Quantification of Primary Metabolites of *Nerium indicum* Mill

Rekha Vijayvergia* and Jitendra Kumar
Plant-Biochemistry and Biotechnology Lab.,
Department of Botany,
University of Rajasthan, Jaipur (INDIA).

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 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.

Keywords : Medicinal plants, Primary metabolites, *Nerium indicums* Mill, Carbohydrates; Starch, Phenols, Proteins.

Introduction :

In recent years, traditional system of medicine has become a topic of global importance. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. It has been mentioned that natural habitats for medicinal plants are disappearing fast and together with environmental and geopolitical instabilities; it is increasingly difficult to acquire plant derived compounds. This has prompted industries, as well as scientists to consider the possibilities of investigation into cell cultures as an alternative supply for the production of plant pharmaceuticals. 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). Latex bearing plants have been widely grown for its ornamental value in frost free area around the 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* have been made for their neuro-protective effects (Man-sham *et al*., 2004).

Nerium indicum 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.

Materials and Method :
Estimation of carbohydrates :
(A) Total soluble sugars : The dried and milled test sample 50 mg each was

* **Corresponding author :** Rekha Vijayvergia, Plant-Biochemistry and Biotechnology Lab., Department of Botany, University of Rajasthan, Jaipur (INDIA).

macerated in a grinder with 20 ml of ethanol and left for 12 hrs. and mixtures was centrifuged (1200 rpm) for 15 min, the supernatants were removed and was concentrated on a water-bath. The volume of these aqueous concentrates were raised to 50 ml with distilled water (Ext. A) and processed further by 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.

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

Quantification of carbohydrates : Aliquot (1 ml) 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 mg/ml) was prepared in distilled water, out of which 0.1 to 0.9 ml was transferred to test tube and the volume was raised to 1 ml 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 5 ml of Conc. H_2SO_4 was rapidly added by agitating gently during the addition of the acid subsequently, the tube was kept on a water-bath ($26° – 30°$ C) for 20 min, and the optical density (ODs) of the yellow orange colours 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 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 : A 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 $4°$ C for 24 hr 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 at $80°$ C 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 (10 ml) and left overnight at room temperature.

Quantification of Proteins : In each of 1 ml extract, total protein content were estimated using the protocol of Lowry *et al.*, 1951. A stock solution (1mg/ml) of bovine serum albumin (Sigma Chemicals) was prepared in 1 N NaOH, from which

0.1 to 0.9 ml of the solution was dispensed separately in a test tube. After this, 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 50 ml of 2% Na_2CO_3 in 0.1 N 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 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocaltcau reagent (CSIR centre for Bio-chemicals, Delhi: diluted with equal volume of distilled water just before use) was rapidly added and after half an hr, the OD of each was measured at 750 nm using a spectrophotometer against the blank. Three replicates of each concentration were taken and there mean values were used to compute a regression curve. The total protein contents in each sample was 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 : One 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-weighted 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. It 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 of 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 test sample was processed in this similar 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 :

N. indicum were evaluated quantitatively for the total levels of soluble sugars, starch, proteins, lipids and phenols by described methods.

Total levels of soluble sugars were found to be higher in *N. indicum* leaves and flowers (4 mg/gdw), as compared to its root and stem. (Fig. 1).

Total levels of starch were found to be higher in *N. indicum* leaves (6 mg/gdw), as compared to its root, stem and flower (Fig. 2).

Total levels of proteins were found to be higher in *N. indicum* leaves and flowers (40 mg/gdw), as compared to its root, stem and flower (Fig. 3).

Total levels of lipids were found to be higher in *N. indicum* flowers (50 mg/gdw), as compared to its root, stem and leaves (Fig. 4).

Total levels of phenols were found to be higher in *N. indicum* stem (85 mg/gdw), as compared to its root, leaves and flower (Fig. 5).

Discussion :

We quantify that *N. indicum* contain many primary metabolites like carbohydrates, proteins, phenols, lipids, etc. Numerous polysaccharides purified from Chinese medicinal herbs are bioactive and possesses immuno-modulating, anti-tumour and antibacterial activities (Wong *et al*., 1994).

