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20. Quantitative Biochemical Analysis of Protein and Carbohydrate of *Litsea salicifolia, Centella asiatica, Ocimum sanctum* and *Azadirachta indica*

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Abstract:

Plant stored nutrients in the form of carbohydrate, also referred to as starch which is source of energy and various types of proteins which are important factors for cellular metabolism. There are several studies based upon estimating the different, individual source of protein from various plants species for medicinal purposes are still looking for. The primary objective on the current study was to estimate the plants protein and carbohydrate in Litsea salicifolia, Azadirachta indica, Ocimum sanctum and Centella asiatica. From the present study, it has been found that Centella asiatica shows the highest (39.768%) and Ocimum sanctum shows the lowest (13.21%) amount of protein content. Similarly, on analyzing all the samples for carbohydrate content it was also found that the sample of Centella asiatica shows the highest (1.5725 mg/ml) and Ocimum sanctum shows the lowest (0.292 mg/ml) amount of carbohydrate. The study results showed that Centella asiatica contains highest and Ocimum sanctum contains lowest amount of both protein and carbohydrate among all the 4 selected plants.

Keywords:

Litsea salicifolia, Azadirachta indica, Ocimum sanctum, Centella asiatica, Nutrition.

20.1 Introduction:

Medicinal plants are referred to be a miracle herb as its remedial healing powers are unique to nature. All over the world greenery filled with plants belongs to various families with its species specificity. The Sanskrit term for Ayurveda translates to "the scripture for longevity". It is an example of an ancient system of traditional medicine that is still in use in India and many other South Asian nations (Murthy et al., 2010). Medicinal plants play an important role in livelihood of human being and are considered as a rich source in bioactive chemicals that produce define physiological action on human body. Uses of traditional remedies by people may not understand the scientific reason behind their medicines, but from their personal experience they know that some medicinal plants can be highly effective if they used at therapeutic doses (Fakim, 2006). Nitrogen is the major element found in living things next to carbon, hydrogen and oxygen. Nitrogen occurs in amino acids, purine and pyrimidine bases, vitamins, amino sugars, alkaloids, compound lipids etc. (AOAC official method 2002). Proteins are one of the most important components of the plant (Patel et al., 2022). However, major nitrogen source is proteins. Nitrogen constitutes 16% of the total makeup in most proteins.

Hence, the total nitrogen content of a sample can be multiplied by factor 6.25 to arrive at the value of the crude protein. To achieve true protein content, the non-protein nitrogen was deducted from the total nitrogen and then multiplied with the factor (AOAC official method 2002). Carbohydrates are important for giving strength to plants and are present in the form of cellulose and are mainly used by plants for food production (Patel *et al*., 2022). Carbohydrates make up a large portion of plant biomass, e.g. cellulose is part of the cellular framework and starch as food reserves (Fakim, 2006). The presence of carbohydrate is not noticed when the plant is taken into account for medicine but carbohydrates are widely prevalent in the plant kingdom, comprising the mono-, di-, oligo-, and polysaccharides (Dash et al., 2017).

There are several studies based upon estimating the different, individual source of carbohydrates for energy and protein from various plants species for medicinal purposes are still looking for. The primary objective on the current study was to estimate the plants protein and carbohydrate in the four selected plants *Litsea salicifolia*, *Azadirachta indica*, *Ocimum sanctum* and *Centella asiatica*. These plants are consumed by local people as medicinal nutritional plants. People believe that these plants have high nutritional content though these are consumed in fewer quantities. So, the present work was conducted to estimate the actual nutritional contents of the plants.

20.2 Materials and Methods:

20.2.1 Collection and Preparation of Plant Samples:

Leaves of four different medicinal plants, *Litsea salicifolia*, *Centella asiatica*, *Ocimum sanctum* and *Azadirachta indica* were collected from Ambari village, Goalpara district, Assam. The samples were nicely washed with tap water, sun dried and ground into powder using mechanical grinder and again sieved to get a fine powdery form.

20.2.2 Protein Analysis (Kjeldahl method, 1883):

0.2g of powdered sample was digested in a Kjeldahl digestion flask by boiling with 10ml of concentrated H_2SO_4 and a catalyst until the mixture was clear. The digest was filtered by adding 40ml of distilled water and connected for distillation. Ammonia was steam distilled from the digest to which 40% of sodium hydroxide solution was added. The distillate was collected in a beaker containing 5 drops of bromocresol green and 5 drops of methyl red indicator. The ammonia that distilled into the receiving beaker reacted with the acid and the excess acid in the flask was estimated by titration against 0.1N H_2SO_4 with colour change from green to pink. Determinations were made alone for all the reagents (AOAC official method, 2002).

$$N\% = \frac{(ml\ of\ H2SO4\ for\ sample - ml\ of\ H2SO4\ for\ blank) \times normality\ of\ H2SO4 \times 14.01}{weight\ of\ sample\ (g) \times 10}$$

Crude protein (%) = total N% × Conversion factor (6.25)

