Analysis of Certain Nutritional Parameters of Some Edible Lesser Known Legumes of Nagaland, India

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Abstract

Nagaland, a hilly state of India, is the home for large number of wild edible legumes which has the potential to add to the food security. In the present study, effort was invested to evaluate certain nutritional parameters of 13 wild as well as cultivated lesser known edible legume species. Study finds that most of these species are very rich nutritionally. In this study, the maximum moisture content was observed in the fruit of *Psophocarpus tetragonolobus* (90.48%) followed by seeds of *Lablab purpureus* (82.57%); highest total protein content was observed in *Vigna umbellata* (76.50 mg/g) followed by *Lablab purpureus* (76.19 mg/g). Fruit of *Canavalia gladiata* showed the highest total carbohydrate content (944.52 mg/g) and reducing sugar content (717.78 mg/g) followed by seeds of *Pisum sativum* where in the total carbohydrate content was 807.62 mg/g and the non-reducing sugar content was observed to be 688.19 mg/g. Both the total phenolic content and total flavanoid content were observed to be highest in flowers of *Bauhinia variegata* with 26.94 mg GAE/g and 265.69 mg QE/g respectively. Highest antioxidant activity was observed in *Mucuna pruriens* with IC50 value of 127 µg/mL.

Keywords

Antioxidant activity, Lesser known edible legumes, Underutilized legumes, Nagaland, Nutritional value

Abbreviations

DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; DNSA: 3,5 Dinitro salicylic acid; GAE: Gallic acid equivalents; HCL: Hydrochloric acid; N: Normal; TFC: Total flavonoid content; TPC: Total phenolic content; QE: Quercetin equivalents.

Introduction

Legumes are known for their protein content and are easily digestible [1]. Dried seed of legumes called pulses are major sources of plant protein and carbohydrates. Other nutrients like phosphorus, minerals, vitamin C, riboflavin and essential amino acids are also major constituents besides having medicinal properties. There are many lesser known legumes and local rural population use these pulses in their daily diet [2]. These plant resources have the potential to uplift the economic condition of the local people and add to food security. Along with the nutritional factors, pulses also contain flatulence causing raffinose family oligosaccharides, lectins, enzyme inhibitors, phytate, heat-stable and heat-labile anti-nutritional and toxic factors [3]. Several processing methods like presoaking, germination or fermentation of pulses reduce these factors, making legumes as an inexpensive and nutritious human food. Members of the legume family are
also capable of biological nitrogen fixation through association with specialized soil bacteria for use as a natural fertilizer, thus providing sustainability of agriculture to the people.

According to Erbersdobler et al. [4], the vitamin and mineral content of peas, faba beans, sweet lupines, and soybeans are relatively high, particularly for potassium and calcium (in lupines and soybeans), magnesium, iron, and zinc, and also vitamin B1 (thiamine) and folates. Legumes are also known for their wide variety of non-nutritional compounds, which are responsible for their different biological activities, such as antitumor activity particularly in colon cancer [5].

According to the ‘Status Paper on Pulses’ Ministry of Agriculture, Directorate of Pulses Development, Government of India, Bhopal, the domestic consumption of pulses in India was 186.5 lakh tonnes for the triennium ending 2010-11. Against this, India produced an average quantity of 158 lakh tonnes. During this period, there was a gap of 28.5 lakh tonnes of pulses in demand and supply. This gap was due to higher growth of population as compared to pulse production and warranted to look for exploration of lesser known legumes which has the potential to fill up the gap and meet the requirement.

Many crops are considered to be neglected at a global level but are staple at a national or regional level and contribute significantly to food supply in certain periods (e.g., indigenous fruits) or are important for a nutritionally well-balanced diet (e.g., indigenous vegetables, cereals, pulses etc.). The preservation of plant genetic resources of promising as well as threatened types for posterity needs top priority. Nagaland is a remote state of North Eastern region of India and exhibits a great deal of plant diversity. Nagaland is basically an agricul
ture dependent state and cultivation of legume crops play a role in the agricultural system of the state. The scope of fresh edible legumes and pulses is limitless and through nutritional assessment the value of legumes and pulses as a dietary supplement will be enhanced which will benefit the local people. Pulses play major role in balanced nutrition as they are major sources of proteins and carbohydrates. There are many wild legumes in Nagaland of which some of them are edible and medicinal and have the potential to be used as pulses. Pulses are considered as the sustainable seed grains for the future which will be helpful in overcoming the nutritional deficiencies in rural area and boost the socio-economic conditions of local people and add to their food security [2].

