Studies on Some Primary Metabolites Extraction and Quantification in Different Plant Parts of *Eupatorium triplinerve* Vahl.

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ABSTRACT

Medicinal plant is most exclusive sources of life saving drugs for the majority of the world population. Laboratory evaluations were made assess the study of primary metabolites in various plants in selected species *Eupatorium triplinerve* Vahl. During the present investigation comparative study of primary metabolites Carbohydrates, Protein, Ascorbic acid, Starch, Sugar, Chlorophyll, Carotenoids and Total phenol of genus *Eupatorium triplinerve* was undertaken for study and all the their chemical composition. The leaf highest amount of chlorophyll-a (4.95gm), chlorophyll-b (4.05gm), carotenoids (3.29gm), ascorbic acid (41.13gm/gdm), sugar (3.92gm/gdm), starch (5.89gm/gdm) protein (67.82µg/mg), total phenol (84.00gm/gdm). The root lowest amount of sugar (2.00gm/gdm), starch (3.15gm/gdm), protein (6.01µg/mg), ascorbic acid (43.09gm/gdm), total phenol (65.13gm/gdm).

1. INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plants provided food, clothing, shelter and medicine. Recent estimates suggest that over 9,000 plants have known medicinal applications in all cultures of various countries and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth, 1998). The potential of these plants depends upon the presence of photochemical inside those may be primary metabolites or secondary metabolites those are secreted by plants during life. In the present study, the primary metabolites Carbohydrates, Protein, Ascorbic acid, Starch, Chlorophylls and Carotenoids, of genus *Eupatorium triplinerve* were studied. Primary metabolite are those organic substances which are synthesized during photosynthesis and these organic compound is essential for plant life, growth and development. Out of the known species of the genus *Eupatorium triplinerve* any systematic study on the primary metabolites viz. Starch, ascorbic acid, proteins, chlorophylls, carotenoids and phenols was not carried out in details so far (Sayeed *et al.*, 1999).

The Asteraceae is the second largest family in its division with some 1,620 genera and over 23,000 recognized species. Many species have been used medicinally and these tropical plants have a rich history in natural medicine. Indigenous to Brazil, it is also found in warmer climates and tropical area of South and North America (Pari and Latha, 2002). It is in the same genus as Senna and in same times called "Coffee Senna" since its seeds, found in long seed pods are sometimes roasted and made into a coffee-like beverage (Souk up, 1970). The three species *Cassia pumila*, *C. nodosa* and *C. renigera* were undertaken for study. As their name implies carbohydrates are composed of the elements of water and carbon so their formula approximates to a multiple of CH₂O. Most of the dry weight of plants is carbohydrate of one kind or another. All carbohydrates are polar and the low molecular forms are what we commonly known as sugars (Sambaiah and Lokesh 1998).

Starch is a substance that plants use to store energy. It is the end product of photosynthesis and can be stored for later use in seeds, tubers, and roots. Chemically, starch is a polysaccharide comprised of glucose molecules linked together in long chains (**Barthakur et al., 1995**). Ascorbic acid is a major metabolite in plants. It is an antioxidant and in association with other components of the antioxidant system, protects plants against oxidative damage resulting from aerobic metabolism, photosynthesis and a range of pollutants (**Lange, 2002**). Proteins are also derived partly from carbohydrates through the formation of amino acids. These latter simple compounds are then combined with nitrates from the soil and other substances to form the highly complex protein molecule. The main characteristic of proteins is their high nitrogen content (**Fairbairn and Shrestha, 1967**). Chlorophylls are greenish pigments which contain a porphyrin ring. This is a stable ring-shaped molecule around which electrons are free to migrate. There are several kinds of chlorophyll, the most important being chlorophyll "a" i.e. Universal is occurrence. This is the molecule which makes photosynthesis possible. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in other higher plants except fungi. A third form of chlorophyll which is common (not surprisingly) called chlorophyll "c", d – red and e – yellow green and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. Carotenoids are usually red, orange, or yellow pigments and include the familiar compound carotene (**Khan et al., 1967**).

