India. (Arinathan *et al.,* 2007).

MATERIAL AND METHODS

The mature seeds of *Dolichos trilobus, Entada rheedi, Mucuna atropurpurea Rhynchosia cana, R. suaveolens, Tamarindus indicus, Teramnus labialis, Vigna radiata* var. *sublobata* and *V. unguiculata* subsp. *cylindrica* (each sample nearly 1Kg) were collected during the months of March to April from Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, South-Eastern Slope of Western Ghats, Tamil Nadu. This Wildlife Sanctuary comes under the agroclimate of semi evergreen forest and the soils are sandy loam. This sanctuary is situated mostly in the Virudhunagar district and partly in Madurai district, Tamil Nadu, South India.

Proximate composition

The moisture content of the seed was estimated by taking 50 transversely cut seeds at a time and the weight was taken before and after incubation in a hot-air oven at 80°C for 24 hr, followed by cooling in a desiccator. The oven-dried and air-dried seeds were powdered separately in a Wiley mill to 60 - mesh size and stored in screw capped bottles at room temperature for further analysis. Three samples for each accession were analysed and the results were expressed on a dry weight basis. The nitrogen content was estimated by the micro Kjeldahl method (Humphries, 1956) and the crude protein content was calculated as N * 6.25. The ash content was determined by heating 2g dried sample in a silica dish at 600° for 6hr (AOAC, 1990, 14.0060). Crude lipid content was determined using Soxhlet apparatus (AOAC, 1990, 14.0180).

The nitrogen free extract (NFE) was obtained by difference (Muller & 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).

Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method proposed by Li BW 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 Aluminum 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 $\geq$2 hr at 105°C and the cooled $\geq$ 2 hr in a desiccation and weighed. One crucible-containing residue was used for ash determination at 525° for 5 hr. The ash-containing crucible was cooled for $\geq$ 2hr in a desiccation 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:

$$\text{TDF \%} = 100 \times \frac{W_r - \frac{P+A}{100} \times W_r}{W_s}$$

Where:

W_r : milligrams of residue,
P: percentage protein in the residue;
A : percentage ash in the residue,
W_s : milligrams of samples

Minerals and vitamins analysis

Five hundred milligrams of the ground legume seed was digested with a mixture of 10 ml concentrated nitric acid, 4 ml of 60% perchloric acid and 1 ml concentrated sulphuric acid. After cooling, the digest was diluted with 50 ml of deionized distilled water, filtered with Whatman No. 42 filter paper and the filtrates were made up to 100 ml in a glass volumetric flask with deionized distilled water. All the minerals except phosphorus were analysed from a triple acid-digested sample by an absorption spectrophotometer-ECIL (Electronic Corporation of India Ltd.), India (Issac and Johnson, 1975). The phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman and Bray, 1940). Ascorbic acid and niacin content were extracted and estimated as using the method of Sadasivan and Manickam (1992).

For the extraction of ascorbic acid, 3 g air-dried powdered sample was ground with 25 ml of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10 ml filtrate until it turned orange-yellow to remove the enolic hydrogen atoms. The excess of bromine was expelled by blowing in air. This filtrate was made up to 25 ml with 4% oxalic acid and used for ascorbic acid estimation. 2ml of the extract was made up to 3 ml with distilled H_2O in a test tube. 1ml of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. The contents of the test tube were mixed thoroughly. After 3 hr incubation at 37°C, 7 ml of 80% H_2SO_4 was added to dissolve the osazone crystals and the absorbance was measured at 540 nm against a reagent blank.

For the extraction of niacin, 5g air-dried powdered sample was steamed with 30 ml concentrated H_2SO_4 for 30 min. After cooling, this suspension was made up to 50 ml with distilled H_2O and filtered. 5ml of 60% basic lead acetate was added to 25 ml of the filtrate. The pH was adjusted to 9.5 and centrifuged to collect the supernatant. 2ml of concentrated H_2SO_4 was added to the supernatant. The mixture was allowed to stand for 1 hr and centrifuged. The 5 ml of 40% $ZnSO_4$ was added to the supernatant. The pH was adjusted to 8.4 and centrifuged again. Then the pH of the collected supernatant was adjusted to 7 and used as the niacin extract. For estimation, 1 ml extract was made up to 6 ml with distilled water in a test tube, 3 ml cyanogen bromide was added and shaken well, followed by addition of 1 ml of 4% aniline. The yellow colour that developed after 5 min was measured at 420 nm against a reagent blank.

