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NUTRITIVE AND ANTIOXIDANT CONTENTS OF WILD-LEGUME PROTEIN-RICH PLANT PRODUCTS

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ABSTRACT

Mature seeds of 20 species of the family Leguminoseae belonging to three subdivisions (Mimosoideae, Caesalpinoideae, Papilonoideae) were collected and were analysed for their nutritive and antioxidant contents. Crude protein values varied between 17.2% in *Cassia hirsuta* and 35% in *Canavalia ensiformis*. The highest crude lipids was recorded in *Xylia xylocarpa* (23.3%) and *Bauhinia vahlii* (23.3%). Among the 20 species, the highest content of crude fiber was recorded in *Leucaena leucocephala* (9.6%). Among the macro-minerals, potassium was the most abundant mineral, followed by calcium. The highest levels of iron was reported in *Mucuna atropurpurea* (17.4%). The antioxidants, phenols and L-DOPA were high in *Mucuna pruriens* var. *pruriens*.

INTRODUCTION

The growing third world population and its domestic animals need more protein. The most cost-effective proteins are those derived from plant materials, which although in abundance in many developing countries, are under-utilized. The search for novel high-quality but cheap sources of protein and energy has continued to be a major concern to governments and bodies charged with the responsibility for food and nutrition in many parts of the developing world (Janardhanan *et al.*, 2003). The aspiration of developing countries to be industrially self-sufficient has demanded huge financial spending in agriculture and the search for locally utilizable raw materials among the neglected agricultural crops. However, before such crops could be used, information is needed on their chemical characteristics.

In India, chickpea, pigeon pea, lentil, mungbean, urdbean, peas and peanuts are widely cultivated. Nutritional composition on these legumes have been reported (Ali and Kumar, 2000). Nonetheless, the production rates of these pulses failed to keep pace with the requirement of growing population (Narasinga Rao *et al.*, 1989). The work reported here was aimed at providing basic information on the proximate and mineral composition and certain antioxidant composition of some wild leguminous crop seeds in South India.

MATERIALS AND METHODS

Mature seeds of 20 species of the family Leguminoseae belonging to three subdivisions (Mimosoideae, Caesalpinoideae, Papilonoideae) were used in this study. A list of species investigated is presented in Table 1. The seeds were collected from various locations in the Western and Eastern Ghats regions of South India during the fruiting seasons. The leguminous seeds/plants were identified by the methods of Duke (1981), Wilmot-Dear (1987), Singh and Premananth (1992) and Sudhir *et al* (1994).

About 100g of seeds for each species were milled in a Wiley mill to pass through a 60-mesh size sieve and stored in screw-capped bottles at room temperature (25°C ± 2°C). Samples were withdrawn as required for chemical analysis. All analyses were carried out on triplicate samples.

Table 1 Species of the Leguminoseae investigated

Species	Subdivision
Leucaena leucocephala (Lamk.) de Wit	Mimosoideae
Xylia xylocarpa (Roxb.) Tanb.	Mimosoideae
Bauhinia vahlii W and A	Caesalpinoideae
Cassia floribunda Cav.	Caesalpinoideae
Cassia fistula L.	Caesalpinoideae
Cassia hirsuta L.	Caesalpinoideae
Cassia obtusifolia L.	Caesalpinoideae
Senna occidentalis (L.) Link	Caesalpinoideae

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Abrus precatorius L.	Papilionoideae
Canavalia ensiformis DC.	Papilionoideae
Canavalia gladiata (Jacq.) DC	Papilionoideae
Lathyrus sativus L.	Papilionoideae
Mucuna atropurpurea (Roxb.) DC	Papilionoideae
Mucuna monosperma DC ex. Wight	Papilionoideae
Mucuna pruriens var. *pruriens* (L.) DC	Papilionoideae
Mucuna pruriens var. *utilis* Wall ex Wight	Papilionoideae
Phaseolus lunatus L.	Papilionoideae
Vigna sinensis (L.) Savi. Ex Hassk	Papilionoideae
Vigna trilobata (L.) Verdc.	Papilionoideae
Vigna umbelata Ohwi & Ohashi	Papilionoideae

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 24h, followed by cooling in a desiccator. Nitrogen content in the powdered seed samples was estimated by the micro-kjeldahl method (Humphries, 1956) and crude protein was calculated (*N* x 6.25). The recommended methods of Association of Official Analytical Chemists (1970) were used for the determination of ash, crude lipid and crude fibre. Ash content was determined by incineration of 2g of sample in a muffle furnace kept at 550°C for 6h. Crude lipid was determined by exhaustively extracting 2g of sample with petroleum ether, using a Soxhlet apparatus. Crude fibre was determined by acid and alkaline digestion methods. The carbohydrate content was calculated by subtracting the total of the percentages of crude protein, crude lipid, crude fibre and ash on moisture-free basis from 100. The energy value of the seed was estimated (in kJ) by multiplying the percentages of crude protein, crude lipid and nitrogen-free-extract (NFE) by the factors 16.7, 37.7 and 16.7, respectively. The moisture was determined for dry-mater estimation. All constituents were therefore expressed on dry weight basis.