Leaf and flower of *N. indicum* contain higher soluble sugar as compared to other plant parts in sufficient amount. Sugar has large numbers of stereo-isomers, because

they contain several asymmetric carbon atoms. (Lindhorst and Thisbe, 2003). *N. indicum*'s stem contain higher level of phenol and phenol have immuno-modulating, anti-tumour and antibacterial activities (Wong *et al*., 1994). Leaf and flower of *N. indicum* contain higher soluble sugar as compared to other plant parts in sufficient amount. Sugar has large number of stereo-isomers, because they contain several asymmetric carbon atoms (Lindhorst and Thisbe, 2003). The stem *N. indicum* contain higher level of phenol and phenol have antibacterial and anti-inflammatory activities. Therefore, biochemistry and medicine are intimately related health depends on harmonious balance of biochemical reaction occurring in the body, disease reflects abnormalities in biomolecules in biochemical reaction. (Robert, 2006). The *N. indicum* flower contain higher level of lipids. Lipids are insoluble in water the problem how to transport them in a aqueous blood plasma is solved by associating nonpolar lipids with amphipathic lipids and proteins to make water miscible lipoproteins (Kathleen, 2006).
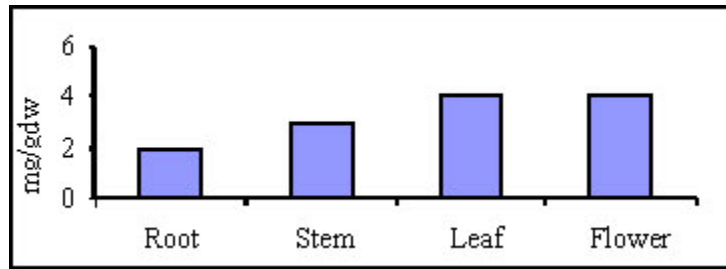
Fig. 1 : Solouble Sugar concentration mg/gdw of Nerium indicum
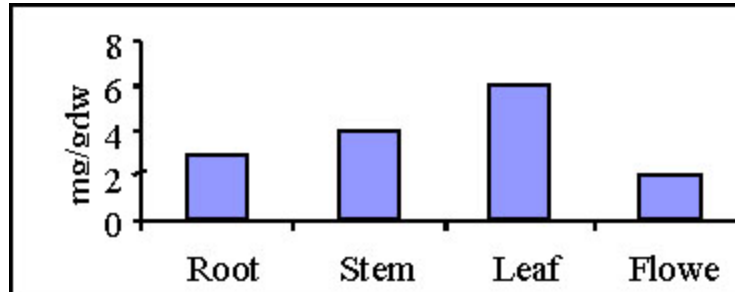

Fig. 2 : Starch concentration mg/gdw of Nerium indicum
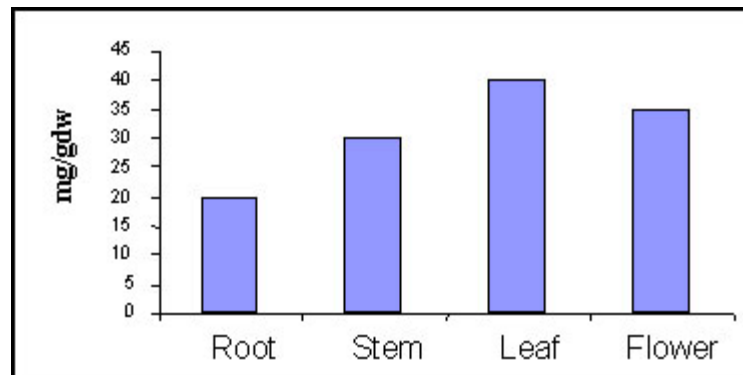

