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Estimation of two primary metabolites from medicinally valuable plant "Ashwagandha"

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Abstract
Medicinal plants are the most exclusive source of life saving drug for the majority of the world's population. Laboratory evaluations were made to assess the study of primary metabolites of various plant parts in selected plant. *Withania somnifera* plant was collected from local area of Bikaner (Sagar) and used for estimation of two primary metabolites (Protein and ascorbic acid). The highest amount of protein (13.8 mg/100 g.d.w.), ascorbic acid (74.46 mg/100 g.d.w.) was observed in the stem. Similarly the lowest amount of protein (5.51 mg/100 g.d.w.) ascorbic acid (39.31 mg/100 g.d.w.) was observed in stems and leaves.

Keywords: Primary metabolites, Protein, Ascorbic acid, medicinal plant, Ashwagandha.

1. Introduction
The plant has been an integral part of traditional medicine across the continents since time immemorial. Medicinal plants have their values in the substances present in various plant tissues with specific physiological action in Human body. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. India is endowed with a rich wealth of medicinal plant. India recognizes more than 2500 plant species which have medicinal value. Plants are like natural laboratories where a great number of chemicals are biosynthesized and in fact they may be considered the most important source of chemical compounds.
Withania somnifera is considered as a highly nutritious plant, but along with nutritional values. It has medicinal values also. It helps in enhancing the mental functioning. It is useful in sexual and general weakness. It gives vitality and vigor and helps in building greater endurance. It is used to cure disease like rheumatism leprosy and arthritis. The leaves and the root bark of plant or abortifacient adaptogen antibiotic, aphrodisiac, diuretic paracolic and tonic. It is also used to tone the uterus after the miscarriage. The fruit and seeds are diuretic. All the parts of the plants are used in the herbal medicines. According to the Ayurveda studies Ashwagandha increases health and longevity. It is also sometimes used to treat the memory loss. In case of cancer Ashwagandha acts as the adjutant. The present work is to analyze two basic primary metabolites (protein and ascorbic acid) of *Withania somnifera* (family: Solanaceae).

2. Material and Methods
For the estimation of primary metabolites different protocol was used. Leaves, stem and flowers of the mature plant were collected, washed with distilled water, shade dried and powdered. The powder was used for analysis of protein and ascorbic acid of the selected plant species.

2.1 Estimation of Protein
Protein Extraction: Each of the plant parts were homogenized separately in 10% Cold tri chloro acetic acid TCA 10 mg: 5 ml) and were centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and the pellets were saved. Pellets were again suspended in 5 ml of 10% cold TCA and re-centrifuged for 10 minutes supernatant was again discarded and the precipitate was dissolved in 10 ml of 0.1N NaOH. 0.1 ml of this solution was used for protein estimation.

2.2 Quantitative Estimation of Protein

In each of 1 ml extract, total protein content was estimated using the protocol of Lowry *et al*., 1951. A stock solution (mg/ml) of bovine serum albumin was prepared in 1N NaOH; five concentration (0.2, 0.4, 0.6, 0.8 and 1 ml) from the working standard solution were taken in a series of test tubes. In another set of test tubes 0.1 ml and 0.2 ml of the sample extracts were taken and the volume was raised up to 1ml in all the test tubes. To each test sample, 5 ml of Freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1N NaOH and 1 ml of 0.5% $CuSO_4$. $5H_2O$ in 1% sodium potassium Tartrate) was added at room temperature and left undisturbed for a period of 10 minutes subsequently for each of these mixture tubes 0.5ml of Folin ciocalteu reagent (diluted with an equal volume of distilled water just before use) was rapidly added and incubated at room temperature (about 25 ^oC) for 30 minutes until the blue colour developed. The spectronic calorimeter (Bausch and Lamb) was adjusted at a Wavelength of 750 nm and set at 100% transmittance using blank before taking the reading of the standard and the test samples respectively. Five replicates were examined in each case and their mean values were recorded A regression curve was worked out of various concentrations of the standard solution against their respective absorbances, which followed the Beer's law.

2.3 Estimation of Ascorbic Acid

Ascorbic Acid was estimated using the protocol of Chinoy (1962). Dried plant parts were weighed separately crushed in a motor in 2% Metaphosphoric Acid (MPA) and allowed to macerate for one hour. These were then centrifuged separately at low speed (2500 rpm) for fifteen minutes, the residue was discarded and the supernatants were used for the estimation of ascorbic acid following the procedure of Gensen (1962).

Each of the 1 ml test solutions were mixed with 2 ml of 5% MPA and kept for 30 minutes without stirring at room temperature. 5 ml of n-amyl alcohol and 3.2 ml of dye (5 mg/100 ml, 2,4-dichlorophenol, indophenol) were added and air bubbled through the lower layer each of the test tubes was stoppered tightly, the mixture was shaken vigorously and the upper layer was used for the estimation of ascorbic acid. The spectronic 20 colorimeter (Bausch and Lomb) was adjusted at wavelength of 546 nm and set at 100% transmittance using a mixture of 1 ml of the extract, 2 ml of 5% MPA, 5 ml n-amyl alcohol and 3.2 ml distilled water (Bland Solution) before taking test sample. Ascorbic acid content present in 1ml of the extract was measured by using the regression formula.

$$\mathbf{Y = 0.1103- (0.14x0.D)}$$

Where, Y = concentration of ascorbic acid in mg.
O.D. = Optical Density

$$\text{Free Ascorbic Acid} = \frac{A \times V}{W} \times 1000 \times 1000$$

Where, A = y = mg ascorbic acid / ml of original extract
V = Total volume of the original extract (in ml)
W = Weight of the plant sample (in mg) used for analysis.

