



BIOCHEMICAL ESTIMATION OF THREE PRIMARY METABOLITES FROM MEDICINALLY IMPORTANT PLANT *MORINGA OLEIFERA*

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ABSTRACT

Medicinal plants are the most exclusive source of life saving drugs 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 species *Moringa oleifera*. Plants of same age group were collected from local areas of Bikaner region and used for estimation of three primary metabolites (protein, chlorophyll and ascorbic acid). The highest amount of protein (4.15mg/ 100g.d.w.), ascorbic acid (77.68 mg/100g.d.w.) was observed in pods and chlorophyll (12.68 mg/g) in leaves. Similarly lowest amount of protein (3.30 mg/100g.d.w), ascorbic acid (39.27mg/100g.d.w.) was observed in stem and chlorophyll in pods (5.17 mg/g).

Keywords: Primary metabolites, Protein, Chlorophyll, Ascorbic acid, medicinal plants, *Moringa oleifera*.

INTRODUCTION

Plants have 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 plants. India recognizes more than 2500 plant species which have medicinal values¹. 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.

M.oleifera (Lam.) is considered as highly nutritious plant but along with nutritional values it has medicinal values also². Leaves are used in the treatment of fever, bronchitis, eye and ear infection, inflammation of mucous membrane, diarrhoea and gastric ulcer. Flower juice is useful in urinary infections. Roots and bark are used for cardiac and circulatory problems as tonic. Bark is appetizer and digestive.

Primary metabolites are of prime importance and essentially required for growth of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs³⁻⁴. The present work is to analyze three basic primary metabolites (protein, chlorophyll and ascorbic acid) of *Moringa oleifera* (family- Moringaceae).

MATERIALS AND METHODS

For the quantitative estimation of primary metabolites different protocols were used. Leaves, stem, flowers and pods of the mature plant were collected, washed with

distilled water, shade dried and powdered. The powder was used for analysis of protein⁵, chlorophyll⁶, and ascorbic acid⁷, of the selected plant species.

1. Estimation of proteins

Proteins 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 pellets were saved. Pellets were again suspended in 5 ml of 10% cold TCA and recentrifuged for 10 minutes. Supernatant was again discarded and the precipitate was dissolved in 10 ml of 0.1 N NaOH. 0.1 ml of this solution was used for protein estimation.

Quantitative estimation of Proteins: In each of 1 ml extract, total protein content was estimated using the protocol of Lowry *et al.*, 1951. A stock solution (1mg/ml) of bovine serum albumin was prepared in 1 N NaOH; five concentrations (0.2, 0.4, 0.6, 0.8 and 1ml) from the working standard solution were taken in 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 1 ml in all the test tubes. To each test sample, 5ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na₂CO₃ in 0.1 N NaOH and 1 ml of 0.5%CuSO₄. 5H₂O in 1% sodium potassium Tartrate) was added at room temperature and left undisturbed for a period of 10 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocalteu reagent (diluted with equal volume of distilled water just before use) was rapidly added and incubated at room temperature (about 25°C) for 30 minutes until the blue colour developed. The spectronic colorimeter (Bausch and Lomb) was adjusted at wavelength of 750 nm and set at 100% transmittance using blank before taking the readings 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 solutions against their respective absorbances, which followed the Beer's law.

2. Estimation of chlorophyll

Chlorophyll was estimated using the protocol of Holden (1960). Each of the plant samples were weighed 100 mg and homogenized separately in a mortar in the presence of excess of 80% acetone until all the color was released from the tissue. CaCO_3 was added to prevent pheophytin formation and this was then centrifuged at 5000 rpm for 10 minutes at room temperature. The clear supernatant was collected and then made up to a known volume (10 ml). The test tubes were wrapped with black paper to protect chlorophyll degradation.

The spectronic colorimeter (Bausch and Lomb) was adjusted at wavelength of 663 nm for chlorophyll 'a' and 645 nm for chlorophyll 'b' set at 100% transmittance using 80% acetone as blank before taking the readings of the samples respectively.

The optical density was measured and the chlorophyll contents in the original extract was estimated using the formula:

$$\text{Total chlorophyll (mg/L)} = 20.20A_{645} + 08.02 A_{663}$$

$$\text{Chlorophyll 'a' (mg/L)} = 12.70A_{663} - 02.69 A_{645}$$

$$\text{Chlorophyll 'b' (mg/L)} = 22.90A_{645} - 04.68 A_{663}$$

These can be converted to chlorophyll content in mg/g dry weight as follows:

$$\text{Chlorophyll 'a' (mg/g)} = \frac{12.3 \times \text{O.D. at } 663 \text{ nm} - 0.86 \times \text{O.D. at } 645 \text{ nm}}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll 'b' (mg/g)} = \frac{19.3 \times \text{O.D. at } 645 \text{ nm} - 3.6 \times \text{O.D. at } 663 \text{ nm}}{a \times 1000 \times W} \times V$$

$$\text{Total Chlorophyll} = a + b$$

Here,

O. D. = Optical Density

V = Final volume of chlorophyll extract in 80% acetone

W = Dry weight of plant material

a = the length of light path in the cell (usually 1 cm)

3. Estimation of Ascorbic acid

Ascorbic acid was estimated using the protocol of Chinoy (1962). Dried plant parts were weighed separately crushed in a mortar in 2% Meta Phosphoric Acid (MPA) (100 mg tissue and seed sample in 1 ml of MPA) and allowed to macerate for one hour. These were then centrifuged separately at low speed (2500 r.p.m.) for fifteen minutes, the residues were discarded and the supernatants were used for the estimation of ascorbic acid following the procedure of Jensen (1962).