2. MATERIALS AND METHOD

2.1. Plant material

The fresh leaf of the plant *Eupatorium triplinerve* Vahl was collected from State Forest Research Institute Vandalore, Kanchipuram District, Tamil Nadu. It was identified and authenticated in P.G Department of Plant Biology & Plant Biotechnology, Presidency College Chennai, Tamil Nadu.

2.2. Extraction Procedure

50 gms each of the experimental materials was soxhlet extracted successively with petroleum ether (60°-80°), benzene, chloroform acetone, alcohol and water 24-36 h. Each of the resultant extract was filtered, dried in vacuum and weighed to calculate the extractive value on dry weight basis. Later, following the established protocols (**Paech and Tracey, 1955**) each of the test sample was processed further to used to evaluate the presence of carbohydrates, proteins, tannins and Flavonoids. Before doing so, each test sample was reconstituted in the respective solvent.

3. CARBOHYDRATES

Extraction Procedure

A. Total soluble sugars

Each of the dried and powdered test sample (50 gm) was macerated in a mortar and pestle with 200 ml of 80% ethanol and left overnight. Each of the homogenates was centrifuged (1200 rpm, 15 min) the supernatants were removed and concentrated on a water bath. Later each resultant concentrate was raised to 50 mls with distilled water and processed further following the method of (**Loomis and Shull, 1937**) for total soluble sugars.

B. Starch

Starch, the residual pellet obtained out of the above process in each case was suspended in 5 ml of 52% perchloric acid and 6.5 ml of distilled water, shaken vigorously (5 min) and then centrifuged (2500 rpm; (**Macready, et al., 1950**)). This step was repeated thrice and the supernatant of each sample were pooled together and the volume was raised to 100 ml with distilled water (Ext. II). Out of this, 1 mL aliquot was measured separately to estimate starch quantitatively.

Quantification

Aliquots (1 ml) of each test sample were used to estimate quantitatively the total levels of carbohydrates following the protocol of (**Dubois *et. al.*, (1951)**) using phenol-sulphuric acid reagent, which included the preparation of a regression curve for the standard glucose. A stock solution of glucose (100 mg/ml) was prepared in distilled water, out of which 0.1 to 0.8 ml were separately pipette into the test tubes and the volume of each was raised to 1 ml with distilled water. Each of these were kept in an ice-chest, 1 ml of 5% aqueous phenol was added and shaken gently. Later, 5 ml of conc. H₂SO₄ was added rapidly, accompanied with gentle agitation during the addition of the acid. These were allowed to stand in a water bath at 26-30°C for 20 min before taking the optical densities (ODs) of the yellow-orange colours thus developed at 490 nm in a spectrophotometer after setting for 100% transmission against the blank (which was prepared by substituting distilled water for the sugar solution). Five replicates in each were run and their mean values were calculated. A regression curve was computed between its known concentration and the respective OD, which followed Beer's Law. The concentration values of the total soluble sugar in the test samples was directly worked out from the regression curve of the standard glucose. Five replicates of each experimental sample were taken and their mean values were recorded. The sugar content in terms of glucose equivalent and the use of conversion factor 0.9 to convert the values of glucose to starch was made in each case.

4. ASCORBIC ACID

Extraction procedure

Each of the fresh experiment materials (400 mg) was homogenize thoroughly with 10 ml of acetate buffer (pH 4.8) and centrifuged (12000 rpm, 20 min.). The supernatants were separately collected, out of which 1 ml was measured to other test tube, 4 ml of 4% trichloroacetic acid (TCA) was added, left overnight and later, centrifuged (**Roe and Kuenthar, 1943**). To the supernatant of each sample, 1 ml of the colour reagent (prepared by mixing 90 ml of 2.2%, 2,4-dinitrophenylhydrazine in 10N H₂SO₄, 5 ml of 5% theorem and 5 ml of 0.6% CuSO₄ solution), was added and incubated at 57°C for 45 min. Later, on cooling 7 ml of 65% H₂SO₄ was added to each test tube and cooled again.

Quantification

The stock solution of ascorbic acid (10 mg/100 ml in 4% TCA), varied concentrations (0.01 to 0.09 mg/ml) were prepared in different test tube. The volume of each was raised to 5 ml by adding 4% TCA solution and left overnight at the room temperature. To these, 1 ml of the colour. Later, to each of these 7 ml of 65% H₂SO₄ was added, brought to the room temperature and the ODs were measured at 540 nm in a spectrophotometer against a blank. A regression curve was computed between the main optical density and the concentration of standard ascorbic acid which followed Beer's Law.

