

Comparative quantification of primary metabolites of some latex bearing plants

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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 comparative study of primary metabolites of some latex bearing plants of Apocynaceae. Amount of soluble sugar is found higher in leaves of *Nerium indicum* Mill and *Cryptostegia grandiflora* R.Br. and also in Flower of *Nerium indicum* Mill, as compared to other plants parts. Starch is found in leaves of *N. indicum* Mill. Protein is found in leaves of *Tabernaemontana divericata*, lipids in roots of *Asclepias curassavica* and *C. grandiflora* R.Br. and phenol is seen in stem and root of *N. indicum* Mill.

Key words: Medicinal plants, Apocynaceae, primary metabolites, carbohydrates, starch, phenols and proteins etc.

INTRODUCTION

It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). A relatively small percentage (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996). Hippocrates (in the late fifth century B.C.) mentioned 300 to 400 medicinal plants (Schultes, 1978). A number of chemical constituents including phenols and sterols from the leaves, stems, and roots of *Tabernaemontana divericata* have previously been reported (Atta-ur-Rahman, *et al.*, 1986, Kam, *et al.*, 1992). Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. The molecules are known to play a major role in the adaptation of plant to their environment, but also represent an important source of pharmaceuticals (Ramchandra and Ravishankar, 2002). All of these latex bearing plants those have bioactive compounds against microbes are widely grown for

its ornamental value in frost free area around world. In traditional Chinese medicine the flower and leaves of *N. indicum* have been used to stimulate cardiac muscles, relieve pain and eliminate blood stasis. (Jangsu, 1985) Immunological active pectic polysaccharides have been isolated from *N. indicum* (Muller *et al.*, 1991). Characterization of polysaccharides from the flower of *N. indicum* and their Nuro-protective effects (Man-sham *et al.* 2004)

Tabernaemontana divericata R.Br is a glabrous, evergreen, dichotomously branched shrub, belonging to the family Apocynaceae. It is distributed in upper Gangetic plain, Garhwal, East Bengal, Assam, Karnataka, Kerala, and in Burma. In Ayurveda, the root is acrid; bitter with a flavour; digestible; useful in kapha, biliousness, and diseases of the blood. The root has a bitter bad taste. It is aphrodisiac; tonic, especially to the brain, liver, and spleen; and purgative. The milky juice mixed with oil is rubbed onto the head to cure pain in the eye; it kills intestinal worms, and its root, chewed, relieves toothache when rubbed with water; it is applied to

wounds to prevent inflammation (Kirtikar and Basu 1987).

Nerium indicum

Nerium indicum is a white – flower oleander growing in china. Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population.. It is an erect, smooth shrub, 1.5 to 3 meters high with a cream-colored sticky resinous juice. Leaves are in whorls of 3 or 4, linear-lanceolate, 10-15 cm long, with numerous horizontal nerves. Flowers are showy, sweet-scented, single or double, 4-5 cm in diameter, white, pink or red, borne in terminal inflorescence (cymes). Fruit is cylindrical, paired, with deep linear striations, 1.5-2.0 cm long. Seeds are numerous and compressed, with a tuft of fine, shining, white, silky hairs. Laboratory evaluations were made to asses the study of primary metabolites of *Nerium indicum* Mill. It contains soluble sugar and starch in leaves, protein in leaves and flower, lipids in flower and phenol in stem and root showed higher concentration as compared to other parts of the plant.(Vijayvrgia and Kumar 2007) *Asclepias curassavica* has been used for bronchitis, pumonits, influenzaand specially for pleuristy (Jonne *et al.*, 2002)

MATERIAL AND METHOD

Estimation of carbohydrates

Total soluble sugars

The dried and milled test sample 50 mg each was macerated in a grinder with 20 ml of ethanol and left for 12 hrs. each of the mixtures was centrifuged (1200 rpm) for 15 min, the supernatants were removed and each was concentrated on a water-bath. The volume of these aqueous concentrates was raised to 50 ml with distilled water (Ext.A) and processed further following the method of Loomis and Shull (1937) for soluble sugars. However the residual pellet obtained by centrifugation was used for the estimation of starch.

Starch

The above Residue of each test sample was suspended in a mixture of 5 ml of 52% perchloric acid solution and 6.5 ml of distilled water, shaken vigorously (5 min) and centrifuged (2500 rpm). This step was repeated (3x) and the

supernatants of each sample were pooled and the volume was raised to 100 ml with distilled water (Ext B). out of this (Ext. B), 1ml aliquot was taken separately to estimate starch quantitatively (McCready *et al.*, 1950).

Quantification of carbohydrates

1ml of aliquot of each of the test sample from Ext. A and B were used to quantifying the total levels of carbohydrates using phenols-sulphuric acid method (Dubois *et al.*, 1951), a regression curve for standard sugar (glucose) was also prepared.