Mineral 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 de-ionised distilled water, filtered with Whatman No. 42 filter paper and filtrates made up to 100ml in a glass volumetric flask with de-ionised distilled water. All the minerals except phosphorus were analysed from triple acid digested sample by an atomic absorption spectrophotometer (Issac and Johnson, 1975). Phosphorus content in the triple acid digested extract was colorimetrically analysed (Dickman and Bray, 1940) at 650nm using a spectrophotometer.

Analysis of antioxidant compounds

The antioxidant compounds, phenols (Bray and Thorne, 1954), tannins (Burns, 1971) and the non-protein amino acid L-DOPA (3,4-dihydroxyphenylalanine) (Brain, 1976) were quantified.

RESULTS AND DISCUSSION

The proximate composition of 20 species of the Leguminoseae is presented in Table 2. Crude protein values varied between 17.2% in *Cassia hirsuta* and 35% in *Canavalia ensiformis*, with mean value of 24.88 ± 5.22%. The two members of the subdivision Mimosoideae had their crude protein higher than the average obtained for the family in this investigation. Among the members of the subdivision Caesalpinoideae, *Bauhinia vahlii* had the highest crude protein content of 24.6%. The results presented revealed that all of the leguminous members investigated contained high protein levels when compared with values of 17.1, 24.0, 24.1, 24.9, 24.0, 22.0, 25.1, 23.6, 19.7 and 22.3%, respectively for the Bengal gram, black gram (dhal), cowpea, field bean, green gram, horse gram, lentil, moth bean, peas and red gram (dhal) seeds by Narasinga Rao *et al* (1989).

Table 2 Proximate composition (g $100g^{-1}$ seed flour) [a]

Species	**Subdivision**	**Moisture**	**Crude protein**	**Crude lipid**	**Crude fiber**	**Ash**	**NFE**	**Energy (kJ 100^{-1}g DM)**
L. leucocephala	Mimosoideae	3.6	26.7	6.7	9.6	3.4	53.6	1593
X. xylocarpa	Mimosoideae	10.1	33.1	23.3	6.4	6.4	30.7	1944
Mean		6.8	29.9	15.0	8.0	4.9	42.1	1768
B. vahlii	Caesalpinoideae	9.7	24.6	23.3	6.2	4.2	41.7	1984
C. floribunda	Caesalpinoideae	4.4	19.9	2.7	8.6	5.6	63.3	1491
C. fistula	Caesalpinoideae	6.2	19.9	4.7	6.0	3.9	65.5	1604
C. hirsute	Caesalpinoideae	8.6	17.2	7.0	4.7	6.1	64.7	1634
C. obtusifolia	Caesalpinoideae	11.2	18.7	7.4	7.1	4.5	62.2	1630
S. occidentalis	Caesalpinoideae	4.5	21.4	8.8	6.8	4.7	58.3	1662
Mean		7.4	20.3	9.0	6.6	4.8	59.3	1667
A. precatorius	Papilionoideae	5.8	21.1	5.1	5.4	3.2	65.3	1633
C. ensiformis	Papilionoideae	8.5	35.0	4.3	7.7	3.9	49.2	1568
C. gladiata	Papilionoideae	8.5	25.5	3.3	5.9	3.5	61.8	1582
L. sativus	Papilionoideae	7.1	21.3	2.6	7.0	3.0	65.9	1557
M. atropurpurea	Papilionoideae	7.6	19.6	9.0	4.5	2.8	64.1	1738
M. monosperma	Papilionoideae	10.8	21.4	7.3	9.4	2.7	59.2	1620
M. pruriens var. *pruriens*	Papilionoideae	3.2	25.0	5.9	6.5	3.7	58.9	1623
M. pruriens var. *utilis*	Papilionoideae	7.9	26.9	7.4	8.7	3.3	53.7	1625
P. lunatus	Papilionoideae	9.3	32.1	4.9	3.8	5.1	54.0	1624
V. sinensis	Papilionoideae	8.3	28.6	6.4	2.5	6.8	55.7	1651
V. trilobata	Papilionoideae	5.6	30.3	4.9	5.8	5.3	53.7	1589
V. umbelata	Papilionoideae	6.4	29.3	5.4	7.7	4.1	53.5	1586
Mean		7.4	26.3	5.5	6.2	3.9	57.9	1616
Mean for Leguminoseae[b]		7.4 (2.3)	24.9 (5.2)	7.5 (5.7)	6.5 (1.8)	4.3 (1.2)	56.7 (8.8)	1646 (118.8)