The ascorbic acid / niacin content present in the sample was 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 phenolic (Bray and Thorne, 1954), tannins (Burns, 1971), the non-protein amino acid, 3, 4-dihydroxyphenylalanine (DOPA) (Brain, 1967) and hydrogen cyanide (Jackson, 1967) were quantified.

Statistical analysis

Proximate composition, minerals, vitamins (niacin and ascorbic acid) antinutritional factors like total free phenolics, tannins, L-DOPA and hydrogen cyanide were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 10.0; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS AND DISCUSSION

The proximate compositions of tribal pulses are shown in Table 1. The crude protein content of *Mucuna atropurpurea, Tamarindus indica* and *Teramnus labialis* is higher than the commonly cultivated legumes like *Cajanus cajan* (Nwokolo, 1987; Kumar *et al.,* 1991); *Cicer arietinum* (Srivastava *et al.,* 1990; Hira and Chopra, 1995); tribal pulses like *Bauhinia racemosa* and *B. vahilii* (Rajaram and Janardhanan, 1991); *Parkia roxburghii* and *Entada phaseoloides* (Mohan and Janardhanan, 1993). Similarly, *Entada rheedi* and *Mucuna atropurpurea* contained higher lipids than those in other tribal pulses, *Bauhinia racemosa* (Mohan and Janardhanan 1994a); *Mucuna pruriens* var. *utilis* (Vadivel and Janardhanan, 2000). Due to the lipid rich nature, the seeds of *Entada rheedi* and *Mucuna atropurpurea* registered high food energy values than those of *Phaseolus vulgaris*, *P. limensis, Vigna unguiculata, Cicer arietinum*, *Pisum sativum* and *Lens culinaris* (Meiners *et al.,* 1976a).

Food legumes have been recognized as important sources of several minerals in Indian diets (Gopalan *et al.,* 1978). Table 2 shows the mineral composition of the samples. The seeds of *Dolichos trilobus, Entada rheedi, Rhynchosia suaveolens, Tamarindus indica, Teramnus labialis, Vigna radiata* var. *sublobata* and *V. unguiculata* subsp. *cylindrica* contained higher levels of sodium, potassium and calcium, when compared with other legumes, *Phaseolus vulgaris, P. limensis, Vigna unguiculata, Cicer arietinum*, *Pisum sativum* and *Lens culinaris* (Meiners *et al.,* 1976b). In the present investigation, all the pulses register a higher level of potassium when compared with 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). The iron content of *Entada rheedi* and *Teramnus labialis* is found to be higher than that of recommended dietary allowance of iron (19 mg) by the ICMR (1992).

The presently investigated tribal pulse exhibits the highest level of niacin content (Table 3). Which is found to be higher than that of an earlier report in *Cajanus cajan, Dolichos lablab, D. biflorus, Mucuna pruriens, Phaseolus mungo, Vigna catjang* and *Vigna* sp. (Rajyalakshmi and Geervani, 1994). The tribal pulses also register higher level of ascorbic acid content than *Cicer arietinum* (Fernandez and Berry, 1988).

The presence of anti-nutritional factors is one of the major drawbacks limiting the nutritional and food qualities of the legumes (Salunkhe, 1982). For this reason, a preliminary evaluation of some of these factors in raw pulses is made (Table 4). Phenolic compounds inhibit the activity of digestive as well as hydrolytic enzymes such as amylase, trypsin, chymotrypsin and lipase (Salunkhe *et al*., 1982). The content of total free phenolics of currently investigated tribal pulses appears to be higher than the earlier reports in *Canavalia gladiata* (Rajaram and Janardhanan, 1992; Mohan and Janardhanan, 1994b); *Vigna sesquipedalis*, *V. sinensis* (two different germplasms), *Vigna umbellata* var. RBL 40 and K1 (Rajaram and Janardhanan, 1990) and different varieties of *Vigna umbellata* (Mohan and Janardhanan, 1994c). Recent researches report that the phenolic compounds in the main human dietary antioxidant and has a decreased incidence of chronic diseases (Padmaja *et al*., 2005). The tannin content of the investigated samples was relatively lower than the domesticated legumes like black gram, chick pea, cow pea and green gram (Khan *et al*., 1979; Rao and Deosthale 1982). Tannins are known to inhibit the activities of digestive enzymes (Jambunathan and Singh, 1981) and hence the presence of even a low level of tannin is not desirable from nutritional point of view.

