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# Extraction And Estimation of Proteins of Selected Medicinal Plants Using Bradford's Method

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*Abstract:*

Protein quantification is necessary to understand the total protein content in a sample or in a formulated product. Accurate protein quantification is important as a range of other critical assays require precise total protein content results in order to generate data. The estimation of protein is done for four different plants *Carica papaya, Ananas cosmosus, Wrightia tinctori, Milletia pinnata.* Determination of protein concentration is necessary and widely used in protein biology, molecular biology, and other research applications. The concentration of protein samples have to be estimated before proceeding to isolation, purification, and analysis. The Bradford protein assay which was developed by Marion. M. it is a quick and accurate spectroscopic analytical procedure used to measure the concentration of protein in a solution. The reaction is dependent on the amino acid composition of the measured proteins. The protein content of pineapple leaves is found to be the highest whereas the lowest protein content is found in pongamia.

***Index Terms*** **- Protein, Bradford assay, Carica *papaya, Ananas cosmosus Wrightia tinctoria, Milletia Pinnata.***

## I. INTRODUCTION

Carica *papaya:*

Papaya is a fast growing, short-lived, tropical tree cultivated for its fruit, papain, pectin, and antibacterial properties (Niklas and Marler, 2007). Nowadays papaya is grown widely in tropical and subtropical lowland regions around the world (Evans and Ballen, 2012). Papaya is the third most cultivated tropical crop worldwide, Brazil and India are the largest producers of papaya although Mexico is the main exporter (Evans and Ballen, 2012)

*Ananas cosmosus:*

Pineapple is grown in tropical climates where the best fruit production is achieved. Pineapple bran and by-products of pineapple processing have been used in livestock feed. Bromelain extracted from pineapple may be used as a meat tenderizer. The stems and leaves are source of fiber and can be processed into paper or 'pina cloth'. A mixture of several proteases, bromelain is used in chill- proofing beer, manufacturing precooked cereals, in certain cosmetics, and in preparations to treat edema and inflammation (James A. Duke 1983). Bromelain is nematicidal (Duke, 1984b).

*Wrightia tinctoria:*

Sweet Indrajao is called dhudi (hindi) because of its preservative nature. Ethnomedically, the bark of this plant is used as a galactogogue to treat abdominal pain, skin diseases and wounds, as an anti-pyretic,anti-dysenteric, anti-diarrheal and anti-hemorrhagic agents, and as an antidote for snake poison. Seeds of the plant are also used as an aphrodisiac (Rajani Srivastava, 2014). It is effective in severe viral diseases like HIV (AIDS). In the Siddha system of medicine it is used for the treatment of psoriasis skin diseases (Ashish Dixit, A K Jain.,at al).

*Pongamia Pinnata:*

The tree is planted for shade and it is grown for ornamental puposes. It acts as Antioxidant, it has Antihyperammonemic activity, Anti-diarrhoel Activity, Anti-ulcer activity, Anti-hyperglycaemic and Anti-lipidperoxidative (V.V Chopade, A.N Tankar et al.,). Juice of roots is used for cleansing foul ulcers and closing fistulous sores. Its oil is a source of biodiesel (Savita Sangawan et al,. 2010). Juice of leaves is used for cold, cough, diarrhea, dyspepsia, flatulence, gonorrhea, leprosy ( Ambasta et al., 1992; Oommen, et al., 2000. and Bhattacharjee, 2001)

## II. MATERIALS REQUIRED

Bradford's reagent and BSA was purchased from Sigma Aldrich. 96 well plate was purchased from Tarson, India.

BSA standard stock 50mg/ml

Phenol buffer Sucrose

TCA-Acetone buffer TCA, Phosphate-TCA-Acetone buffer Phosphate buffer

## III. METHODOLOGY

The protein from the abovementioned medicinal plants were extracted by the following method.

Extraction Buffers

Protein extractions from different tissues were carried out using three extraction/ buffers:

• Phenol buffer Sucrose (0.7 M), Tris (0.5 M), HCI (30 mM), EDTA (50 mM), KCl (0.1 M).

• TCA-Acetone buffer TCA (10%, w/v) in acetone with 2-mercaptoethanol (0.07%).

• Phosphate-TCA-Acetone buffer Phosphate buffer (0.1 M, pH 7.5), TCA (10%, w/v) in acetone with 2-mercaptoethanol (0.07%).

Samples were grounded and then homogenized with 3 ml solution comprising TCA (10%) in acetone with 2 ME (0.07%). The total protein was precipitated overnight at −20°C. The precipitate was vortexed and centrifuged at 13,000 rpm at 4°C for 15 min. The pellet obtained was rinsed thrice with acetone supplemented with 2 ME (0.07%), EDTA (2 mM) and 1 tablet of complete EDTA free protease inhibitor. For every washing, 500 µl of chilled wash buffer was added, vortexed briskly and centrifuged at 13,000 rpm at 4°C for 15 min. Final washing was carried out with pre-chilled acetone (100%). Airdried pellet was kept overnight at −80°C for removing remaining traces of acetone. The pellet was then dissolved in rehydration buffer.