a - Average of triplicate analysis

b - Mean and standard deviation in brackets

The mean crude lipid value for the family was 7.52 ± 5.69%. Noticeably, high values were obtained in *Xylia xylocarpa* (23.3%) and *Bauhinia vahlii* (23.3%). These values were higher than the value reported for soybean by Narasinga Rao *et al* (1989). Thus, barring other factors and taking cognisance of their protein content, these species may be useful in the formulation of high protein caloric diets. They may also serve as new sources of oil for other nutritional and industrial uses. The mean crude lipid values in the subdivision Mimosoideae, Caesalpinoideae and Papilionoideae were 15.00%, 8.98% and 5.54%, respectively.

Crude fiber levels in the subdivision Mimosoideae varied between 6.4% in *Xylia xylocarpa* and 9.6% in *Leucaena leucocephala*. Values of 4.7% in *Cassia hirsuta* and 8.6 in *Cassia floribunda* were the lowest and the highest fiber levels, respectively, in the subdivision Caesalpinoideae. Among the members of the subdivision Papilionoideae, *Mucuna monosperma* had the highest crude fiber content of 9.4%. However, a grand mean of 6.51 ± 1.83% was obtained for the family. Relative to the levels reported for conventional edible legumes by Narasinga Rao *et al* (1989), a high range (2.5 - 9.6%) of fiber was obtained in the wild leguminous seeds. High crude fiber could effectively trap and protect a greater proportion of nutrients (protein and carbohydrates) from hydrolytic breakdown, thus reducing digestibility and utilization of the products of digestion (Vadivel and Janardhanan, 2000). However, recently, World Health Organization (WHO) has recommended an intake of 22-23g of fiber for every 1000 kcal of diet (Kanwar *et al*., 1997). Though it does not contribute to the nutritive value of foods, the presence of fiber (i.e., roughage) in the diet is necessary for digestion and for elimination of wastes. The contraction of muscular walls of the digestive tract is stimulated by fiber, thus counteracting constipation (Vadivel and Janardhanan, 2000). Therefore, the high range of fiber noted in the wild leguminous seeds is a desirable characteristic since fiber plays an important role in the diet of humans and animals.

Ash ranged between 2.7% in *Mucuna monosperma* and 6.8% in *Vigna sinensis* in the 20 species in the Leguminoseae. The ash values were indicative of these species as far sources of minerals. The average NFE content for the Leguminoseae was 56.75 ± 8.80%.

The mineral elements analysed and presented in Table 3 are important nutritionally.

Table 3 Macro-mineral composition (g 100g^{-1}seed flour) [a]

Species	Subdivision	Sodium	Potassium	Calcium	Magnesium	Phosphorus
L.leucocephala	Mimosoideae	162	608	444	333	319
X. xylocarpa	Mimosoideae	116	791	534	373	326
Mean		139	699	489	353	322
B. vahlii	Caesalpinoideae	77	456	356	476	473
C. floribunda	Caesalpinoideae	42	913	549	318	503
C. fistula	Caesalpinoideae	127	624	597	290	455
C. hirsute	Caesalpinoideae	175	1029	620	1112	685
C. obtusifolia	Caesalpinoideae	58	1014	413	765	596
S. occidentalis	Caesalpinoideae	38	796	447	353	244
Mean		86	805	497	552	493
A. precatorius	Papilionoideae	81	591	425	170	290
C. ensiformis	Papilionoideae	57	1017	498	192	240
C. gladiata	Papilionoideae	109	1640	510	481	601
L. sativus	Papilionoideae	73	508	287	327	351
M.atropurpurea	Papilionoideae	50	931	609	206	331
M.monosperma	Papilionoideae	37	681	471	214	138
M.pruriens var.*pruriens*	Papilionoideae	104	1588	780	68	634
M.pruriens var. *utilis*	Papilionoideae	43	1846	507	378	326
P. lunatus	Papilionoideae	123	876	323	229	330
V. sinensis	Papilionoideae	118	816	582	262	574
V. trilobata	Papilionoideae	140	695	311	237	353
V. umbelata	Papilionoideae	98	714	456	239	385
Mean		86	992	480	250	379
Mean for Leguminoseae[b]		91.4 (42.1)	906.7 (378.5)	485.9 (120.4)	351.1 (231.3)	407.7 (149.6)

