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Estimation of protein content in Lens culinaris, Vigna unguiculata, Dolichos biflorus and Phaseolus vulgaris seeds using ultraviolet visible spectrophotometry

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ABSTRACT
The present study was carried out to estimate the total amount of secondary metabolites (protein) in four important valuable medicinal plants, viz. *Lens culinaris*, *Vigna unguiculata*, *Dolichos biflorus* and *Phaseolus vulgaris* using ultraviolet visible spectrophotometry. The result decribed that the all four seeds belonging to family Leguminosae have sufficient amount of protein which prove all four of them possess high nutritive values.

KEY WORDS: *Lens culinaris*, *Vigna unguiculata*, *Dolichos biflorus* and *Phalaseous vulgaris*

INTRODUCTION:

Since the beginning of human civilization, medicinal plants have been used by humanity for its therapeutic value Plants are known to provide a source of inspiration for novel drug compounds and this is sequel to the fact that medicines derived from plants have made large contributions to human health and well being. Plants have been the major source of therapeutic agents for curing the human diseases. Tribals as well as the rural people depend for all their medicinal and other necessities on the surrounding plant wealth. Fabaceae is the third largest family of flowering plants, which is commonly known as the legume family, pea family, bean family or pulse family.[1] With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems. The Leguminosae family includes about 600 genera and 13,000 species, making it the third largest family within the plant kingdom. *Lens culinaris* which claimed to have blood purifying property, to get rid of old skin marks, treats various kidney and gastric ailments and exhibit antifungal properties, the seeds of *Dolichus biflorus* are anthelmintic, astringent, diaphoretic, diuretic, emmenagogue, expectorant, febrifuge, ophthalmic and tonic in activity, *Vigna unguiculata* seeds have astringent, laxative, diuretic, anthelmintic, antibacterial and galactogogue properties and *Phseolus vulgaris* seeds have a notable place in the folklore throughout the world and in the traditions of many cultures such as pharmacotherapeutic effects, diabetes and obesity.[2-5] The present study focussed to estimate quantitatively protein in all four plant seeds belonging to family Leguminosae.

MATERIAL AND METHODS:

Preparation of plant extract:

The samples were collected and authenticated and were coded as follows:

S no.	Plants studied on	Code
1	*Lens culinaris*	*Lc*
2	*Vigna unguiculata*	Vu
3	*Dolichos biflorus*	Db
4	*Phalaseous vulgaris*	Pv

The collected plant material was washed and dried under room temperature and processed for extraction. With the help of soxhlet apparatus extraction of dried plant material was carried out using ethanol for 72 hours or till the decolourisation of the solvent in the siphon tube whichever is earlier.

Reagent used

In Lowry's method - Complex forming reagent (sodium carbonate-2%, copper sulfate-1%, sodium tartrate-2% [100:1:1]), folin's reagent, 2M NaOH(80 gm NaOH dissolve in 1000 ml distilled water).

In Biuret method – Biuret reagent (prepared by dissolving 6 gm of sodium potassium tartrate and 1.5 of cupric sulphate in 500 ml of distilled water), Potassium iodide, 10% NaOH.

In Bradford method- Bradford reagent: 100 mg Coomassie Brilliant Blue G-250 was dissolved in 50 ml 95% ethanol, added 100 ml 85% (w/v) phosphoric acid. Diluted to 1 liter when the dye had completely dissolved, and filtered through Whatman #1 paper just before use.[6, 7]

Preparation of Serum

A 10 ml tube of whole blood was collected for standard procedures using a serum separator tube (SST, tiger top tube). The samples were allowed to clot for one hour at room temperature. The samples were centrifuged for 10 minutes at approximately 1000 g. A clean pipette was used to take aliquot of 210 serum into labeled cryovials. After that the vials of serum were freezed at –80 degree freezer

PROCEDURE

Preparation of Standard Curve

(Standard Assay, 20-150 µg protein; 200-1500 µg/ml)

A series of protein standards diluted with 0.15 M NaCl to final concentrations of 0 (blank = NaCl only), 250, 500, 750 and 1500 µg/ml were prepared. The serial dilutions of the unknown sample to be measured was also prepared. 100 µL of each of the above to a separate test tube (or spectrophotometer tube if using a Spec 20) was added. 5.0 ml of coomassie blue was added to each tube and mixed by vortex, or inversion. The spectrophotometer was adjusted to a wavelength of 595 nm, and blank used in tube which contained no protein. After 5 minutes each of the standards and samples were read at 595 nm wavelength. The standard curve was plotted between absorbance and their concentration. The extinction coefficient was computed and the concentrations of the unknown samples were calculated.[8]

Lowry's method- 0.1 ml of test sample or standard protein was taken 0.1 ml of 2M NaOH was added and hydrolyzed the mixture at 100^{o}C for 10 min in a heating block or boiling water bath. The mixture was cooled to room temperature and 1 ml of freshly mixed complex forming reagent was added. The solution was allowed to stand at room temperature for 10 min. 0.1 ml of folin's reagent was added and mixed well. Mixture was allowed to stand for 30 to 60 min at room temperature. The absorbance was taken at 750 nm.