Table 1. Proximate composition of tribal pulses (g $100g^{-1}$ seed flour)[a].

Botanical Name	Moisture	Crude protein (N * 6.25)	Crude Lipid	Dietary fibre	Ash	Nitrogen Free Extract	Gross energy (KJ $100g^{-1}$ DM)
Dolichos trilobus	7.9 ± 0.12	17.8±0.05	6.3±0.12	6.6± 0.11	4.9±0.11	64.4	1612.09
Entadar heedi	4.5 ±0.37	17.0±0.44	10.2±0.21	9.4±0.04	2.7±0.31	60.7	1682.38
Mucuna atropurpurea	9.6 ± 0.23	24.4±0.59	13.9±0.07	9.2±0.13	2.6±0.18	49.8	1765.09
Rhynchosia cana	10.5 ±0.52	12.8±0.36	3.3±0.12	9.8±0.38	2.4±0.07	71.6	1535.31
Rhynchosia suaveolens	5.1 ±0.11	14.8±0.07	3.2±0.13	8.4 ±0.17	4.1±0.14	69.5	1527.78
Tamarindusindica	9.7±0.23	26.2 ±0.55	5.5±0.22	10.3±0.12	1.9±0.24	56.1	1580.59
Teramnus labialis	11.6 ±0.15	23.8±0.44	4.4 ±0.22	9.2±0.16	4.9±0.21	57.6	1527.02
Vigna radiata var. sublobata	5.3±0.04	18.5 ±0.07	5.3±0.11	7.4±0.18	4.6 ±0.07	64.2	1582.58
V. unguiculata subsp. cylindrica	3.2±0.32	14.0 ±0.11	3.4±0.28	3.4±0.28	3.6±0.33	75.6	1625.3

[a] all the values are means of triplicate determinations expressed on dry weight basis.
± denotes standard error.

Table 2. Mineral composition of tribal pulses (mg $100g^{-1}$ seed flour) [a].

Botanical Name	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Zinc	Manganese	Iron	Copper
Dolichos trilobus	26.5 ±0.52	1856.1 ±0.36	260.5 ±0.33	66.3 ±0.28	154.1 ±0.28	2.1 ±0.01	1.3 ±0.01	18.4 ±0.18	0.5 ±0.01
Entada rheedi	35.0 ±0.14	2637.7 ±1.50	284.0 ±0.31	640.3 ±0.08	244.1 ±0.11	1.1 ±0.11	5.2 ±0.08	22.3 ±0.12	1.2 ±0.04
Mucuna atropurpurea	20.0 ±0.14	2139.3 ±1.33	189.7 ±0.34	98.9 ±0.148	152.2 ±0.12	3.1 ±0.05	6.0 ±0.04	6.7 ±0.08	0.9 ±0.01
Rhynchosia cana	21.5 ± 0.08	1562.2 ±0.88	160.3 ±0.78	160.5 ±0.75	174.1 ±0.32	4.1 ±0.11	8.4 ±0.13	6.2 ±0.14	1.8 ±0.01
Rhynchosia suaveolens	24.3 ±0.76	1678.2 ±0.94	210.2 ± 0.30	94.3 ±0.66	278.2 ±0.21	3.5 ±0.01	7.4 ±0.11	5.3 ±0.16	1.3 ±0.10
Tamarindus indica	25.6 ±0.34	2038.1 ±1.34	240.0 ±0.19	248.0 ±0.68	153.2 ±0.16	4.2 ±0.03	3.4 ±0.03	9.2 ±0.07	2.4 ±0.11
Teramnus labialis	23.0 ±0.22	1866.2 ±1.06	230.2 ±0.18	520.3 ±0.86	162.1 ±0.12	3.2 ±0.03	0.5 ±0.01	44.0 ±0.12	2.2 ±0.08
Vigna radiata var. sublobata	28.7 ±0.34	2416.2 ±0.88	244.4 ±0.58	174.1 ±0.98	146.4 ±0.14	1.1 ±0.01	0.6 ±0.02	9.4 ±0.03	0.3 ±0.02
Vigna unguiculata subsp. cylindrica	20.0 ±0.10	1734.5 ±0.74	240.5 ±0.18	84.1 ±0.34	166.3 ±0.12	3.9 ±0.12	1.5 ±0.01	15.1 ±0.06	0.3 ±0.01

[a] all the values are means of triplicate determinations expressed on dry weight basis.
± denotes standard error.

