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Quantification of total protein content from some traditionally used edible plant leaves: A comparative study

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Abstract
Protein is an important primary metabolite of our living systems. There are several studies based upon analysing the different, individual protein sources from various plant species are still on searching mode. On parallel with this context, traditionally edible plants has been proven to be an enriched source of protein based nutrition and largely utilized by most of the ethnic communities for their survival. The primary objective of the current research investigation is to estimate the total protein content from 33 traditionally used edible plant leaves categorized in trees, shrubs and herbs and to draw a comparative conclusion that which plant leaf contains better protein concentration. The protein content was estimated by UV-Vis spectrophotometric technique using the conventional Lowry's method. Bovine Serum Albumin (BSA) was used as standard reagent against which unknown protein concentration of plants had been estimated. All the proteins were extracted by using phosphate buffer (pH 7.4). From the results, it has been found that under tree category, *Psidium guajava* shows the highest (98.51 mg BSA Equivalent/ g of Fresh Weight) and *Dillenia indica* shows the lowest (13.73 mg BSAE/ g of FW) amount of protein content. In case of shrubs, *Justicia adhatoda* showed the maximum (86.37 mg BSAE/ g of FW) and *Ocimum canum* shows the minimum (10.59 mg BSAE/ g of FW) amount of protein content. Among the herbs, red *Amaranthus viridis* contains highest (97.43 BSAE/ g of FW) and *Marsilea quadrifolia* contains the lowest (15.04 mg BSAE/ g of FW) content of protein. The study results showed that *Psidium guajava* contains highest amount of protein among all the 33 plants. The study findings conclude that the protein content obtained from the leaves of different plant categories varies in their quantity and further supports the fact that plant leaves can be used as a potential source of nutrient consumption in near future.

Keywords: Traditional edible leaves, plant protein, UV-Vis, Lowry's method

Introduction
Protein is one of the essential macronutrient, playing a vital role in the growth and maintenance of the human body and supposed to be one of the energy giving component in the diet along with carbohydrates and lipids [1, 2]. In addition, proteins also have a wide range of substitute actions such as enzymatic activity and nutrients transportation and other biochemical activities across the cell [3]. To maintain these necessary functions, it is important to provide the human body with sufficient amount of proteins through diet. There are two basic source of protein which comes from both the plant and animal [4, 5]. Despite of the challenges and limitations posed by plant proteins including low solubility, coarse texture and additional plant like flavours plant-based nutrition has received much attention in the past decade. There is a huge importance for generating high quality products through plant proteins [6]. Wild traditionally utilized edible plants have been consumed since pre-historic times as food, subsidiary food source, folk medicine etc. However, the century old traditional knowledge of using such edible plants is depleting very quickly [7, 8]. Modern scientific researchers are also trying to value these traditional food items to fill the gaps between growing population and food production [9].
Here in our study, 33 edible plant leaves belonging to various families have been selected, which we take as our food on regular basis from ancient time in different parts of the world. The plant species are divided into three main types: tree, shrub and herb.

This plant leaves are low cost and easily available in West Bengal, India. From ancient times these plant leaves are consumed in various parts of West Bengal and to our knowledge no such adverse side effects has been noticed from these leaves as well. In addition of proteins, most of these selected plants contain huge amount of plant based bioactive compounds such as alkaloids, flavonoids, terpenoids and polyphenols [10]. Presence of these secondary biomolecules along with primary biomolecules such as carbohydrates, proteins and lipids enhances their nutrient efficiency and disease preventive measures. This kind of research may help researchers to further work and evaluate more healthy food choices and to inform the development of nutritionally balanced products that promote healthy ageing [11].

In our present course of work, we aim at the extraction and estimation of total protein content from the fresh leaves of 33 traditionally edible easily available plants by using Lowry's method and to observe the highest plant protein containing plant leaves for future nutritional uses. The overall workflow is being represented in Figure 1.