With keeping in mind the above; present study was undertaken for exploration of lesser known edible legumes and nutritional analysis of edible parts. In the present study, 13 species of lesser known legumes which are easily available for consumption by rural masses were selected for assessment of different nutritional parameters viz., total phenol content, flavonoid content, protein content, carbohydrate content, reducing sugar, moisture content and antioxidant activity.

**Materials and methods**

**Collection of samples**
Biochemical analysis

Moisture content

Determination of moisture content was done following AOAC [6]. To calculate moisture content, pre-weighed legume seeds of the selected species except for Bauhinia variegata and Crotolaria tetragona for which the flowers are the edible part and the whole pod of Canavalia gladiata and Pseudopropocarpus tetragonolobus were maintained in the hot air oven at 80 ± 5°C and weights were measured at regular intervals till constant weights were achieved. The dry matter content was taken as the final weight obtained after the samples have been dried in the hot air oven at 80 ± 5°C for 6-12 h, followed by calculation of moisture contents using the formula:

Moisture content (%) = \((W_1 - W_f)/W_1 \times 100\);

where \(W_1\) = Weight (g) of sample before drying and \(W_f\) is weight (g) of sample after drying.

Total protein content

Total protein content was determined using the partially modified colorimetric Bradford's method [7]. One gram each of the selected samples was ground in 20 mL of 0.1 M phosphate buffer (pH 7.0) and centrifuged at 12000 rpm for 10 min followed by filtration. The filtrate/extract was then used for the analysis. To 4 mL of the extract, 2 mL of Bradford's solution [prepared by dissolving 100 mg of coomassie brilliant blue G 250 in 50 mL of 95% ethanol (v/v), 100 mL of concentrated (ortho) phosphoric acid and making the volume to 200 mL with pure water] was added. The mixture was neutralized with solid sodium carbonate until the effervescences were ceased. The volume was made up to 100 mL with pure water and centrifuging at 10000 rpm for 10 min. The absorbance was measured at 562 nm and standard curves were prepared with 'Bovine Serum Albumin'.

Total carbohydrate

Total carbohydrate was determined following Anthrone method [8]. For extraction, 100 mg of sample species was taken in test tubes and hydrolyzed it with 5 mL of 2.5N HCl by keeping in a boiling water bath for 3 h after which it was neutralized with solid sodium carbonate until the effervescences were ceased. The volume was made up to 100 mL with pure water and centrifuged at 12000 rpm for 10 min and supernatant was used as extract for analysis. To 3 mL of the sample, 4 mL of anthrone reagent (200 mg anthrone in 100 mL of ice-cold 95% (v/v) sulphuric acid) was added and kept in a boiling water bath for 8 min followed by cooling. The absorbance was then read at 630 nm with the standard curve using glucose.

Reducing sugar

Reducing sugar was determined following DNS method [9]. Reducing sugar was estimated using DNSA reagent. The extraction was done by taking one gram of each of the fresh samples with 80% (v/v) ethanol. For each sample, 4 mL of extract was mixed with 2 mL of DNS reagent and incubated in boiling water bath for 5 min. It was then brought to room temperature and added 0.5 mL of Rochelle salt solution (40%, v/v). The absorbance was measured at 540 nm and glucose was taken as the standard.

Total phenolic, flavonoid content and antioxidant activity

Methanol extract preparation

One-gram fresh sample of each legume species was ground and extracted in 20 mL of 80% (v/v) methanol by centrifuging at 10000 rpm for 10 min. The extract was then filtered over with Whatman No. 4 filter paper [10]. The filtrate obtained was used for analysis.