5. PROTEINS

Extraction Procedure

Each of the dried test samples (60 mg) was measured in 10 ml of cold 10% TCA solution (30 min), kept at 4°C overnight and centrifuged. The supernatants were discarded in each case and the resultant pellet of each was re-suspended in 10 ml of 5% TCA solution and heated at 80°C in a water bath for 30 min. These samples were cooled, re-centrifuged and the supernatant so obtained were discarded each time. The pellet was then washed with distilled water and centrifuged. Each of the residues left after the centrifugation was dissolved in 10 ml of 1 N NaOH and left overnight at room temperature (**Osborne, 1962**).

Quantification

Using 1 ml aliquot of extract, total phenol contents were estimated following the method of (Lowry *et al.*, 1951).

A stock solution of Bovine serum albumin (BSA; Sigma chemical Co., St. Louis, USA) was prepared in 1N NaOH (1 mg/ml), out of which 0.1 to 0.8 ml of the solution was separately pipetted in the test tubes and the volume in each case was raised to 1 ml by adding distilled water. To each, 5 ml of the alkaline solution (prepared freshly by mixing 50 ml of 2% Na₂CO₃ in 0.1 N NaOH and 1 ml of 0.5% CuSO₄.5H₂O in sodium potassium tartarate) was added and kept at room temperature (10 ml). Later, to each of these tubes 0.5 ml of Folin-Ciocalteu reagent (CSIR Centre for Biochemical's, Delhi, India; diluted with equal volume of distilled water, just before use) was added rapidly with immediate mixing and after 30 min, the ODs were measured at 750 nm of using a spectrophotometer against the appropriate blank. Five replicates of each concentration were taken and their mean values plotted against their respective concentration to compute a regression curve.

All the test samples were similarly processed as above and the level of total proteins individually was calculated by referring the ODs of the test sample with the standard curve (BSA). Five replicates were examined in each case and the mean values were recorded.

6. PHENOLS

Extraction Procedure

Each of the deproteinized test samples (200 mg) was homogenized in 10 ml of 80% ethanol for 2 hrs. And left overnight at the room temperature. Each of these was centrifuged and the supernatant was collected separately, the volume of which was raised 40 ml with 80% ethanol in each case.

Quantification

Total phenols were quantified in each test sample, following the protocol of (Bray and Thorpe, 1954) which included the preparation of a regression curve of standard phenol (Caffeic acid). A stock solution of the standard phenol (Caffeic acid) was prepared in 80% ethanol (400 mg/ml) out of which 0.1 to 0.9 ml was taken into separate test tubes and the volume of each was raised to 1 ml with 80% ethanol. To each tube, 1 ml Folin-Ciocalteu reagent (diluted with distilled Water in 1:2 ratio just before use) was added followed by 2 ml of 20% Na₂CO₃ solution and this mixture was shaken vigorously. Such samples were placed in a boiling water bath for exactly 5 min and later, cooled under 3 times running tap water. Each of the reaction mixture was diluted to 25 ml with distilled water and ODs were taken at 750 nm against a blank using a spectrophotometer. Five replicates were taken for each concentration and the average OD was plotted against the respective concentration to compute a regression curve which followed the Beer's Law. Similarly various experimental plant samples were processed and the ODs were measured. From the mean values, total levels of phenols were calculated (with reference to caffeic acid) by referring the ODs experimental samples with the standard regression curve.

7. PHOTOSYNTHETIC PIGMENTS (CHLOROPHYLLS AND CAROTENOIDS)

Extraction Procedure

The fresh experimental materials (1 g each) were homogenized in 40 ml of 80% acetone, to which a pinch of NaHCO₃ was added to prevent any pheophytin formation (Sunderland, 1966). This extraction was carried out in the dim light conditions to avoid any photo bleaching (Holden, 1976). Each sample was centrifuged and the supernatant was collected separately. For the complete extraction of pigments, this step was repeated thrice and the supernatants of each were pooled separately. For the volume of which was raised to 80% acetone individually.

