

Estimation of Protein content in some selected plants using the Lowry's method

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Abstract

This study aims to conduct a comprehensive phytochemical analysis of protein content and its concentrations derived from selected plant species through the utilization of acetone extraction, adhering strictly to the Lowry's method. The acetone extract from various parts of the selected plants on the college campus was meticulously prepared through a systematic process involving thorough cleaning, precise chopping, careful drying, and ultimately grinding into a fine powdered form. The presence of protein content was diligently assessed, and the concentration of unknown proteins in the samples was accurately determined, employing a standard curve derived from BSA as a benchmark, utilizing the Lowry's method. A comprehensive selection of 12 plants, representing 4 distinct families, was meticulously gathered, revealing that the protein concentration was maximally recorded in *Albizia lebbek* (L.) Benth. at 0.85 mg/ml, while the minimum was observed in *Cucurbita maxima* Duchesne at 0.11 mg/ml. Furthermore, the other selected plants exhibited notable levels of protein content. The selected plants possess the potential to serve as a significant source of protein, with applications in various pharmaceutical industries and as components of vegan protein products.

Key words : Plants, Protein, Phytochemical, Lowry's Method, UV-Vis.

Numerous plants are engaged in the production of active components through secondary metabolites within their metabolic pathways^{1,3}. These components are critically important for human health and are directly utilized in the pharmaceutical industry³; furthermore, they serve as a source of essential nutrients, including provitamin A (B-carotene), vitamin C (ascorbate), and vitamin E (tocopherol). Additionally, they provide phytochemicals linked to health advantages, such as Glycosylates, carotenoids, tocopherols, and flavonoids⁶. Furthermore, secondary metabolites represent a chemically and taxonomically vast array of compounds whose functions remain largely unexplained. They are extensively applied in

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human therapy, veterinary practices, agriculture, scientific research, and innumerable other fields. Plant-derived products have constituted a fundamental aspect of phytomedicines throughout history. These compounds can be sourced from various plant parts, including barks, leaves, flowers, roots, fruits, and seeds. Acquiring knowledge about the chemical constituents of plants is imperative, as such information is invaluable for the synthesis of complex chemical substances.

Protein, alongside carbohydrates and fats, constitutes one of the essential macronutrients imperative for human growth and maintenance, and it is recognized as a significant contributor to energy production within the dietary framework^{2,16}. In recent years, consumer demand for plant-based proteins has surged dramatically. A survey involving 1000 randomly selected individuals revealed that grocery sales of plant-based meat alternatives experienced a remarkable increase of 12.1% from July 2020 to July 2021⁷. By the year 2050, it is projected that the global population will either grow or surpass 9 billion, subsequently driving a 70% escalation in the demand for food, feed, and fiber worldwide⁸. In order to address this mounting demand, it is imperative to explore new sources. Presently, plant-based proteins are classified as vegan food, offering an abundant supply of amino acids, being readily absorbed by the body, and playing a pivotal role in alleviating various health conditions. Furthermore, proteins originating from plant-based sources are abundant in fiber, polyunsaturated fatty acids, oligosaccharides, and carbohydrates. Various sources of plant-based protein, including cereals (such as wheat, rice, millet, maize, barley, and sorghum), legumes (including pea,

soybean, bean, faba bean, lupin, chickpea, and cowpea), pseudo cereals (such as buckwheat, quinoa, and amaranth), nuts, almonds, and seeds (flaxseed, chia, pumpkin, sesame, and sunflower) have been thoroughly investigated⁹. As indicated by the nomenclature, plant-based proteins are those proteins derived from plant food sources, including whole grains, legumes, and nuts.

Thus, this investigation is dedicated to ascertaining the concentration of protein content in designated plant parts sourced from M.N. College, employing the acetone extract in accordance with the Lowry method. The Lowry method integrates the biuret method with the reduction of the Folin–Ciocalteu phenol reagent. This reduction transpires through interactions with tyrosine and tryptophan residues present in the proteins post-hydrolysis, resulting in the reduced form of the reagent displaying a bluish color^{7,8}. Depending on the desired protein concentration and sensitivity, absorbance is meticulously measured at 750 nm or 500 nm (with absorbance at 750 nm being utilized for heightened sensitivity and lower protein concentration)⁸. Our current research rigorously applies the Lowry method to quantify the total protein content from the fresh leaves of 12 commonly accessible plant species.