Total phenolic content (TPC)

For estimation of TPC, Folin-Ciocalteau method [11] was followed. About 0.2 mL methanol extract was added to 0.5 mL Folin-Ciocalteau reagent and 2.8 mL of pure water and allowed to stand for 5 min followed by mixing of 2 mL of saturated sodium carbonate solution and 2 mL of water. The mixture was incubated under dark condition with intermittent shaking. The absorbance was measured at 765 nm against gallic acid as the standard and expressed as mg Gallic Acid Equivalents (GAE)/g of extract.

Total flavonoid content (TFC)

Total flavonoid content was determined following technique of Sahreen et al. [12] with slight modification. To 1 mL of methanol extract, 2.7 mL of methanol (30%), v/v 0.15 mL of sodium nitrite (0.5 M) and 0.15 mL of aluminium chloride (0.3 M) were added. The mixture was then allowed to stand for 5 min and then added 1 mL of 1 M NaOH. The absorbance was measured at 510 nm and standard curve was prepared using Quercetin and expressed as mg Quercetin Equivalents (QE)/g.

Antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

For study of DPPH radical scavenging assay, protocol given by Aoshima et al. [13] with suitable modification was followed. To variable volumes of methanol extract [2-10 µL extract (100-500 µg/mL concentration) of Mucuna pruriens, 5-40 µL (250-2000 µg/mL concentration) extract of Bauhinia variegata, 20-200 µL (1000-10000 µg/mL concentration) extract of Cajanus cajan, Crotolaria tetragonla, Lablab purpureus, Phaseolus vulgaris, Pismum sativum, Pseudopropocarpus tetragonolobus, Vigna umbellata, Vigna unguiculata subsp. sesquipedalis and 150-390 µL (7500-21000 µg/mL concentration) extract of Canavalia gladiata and Phaseolus lunatus], 3 mL of 0.1 mM DPPH methanol solution was added. Mixture was mixed thoroughly and allowed to stand in the dark for 30 min at room temperature. The absorbance was read at 517 nm. Inhibition of free radical by DPPH was calculated by the following equation:

DPPH scavenging effect (%) = \([(A_0 - A_1/A_0) \times 100]\)

where \(A_0\) is the absorbance of control reaction and \(A_1\) is the absorbance in the presence of sample.
Results and Discussion

Moisture content

Mohan et al. [14] reported the moisture contents of the dry legumes in their study ranging from 9-13% making them favorable for long storage. In the present study, the moisture contents of the studied legume species were in the range of 45-90%. Among the 13-legume species studied, the moisture content in most of the species was very high with highest in *Psophocarpus tetragonolobus* (90.48%) followed by *Canavalia gladiata* (86.66%), *Lablab purpureus* (82.57%), *Crotolaria tetragona* (80.97%), *Bauhinia variegata* (80.85%), while the lowest moisture content was observed in *Phaseolus vulgaris* (45.99%) (Table 2).

Protein content

Among the species studied, maximum total protein content was found to be in *Vigna umbellata* (76.50 mg/g) followed by *Lablab purpureus* (76.19 mg/g), *Mucuna pruriens* (68.84 mg/g) while lowest protein content was observed in *Bauhinia variegata* (1.95 mg/g) and *Psophocarpus tetragonolobus* (3.65 mg/g) (Figure 2, Table 2).

Carbohydrate content

Carbohydrate content was found to be high in all the samples analyzed (Figure 3, Table 2). The highest total carbohydrate content from the dry weight of the samples was observed in *Canavalia gladiata* (944.52 mg/g) while the lowest content was observed in *Psophocarpus tetragonolobus* (150.39 mg/g). The reducing sugar content ranged from 717.78 mg/g in *Canavalia gladiata* 28.97 mg/g in *Vigna umbellata*. The non-reducing sugar was calculated as the difference between total carbohydrate and reducing sugar and ranged from 688.19 mg/g in *Pisum sativum* to 11.07 mg/g in *Bauhinia variegata* (Figure 3, Table 2).

