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Quantification of the Total Protein Content of Some Wild Edible Plants Used by the Local People of Kangchup Area, Senapati District of Manipur, India

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ABSTRACT

The experiment was conducted during March, 2023 to November, 2024 at the Laboratory of Institutional Biotech Hub, Lilong Haoreibi College, Lilong, Manipur, India to explore various plant protein sources, with traditionally edible plants recognized as rich in protein and widely used by many communities for sustenance. This study analyzed the total protein content in 21 plant species from four categories: trees, shrubs, herbs and creepers, using Lowry's estimation method with Bovine Serum Albumin (BSA) as a standard. All the proteins were extracted by using phosphate buffer (pH 7.4). Results showed that the highest protein content among trees was found in *Wendlandia grandis* flower (209.71±3.66 mg g^{-1} of dry weight) and the lowest in *Albizia myriophylla* bark (12.00±3.34 mg g^{-1} of dry weight). *Curcuma caesia* rhizome contained the highest protein among herbs (108.57±0.48 mg $g^{-1}$of dry weight), while *Alpina officinarum* leaves had the lowest (14.86±0.86 mg g^{-1} of dry weight). In shrubs, *Zanthoxylum oxyphyllum* leaves exhibited the highest protein content (181.43±2.24 mg g^{-1} of dry weight), and *Smallanthuss onchifolius* tubers had the lowest (28.00±0.83 mg g^{-1} of dry weight). Among creepers, *Paederia foetida* galls showed the highest protein content (45.14±0.39 mg g^{-1} of dry weight), while *Hodgsonia heteroclita* fruit had the lowest (20.29±1.19 mg g^{-1} of dry weight).

KEYWORDS: Wild plants, protein, phosphate buffer, Lowry's method, BSA

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1. INTRODUCTION

The practice of consuming wild food plants is as old as human prehistory. With early humans relying on hunting, fishing, and gathering various plant parts like stems, roots, flowers, fruits, leaves, buds, and seeds. Wild edible plants (WEPs) hold significant nutritional and cultural value, particularly for indigenous communities, and have historically been utilized as food, medicine, raw materials, and in religious rituals (Kikim et al., 2024). In developing countries, the scarcity, high costs, and unreliable supply of healthy food have led to a search for affordable and alternative nutritious food sources. WEPs are one such alternative that plays a crucial role in supporting the global food supply. They have significantly contributed to meeting food and nutritional requirements and improving the health of impoverished communities in many rural areas worldwide. Research indicates that many wild edible plants are rich sources of essential nutrients, including proteins, carbohydrates, vitamins, and minerals. Additionally, some of these plants contain substantial amounts of various health-promoting compounds, such as phenolic compounds (Duguma, 2020; Seifu et al., 2017; Elkhatim et al., 2018; Zhang et al., 2018).

Proteins are large organic molecules made of amino acids and are vital for high-quality nutrition. They are essential for maintaining muscle mass, supporting immune responses, and aiding cell repair. Protein can be obtained from two main sources: animal and plant sources (Akshay et al., 2023).

Animal protein, although higher in demand, is generally considered less environmentally sustainable. Therefore, a gradual transition from animal- to plant-based protein food may be desirable to maintain environmental stability, ethical reasons, food affordability, greater food safety, fulfilling higher consumer demand, and combating of protein-energy malnutrition. Due to these reasons, plant-basedproteins are steadily gaining popularity, and this upward trend is expected to continuefor the next few decades. Plant proteins are a good source of many essential aminoacids, vital macronutrients, and are sufficient to achieve complete protein nutrition (Langyan et al., 2022). Among the various dietary protein sources, plant-based origins contribute significantly, comprising 57% of the proteinsupply, while the remaining 43% includes dairy products (10%), shellfish and fish (6%), meat (18%), and other animal products (9%) (Sytar et al., 2024). Plant proteins are nutritionally comprehensive, similar to animal proteins, easily digested and absorbed by the body, and have a variety of physiological health functions (Xiao et al., 2023). Animal proteins have long been used in the food industry for their functional properties, such as foaming and emulsification. However, their environmental impact is greater than that of crop production, prompting research into plant-based proteins as a sustainable, cost-effective alternative (Munialo, 2023).