### 3.1 Estimation of protein by Bradford's method:

The most widely used Bradford method was developed by M. Bradford. It is based on the observation of a shift in wavelength from 465nm to 595nm for Coomasssie Brilliant Blue G-250 dye in an acidic solution as it binds to a protein. When the dye is bonded to the protein it is in the anionic form and has a maximum absorbance around 595nm. When the dye is not bound, it is in the cationic form and has a maximum absorbance around 470nm. The dye interacts with both hydrophobic and basic amino acids of the protein. With increasing protein concentration, the dye changes color brown to blue to darker shades of blue. The dye appears to bind more readily to arginine residues (but not to the free amino acid) of the protein. Hence the absorbance of light by the dye-protein complex at 595 nm is proportional to the amount of protein bound (over a limited range); i.e., there is a linear relationship between absorbance and the total protein concentration of the sample over a narrow range.

### 3.2 Standard preparation:

| Standards | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 |
|---|---|---|---|---|---|---|---|---|
| Dist .H2O | 900µl | 500µl | 500µl | 500µl | 500µl | 500µl | 500µl | 500µl |
| Serial dilution of BSA | 100µl from the stock | 500µl | 500µl | 500µl | 500µl | 500µl | 500µl | 500µl |
| Conc. of standards | 5 mg/ml | 2.5 mg/ml | 1.25 mg/ml | 0.625 mg/ml | 0.312 mg/ml | 0.156 mg/ml | 0.078 mg/ml | 0.039 mg/ml |

**3.3 Procedure:**

Added 10 µl of each standard solution and test samples to the Elisa titter plate.

Then, 100µl of Bradford's reagent was added to the standards and test samples.

All the samples and standard were done in triplicates to avoid any error.

The plate was incubated for a minimum of 10 minutes at dark.

The absorbance was measured at 595nm in a microplate reader.

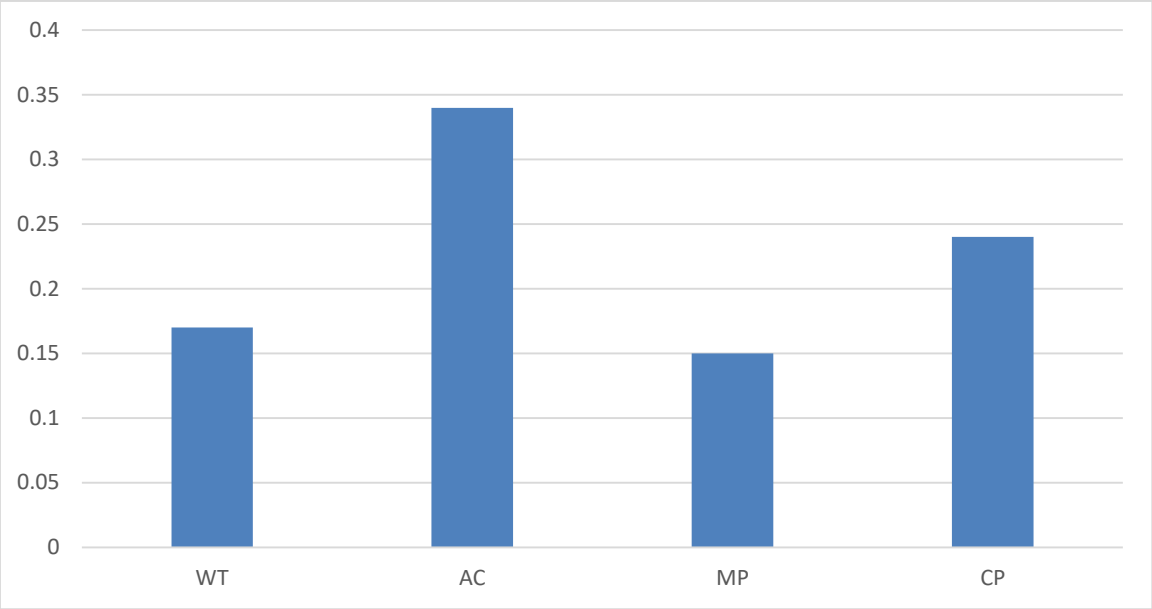
From this the value of unknown concentration is found out.

**IV. RESULTS**

**4.1 Mean value of total protein content**

| Name of the sample | OD value at 595 nm | Total protein content | Mean value of total protein content |
|---|---|---|---|
| WT | 0.695 | 0.26 | 0.17 |
| | 0.622 | 0.03 | |
| | 0.668 | 0.24 | |
| AC | 0.812 | 0.63 | 0.34 |
| | 0.664 | 0.16 | |
| | 0.688 | 0.24 | |
| MP | 0.665 | 0.17 | 0.15 |
| | 0.634 | 0.07 | |
| | 0.685 | 0.23 | |
| CP | 0.666 | 0.17 | 0.24 |
| | 0.647 | 0.11 | |
| | 0.751 | 0.44 | |

**4.2 GRAPH REPRESENTING THE PROTEIN CONTENT OF SELECTED MEDICINAL PLANTS**




## V. DISSCUSSION

The total protein content present in *Wrightia tinctoria* (sweet indrajao) was found to be 0.17 %.

The total protein content present in *Ananas cosmous* (pineapple) was found to be 0.34 %.

The total protein content was present in the sample *Milletia pinnata* (Indian beech) was found to be 0.15 %.

The total protein content was present in the sample *carica papaya* (papaya) was found to be 0.24 %.

The difference in the protein content of the plants may be due to the ability of the plants of fixing nitrogen in soil which in turn helps in synthesis of protein.

## VI. CONCLUSION

In comparison of the selected four medicinal plants, the protein content of the leaves of pineapple was found to be the highest whereas the lowest protein content was found in Indian beech.

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