EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 4
362-364
Year: 2017

QUANTITATIVE ESTIMATION OF PROTEIN USING NATURAL DYES EXTRACTED FROM *CAESALPINIA PULCHERIMA* PLANT FLOWERS

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Article Received on 30/01/2017 **Article Revised on 19/02/2017** **Article Accepted on 12/03/2017**

ABSTRACT
A new photometric method for the quantification of protein using the dye extracted from Purple, Yellow and Red color flowers of *Caesalpinia pulcherima* plant has been developed In the present study, we have used aqueous, acid, alkali and methanolic extract of above said flowers. The extracted dyes were used in the protein-dye binding studies. Out of that the methanolic extract show the stable color of protein-dye complex with increase in concentration of protein. The methanolic extract for three dyes achieved linearity up to the following concentration of protein, Purple color dye (80µg); Yellow color dye (90 µg) and Red color dye (80 µg).

KEYWORDS: *Caesalpinia pulcherima,* Natural dye and protein-dye complex.

INTRODUCTION
Proteins are the class of biochemical compounds having differences in their chemical properties and hence they perform many biological functions such as an enzyme catalyst, metabolic regulation, binding and transport of small molecules, gene regulation and cell structure.[1,2] The basic building blocks of proteins are amino acids. These amino acids of protein are linked together by peptide bonds between their carboxyl and α-amino group to form linear polymers.[3] Protein quantification using Folin-Phenol reagent can detect protein in solution at concentration as low as 5-10 µg. The disadvantages of this method are in presence of surfactants some proteins get solubilized while other show unreliable results[4, 5].

Dyes are chemical substances of chemical or synthetic origin, soluble in a medium used for impart a desired colour to a non-food material like paper, leather, wood, textile and even cosmetics in a process known as dying (Green 1995). Photometric method can be used to quantification of proteins in solution which contains only proteins without any interfering substances. As mentioned above, it is necessary to require a suitable reagent for protein binding estimation. Hence, in the present investigation, the extracts of Purple, Yellow and Red colour flowers of *Caesalpinia pulcherima* were used for quantification of protein.

MATERIALS AND METHODS
1. Chemicals
All chemicals used are of Analytical grade. The UV analysis was carried out using UV-Visible Spectrophotometer Chemito 2100.

2. Sampling of Plant Sources.
The flowers of *Caesalpinia pulcherima* were collected from the local area of Sangamner Tehsil, District-Ahmednagar, (M.S.), India (Figure 1).

Figure 1: Red, Purple and Yellow Colour Flowers of *Caesalpinia pulcherima*

3. Extraction of Dye from the Plant Flowers

Following are the extrcation methods employed in extraction of the dye intermediates.

3.1 Aqueous Method

5 g of the Purple, Yellow and Red colour flowers of *Caesalpinia pulcherima* were soaked in 100 mL of distilled water and boiled for 10 minutes. The solution was allowed to cool and then filtered to obtain clear solution.

3.2 Alcoholic Method

5 g of the Purple, Yellow and Red colour flowers of *Caesalpinia pulcherima* were soaked in 100 mL 50% of methanol and boiled in water bath for 10 minutes. The solution was allowed to cool and then filtered to obtain clear solution.

3.3 Acidic Method

5 g of the Purple, Yellow and Red colour flowers of *Caesalpinia pulcherima* were soaked in 100 mL of 1% Hydrochloric acid and boiled for 10 minutes. The solution was allowed to cool and then filtered to obtain clear solution.

3.4 Alkaline method

5 gm of the Purple, Yellow and Red colour flowers of *Caesalpinia pulcherima* were soaked in 100 mL of 1% Na_2CO_3 and bolied for 10 minutes. The solution was allowed to cool and then filtered ao obtain clear solution.

4. Preparation of Standrad Protein Solution

Stock 100 µg/mL bovine serum albumin solution was prepared in 100 mL distilled water.

5. Absorption measurment of extracted dyes

The filtered dyes were diluted with the respective solvents used for extract and absorption was measured in visible region using UV-Visible Spectrometer Chemito 2100.

6. Protein Quantification using Extracted Dyes

Pipette out 10-100 µg/mL protein concentration in each labeled test tube. Tube 1 as a control with distilled water. 2 mL of the extracted dye was pipette out into each tube with distilled water to make final volume. The mixture was shaken and the absorbance was measured at a wavelength where the protein-dye complex shows maximum absorbance.