Where,

Atomic weight of nitrogen = 14.01

Factor to convert mg/g to percent = 10

Factor to convert N to protein = 6.25

20.2.3 Carbohydrate Analysis (Anthrone method, 1952):

0.1g of sample was weighed out and collected in test tubes with addition of 5ml HCl. The solution was kept on water bath for 10min at 70°c and cooled at room temperature. After cooling down, solution was neutralised with solid sodium carbonate until the bubble in liquid ceases. As the effervescence ceases, the volume was made up to 100 ml with distilled water in beaker and half was taken for centrifuge at 5000 rpm for 20 minutes. The supernatant was then collected for analysis (Hedge et al., 1962). For the standard glucose solution, 0.2, 0.4, 0.6, 0.8 and 1ml of the standard solution were added in tubes with the help of micropipette and made the volume up to 1ml with distilled water in the required tubes. The test tubes marked with blank was prepared by taking 1ml of distilled water. In four test tubes, 0.5ml of sample was taken and the total volume was increased to 1ml by adding 0.5ml distilled water. To all the test tubes, 4ml of Anthrone reagent were added and incubated in hot boiling water for 8 minutes at 65°C. Then after cooling the test tubes, the absorbance of the green coloured complex was measured in UV-Spectrophotometer by taking the optical density at 630nm (Patel et al., 2022).

20.3 Result and Discussion:

Among all the 4 plant samples *Centella asiatica* contained the highest (39.768%) and *Ocimum sanctum* showed the lowest (13.21%) amount of protein content (Figure 20.1). Whereas, in case of carbohydrate content the highest was also observed in *Centella asiatica* (1.5725mg/ml) and *Ocimum sanctum* showed the lowest (0.292mg/ml) (Table 20.1). The present study highlighted the value of plant proteins in relation to human nutrition. Plant protein is used as an alternative source of protein for daily life. Carbohydrates are said to be important for giving strength as they are present in the form of cellulose which are mainly used by plant for food production. It can be stated that carbohydrate and plant protein can provide a necessary and balanced source of amino acids which would fulfills some of the physiological requirements in human.

Table 20.1: Total carbohydrate content of selected medicinal plants.

Sr No.	Name of the sample	Family	Protein (%)	Carbohydrate (mg/ml)
1	*Litsea salicifolia*	Lauraceae	17.58%	0.431mg/ml
2	*Ocimum sanctum*	Lamiaceae	13.21%	0.292mg/ml
3	*Centella asiatica*	Apiaceae	39.77%	1.5725mg/ml
4	*Azadirachta indica*	Meliaceae	13.43%	0.6331mg/ml

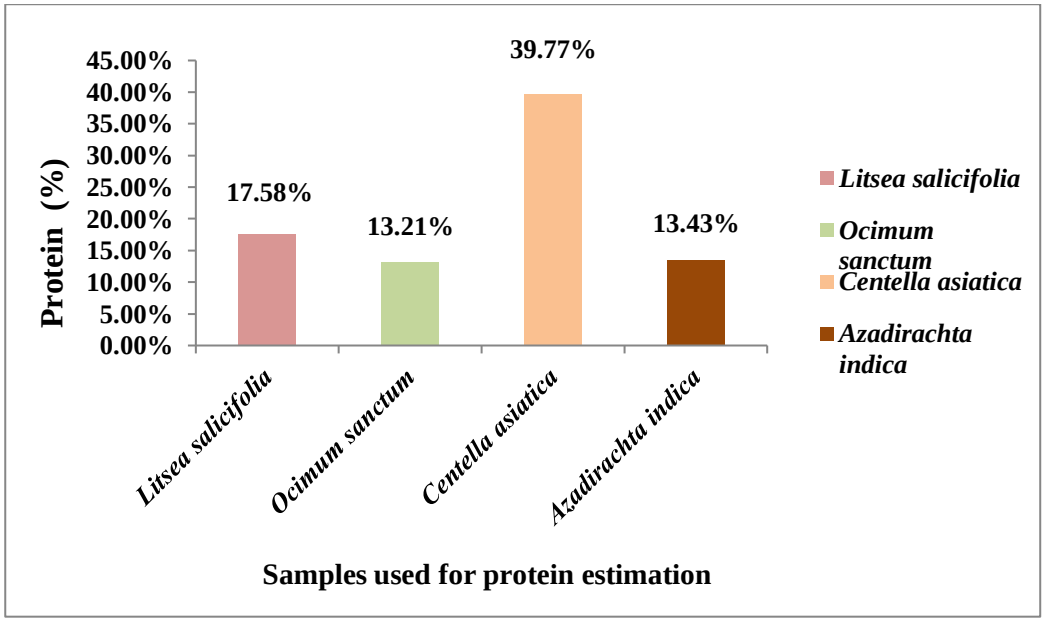

Figure 20.1: Total protein content of selected medicinal plants.

20.4 Conclusion:

Based on the present results of protein and carbohydrate estimation of leaves from different types of plant it can be concluded that all the selected plants contain a reasonable number of proteins and carbohydrate. The study results observed that the *Centella asiatica* leaves contains highest amount of both protein and carbohydrate among all the 4 plants. So, this plant leaves can be used as a low cost and easily available natural protein for future.

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