A stock solution of glucose (100 µg/ml) was prepared in distilled water, out of which 0.1 to 0.9ml was transferred to test tube and the volume of each was raised to 1ml with distilled water. To each of these, 1 ml of 5% aqueous phenol was added rapidly having kept in an ice chest and shaken gently. Later 5ml of conc. H₂SO₄ was rapidly added in agitated gently during the addition of the acid subsequently, the tube was kept on a water-bath (26 – 30°C) for 20min, and the optical density (ODs) of the yellow orange colors thus developed were taken at 490 nm in a Spectrophotometer after having set it for 100% transmission against the blank. Four replicates of each sample were run and there mean values were calculated. A regression curve was computed between its known concentrations and their respective ODs. Which followed the Beer's Law. The concentration (mg/gdw) of the total soluble sugars was directly worked out from the regression curve of the standard glucose. Four replicates of each experimental sample were taken and their mean values 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.

Extraction of proteins

60 mg of the dried test sample was macerated (Osbrone, 1962) in 10 ml of cold TCA (10%) for 30 min kept at low temperature 4úC for 24 h and then centrifuged. Each of the supernatants was discarded and the resultant pellet was re-suspended in 5% TCA (10 ml) and heated on a water bath 80úC for 30 min. Each of these samples was cooled, re-centrifuged and each time the supernatant discarded, Later the pellet was washed with distilled water, centrifuged and each of the

residues was dissolved in 1N NaOH (10ml) and left overnight at room temperature.

Quantification of Proteins

In each of 1 ml extracts, total protein contents were estimated using the protocol of Lowry *et al.* (1951). A stock solution (1mg/ml) of bovine serum albumin (BSA; Sigma Chemicals) was prepared in 1 N NaOH, from which 0.1 to 0.9 ml of the solution was dispensed separately in the test tube. Later, the volume of each was raised to 1 ml by adding distilled water. To each test sample, 5ml of freshly prepared alkaline solution (prepared by mixing 50ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1% sodium potassium tartrate) was added at room temperature and left undisturbed for 10 min. Subsequently, to each of these mixture tubes 0.5 ml of folin-ciocalteau reagent (CSIR centre for Bio-chemicals, Delhi: diluted with equal volume of distilled water just before use) was rapidly added and after $\frac{1}{2}$ h, the OD of each was measured at 750 nm using a spectrophotometer against the blank. Three replicates of each concentration were taken and their mean values were used to compute a regression curve. The total protein contents in each sample were calculated by referring the ODs of test sample with the standard curve of BSA. Three replicates were examined in each case and their mean values were recorded.

Extraction of Lipids

1 g of each of the dried and milled test sample was macerated with 10 ml distilled water (Jayaraman., 1981). To this, 30 ml of chloroform-methanol (2:1, v/v) was added and mixed thoroughly. Each mixture was left overnight at room temperature, 20 ml of chloroform and the equal volume of distilled water was added and centrifuged. Out of the three layers. A clear lower layer of chloroform containing all lipids was collected in pre-weighed beaker, the solvent evaporated completely and weighed. Which was taken as the weight of total lipids/g of the dried tissue sample.

Extraction of Phenols

Each of 200 mg dried and milled test samples was homogenized in 80% ethanol (10 ml) for 2 hrs and left over night at room temperature. Later, each was centrifuged, the supernatants were

collected individually and the volume of each was raised to 40 ml with 80% ethanol.

Quantification of Phenols

To estimate total phenols in each of the test sample, the protocol (Bray and Thorpe., 1954) was followed, wherein a standard curve of caffeic acid (a phenol) was prepared. A stock solution (40 mg/ml) of caffeic acid was prepared in 80% ethanol, from which 0.1 to 0.9 ml was transferred into test-tubes separately and the volume in each case was raised to 1 ml with 80% ethanol. To each of these tubes, 1 ml of folin-ciocalteau reagent (prepared by diluting the reagent with distilled water in 1:2 ratio just before use) accompanied by 2 ml of 20% Na_2CO_3 solution was added and the mixture was shaken vigorously. Each of these were boiled on a water bath (1 min), cooled and diluted to 25 ml with distilled water. The OD was taken at 750 nm using a spectrophotometer against a blank. Three such replicates were taken for each concentration and the average OD was plotted against the respective concentration to compute a regression curve.

Each of the test samples was processed in this manner, ODs were measured and the total level of phenols was calculated from the mean values (with reference to caffeic acid) by referring the OD of the test sample with the regression curve of the standard.