RESULT AND DISCUSSION

1. Absorption of Extracted Dye

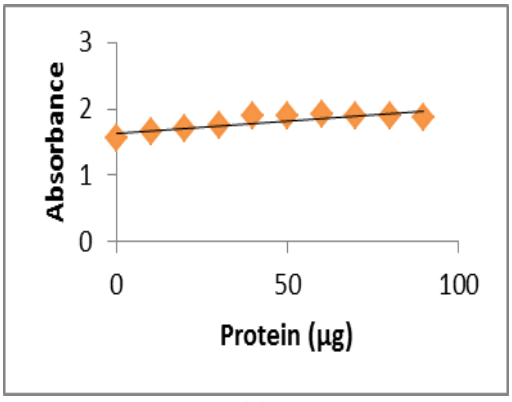
Table 1 includes the λmax and the respective absorbance for extracted dye.

Solvent / Flowers	Aqueous	Alcoholic	Acidic	Alkaline
Purple	432 nm (1.820)	452 nm (1.432)	528 nm (2.298)	524 nm (2.379)
Yellow	416 nm (0.990)	480 nm (2.050)	508 nm (2.329)	528 nm (2.409)
Red	538 nm (0.629)	478 nm (2.195)	522 nm (2.38)	458 nm (2.497)

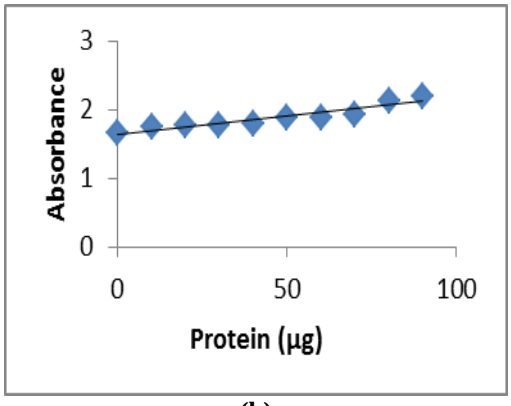
The extracted dyes possess absorbances at their respective λmax wavelengths as shown in Table 1. The aqueous, alkali and acid extracts showed microbial growth and hence only methanolic extract of Purple, Yellow and Red flowers of *Caesalpinia pulcherima* were considered for quantitative assay of protein and the results were represented in the form of calibration curves (Figure 2).

2. Measurement of Protein Concentration

The selected extract was mixed with 0.1-0.9 mL BSA and distilled water used for final volume.Measure the absorbance at λmax of the respected dyes.Some of them shows linearity with increase in the concentration of protein (Figure 2). Plot the graph absorbance against amount of protein for methanolic extract of dye.



(a)



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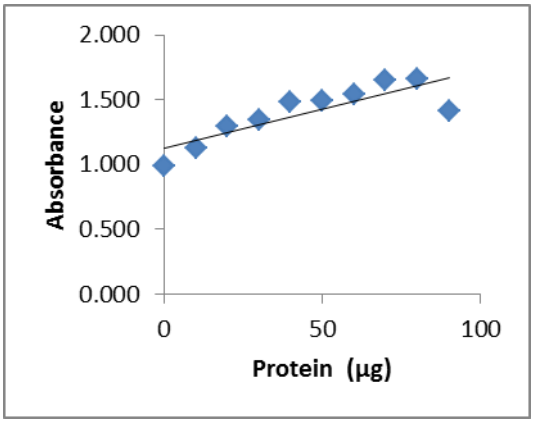

(c)

Figure 2. Calibration curves for protein with dyes (a) Methanolic extract of purple colour flowers; (b) Methanolic extract of yellow colour flowers and (c) Methanolic extract of red colour flowers.

CONCLUSION

The data obtained in this investigation indicate that the plant flower extracts can be used for quantification of proteins in solution. Most of the times precipitaion occurred during the estimation of proteins. To avoid this problem, low concentration of protein may suffice. We are currantly working on ways to optimise the procedure of protein-dye binding using natural dyes.

ACKNOWLEDGMENT

We are thankful to Principal Dr K.K. Deshmukh for providing the facilities and motivation.

REFERENCES

1. Bradford M. M., "A rapid and sensitive method for the quantitation of Microgram quantities of protein utilizing the principle of protein-dye binding", *Analytical Bio-chemistry*, 1976; 72: 248-254.
2. Smith P. K., Krohn R. I., Hermanson G.T., Mallia A. K. and et.al. "Measurment of protein using bicinchoninic acid". *Analytical Biochemistry*, 1985; 150: 76-85.
3. Antharavally B. S., Mallia K. A., Rangaraj P., Haney P. and Bell P. A., "Quantitation of proteins using a dye-metal-based colorimetric protein assay". *Analytical Biochemistry*, 2009; 385: 342-345.
4. Brown R. E., Jarvis K. L., and Hyland K. J., "Protein measurment using bicinchoninic acid: Elimanation of interfering subsatances". *Analytical Biochemistry*, 1989; 180: 136-139.
5. Groves W. E., Davis F. C. and Sells B. H. Spectrophotomertic determination of microgram quantities of protein without nucleic acid interference". *Analytical Biochemistry, 1968;* 22: 195-210.