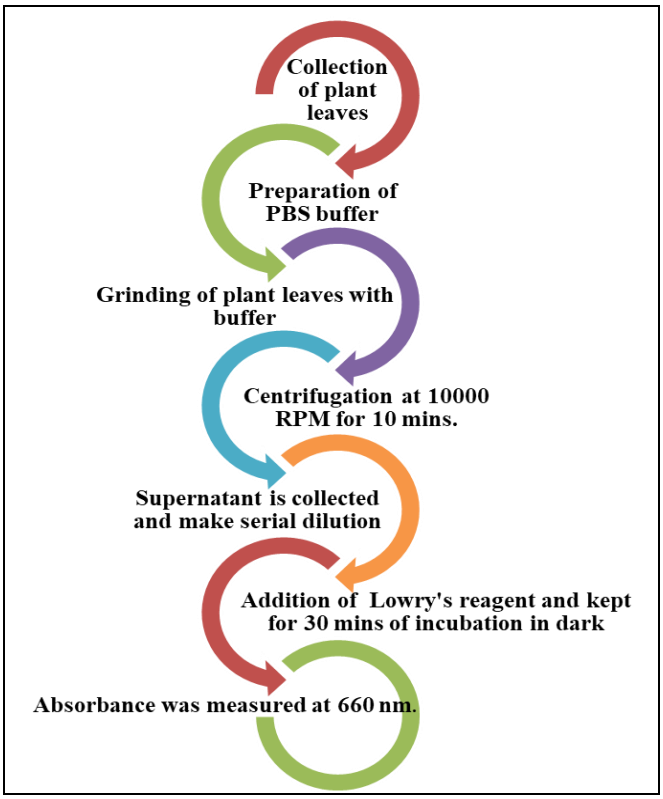

Fig 1: Operative Work Flow

Materials and Methods

Chemicals and Reagents

All the chemicals and reagents used in the experiments were of analytical grade (AR). Potassium dihydrogen phosphate and sodium di hydrogen phosphate were purchased from Sigma- Aldrich Company, Mumbai. Sodium carbonate, potassium chloride was obtained from RFCL Limited, New Delhi. Sodium hydroxide, Sodium potassium tartarate, copper sulphate and sodium chloride were supplied by SD Fine-Chem Limited, Mumbai. Folin-ciocalteu was obtained from Merck Life Science Private Limited, Mumbai.

Preparation of 1X phosphate buffer saline (pH 7.4)

For preparing 100 ml of 1X PBS (pH 7.4), 0.8 g of sodium chloride, 0.02 g of potassium chloride, 0.144 g of sodium dihydrogen phosphate and 0.0245 g of potassium dihydrogen phosphate were mixed with 80 ml of double distilled water. Later volume was made up by adding 20 ml of double distilled water and pH was adjusted by using digital pH meter.

Sample collection and preparation

The fresh leaves of the 33 plants were collected from various regions of Kolkata during the month of August to September, 2019. After collecting the leaves were cleaned by using tap water followed by double distilled water to remove all the dust. 1 g of finely chopped fresh leaves was taken in a mortar pestle and to it approximately 20 ml of freshly prepared phosphate buffer saline of pH 7.4 was added and keep it pasting until a clear plant solution was observed (Figure 2A and 2B). Then the solutions were centrifuged at 10000 rpm for 10 min and the final supernatants were collected in respective tubes (Figure 2C).

Fig 2A & 2B: Grinding of plant leaves with PBS; 2C: Final supernatant collection after centrifugations

Quantification of Total Protein Content

BSA is used as standard reagent for preparing the standard curve (Figure 3) against which the unknown concentration of proteins was estimated. 4.5 ml of reagent 1 (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of 1% sodium potassium tartrate + 1ml of 0.5% copper sulphate) was added to the sample extracts and incubated for 15 min. After this, 0.5 ml of freshly prepared reagent 2 (1 part Folin-Ciocalteau: 1 part water) was mixed with the each sample and left for 30 min of dark incubation. After that the absorbance was measured at 660 nm and the amount of protein is expressed as mg BSAE/ g of fresh weight [12-15].