a - Average of triplicate analysis b - Mean and standard deviation in brackets

The human body contains 24 minerals all of which must be provided through the diet. Sodium, potassium and phosphorus are components of body fluids and regulate the osmotic pressure of the plasma. Calcium is required for the formation and maintenance of skeleton and teeth. Magnesium is required for cellular metabolism. Iron is an essential element for the formation of haemoglobin of red cells of blood and plays an important role in the transport of oxygen. Zinc is a constituent of carbonic anhydrase and a hormone, insulin. Hence, all are essential for life. Average values for sodium, potassium, calcium, magnesium and phosphorus were 91.4±42.1%, 906.7±378.5%, 485.9±120.4%, 351.1±231.3% and 407.7±149.6%, respectively. Potassium was the most abundant mineral, followed by calcium. Sodium levels were generally low in all the species of Leguminoseae investigated.

Composition of the micro-minerals of species presented in Table 4 showed that average iron, zinc and manganese contents were 11.6±6.5%, 6.0±5.7 and 2.8±1.8%, respectively. In the subdivision Caesalpinoideae, iron content varied between 4.4% in *Cassia floribunda* and 31.1% in *Cassia obtusifolia*. High zinc concentration (15.3%) was obtained in *Cassia hirsuta*. In the subdivision Papilionoideae, iron content varied between 3.4% in *Abrus precatorius* and 17.4% in *Mucuna atropurpurea*. The wide variability (as indicated by the standard deviation) obtained in the mineral contents of the species may be ascribed to the differences in the species investigated. It may also be reflection of the difference in the mineral status of the soils of the different locations where these seeds were collected (Vadivel and Janardhanan, 2001).

Table 4 Micro-mineral composition (g 100g^{-1}seed flour) [a]

Species	Subdivision	Iron	Zinc	Manganese
L. leucocephala	Mimosoideae	9.5	2.2	4.3
X. xylocarpa	Mimosoideae	8.7	1.4	3.4
Mean		9.0	1.8	3.8
B. vahlii	Caesalpinoideae	18.2	2.0	0.3
C. floribunda	Caesalpinoideae	4.4	1.7	1.0
C. fistula	Caesalpinoideae	4.8	4.3	6.9
C. hirsute	Caesalpinoideae	15.3	15.3	2.2
C. obtusifolia	Caesalpinoideae	31.1	15.1	4.5
S. occidentalis	Caesalpinoideae	12.0	8.4	2.0
Mean		14.3	7.8	2.8
A. precatorius	Papilionoideae	3.4	1.7	1.2
C. ensiformis	Papilionoideae	5.2	4.3	1.0
C. gladiata	Papilionoideae	11	7	2
L. sativus	Papilionoideae	5.6	2.4	4.7
M. atropurpurea	Papilionoideae	17.3	19.5	4.3
M. monosperma	Papilionoideae	10.5	2.7	2.3
M. pruriens var. *pruriens*	Papilionoideae	17.3	1.8	1.0
M. pruriens var. *utilis*	Papilionoideae	13.0	10.9	4.3
P. lunatus	Papilionoideae	15.9	12.7	4.6
V. sinensis	Papilionoideae	12.4	1.4	2.4
V. trilobata	Papilionoideae	10.6	3.4	4.2
V. umbelata	Papilionoideae	6.4	1.7	0.5
Mean		10.7	4.5	2.7
Mean for Leguminoseae[b]		11.6 (6.5)	6.0 (5.7)	2.8 (1.8)