The fresh plant materials, encompassing *Acalypha indica* L. (leaves, stem, flower), *Albizia lebbeck* (L.) Benth. (seed), *Crotalaria juncea* L. (seed), *Cucurbita maxima* Duchesne (seed), *Dactyloctenium aegyptium* (L.) Willd. (leaves, stem, flower), *Echinochloa crus-galli* (L.) P.Beauv. (leaves, stem, flower), *Eragrostis cilianensis* (All.) Vignolo ex Janch. (leaves,

stem, flower), *Hordeum vulgare* L. (seed), *Panicum miliaceum* L. (leaves, stem, flower), *Senna occidentalis* (L.) Link (stem, seed), *Sesbania bispinosa* (Jacq.) W.Wight (seed), and *Setaria pallide-fusca* (Schumach.) Stapf & C.E. Hubb. (leaves, stem, flower) were systematically collected from various locations within the M.N. College, Visnagar campus, which encompasses both rural and urban sectors of Visnagar. The M.N. College campus is strategically located in the northern region of Gujarat State, precisely positioned at geographical coordinates 23.7 N 72.55 E and at an elevation of approximately 117 meters above mean sea level.

Upon collection, the specimens were rigorously cleaned utilizing tap water, followed by an exhaustive rinse with double distilled water to eradicate any lingering particulate matter. One gram of finely minced fresh plant material was placed into a mortar and pestle, to which approximately 80 ml of Acetone and 20 ml of freshly prepared Distilled water were judiciously added, and the mixture was incessantly ground until a transparent plant solution was attained. Subsequently, the solutions were subjected to centrifugation at 1000 rpm for a duration of 15 minutes, after which the resultant supernatants were meticulously collected into designated tubes. This separately prepared sample of each plant part was amalgamated, culminating in the preparation of the final extract.

Preparation of standard curve :

BSA serves as the definitive standard reagent for constructing the standard curve utilized for estimating the unknown concentrations of proteins. An exact volume of 4.5 ml of reagent 1 (comprising 48 ml of 2% sodium

carbonate in 0.1N sodium hydroxide, 1 ml of 1% sodium potassium tartrate, and 1 ml of 0.5% copper sulphate) was meticulously added to the sample extracts and incubated for precisely 15 minutes. Subsequently, 0.5 ml of freshly prepared reagent 2 (a 1:1 mixture of Folin Ciocalteu and water) was thoroughly combined with each sample and subjected to a 30-minute dark incubation period. Following this incubation, the absorbance was accurately measured at 620 nm, and the protein content was quantified as mg BSAE per gram of fresh weight¹²⁻¹⁵.

All chemicals and reagents employed in accordance with Lowry's Method¹⁰ during the experiments and the determination of the unknown protein concentration of the samples were meticulously documented, and the standard curve was successfully generated¹².

The exhaustive investigations carried out on the twelve meticulously selected plant species, which include *Albizia lebbeck* (L.) Benth. (0.85mg/ml), *Sesbania bispinosa* (Willd) Pers. (0.68 mg/ml), *Acalypha indica* L. (0.51 mg/ml), *Eragrostis cilianensis* (All.) Vignolo ex Janch. (0.47 mg/ml), *Senna occidentalis* (L.) Link (0.38mg/ml), *Dactyloctenium aegyptium* (L.) Willd. (0.35 mg/ml), *Echinochloa crusgalli* (L.) P. Beauv (0.33 mg/ml), *Setaria pallide fusca* (Schumach.) Stapf & C.E. Hubb. (0.32mg/ml), *Panicum antidotale* Retz. (0.34 mg/ml), *Hordeum vulgare* L. (0.15 mg/ml), *Cucurbita maxima* Duchesne (0.11 mg/ml), and *Crotalaria juncea* L. (0.45 mg/ml) respectively, have yielded critical insights. The highest concentration of protein was definitively established in *Albizia lebbeck* L. (42.8 mg/ml), while the lowest protein content was conclusively identified in *Cucurbita maxima* Duchesne (0.11mg/ml).

Table-1. List of plants along with their respective O.D. and concentration.

Sr. no.	Plant Name	O.D. at 765 nm	Concentration (mg/ml)
1	<i>Acalypha indica</i> L.	2.49	14.5
2	<i>Albizia lebbek</i> (L.) Benth.	1.01	5.32
3	<i>Crotolaria juncea</i> L.	1.12	6.02
4	<i>Cucurbita maxima</i> Duchesne	0.67	3.15
5	<i>Dactyloctenium aegyptium</i> (L.) Willd	1.43	7.94
6	<i>Echinochloa crus-galli</i> (L.) P.Beauv.	1.92	10.9
7	<i>Eragrostis cilianensis</i> (All.) Vignolo ex Janch.	1.97	11.3
8	<i>Hordeum vulgare</i> L.	1.04	5.50
9	<i>Panicum antidotale</i> Retz.	1.42	7.89
10	<i>Senna occidentalis</i> (L.) Link	1.28	6.97
11	<i>Sesbania bispinosa</i> (Jacq.) W.Wight.	2.85	16.8
12	<i>Setaria pallidifusca</i> (Schumach.) Stapf & C.E. Hubb.	1.39	7.69

The predominant technique employed for accurately assessing the total protein content of any biological sample, regardless of whether it is already in solution or readily soluble in diluted alkali, is the Lowry's method. This method possesses the capability to detect even extremely low protein concentrations with precision. The fundamental principle underlying the Lowry's method for protein concentration analysis is predicated on the reaction that transpires in an alkaline milieu between the peptide nitrogen and the copper [II] ions. This is subsequently followed by a reduction reaction that occurs between the phosphotungstic acid and Folin-Ciocalteu phosphomolybdic acid, culminating in the formation of heteropoly molybdenum blue. Given the sensitivity of the Lowry technique to pH fluctuations, it is imperative to maintain the test solution's pH within the critical range of 10 to 10.5^{10,12,14,15}.