Quantification

The ODs of each of the, above extracts were recorded at 652, 663 and 480 nm with a spectrophotometer against 80% acetone as the blank. Five such replicates at each wavelength were chlorophyll a band the total present in the samples were calculated in mg/g of plant material from the equations derived by (Arnon, 1949).

8. RESULT AND DISCUSSION

Plant are the source of many bioactive compounds containing many primary metabolites like Carbohydrates (Starch, Sugar), Protein, Phenols, Ascorbic acid.etc... are useful for flavoring, fragrances, insecticides, sweeteners and natural dyes (Kaufman *et al.*,1999). During the present work root, stem, and leaf of the plant *Eupatorium triplinerve* Vahl, were evaluated quantitatively for the analysis of Chlorophyll, Sugar, Starch, Protein, ascorbic acid, and Phenol. Keeping in view the important of these primary metabolites, the present studies for biochemical evaluation of primary metabolites from different parts of *Eupatorium triplinerve* Vahl were taken.

In the present investigation it was observed that maximum amount of chlorophyll a, chlorophyll b, and carotenoids is found in leaf and minimum roots of *Eupatorium triplinerve* Vahl respectively. During the present investigation it was observed that maximum sugar is found in leaf and minimum in roots.

Starch is biodegradable and renewable in nature. They are increasingly being considered as an ecofriendly alternative to the use of synthetic additives. During the present research studies, leaf of *Eupatorium triplinerve* showed higher amount of starch whereas root and stem contain lower amount of starch content.

The proteins are the primary components of living beings. The presence of higher protein level in the plant points towards their possible increase in nutritive value or that a protein base bio active compound could also be isolated in future. During the present studies, stem contain maximum amount of protein followed by leaf and stem. Similar result showing maximum protein content in the stem part was also observed in *Eupatorium triplinerve* Vahl.

The biochemistry and medicine are intimately related to health depend on harmonious balance of biochemical reaction occurring in the body, disease reflects abnormalities in bimolecular in biochemical reaction. Phenol has many modulating anti-tumors; antibacterial activities in the present studies were evaluated for total phenol content. It was observed the leaf contents maximum amount of phenol content, however in contrast to this stem content higher level of phenol content has antibacterial and anti-inflammatory activities.

Ascorbic acid (Vitamin C) is a familiar molecule because of its dietary significance, it is not only an important antioxidant, but it also appears to be linked during flowering time, developmental senescence, programmed cell death and responses to pathogens through a complex signal transduction network. During the present research investigation ascorbic acid was found maximum in leaf and minimum in stem of the species. Similar result showing higher amount of ascorbic acid content in leaf. Primary metabolite analysis is necessary for knowing role as precursors of plants and their role as precursors for the synthesis of secondary metabolites.

Table 1. Biochemical analysis of *Eupatorium triplinerve* Vahl

S. No	Experiment	Root	Leaf	Stem
1	Chlorophyll –a	0.00gm /gdm	4.95 gm/gdm	2.26 gm/gdm
2	Chlorophyll –b	0.00gm /gdm	4.05gm /gdm	1.03gm /gdm
3	Carotenoids	0.26 gm /gdm	3.92gm /gdm	2.63gm /gdm
4	Starch	3.15gm /gdm	5.86gm /gdm	4.03gm /gdm
5	Protein	60.04µg/ mg	67.82 µg/ mg	71.53 µg/ mg
6	Ascorbic Acid	43.09 µg/ mg	41.13 µg/ mg	37.19 µg/ mg
7	Total Phenol	65.13 µg/ mg	84.00 µg/ mg	80.05 µg/ mg

Figure: 1

9. CONCLUSION

Medicinal plant is most exclusive source of many drugs for the majority of the world population. Laboratory evaluations were made to assess the study of primary metabolites of various plants in selected species *Eupatorium triplinerve* Vahl. During the present investigation comparative study of primary metabolites Carbohydrates, Protein, Ascorbic acid, Starch, Sugar, Chlorophyll, Carotenoids and Total phenol in genus *Eupatorium triplinerve* were undertaken for study and all the their chemical composition.

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