Global food challenges have driven research into wild plant species as sources of essential nutrients, while dietary trends shift towards less red meat and more plant-based foods (Ridwane et al., 2022). A shift towards a more sustainable diet involves less reliance on animal-based proteins, prompting the agri-food industry to explore alternative protein sources (Kumar et al., 2022). Plant-based dairy and meat alternatives provide equal nutritional value at lower costs while reducing deforestation and greenhouse gas emissions. Understanding the functional properties of plant-based proteins is essential for their effective use in food products (Sa et al., 2020; Pattnaik et al., 2021). With a growing global population, meeting nutritional needs requires adequate calorie intake and macronutrients, particularly proteins. However, the large-scale production of animal-derived proteins is resource-intensive and unsustainable. Plant-based proteins offer a viable alternative, being cost-effective, environmentally friendly, and easier to cultivate (Mistry et al., 2022). Therefore, this study aims to quantify the total protein content of various wild food plants used by the local population in the Kangchup Chingkhong area of the Senapati District of Manipur.

2. MATERIALS AND METHODS

The experiment was conducted from March, 2023 to November, 2024 at the Laboratory of Institutional Biotech Hub, Lilong Haoreibi College, Lilong, Manipur, India.

2.1. Chemicals and reagents

All the chemicals and reagents used in the experiments were of analytical grade (AR) and are given below:

- Potassium di-hydrogen phosphate
- Potassium mono-hydrogen phosphate
- Sodium carbonate
- Potassium chloride
- Sodium hydroxide,
- Sodium potassium tartarate,
- Copper sulphate
- Folin-ciocalteu
- TCA (Trichloro acetic acid)

2.2. Sample collection and preparation

Various parts of 30 plant species were collected from the Kangchup Chingkhong area, Senapati district of Manipur from March, 2022 to April, 2023. After collecting, the plant samples were cleaned by using tap water followed by double distilled water to remove all the dust and oven-dried at 60°C. The dried samples were ground to powder by using a grinder. 200 mg of each sample was taken in a mortar and pestle and to it, approximately 10 ml of freshly prepared phosphate buffer (0.2 M, pH 7.5) was added and kept pasting until a clear plant solution was observed. Then the solutions were centrifuged at 4000 rpm for 12 min and the final supernatants were collected in respective tubes.

2.3. Quantification of total protein content

Bovine Serum Albumin (BSA) is used as a standard (50 mg/100 ml dry weight) for estimating the unknown concentration of proteins. To prepare the sample, 200 mg of dry tissue was crushed with 10 ml of phosphate buffer (pH 7.5, 0.2 M) using a chilled pestle and mortar. The homogenate was then filtered through four layers of cheesecloth. Next, the filtrate was centrifuged at 4000 rpm for 12 minutes. The supernatant was transferred to a graduated test tube, and its volume was adjusted to 20 ml with the same buffer. The pellet was discarded, leaving the supernatant, which contained the soluble protein.

To precipitate the protein, 1 ml of the supernatant was transferred to another centrifuge tube, and 1 ml of TCA (Trichloroacetic acid) solution (10% w/v) in distilled water was added. Precipitation was completed in 5 minutes, after which the tube was centrifuged, and the supernatant was discarded. A small amount (5 ml) of 95% ethyl alcohol was added to the protein precipitate to remove excess TCA from the surface of the protein. This washing step was repeated twice.

Following the washes, 1 ml of reagent C was added to the protein pellet. Reagent C was freshly prepared by mixing 10 ml of solution A (2% Na_2CO_3 in 0.1 N NaOH) and 1 ml of solution B (0.5% CuSO4·5 H_2O in 1% potassium sodium tartrate). The mixture was vigorously shaken at room temperature and allowed to stand for 10 minutes. Next, 0.2 ml of reagent D (Folin-Ciocalteu phenol reagent diluted with distilled water at a 1:1 ratio) was added, and the mixture was allowed to react for 30 minutes at room temperature. The final volume was then adjusted to 5 ml with distilled water, and the intensity of the blue colour was measured at 750 nm using a spectrophotometer. The amount of protein was expressed as mg g^{-1} of dry weight (Lowry et al., 1951; Sapan et al., 1999; Shakir et al., 1994; Peterson, 1983).

2.4. Statistical analysis

All the experimental measurements were performed in triplicates and expressed as the mean±standard deviations. The magnitude of the means, standard curve, standard errors, and standard deviations were calculated using MS Excel 2019 software.

3. RESULTS AND DISCUSSION

In this study, a total of 32 plant parts were used as protein sources belonging to 21 plant species and categorised into four types i.e. Trees, Shrubs, Herbs, and Creepers. The total protein content was expressed as mg g^{-1} of dry weight of the sample.