The recent research unequivocally highlights the critical significance of plant-

based proteins in human nutrition. Plant protein has now been established as a vital alternative source of protein in everyday diets. The contribution of plant proteins to the total dietary protein intake exhibits considerable variability across different populations, even within the most affluent nations. For vegetarians and those facing economic constraints, the primary protein sourced from plant leaves is indispensable, as it is readily available, cost-effective, and virtually devoid of adverse effects⁵. It can be asserted that combinations of plant proteins provide a complete, essential, and nutritionally rich source of amino acids that effectively meets the physiological requirements of humans¹¹.

A total of 12 plant species were meticulously selected from the precincts of M.N. College, and the analysis conclusively indicated that the highest concentration of protein was recorded in *Albizia lebbek* (L.) Benth. (0.85 mg/ml) and the lowest in

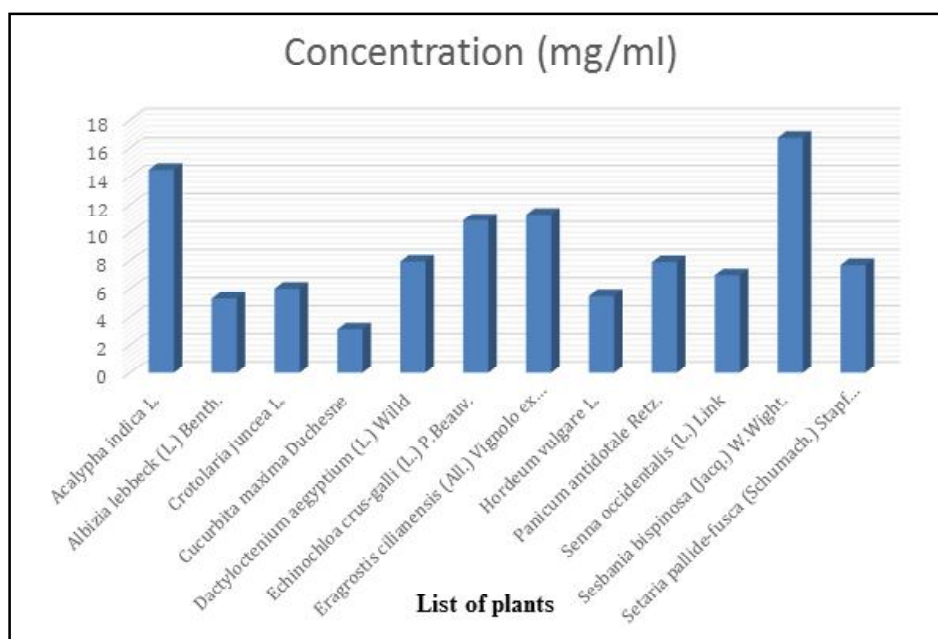


Fig. 1. Total Protein Concentration (mg/ml) among the Plants.

Cucurbita maxima Duchesne (0.11mg/ml). The comprehensive investigations conducted on these 12 selected plant species, encompassing *Albizia lebbbeck* (L.) Benth. (0.85mg/ml), *Sesbania bispinosa* (Willd) Pers. (0.68 mg/ml), *Acalypha indica* L. (0.51 mg/ml), *Eragrostis ciliaris* (All.) Vignolo ex Janch. (0.47 mg/ml), *Senna occidentalis* (L.) Link (0.38mg/ml), *Dactyloctenium aegyptium* (L.) Willd. (0.35 mg/ml), *Echinochloa crusgalli* (L.) P. Beauv (0.33 mg/ml), *Setaria pallidifusca* (Schumach.) Stapf & C.E. Hubb. (0.32mg/ml), *Panicum antidotale* Retz. (0.34 mg/ml), *Hordeum vulgare* L. (0.15 mg/ml), *Cucurbita maxima* Duchesne (0.11 mg/ml) and *Crotalaria juncea* L. (0.45 mg/ml) have incontrovertibly demonstrated the presence of significant protein content. Analysis of selected plants extract for the phytochemicals and active ingredients could provide leads in

pharmaceuticals. The high protein of the selected plants material coupled with a fairly high concentration of the amino acids make it suitable for fortification of foods. In these investigation plants possess a reasonable number of proteins, and these leaves, seed, stem, flower, can be uses as a protein-enriched food material for nutritional purposes.

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Conflict of interest

The authors declare that they have no

known conflict of interest.

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