Table 2: Moisture, total protein content, reducing sugar, non-reducing sugar and total carbohydrate content of the studied species (dry weight basis).

| Species             | Moisture Content (%)* | Total protein content (mg/g) (D/W) | Total carbohydrate content (mg/g) (D/W) | Reducing sugar content (mg/g) (D/W) | Non-reducing sugar content (mg/g) (D/W) |
|---------------------|-----------------------|----------------------------------|---------------------------------------|-----------------------------------|---------------------------------------|
| Bauhinia variegata  | 80.85 ± .01           | 1.95 ± 0.12                      | 297.65 ± 0.1                          | 286.57 ± 0.15                     | 11.07 ± 0.01                          |
| Cajanus cajan       | 63.28 ± .02           | 27.45 ± 0.1                      | 571.8 ± 0.1                           | 128.96 ± 0.12                     | 442.92 ± 0.01                          |
| Canavalia gladiata  | 86.66 ± 0.04          | 15.18 ± 0.1                      | 944.52 ± 0.17                         | 717.78 ± 0.13                     | 226.74 ± 0.01                          |
| Crotolaria tetragona| 80.97 ± 0.05          | 34.89 ± 0.1                      | 732.10 ± 0.1                          | 408.62 ± 0.1                      | 323.48 ± 0.01                          |
| Lablab purpureus    | 82.57 ± 0.01          | 76.19 ± 0.15                     | 693.97 ± 0.1                          | 390.22 ± 0.1                      | 303.75 ± 0.01                          |
| Mucuna pruriens     | 57.47 ± 0.01          | 68.845 ± 0.1                     | 303.96 ± 0.1                          | 178.65 ± 0.12                     | 151.30 ± 0.01                          |
| Phaseolus lunata    | 71.64 ± 0.1           | 54.894 ± 0.1                     | 368.89 ± 0.1                          | 35.68 ± 0.1                       | 333.21 ± 0.01                          |
| Phaseolus vulgaris  | 45.99 ± 0.04          | 67.158 ± 0.1                     | 184.61 ± 0.1                          | 33.47 ± 0.1                       | 151.13 ± 0.01                          |
| Pisum sativum       | 59.8 ± 0.02           | 28.37 ± 0.06                     | 807.62 ± 0.1                          | 119.42 ± 0.1                      | 688.19 ± 0.01                          |

*Data represents the mean of three sample analysis (n = 3) ± SE; D/W: Dry weight basis.
According to Siddiq et al. [15] and Tosh et al. [21] pulses are rich in carbohydrates and the findings of the present study agrees with the past reports particularly in Canavalia gladiata and Pisum sativum. In the present study, Pisum sativum showed high total carbohydrate content of 807.62 mg/g as compared to 120 Kcal per 100 gm of seeds of green pea as reported by Mohan et al. [14]. Peas are rich sources of starch and soybeans are low in utilizable carbohydrates while lupines are high in dietary fiber [4]. While, Madar et al. [22] reported 0.017 mg/g carbohydrates in dry seeds of Vigna radiata.

**Total phenol and flavonoid content**

Besides protein and carbohydrate, most of the studied samples were found to be rich in phenols and flavonoids. As reported in the bark of Malpighia umbellata Rose [23], high phenolic content was reported by Zhao et al. [24] in lentils. Xu and Chang [25] reported high total phenolic acid in light colored beans while Laparra et al. [26] reported black beans with higher phenolic contents, whereas in this study, high phenolic content was seen in white beans of Mucuna prureins as compared to the other colored beans. Ha et al. [27] reported high total phenolic content with methanol extracts of pods of Phaseolus vulgaris (95.41 mg GAE/g). In the present study, the highest phenolic content was observed in Bauhinia variegata (26.94 mg GAE/g) while the lowest was in Phaseolus vulgaris (1.15 mg GAE/g) (Figure 4, Table 3).

