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COMPARISON AND BIOCHEMICAL ESTIMATION OF THREE PRIMARY METABOLITES OF MEDICINALLY IMPORTANT PLANT AMLA (PHYLLANTHUS EMBLICA)

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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 Amla plant parts that were stem, leaves and fruits. Present investigation focussed on the estimation of three primary metabolites (protein, chlorophyll and ascorbic acid). The highest amount of protein (4.70mg/ 100g.d.w.) was observed in fruits extract. Ascorbic acid (72.85 mg/100g.d.w.) was found to be highest in fruits extract and chlorophyll content was found to be highest (14.68 mg/g) in leaves extract.

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 values **(1).** 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. P.emblica (Amla) is considered as highly nutritious plant as well as important medicinal plant. 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 drugs **(2,3,4).** The present work is to analyze three basic primary metabolites (protein, chlorophyll and ascorbic acid) of P.emblica (family- Euphorbiaceae).

Materials and Methods
For the quantitative estimation of primary metabolites different protocols were used. Leaves, stem and fruits of the mature plant were collected, washed with distilled water, shade dried and powdered and different extracts were prepared for estimation.

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:

It was done by using Lowry's method. Five different reagents were prepared. Reagent A was prepared by dissolving 2% sodium carbonate in 0.1 N sodium hydroxide. Reagent B was prepared by dissolving equal amount of 1% copper sulphate and 2% of potassium sodium tartarate. Reagent C was prepared by mixing 50ml reagent A and 1ml reagent B and it was freshly prepared before use. Folin-Ciocalteu reagent was used as Reagent D which was commercially available and it was used after 1:1, v/v dilution with distilled water. Protein standard was prepared by weighing 100 mg bovine serum albumin (BSA) and dissolving it in distilled water, thereafter, the volume was made upto 100 ml by distilled water. Final concentration was made 1 mg/ml.

Estimation of protein was done by pipetting out 50 µl solution of proteins into test tubes in replicates of three and the total volume was made up to 1 ml. A tube with 1 ml distilled water served as a blank. 3 ml reagent C was added to each tube including the blank and after proper mixing the solutions were allowed to stand for 30 min then 0.5 ml reagent D was added and after mixing, the tubes were left at room temperature in the dark for 60 min. Blue colour developed in the solution. The absorbance was taken at 660 nm in UV-visible spectrophotometer. With the help of the standard graphs the amount of protein in different parts were compared.

2. Estimation of chlorophyll

The estimation of chlorophyll was done by using dimethyl sulphoxide (DMSO) extraction procedure. Plant samples were collected at random and were chopped into fine pieces. 50 mg sample from these chopped material were added in replicated tubes each containing 10 ml dimethyl sulphoxide (DMSO). The tubes containing plant pieces and DMSO were incubated at 65^0C for 3 h in an oven by providing gentle shake twice. After complete extraction, clear supernatants were used for measuring the absorbance with the help of a spectrophotometer against DMSO blank. The absorbance was recorded at wavelength of 663 nm for chlorophyll 'a' and 645 nm for chlorophyll 'b'. The optical density was measured and the chlorophyll contents in the original extract was estimated using the formula **(Hiscox and Israelesham, 1979)**.

Total chlorophyll (mg/L) = 20.20A645 + 08.02 A663

Chlorophyll 'a' (mg/L) = 12.70A663 – 02.69 A645

Chlorophyll 'b' (mg/L) = 22.90A645 – 04.68 A663

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

Chlorophyll 'a' (mg/g)

$$= (12.3\times A_{663} - 0.86\times A_{645}) \quad \frac{V}{1000 \times W}$$

Chlorophyll 'b' (mg/g)

$$= (19.3\times A_{645} - 3.6\times A_{663}) \quad \frac{V}{1000 \times W}$$

Total Chlorophyll = a + b
Here,
A = Absorbance or O.D. (Optical density).
V = Final volume of chlorophyll extract in 80% acetone.
W = Dry weight of plant material.

3. Estimation of Ascorbic acid

Ascorbic acid was estimated using the protocol of **Chinoy (1962).** Dried plant parts were weighed separately and crushed in a mortar in 2% Meta Phosphoric Acid (MPA) (100mg 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. 5 ml 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 absorbance was recorded at 546 nm of each 1 ml sample against distilled water as blank solution with the help of u.v. spectrophotometer. Ascorbic acid content present in 1 ml of extract was measured by using the regression formula:

Y = 0.1103 – (0.14 x 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:
Free ascorbic acid = (A x V) / W x 1000 x 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

Plants synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates) by simple substances such as water, carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids etc.,) that are used as drugs **(5).** Plants have great importance due to their nutritive value and they are the major source of medicines which play an important role in the human history **(6).**

4.3.1 Estimation of protein- 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 future **(7).** Protein content of amla plant stem solution was 4.30 mg/ 100g. d.w. Amla plant leaves solution showed 2.43 mg/ 100g. d.w. and amla plant fruits solution showed 4.7 mg/ 100g. d.w. protein content (Table. 1). So, total levels of protein were found to be maximum in fruits i.e. 4.7 mg/100g.d.w. and minimum amount in leaves i.e. 2.43 mg/100g.d.w.