3. Result and Discussion

The various plant parts (leaf, stem and flowers) varied in composition of primary metabolites studied. In the present investigation *Withania somnifera* evaluated qualitatively for the analysis of total soluble protein and ascorbic acid. The results are presented in Table 1.

Protein are the primary components of living things. The presence of higher protein level in the plants points towards their possible increase food value or that a protein base bioactive compound could also be isolated in the future. Total Level of protein were found to be maximum in Fruit (13.81 mg/100 g.d.w.) and minimum amount in Root 5.51 mg/100 g.d.w.

Ascorbic acid (Vitamin C) is a familiar molecule because of its dietary significance, it is not only an important antioxidant, it also appears to link flowering time, developmental senescence, programmed cell death and responses to pathogens through a complex signal transduction network. Total levels of Ascorbic acid were found to be maximum in the stem 77.67 mg/100 g. d.w. and minimum in leaves 39.31 mg/100 g.d.w. (Fig. 1).

Table 1: Estimation of Primary Metabolites in different parts of *Withania somnifera*

Plant Parts	Primary Metabolites	
	Protein	Ascorbic Acid
Stem	5.51	74.46
Leaves	11.69	39.31
Fruit	13.81	71.85

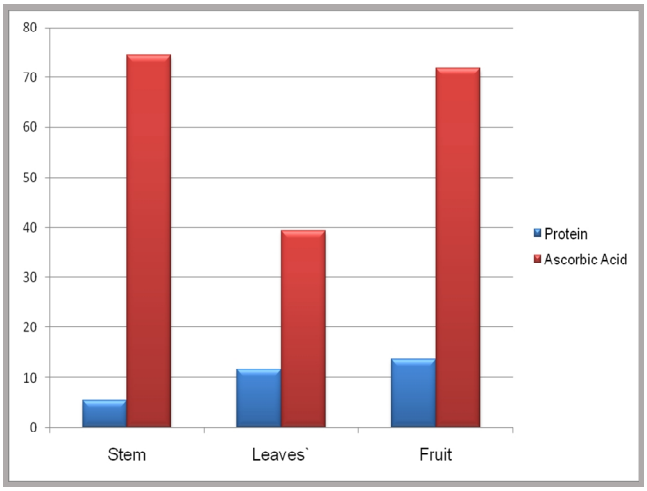

Fig 1: Protein & Ascorbic acid content in different parts of *Withania somnifera* (mg/100 g.d.w.)

4. Conclusion

In the present study it was found that *Withania somnifera* is an outstanding source of protein and Ascorbic Acid. *Withania somnifera* serve as a boon in medicine as well as Ayurveda. Developing country like India, where many people live their lives below the poverty line and suffer from osteoarthritis, anxiety, type 2 diabetes, cancer. *Withania somnifera* can be used as powder form and it can easily available.

5. References

1. Kirtikar KR, Basu BD. Indian Medicinal Plant. Vol I, International Book Distributors, Dehradun, India, 1995, 830-832.
2. Jayaraman J. Laboratory Manual in Biochemistry, New Delhi. Wiley Eastern Limited, New Delhi, 1981.
3. Bray HG, Thrope WV. Analysis of Phenolic compound of interest in Metabolism. Meth Biochem Anal 1954, 127-52.
4. Lowery OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagent. J Biol Chem 1951; 193:265-275.

5. Chinoy JJ. Formation and Utilization of as ascorbic acid in the shoot apex of wheat as Factor of growth and development Ind. J Plant Physiol 1962; 5:172-201.
6. Sagwan DS, Rao V, Sharma RA. Biochemical estimation of Primary metabolites from *Pongamia pinnata* (L); and important biodiesel plant IJPSRR 2010; 5(1):146-149
7. Vijayvergia R, Kumar J. Quantification of Primary metabolites of *Nerium indicum* Mill. Asian J Exp Sci 2007; 21(1):123-128.
8. Rishi A, Sarin R. Estimation of primary metabolites from *Gloriosa superba* L. *in vivo* and *in vitro* Int. J Mendel 2009; 26(1-4):87.
9. Thomson S, Handen HS, Nyman V. Ribosome inhibiting protein from *in vitro* culture of Phytolacea dedecandra. Planta Med 1991; 57:232-236.
10. Nicholas. The Function and metabolism of ascorbic acid in plants. Annals of Botany 1996; 78:661-669.
11. Mapson LW. Metabolism of ascorbic acid system in plants Part I. Function, Ann Rev. Plant Physiol 1958; 2:119-150.