RESULTS

Latex Bearing plants were evaluated quantitatively for the total levels of soluble sugars, starch, proteins, lipids and phenols by described methods. When we studied about total levels of soluble sugar we found that it is higher in leaves and flower of *N. indicum* and *T. diversicata's* leaves (4 mg/gdw), in comparison to other plants parts (Fig. 1.). Total levels of starch is found to be higher in *N. indicum's* leaves (6 mg/gdw), as compared to *C. grandiflora*, *A. Curassavica* and *T. diversicata's* root, stem and flower (Fig. 2). Total levels of proteins were found to be higher in *T. diversicata's* leaves and flowers (70 mg/gdw), in comparison to other plants parts (Fig. 3). Total levels of lipids were found to be higher in *A. curassavica* and *C. grandiflora's* root (80 mg/gdw), in comparison to other plants parts (Fig. 4). Total levels of phenols were found to be

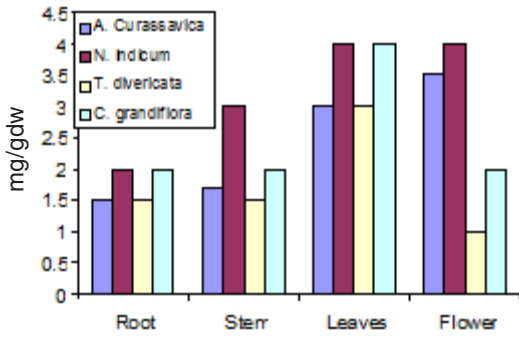


Fig. 1. Soluble sugar concentration (mg/gdw)

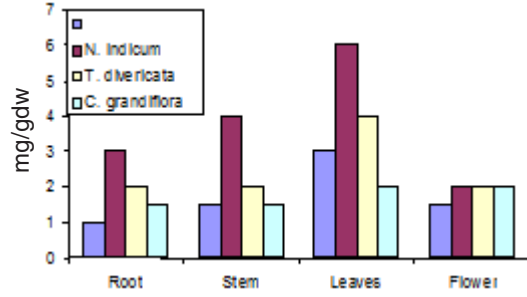


Fig. 2. starch concentration (mg/gdw)

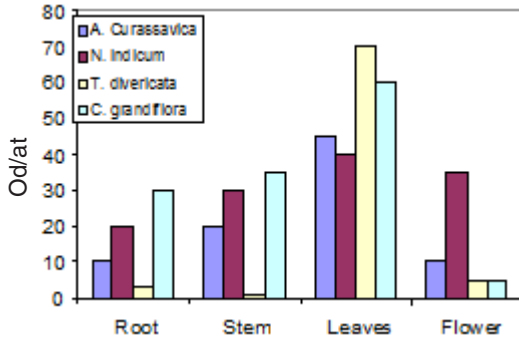


Fig. 3. Protein concentration (mg/gdw)

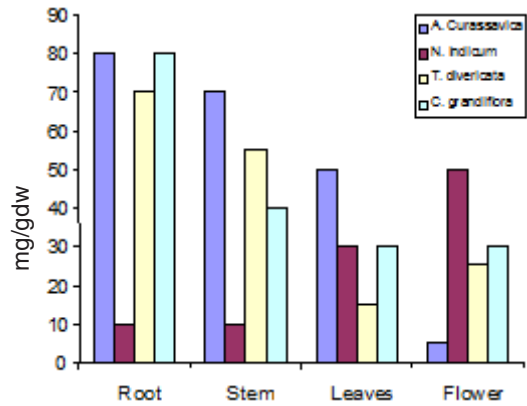


Fig. 4. Lipid (mg/gdw)

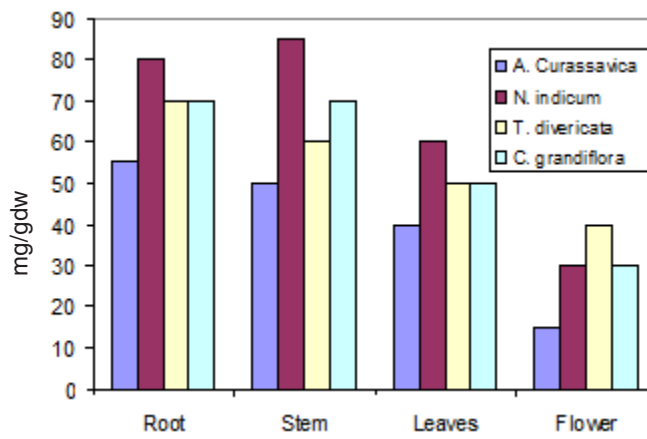


Fig. 5. Phenol concentration (mg/gdw)

higher in *N. indicum's* stem (85 mg/gdw), as compared to other plants root, leaves and flower (Fig. 5).

DISCUSSION

We quantify that Some latex bearing plants (Apocynaceae) contain many primary metabolites like carbohydrates, proteins, phenols, lipids etc. Level of soluble sugar were found to be higher in leaves of *N. indicum* and *C. grandiflora* and Flower of *N. indicum*, as compared to other plants parts and level of Starch is higher in leaves of *N. indicum* Mill, Sugar has large numbers of stereo-isomers because they contain several asymmetric carbon atoms. (Lindhorst and Thisbe, 2003). Total levels of phenols were found to be higher in *N. indicum's* stem (85 mg/gdw), as compared to

other plants parts and Phenol have immunomodulating, anti-tumor and antibacterial activities (Wong *et al.*, 1994) Total levels of proteins were found to be higher in *T. diversicata's* leaves and flowers (70 mg/gdw), in comparison to other plants root, stem, leaves and flower.

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