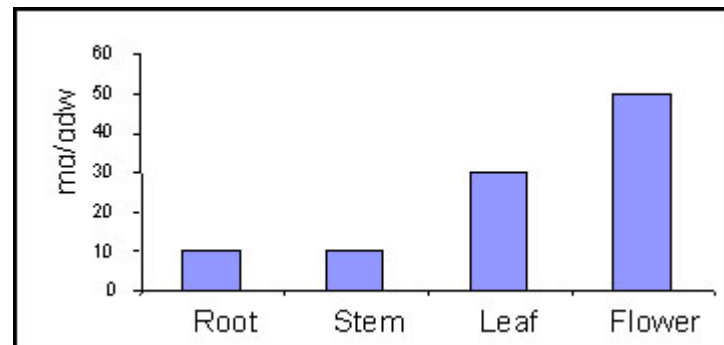**Fig. 3 : Protein concentration mg/gdw of Nerium indicum**

Fig. 4 : Lipid concentration mg/gdw of Nerium indicum
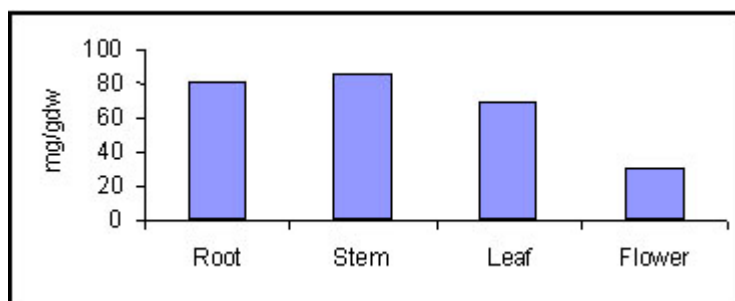
127

Fig. 5 : Phenol concentration mg/gdw of Nerium indicum

References :

Bray H.G. and Thorpe W.V. (1954) : Analysis of Phenolic compounds of interest in metabolism. *Meth. Biochem. Anal.* **1,** 27-52.

Dubois M., Gilles K., Hamilton J.K., Rebers P.A., and Smith F. (1951) : A colorimetric method for the determination of sugar. *Nature* **168,** 167.

Jangsu New Medical College (1985) : In: The Dictionary of Chinese Herbal Medicine. P 867.

Jayaraman J. (1981) : Laboratory Manual in Biochemistry. New Delhi : Wiley Eastern Limited.

Kathleen M. B. (2006) : Harper's Illustrated Biochemistry, London : Mc Graw Hill.

Lindhorst T.K. and Thisbe K. (2003) : Essentials of Carbohydrate Chemistry and Biochemistry. New Delhi : Wiley Eastern Ltd.

Loomis W.E. and Shull C.A. (1937) : Methods in Plants Physiology, New York : McGraw Hill Book Co.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951) : Protein measurement with the Folin-Phenol Reagent. *J. Biol. Chem.* **193,** 265–275.

Man-sham Yu, Sau-wan L., Kim-Fung L., Ji-Nian F., Wai-Hung Y. and Raymond C.C.C. (2004) : Characterization of polysaccharides from the flower of *Nerium indicum* and their neuroprotective effects. "*Int. J. Mol. Med.* **14,** 917-924.

McCready R.M., Guggoiz J., Silviera V. and Owens H.S. (1950) : Determination of starch and amylase in vegetables. *Anal. Chem.* **22,** 1156-1158.

Muller B.M., Rosskopf F., Paper D. H., Kraus J. and Franz G. (1991) : Polysaccharides from *Nerium oleander:* Structure and biological activity. *Pharmazie* **46,** 657-663.

Osborne D.J. (1962) : Effects of Kinetin on protein and nuclic acid metabolism in *Xanthium* leaves during senescence. *Plant Physiol.* **37,** 595-602.

Ramchandra R. S. and Ravishankar G.A. (2002) : Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol. Adv.,* **20,** 101-153.

Robert K.M. (2006) : Biochemistry and Medicine Harper's. Illustrated Biochemistry. Mc Graw Hill.

Wong C.K., Leung K.N., Fung K.P. and choy Y.M. (1994) : Immuno-modulatory and anti-tumor polysaccharides from medicinal plants. *J. Int. Med. Res.* **22,** 299-311.