a - Average of triplicate analysis b - Mean and standard deviation in brackets

Phenols have gained extensive attention because of their physiological functions, including free radical scavenging, anti-mutagenic, anticarcinogenic and anti-inflammatory effects (Biju *et al*., 2014). According to Pietta (2000), the antioxidant activity of phenols is largely due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators. These compounds have demonstrated higher *in vitro* antioxidant capacity than other antioxidants, such as ascorbic acid and α-tocopherol (Pulido *et al*., 2000), emphasising the importance of polyphenols as antioxidants in the diet. Phenolic compounds may exert their antioxidant activity in different ways. They may directly scavenge some reactive species, including hydroxyl, peroxyl and superoxide radicals, acting as chain breaking antioxidants. They may suppress lipid peroxidation recycling other antioxidants, such as α-tocopherol. Some phenolic compounds may bind pro-oxidant metals, such as iron and copper, preventing the formation of free radicals from these pro-oxidants while simultaneously maintaining their capacity to scavenge free radicals (Kris-Etherton *et al*., 2002; Halliwell, 2007). Besides, the effects of some phenolics are related to the increase in the activity of antioxidant enzymes and induction of the synthesis of antioxidant proteins (Chung *et al*., 2006). In this study, a considerable levels of phenols were observed in the 1% HCl methanol extracts of the wild-legume seeds (Table 5). The mean phenolic values in the subdivisions Mimosoideae, Caesalpinoideae and Papilionoideae were 0.89%, 0.66% and 1.61%, respectively.

Table 5 Antioxidant composition (g $100g^{-1}$seed flour) [a]

Species	Subdivision	Phenols	Tannins	L-DOPA
L.leucocephala	Mimosoideae	1.37	0.23	ND
X. xylocarpa	Mimosoideae	0.41	0.16	ND
Mean		0.89	0.19	
B. vahlii	Caesalpinoideae	1.70	1.79	2.3
C. floribunda	Caesalpinoideae	0.3	0.30	1.9
C. fistula	Caesalpinoideae	0.74	1.03	1.8
C. hirsuta	Caesalpinoideae	0.62	0.98	2.63
C. obtusifolia	Caesalpinoideae	0.22	0.16	0.80
C. occidentalis	Caesalpinoideae	0.36	3.46	1.86
Mean		0.66	1.29	1.88
A. precatorius	Papilionoideae	0.61	0.04	1.03
C. ensiformis	Papilionoideae	1.23	0.16	2.64
C. gladiata	Papilionoideae	1.94	0.20	2.83
L. sativus	Papilionoideae	0.28	0.29	ND
M.atropurpurea	Papilionoideae	2.89	0.34	2.13
M.monosperma	Papilionoideae	2.2	0.40	4.5
M.pruriens var.*pruriens*	Papilionoideae	5.29	0.14	7.62
M.pruriens var. *utilis*	Papilionoideae	3.44	0.05	6.98
P. lunatus	Papilionoideae	0.25	0.29	3.64
V. sinensis	Papilionoideae	0.37	0.43	0.58
V. trilobata	Papilionoideae	0.30	0.24	0.65
V. umbelata	Papilionoideae	0.59	0.49	0.11
Mean		1.61	0.25	2.97
Mean for Leguminoseae		1.2 (1.3)	0.6 (0.8)	2.6 (2.1)

a - Average of triplicate analysis b - Mean and standard deviation in brackets
ND - Not detected

Studies confirmed that the tannins exhibit anti-oxidant, anti-microbial, and anti-inflammatory properties. The consumption of tannins rich food will offer a lot of remedial and beneficial effects to human being. The tannin can also be used as drugs to heal the burning injury and on cuts to stop bleeding (Chung *et al*., 1998). The tannins were 15-30 times more effective at quenching peroxyl radicals than simple phenolics or Trolox. One of the tannins, polygalloyl glucose, reacted an order of magnitude more quickly with hydroxyl radical than mannitol. Tannins are found in many plant-based foods and beverages and are potentially very important biological antioxidants (Hagerman *et al*., 1998). Hence, the contents of tannins were shown in Table 5. The highest amount of tannin was found in *Senna occidentalis* (3.46%); whereas, the lowest levels of tannin was found in *Abrus precatorius* (0.04%).

Natural L-DOPA had a more rapid onset of action and longer effect without increases in dyskinesias, when compared to synthetic L-DOPA formulations (Katzenschlager *et al*., 2004). In the present study, the highest levels of L-DOPA was recorded in *M. pruriens* var. *pruriens* (Papilionoideae) (7.62%).

CONCLUSION

The present investigation indicated that compared to other commercial cultivated legume seeds, wild-legumes contained considerably higher amounts of proteins, minerals and antioxidants. This should reflect on overall better nutritive value of seeds of these wild-legumes. It should be pointed out, however, that animal feeding experiments are needed for the final answer as to the nutritional significance of the findings.

The work with wild legume species will assume greater importance in the coming years, as gene transfer between the cultigens and wild species is improved by new biotechnological techniques which will drive the need for further collection of these important species.

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