3.1. Total protein content among the tree

The total protein content among the tree species is shown in Table 1. The highest protein concentration was found in *Wendlandia grandis* flower (209.71±3.66 mg g^{-1} of dry weight) followed by *Leucaena leucocephala* seed, *Dysoxylum excelsum* leaves, and *D. excelsum* flower with a total protein content of 183.43±1.03, 121.43±2.40 and 115.7±2.50 mg g^{-1} of dry weight respectively. The lowest protein concentration was recorded in *Albizia myriophylla* bark (12.00±3.34 mg g^{1} of dry weight) followed by *Clerodendrum colebrookianum*

Table 1: Total protein content among the trees

Sl. No.	Plant sample	Family	Plant part	Protein concentration (mg g^{-1} of dry weight)
1.	*Clerodendrum colebrookianum*	Verbenaceae	Stem	22.57±0.67
			Leaves	19.14±2.42
2.	*Dysoxylum excelsum*	Mileaceae	Flower	115.7±2.50
			Leaves	121.43±2.40
			Stem	75.43±4.63
3.	*Parkia timoriana*	Fabaceae	Fruit pulp	58.29±1.47
			Seed	76.57±1.60
4.	*Leucaena leucocephala*	Mimosaceae	Fruit pulp	108.00±0.92
			Seed	183.43±1.03
5.	*Wendlandia grandis*	Rubiaceae	Flower	209.71±3.66
6.	*Albizia myriophylla*	Fabaceae	Bark	12.00±3.34

The data represent are mean of 3 replications leaves (19.14±2.42 mg g^{-1} of dry weight), *C. colebrookianum* stem (22.57±0.67 mg g^{1} of dry weight), *Parkia timoriana* fruit pulp (58.29±1.47 mg g^{-1}), *D. excelsum* stem (75.43±4.63 mg g^{1} of dry weight) and *P. timoriana* seed (76.57±1.60 mg $g^{-1}$of dry weight).

3.2. Total protein content among the shrubs

The total protein content among the Shrubs is shown in Table 2. The highest total protein content was found in *Zanthoxylum oxyphyllum* leaves (181.43±2.24 mg g^{-1} of dry weight) followed by *Clerodendrum serratum* leaves (83.43±2.45 mg g^{-1} of dry weight), *C. serratum* stem (78.57±7.70 mg g^{-1} of dry weight) and *Accacia pennata* leaves (62.57±1.39 mg g^{-1} of dry weight). The lowest protein content was recorded in *Smallanthuss onchifolius* tuber (28.00±0.83 mg g^{-1} of dry weight) followed by *C. serratum* root (42.29±0.49 mg g^{-1} of dry weight), *A. pennata* stem (46.86±1.04 mg g^{-1} of dry weight) and *C. serratum* flower (52.00±1.06 mg g^{-1} of dry weight).

3.3. Total protein content among the herbs

The total protein content among the Herbs is shown in Table 3. The highest protein concentration was found in *Curcuma caesia* rhizome (108.57±0.48 mg g^{-1} of dry weight) followed by *Brachycorythis obcordata* leaves (99.14±0.95 mg g^{-1} of dry weight), *Maranta arundinaceae* rhizome (65.43±2.94 mg g^{-1} of dry weight). *Curcuma amada* rhizome (65.43±1.32 mg g^{-1} of dry weight) and *Kaempfera parviflora* rhizome (54.00±0.30 mg g^{-1} of dry weight). The lowest protein concentration was recorded in *Alpina officinarum* leaves (14.86±0.86 mg g^{-1} of dry weight) followed *by Alpinia galanga* rhizome (19.14±1.69 mg g^{-1} of dry weight), *Siphonochilus aethiopicus* rhizome (24.29±0.18 mg g^{-1} of dry weight) and *Zinziber striolatum* rhizome (49.43±0.51 mg g^{-1} of dry weight).

Table 2: Total Protein content among the Shrubs

Sl. No.	Plant sample	Family	Plant part	Protein concentration (mg g^{-1} of dry weight)
1.	*Kaempfera parviflora*	Zingiberaceae	Rhizome	54.00±0.30
2.	*Curcuma amada*	Zingiberaceae	Rhizome	65.43±1.32
3.	*Brachycorythis obcordata*	Orchidaceae	Leaves	99.14±0.95
4.	*Alpina officinarum*	Zingiberaceae	Leaves	14.86±0.86
5.	*Siphonochilus aethiopicus*	Zingiberaceae	Rhizome	24.29±0.18
6.	*Alpinia galanga*	Zingiberaceae	Rhizome	19.14±1.69
7.	*Maranta arundinaceae* L.	Marantaceae	Rhizome	65.43±2.94
8.	*Zinzibe rstriolatum*	Zingiberaceae	Rhizome	49.43±0.51
9.	*Curcuma caesia*	Zingiberaceae	Rhizome	108.57±0.48