Table 3. Niacin and ascorbic acid content of the tribal pulses (mg 100g^{-1} seed flour)[a]

Botanical Name	Niacin	Ascorbic Acid
Dolichos trilobus	48.11 ±0.13	34.18 ±0.08
Entada rheedi	27.32 ±0.39	108.19 ±0.19
Mucuna atropurpurea	86.32 ±0.09	75.39 ±0.43
Rhynchosia cana	41.38 ±0.03	76.94 ±0.18
Rhynchosia suaveolens	24.11 ±0.09	56.24 ±0.08
Tamarindus indica	76.67 ±0.09	50.42 ±0.17
Teramnus labialis	53.00 ±0.02	21.25 ±0.17
Vigna radiata var. sublobata	38.14 ±0.14	64.38 ±0.13
Vigna unguiculata subsp. cylindrica	14.62 ±0.15	127.67 ±0.14

± denotes standard error.
[a] all the values are means of triplicate determinations expressed on dry weight basis.

In the currently investigated samples, the content of the non-protein amino acid, L-DOPA is low compared with those of other tribal pulses such *as Mucuna utilis, M. monosperma, M. pruriens,* and *M. pruriens* var. *utilis* (Janardhanan and Lakshmanan, 1985; Mohan and Janardhanan, 1995b; Mary Josephine and Janardhanan, 1992; Arulmozhi and Janardhanan, 1992; Vadivel and Janardhanan, 2000). It has been demonstrated that in *M. pruriens*, the level of L-DOPA is significantly eliminated by dry heat treatment (Siddhuraju *et al*., 1996), cooking and autoclaving (Vijayakumari *et al.,* 1996).

Hydrogen cyanide is known to cause acute or chronic toxicity. The content of HCN level in the presently investigated tribal pulses is far below the lethal level i.e. 0.36 mg / 100 g (Oke, 1969) and comparable with those of *Vigna sinensis* and *Pisum sativum* (Montgomery, 1980); *Dolichos lablab* var. *vulgaris, Bauhinia purpurea* (Vijayakumari *et al.,* 1995, 1997); and *Entada phaseoloides* (Siddhuraju *et al.,* 2001).

CONCLUSION

The processing methods used by the *Palliyar* tribals mostly eliminate the anti-nutritional factors such as total free phenolics, tannins and hydrogen cyanide which are heat labile; thus potentially increasing the *in-vitro* protein digestibility of the seed meal. However the L-DOPA could only 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 with problem of protein deficiency in most of the developing countries which may result in many child killer diseases. The presence of anti-nutritional factors identified in the current report should not pose a problem for humans if the beans are properly processed.

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Table 4. Data on anti-nutritional factors of tribal pulses evaluated [a].

Botanical Name	Total Free Phenolic (g 100g^{-1})	Tannin (g 100g^{-1})	L-DOPA (g 100g^{-1})	Hydrogen cyanide (mg 100g^{-1})
Dolichos trilobus	1.34 ±0.19	0.58 ±0.07	1.74 ±0.11	0.33 ±0.04
Entada rheedi	3.13 ±0.15	0.63 ±0.04	0.23 ±0.36	0.20 ±0.05
Mucuna atropurpurea	2.46 ±0.15	0.03 ±0.01	1.09 ±0.07	0.28 ±0.07
Rhynchosia cana	1.60 ±0.12	0.44 ±0.03	1.09 ±0.07	0.27 ±0.03
Rhynchosia suaveolens	1.84 ±0.10	0.58 ±0.06	1.24 ±0.11	0.25 ±0.12
Tamarindus indica	3.76 ±0.14	0.03 ±0.01	4.12 ±0.36	0.09 ±0.06
Teramnus labialis	2.01 ±0.07	0.21 ±0.02	1.52 ±0.03	0.31 ±0.06
Vigna radiata var. sublobata	1.38 ±0.03	0.19 ±0.03	0.62 ±0.07	0.26 ±0.02
Vigna unguiculata subsp. cylindrica	2.77 ±0.12	0.27 ±0.09	1.96 ±0.05	0.29 ±0.05

[a] all the values are means of triplicate determinations expressed on dry weight basis.
± denotes standard error.

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