Biuret method-

An appropriate volume was diluted with distilled water to give a solution containing about 5 mg of protein per ml. To 1.0 ml of resulting solution in the test tube, 4 ml of biuret reagent was added. With constant stirring 300 ml of 10% NaOH was added and the final volume was made to 1000 ml by adding 5 mg of potassium iodide.

The absorbance was measured of all resulting test solution at 550 nm. Blank was prepared in the same manner by taking 1.0 ml of distilled water. The protein content was calculated from the absorbance obtained using calibration curve prepared by repeating the operation using 1.0 ml of each of a series of bovine serum albumin solution containing 1.0 mg, 2.0 mg, 3.0 mg, 4.0 mg, and 5.0 mg per ml of protein.

Bradford method- 3 ml of bradford reagent was taken in a test tube and 1 ml of test solution was added. Then the volume was made upto 10 ml. Then allowed to stand for 45 min. After 45 min the absorbance was observed at 595 nm.

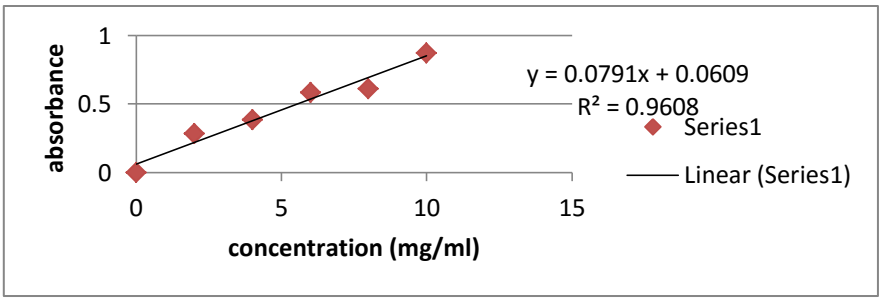
RESULTS

Quantitative estimation of protein in ethanolic extract of seeds of Lens culinaris, Vigna unguiculata, Dolichos biflorus and Phalaseous vulgaris was done by three methods Biuret method, Lowry method and Barford method. The results are given in following table accordingly.

Biuret method

Observation for absorbance for the standard curve

S.No.	BSA Concentration (mg/1000ml)	Absorbance 550nm
1	0	0.47
2	2	0.48
3	4	0.49
4	6	0.50
5	8	0.50
6	10	0.52



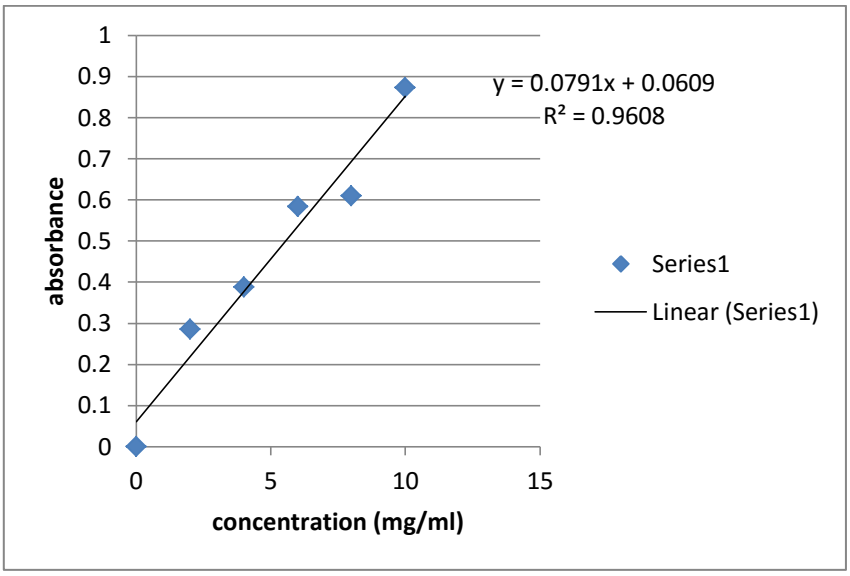
According to the standard curve, following regression equation was obtained and using the equation the concentration of sample was calculated.