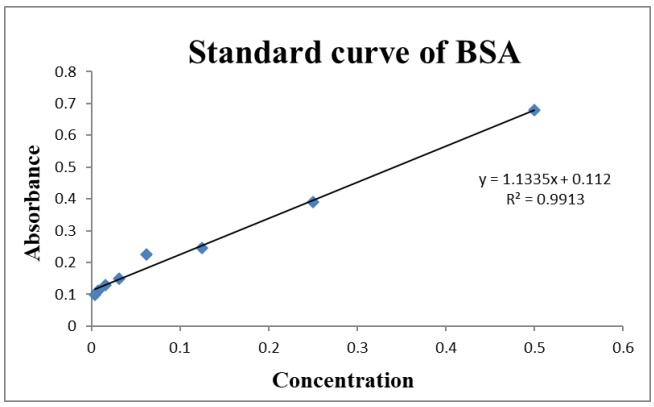

Fig 3: Standard Curve of BSA

Statistical Analysis

All the experimental measurements were performed in triplicates and expressed as the average ± standard deviations. The magnitude of the means, standard curve, standard errors, and standard deviations were calculated by using MS Excel 2010 Software.

Results and Discussions

Among all the 33 plant samples divided in to three categories, it was clearly noticed that in case of trees (Figure 4 and Table

1), leaves of *Psidium guajava* contained the highest (98.51 mg BSAE/g of FW) and *Dillenia indica* showed the lowest (13.73 mg BSAE/g of FW) amount of protein content. Whereas, in case of shrubs (Figure 5 and Table 2), the highest and lowest protein contents were observed in the leaves of *Justicia adhatoda* (86.37 mg BSAE/g of FW) and *Ocimum canum* (10.59 mg BSAE/g of FW), respectively. Similarly, Red *Amaranthus viridis* (97.43 mg BSAE/g of FW) and *Marsilea quadrifolia* (15.04 mg BSAE/g of FW) contained the highest and lowest amount of protein content, respectively among all the herbs (Figure 6 and Table 3).

Estimation of total protein content by Lowry's method is the most widely accepted procedure when it comes to determine the amount of protein present in any biological sample (either already in solution or easily soluble in dilute alkali). This method is sensitive to even low concentrations of protein. The main perception behind the Lowry method of determining protein concentrations is based on the reaction between the peptide nitrogen and the copper [II] ions under alkaline environment, followed by reduction reaction of the Folin-Ciocalteau phosphomolybdic and phosphotungstic acid that turns into heteropolymolybdenum blue. The Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained at 10 - 10.5 [12-15]. The sensitivity and applicability of the Lowry method was also observed in present study which supports the previous experimental studies, too.

The current research study highlighted the value of plant proteins in relation to human nutrition. Now a day's plant protein is used as an alternative protein source for daily life.There is a large variation in the contribution made by plant proteins to the availability and intake of total dietary protein among populations both within the advanced regions of the world [16]. The primary protein which human beings get from leaves is necessary for vegetarians and poor people, because it is easily available and affordable or low cost with almost no side effects [17-19]. It can be stated that the combinations of plant proteins can provide a complete, necessary and balanced source of amino acids which effectively fulfills human physiological requirements [20-22].