Each of the 1 ml test solutions were mixed with 2ml of 5% MPA and kept for 30 minutes without stirring at room temperature. 5ml of n-amyl alcohol and 3.2 ml of dye (5mg/100ml, 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 546nm and set at 100% transmittance using a mixture of 1ml of the extract, 2ml of 5% MPA, 5 ml n-amyl alcohol and 3.2ml distilled water (bland solution) before taking test samples. Ascorbic acid content present in 1ml of extract was measured by using the regression formula:

$$Y = 0.1103 - (0.14 \times \text{O.D})$$

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

Ascorbic acid content per 100 gm dry weight was calculated as follows:

$$\text{Free ascorbic acid} = \frac{(A \times V)}{W} \times 1000 \times 100$$

Where, A = Y = mg ascorbic acid / ml of original extract

V = total volume of the original extract (in ml)

W = weight of the plant tissue sample (in mg) used for analysis

RESULTS AND DISCUSSION

The various plant parts (leaf, stem, flowers and Pods) of *M. oleifera* varied in composition of primary metabolites studied⁸⁻¹⁰. In the present investigation, *M. oleifera* was evaluated quantitatively for the analysis of total soluble protein, chlorophyll and ascorbic acid. The results are present in **Table-1**.

Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future¹¹. Total levels of protein were found to be maximum in pods i.e. 4.15mg/100g.d.w. and minimum amount in leaf i.e. 3.30 mg/100g.d.w. (Fig.1)

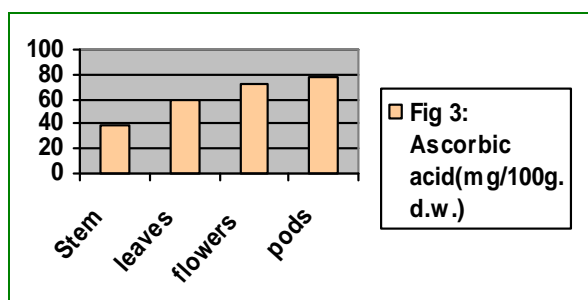
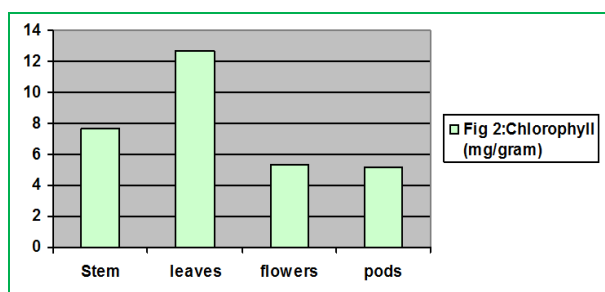
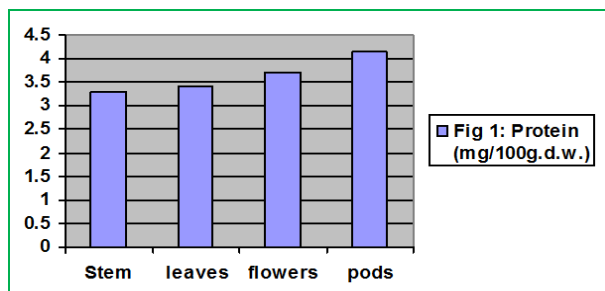
Chlorophyll is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation on photosynthesis¹². Total levels of Chlorophyll were found to be maximum in leaf i.e. 12.68 mg/g and minimum in pods i.e. 5.17 mg/g (Fig.2).

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^{13,14}. Total levels of ascorbic acid were found to be maximum in pods i.e. 77.67 mg/100 g.d.w. and minimum in stem i.e. 39.27mg/100g.d.w. (Fig.3).



Table 1: Estimation of primary metabolites in different parts of *Moringa oleifera*

Plant Parts	Primary metabolites		
	Protein (mg/100g.d.w.)	Chlorophyll (mg/gram)	Ascorbic acid (mg/100g.d.w.)
Stem	3.30	7.68	39.31
Leaves	3.43	12.68	58.48
flowers	3.7	5.31	71.85
Pods	4.15	5.17	77.68



CONCLUSION

In the present study, it was found that *M.oleifera* is outstanding source of Protein, Ascorbic Acid and Chlorophyll, which suggests that the plant *M. oleifera* can serve as a boon for curing 'protein-malnutrition' as well as a means to help in blood formation (because chlorophyll is the precursor of hemoglobin) and as a cure of scurvy, particularly in a developing country like India where many people live their lives below poverty line and

suffer from P.E.M. (Proteins Energy Malnutrition). *Moringa* can be used to get the required nutritional requirement for lactating women and weakened children of 1-3 years and can easily get it by consuming its pods, leaves or leaf powder. For this, people can be made well aware of its remarkable benefits in counter acting such ailments.

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Miss Tamanna Talreja graduated, post graduated and did Ph.D. at M.G.S University, Bikaner (Rajasthan). At post graduation level taken specialization in plant physiology and at the level of Ph.D. taken specialization in plant tissue culture and biochemical analysis, completed thesis in "*In vivo* and *in vitro* biochemical studies of economically and medicinally useful tree *Moringa oleifera*."