S.No.	Samples	Absorbance at 550 nm	Concentration (ug)
1.	Lc	0.786	10.00
2.	Pv	0.731	9.31
3.	Vu	0.649	8.27
4.	Db	0.571	7.28

Lowery method

Observation for absorbance for the standard curve

S.No.	BSA Concentration (mg/1000ml)	Absorbance 750nm
1	0	0
2	2	0.285
3	4	0.387
4	6	0.583
5	8	0.610
6	10	0.873



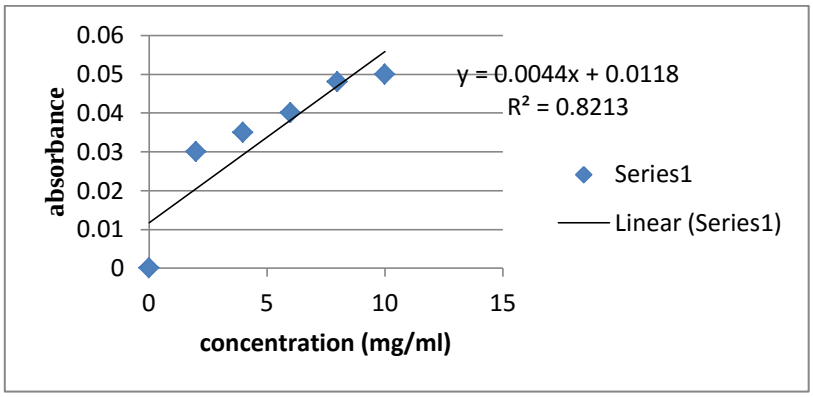
According to the standard curve, following regression equation was obtained and using the equation the concentration of sample was calculated.

S.No.	Samples	Absorbance at 550 nm	Concentration (ug)
1.	Lc	0.862	10.97
2.	Pv	0.594	7.57
3.	Vu	0.649	8.21
4.	Db	0.483	6.17

Bradford method

Observation for absorbance for the standard curve

S.No	BSA Concentration (mg/1000ml)	Absorbance 595nm
1	0	0.00
2	2	0.030
3	4	0.035
4	6	0.040
5	8	0.048
6.	10	0.050



According to the standard curve, following regression equation was obtained and using the equation the concentration of sample was calculated.

S.No.	Samples	Absorbance at 595 nm	Concentration (ug)
1.	Lc	0.098	24.51
2.	Pv	0.099	24.73
3.	Vu	0.059	14.73
4.	Db	0.064	15.98

CONCLUSION:

The present study deals with the quantitative estimation of protein content in the four seeds which consist of nutritional value. These could be considered as a valuable source of dietary fibers (most of which (93–99.7 %) is insoluble and less than 7 % soluble. The b-glucan component of soluble fibers in lentils is relatively low as compared with its good sources such as oats. However, it is relatively higher than that of peas, winter wheat and flaxseeds. The present study concluded that protein is present in all four Lens culinaris, Vigna unguiculata, Dolichos biflorus and Phalaseous vulgaris seeds.

REFERENCES:

1. A.N. Korpenwar, S.U. Borkar, 'Indigenous Medicinal Plants Used By Traditional Healers of Buldhana Tahsil, Dist. Buldhana (M.S.)', International Journal of Recent Trends in Science and Technology, Vol 1(1), (2011) 14-19.
2. C. Menaka and A. Mary Helitha, 'Role of methanolic extract of Dolichos biflorus seeds in hyperlipidaemic models of wistar albino rats', International Journal of ModernResearch and Reviews, Vol. 1(1), (2013) 35-43.
3. D. Sicard , L. Nanni , O. Porfiri , D. Bulfon and R. Papa, 'Genetic diversity of Phaseolus vulgaris L. and P. coccineus L. landraces in central Italy', Plant Breeding 124, (2005) 464 - 472.
4. Gangarao Battu, Ch.K.V.L.S.N.Anjana Male, T.Hari priya, Venna Naga Malleswari, S .K. Reeshma, 'A Phytopharmacological Review On Vigna Species', Pharmanest - An International Journal of Advances In Pharmaceutical Sciences Vol. 2 (1) (2011), 62-67.
5. Kripi Vohra, Vivek Kumar Gupta, 'Pharmacognostic evaluation of Lens culinaris Medikus seeds' Asian Pacific Journal of Tropical Biomedicine (2012) S1221-S1226.
6. Bradford, MM. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: (1976), 248-254.
7. Stoscheck, CM. Quantitation of Protein. Methods in Enzymology 182: (1990), 50-69 .
8. Government of India. Ministry of health and family welfare. Indian Pharmacopoeia Vol I . The Controller of Publication, Ghaziabad, Uttar Pradesh, 2007 p no. 99