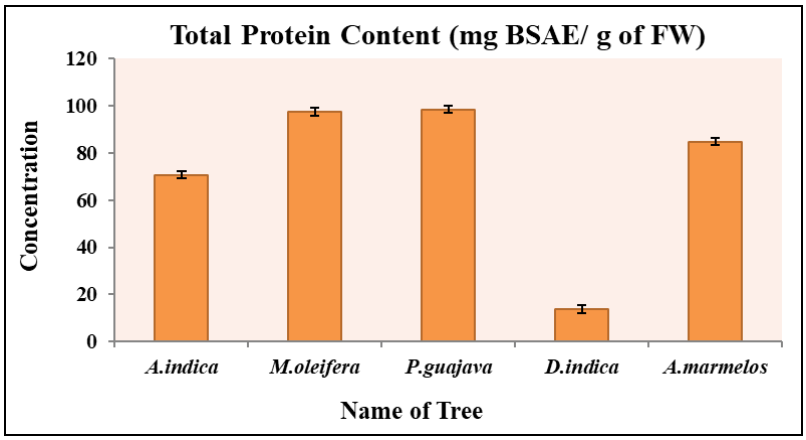

Fig 4: Total protein content (mg BSAE/g of FW) among the trees

Table 1: Total protein content (mg BSAE/g of FW) among the trees

Sl No	Type	Name of the Plant	Family	Concentration (mg BSAE/g of FW)
1	Tree	*Azadirachta indica*	Meliaceae	70.72±1.39
2		*Moringa oleifera*	Moringaceae	97.53±1.69
3		*Psidium guajava*	Myrtaceae	98.51±1.55
4		*Dillenia indica*	Dilleniaceae	13.73±1.78
5		*Aegle marmelos*	Rutaceae	84.73±1.55

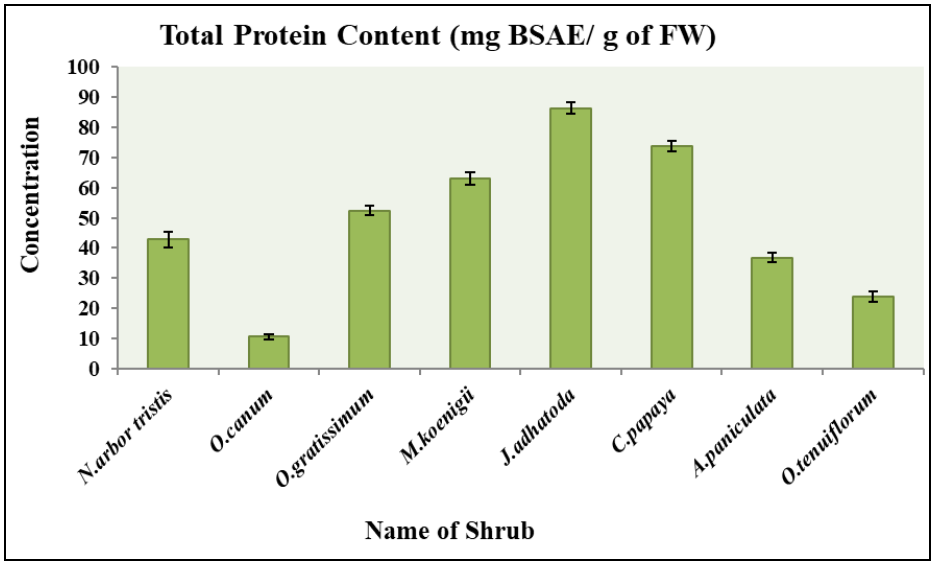

Fig 5: Total protein content (mg BSAE/g of FW) among the shrubs

Table 2: Total protein (mg BSAE/g of FW) among the shrubs

Sl No	Type	Name of the Plant	Family	Concentration (mg BSAE/g of FW)
1	Shrub	*Murraya koenigii*	Rutaceae	62.94±2.06
2		*Ocimum gratissimum*	Lamiaceae	52.40±1.64
3		*Ocimum canum*	Lamiaceae	10.59±0.77
4		*Ocimum tenuiflorum*	Lamiaceae	23.95±1.62
5		*Nyctanthes arbor-tristis*	Oleaceae	42.79±2.73
6		*Andrographis paniculata*	Acanthaceae	36.84±1.69
7		*Justicia adhatoda*	Acanthaceae	86.37±2.06
8		*Carica papaya*	Caricaceae	73.72±1.76