4.3.2 Estimation of chlorophyll- 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 photosynthesis **(8).** Chlorophyll content of amla plant stem solution was 8.68 mg/gram. Amla plant leaves solution showed 14.68 mg/gram and amla plant fruits solution showed 7.31 chlorophyll content (Table. 2). So, total levels of Chlorophyll were found to be maximum in leaf i.e. 14.68 mg/g and minimum in fruits i.e. 7.31 mg/g.

4.3.2 Estimation of ascorbic acid- 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 **(9).** Ascorbic acid content of amla plant stem solution was 37.31 mg/100g.d.w. Amla plant leaves solution showed 54.48 mg/100g.d.w. and amla plant fruits solution showed 72.85 mg/100g.d.w. ascorbic acid content (Table. 3). Total levels of ascorbic acid were found to be maximum in fruits i.e. 72.85 mg/100 g.d.w. and minimum in stem i.e. 37.31mg/100g.d.w.

Table- 1 (Protein content)

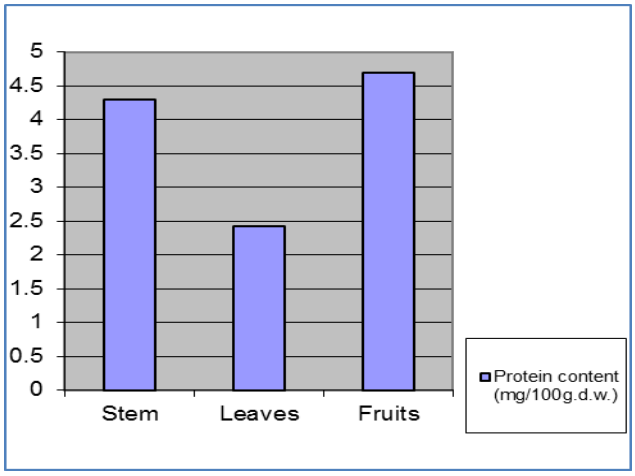
S.No.	Amla plant parts	Protein content (mg/100g.d.w.)
1.	Stem	4.30
2.	Leaves	2.43
3.	Fruits	4.70

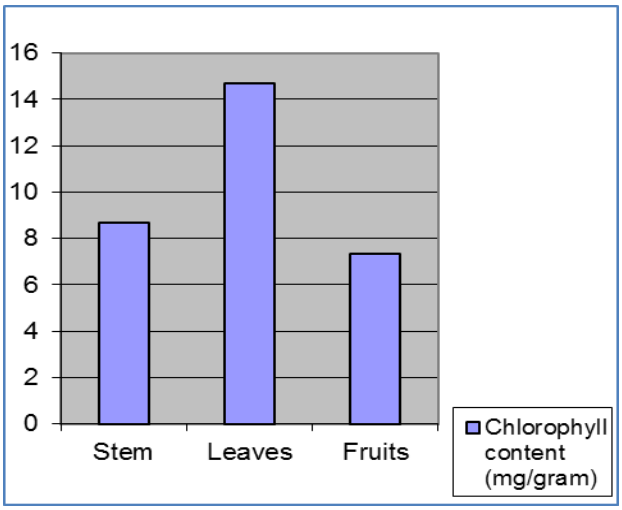
Table-2 (Chlorophyll content)

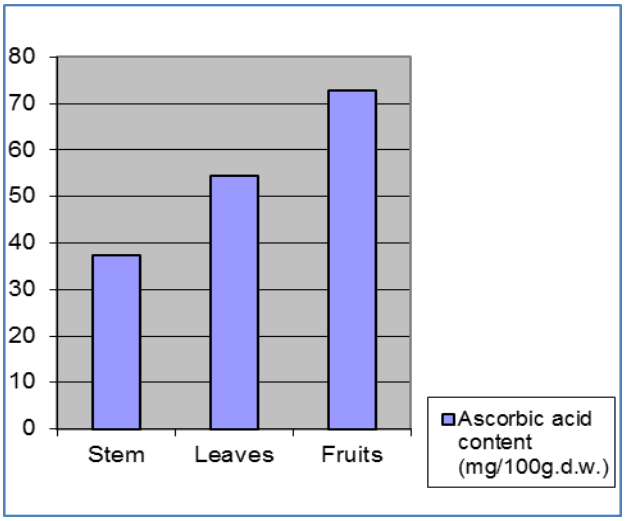
S.No.	Amla plant parts	Chlorophyll content (mg/gram)
1.	Stem	8.68
2.	Leaves	14.68
3.	Fruits	7.31

Table-3 (Ascorbic acid content)

S.No.	Amla plant parts	Ascorbic acid content (mg/100g.d.w.)
1.	Stem	37.31
2.	Leaves	54.48
3.	Fruits	72.85







Conclusion- In the present study, it was found that P.emblica is a strong source of protein, ascorbic acid and chlorophyll, which suggests that the plant P.emblica can used in cure of various diseases like protein malnutrition, scurvy etc. Protein content and ascorbic acid was found to be highest in amla plant fruits solution. Chlorophyll content was found to be highest in amla plant leaves solution. With the help of these results we can conclude that use of herbal plants, mainly Amla plant, in medical field can protect the growth of microorganisms to a certain level, which can be a measure of precaution from infectious diseases. To a certain level this plant can also destroy pathogenic organisms. Regular use of herbal medicines can also eliminate the chances of infections.

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