The data represent are mean of 3 replications

Table 3: Total Protein content among the herbs

Sl. No.	Plant sample	Family	Plant part	Protein concentration (mg g^{-1} of dry weight)
1.	*Clerodentrum serratum*	Verbenaceae	Flower	52.00±1.06
			Stem	78.57±7.70
			Root	42.29±0.49
			Leaves	83.43±2.45
2.	*Accacia pennata*	Fabaceae	Leaves	62.57±1.39
			Stem	46.86±1.04
3.	*Smallanthuss onchifolius*	Asteraceae	Tuber	28.00±0.83
4.	*Zanthoxylum oxyphyllum*	Rutaceae	Leaves	181.43±2.24

*The data represent are mean of 3 replications

3.4. Total protein content among the creepers

The total protein content among the Creepers is shown in Table 4. The highest total protein content was found in *Paedaria foeteda* gall (45.14±0.39 mg g^{-1} of dry weight) followed by *P. foeteda* stem (40.86±0.60 mg g^{-1} of dry weight). The lowest protein content was recorded in *Hodgsonia heteroclita* fruit (20.29±1.19 mg g^{-1} of dry weight) followed by *P. foeteda* leaves (34.86±0.10 mg g^{-1} of dry weight).

The current findings have brought attention to a variety of plant-based protein sources in wild edible plants, highlighting their potential benefits for nutrition and sustainability. This information can assist both researchers and vegetarians in selecting diverse plant-based protein options.

Table 4: Total protein content among the creepers

Sl. No.	Plant sample	Family	Plant part	Protein concentration (mg g^{-1} of dry weight)
1.	*Paedaria foeteda*	Rubiaceae	Leaves	34.86±0.10
			Gall	45.14±0.39
2.	*Hodgsonia heteroclita*		Stem	40.86±0.60
			Fruit	20.29±1.19

*The data represent are mean of 3 replications

Today, plant protein is widely used as an alternative source of protein in everyday life. The contribution of plant proteins to overall dietary protein availability and intake varies significantly among different populations, even within advanced regions of the world (Fasuyi, 2006). In recent years, plant proteins have received increasing interest in the food industry, due to their various health benefits and essential functional properties. Plant proteins have been used with other edible polymers in forming films, gels, emulsions, and foams to improve their characters. Plant proteins have also been used to produce food products (e.g., meat, flour, and extruded food products) with enhanced nutritional value and quality. Interactions among different components in these food systems usually result in various structures, which have a significant influence on the properties of final food products. (Duanquan and Song, 2021)

Plant proteins are generally preferred over animal proteins, as the latter may lead to adverse health effects in humans. Additionally, proteins derived from plant sources are usually less expensive, making them a more cost-effective option. However, each plant source often lacks one or more essential amino acids, so it is recommended to incorporate a variety of plant-based foods into the diet (Mistry et al., 2022). Moreover, an increased consumption of animal proteins is linked to the depletion of natural resources, harm to biodiversity, the climate crisis, and freshwater scarcity. (Almeida et al., 2020). The main plant-based proteins derived from leaves are crucial for vegetarians and individuals with limited resources, as they are both easily accessible and affordable, with minimal side effects (Ghosh et al., 2020).

The growing global population presents a significant challenge for food and nutritional security. This increase, combined with changing demographics, will strain resources needed to provide nutritious food. Rising demand for protein, driven by urbanization, increased incomes, and aging populations, highlights protein's role in healthy aging and diets. Economic growth has altered dietary patterns, particularly in middle- and low-income countries, while health concerns about meat consumption have led organizations like the WCRF and WHO to recommend plant-based diets. However, producing high-quality animal protein is resource-intensive, requiring 6 kg of plant protein kg^{-1} of animal protein, which strains land, water, and contributes to greenhouse gas emissions. Encouraging more plant protein consumption can help meet dietary needs and mitigate environmental impacts. (Lehikoinen and Salonen, 2019; Hertzler et al., 2020)

4. CONCLUSION

This study highlighted the significant variation in protein content among different wild edible plant species, emphasizing their potential as valuable plant-based protein sources. The highest protein concentrations were found in *Wendlandia grandis* flower (trees), *Zanthoxylum oxyphyllum* leaves (shrubs), *Curcuma caesia* rhizome (herbs), and *Paederia foetida* galls (creepers).

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