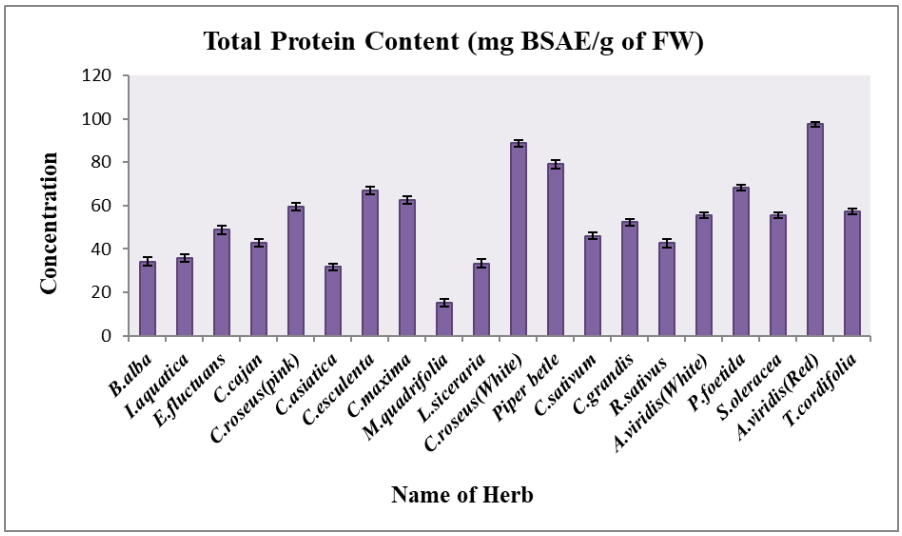

Fig 6: Total protein content (mg BSAE/g of FW) among the herbs

Table 3: Total protein content (mg BSAE/g of FW) among the herbs

Sl No	Types	Name of the Plant	Family	Concentration (mg BSAE/g of FW)
1	Herb	*Basella alba*	Basellaceae	34.26±2.11
2		*Ipomoea aquatica*	Convolvulaceae	35.81±1.60
3		*Enhydra fluctuans*	Asteraceae	48.79±1.84
4		*Centella asiatica*	Apiaceae	31.68±1.50
5		*Coriandrum sativum*	Apiaceae	46.07±1.42
6		*Catharanthus roseus* (White)	Apocynaceae	88.72±1.48
7		*Catharanthus roseus* (Pink)	Apocynaceae	59.33±1.69
8		*Marsilea quadrifolia*	Marsileaceae	15.04±1.71
9		*Coccinia grandis*	Cucurbitaceae	52.26±1.38
10		*Langenaria siceraria*	Cucurbitaceae	33.28±2.01
11		*Cucurbita maxima*	Cucurbitaceae	62.47±1.77
12		*Cajanus cajan*	Fabaceae	42.84±1.76
13		*Colocasia esculenta*	Araceae	66.92±1.62
14		*Raphanus sativus*	Brassicaceae	42.74±1.90
15		*Paederia foetida*	Rubiaceae	68.19±1.34
16		*Amaranthus viridis* (White)	Amaranthaceae	55.54±1.13
17		*Amaranthus viridis* (Red)	Amaranthaceae	97.43±1.13
18		*Spinacia oleracea*	Amaranthaceae	55.49±1.21
19		*Piper betle*	Piperaceae	79.06±1.97
20		*Tinospora cordifolia*	Menispermaceae	57.36±1.34

Conclusion

Based on the present comparative study results of protein estimation of leaves from different types of plant it can be concluded that the current experimental plants contain a reasonable amount of proteins and these leaves can be a used as a protein enriched food materials for nutritional purposes. The study results also observed that the *Psidium guajava* leaves contains highest amount of protein among all the 33 plants. So this plant leaves can be the used as a source of low cost and easily available natural protein for future and we can incorporate this plant leaves in our daily diet to get maximum amount of protein alternative to high amount of animal protein sources.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research article.

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