The total flavonoid content from the flowers of Bauhinia variegata (265.69 mg QE/g) was found to be highest among the studied species followed by seeds of Phaseolus lunata (69.89 mg QE/g) while the lowest flavonoid content was observed in seeds of pisum sativum (4.64 mg QE/g) and Cajanus cajan (5.26 mg QE/g) (Table 3). According to Prakash and Kaskaran [28], acerola is one of the few fruits, which shows a plethora of phytonutrients like phenolics, flavonoids etc. However, Fidrianny et al. [29] reported highest total flavonoid content (13.37 g QE/100 g) in Arachis hypogaea and lowest in Glycine max (1.64 g QE/100 g) and Ha et al. [27] also reported less flavonoid content (9.29 mg RE/g in seeds of Phaseolus vulgaris.

**Antioxidant activity**

The antioxidant capacity was analyzed in the selected legume species by DPPH scavenging assay which showed significant activity. The IC50 values determined from the plotted graph of scavenging activity against various concentrations of extracts are in the order of 127, 1088, 1186, 1929, 3674, 5991, 6950, 12489, 13498, 24450, 24827, 24895, 43887 µg/ml of Mucuna prureins, Lablab purpureus, Bauhinia variegata, Vigna unguiculata sub-sp. sesquipedalis, Crotolaria tetragona, Phaseolus vulgaris, Vigna unguiculata, Vigna umbellata, Phaseolus lunata, Pisum sativum, Psophocarpus tetragonolobus, Cajanus cajan and Canavalia gladiate respectively (Figure 5, Table 3). Among the 13 legume species studied, the methanolic extracts of seeds of Mucuna prureins showed the highest antioxidant activity with

![Figure 4: Total phenol and flavonoid content of the selected edible legumes.](image)

![Figure 5: Percent inhibition of DPPH of the studied edible legumes.](image)
Higher antioxidant activity and DPPH scavenging activity in *Mucuna pruriens* and *Lablab purpureus* as in the present study was also was reported in the legume of fermented soybean due to presence of polyphenols and phytochemicals in soya fermented foods [30, 31], lentils [24] and *Phaseolus vulgaris* [32]. Because of antioxidant properties, polyphenols shows positive effect and should be included in the diets and it has been reported that regular intake of flavonoids rich food plays a protective role against coronary and cardiovascular diseases [33, 34]. DPPH scavenging activity in some legumes with promising antioxidant and free radical scavenging activity was reported by Dordevic et al. [35]. Studies on the antioxidant constituents of various legume seeds have reported that they contain potential medicinal properties including antioxidant activities [29, 36, 37] as was also reported in *Malpigia emarginata* which is extremely rich in vitamin C and also contains vitamins A, B₂, B₉, and B₁₂ as well as carotenoids and bioflavonoids, and have antioxidant uses [38]. According to Craig and Beck [39], vegetables and fruits play vital roles in human nutrition as sources of vitamins, minerals and dietary fiber. Consumption of fruit and vegetables decreases blood pressure [40] and lower the risk of stroke development [41].

**Conclusion**

Legumes can have a prospective role in improving the nutritional status of the people especially the undernourished group of people. This study has given us the basic information regarding nutritional content of edible legumes which will be an eye opener especially for the rural people of Nagaland and worldwide as well. On the basis of the above findings, it can be concluded that the fresh edible legumes studied are good sources of phenol, flavonoids, antioxidants etc. besides proteins and carbohydrates. These legumes can be useful sources of low-cost protein especially for the under developed countries and under privileged rural population. In addition to being a highly nutritious food, there is also evidence that edible legumes can play a major role in managing a number of health conditions and therefore, there is the need for more exploration, and conservation of the edible legumes. Cultivation of legumes and pulses should be encouraged and can be used not only as meat replacers but also as components of rational nourishment and food for vegetarians. The isolated proteins, starch and fibers from legume seeds have good physico-chemical and health protecting properties and are promising basic materials for food industrial use. Leguminous seeds are important in the traditional diet of large populations particularly in the rural areas. However most of the people particularly in Nagaland are unaware of the nutritional components and importance of the legumes for which awareness should be initiated and cultivation in large scale should be encouraged which will also be a way of providing food security.

**Declaration of conflicting interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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