Research Article

In vivo and *In vitro* Comparative Study of Primary Metabolites of *Citrullus colocynthis* (linn.) Schrad

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ABSTRACT

One of very important medicinal herb *Citrullus colocynthis* belonging to family Cucurbitaceae has also become threatened due to overexploitation. It is commonly known as Bitter apple. All plant parts are used in Ayurvedic system of Indian medicine. In the present set of investigations, biochemical changes of various cellular metabolites/enzymes viz. protein, peroxidase activity and ascorbic acid were observed from *in vivo* (leaf, stem, fruit and root) and *in vitro* (callus and differentiating callus) of *Citrullus colocynthis.* The callus obtained by the culture of nodal stem segment of the plant on MS medium supplemented with BAP (2.0 mg/l) and NAA (2.0 mg/l) and differentiating callus achieved on MS medium supplemented with BAP (1.0 mg/l) and Kn (1.0 mg/l) in tissue culture experiment, was used for present biochemical analyses. Maximum amount of protein was observed in leaf samples and minimum amount was observed in differentiating callus. Higher peroxidase activity was observed in callus and minimum in leaf sample. Maximum amount of ascorbic acid was observed in differentiating callus and minimum amount was recorded in root sample. It further concludes that *Citrullus colocynthis* serves as a rich source of primary metabolites which can be used as raw material in industries.

Keywords: Ascorbic acid, *Citrullus colocynthis,* Primary metabolites, Protein, Peroxidase.

INTRODUCTION

The World Health Organization (WHO) estimated that 80% of the population of developing countries depend on production and utilization of traditional medicine. The drugs obtained from medicinal plants are known to have many advantages than synthetic drugs. The important advantages are, being natural they are non-necrotic, having no side effects, of a range of safe, cost effective preventive and curative agents for many diseases.

Plants are efficient chemical factories that produce a wide variety of chemical compounds called phytochemicals. Two types of metabolites are produced from plants i.e. primary metabolites viz., carbohydrates, proteins, phenols, lipids etc. and secondary metabolites viz., alkaloids, flavonoids, sterols etc. Research on primary and secondary metabolites provide evidences for producing commercially and medicinally important plant products. Primary metabolites are substances widely distributed in nature, occurring in one form or another in virtually all organisms. Primary metabolites are mainly used as industrial raw materials, food or food additives. Many plants such as *Clitoria ternatea*[1], *Phyla nodiflora*[2], *Jatropha curcas*[3], *Sesamum indicum*[4], *Terminalia arjuna*[5], *Cassia nodosa*[6], *Digera muricata*[7], *Withania Somnifera*[8], *Nerium indicum*[9], *Alangium salviifolium*[10], *Pongamia pinnata*[11] have been evaluated for their composition of primary metabolites.

Citrullus colocynthis is an important medicinal herb belonging to family Cucurbitaceae. It is commonly known as 'Indrayan'. It is a xerophytic plant common in sandy soils and often found on the slopes in gregarious patches. It is found in warmer climate and tropical areas. Roots are perennial, fibrous, tough and stringy, of a yellowish-white color with a broad crown. Stem is slender, tough, angular, branched, greyish, prostrate, very rough with numerous short scabrous hairs. Leaves are alternate, on long hispid petioles which are about as long as the slender, slightly branched, lateral tendrils. Fruits are Globose or ellipsoid, smooth, fleshy indehiscent, 5-7.5 cm diameter, variegated green and white, glabrous ripe, filled with a dry spongy pulp, epicarp thin

Citrullus colocynthis (Linn.) Schrad is an important medicinal plant belonging to family Cucurbitaceae. It is also known as Indrayan or bitter apple. *C. colocynthis* is used in folk medicine by people in rural areas a purgative, antirheumatic, anthelmintic and as remedy for skin infection. In addition to above medicinal properties the root has a beneficial action in opthalia, uterine pains, jaundice and also useful against boils, pimples and enlarged abdomen. Seeds are purgative and leaves are used in migraine and neuralgia. Pulp of fruit has antibacterial activity. The dried and powdered pulp taken orally to cause abortion. It is a marvel desertic plant that could be exploited as to supply a specific product in demand nationally and internationally, oil for cooking, industries and in automobiles as a source of biodiesel, seeds for human and animal consumption, cucurbitacins

in medicinal and agropesticidal industries, oilcakes for animal feed and as a source of nutrient supplement in agriculture. *Citrullus colocynthis* is an important sand-dune stabilizer, found throughout the desert spreading on the loose sand. However, in the last nearly 15 years ever since its seeds have been collected for oil, it has become a threatened plant in the desert.[12] The present study was conducted to investigate biochemical estimation of primary metabolites viz. Protein, Peroxidase activity and Ascorbic acid of *Citrullus colocynthis*.

MATERIALS AND METHODS

In the present set of investigations, biochemical changes of various cellular metabolites/enzymes viz. protein, peroxidase and ascorbic acid were observed from *in vivo* (leaf, stem, fruit and root) and *in vitro* (callus and differentiating callus) of *Citrullus colocynthis*. The callus obtained by the culture of nodal stem segment of the plant on MS medium supplemented with BAP (2.0 mg/l) and NAA (2.0 mg/l) and differentiating callus achieved on MS medium supplemented with BAP (1.0 mg/l) and Kn (1.0 mg/l) in tissue culture experiment, was used for present biochemical analyses.

Estimation of protein

During the present studies, protein contents were estimated by Lowry's method (1951).[13]

Protein reacts with folin ciocalteau reagent to give a colored complex. This colour is produced by the reduction of phosphomolybdate by tyrosine and tryptophan of protein by the action of alkaline copper. The intensity of colour depends upon the amount of these aromatic amino acids and thus will vary for different proteins.

500 mg of fresh both *in vivo* and *in vitro* tissues were ground with 10.0 ml of 5% trichloro acetic acid (TCA), using a mortar and pestle. The homogenate was centrifuged at 2000 rpm for 20 minutes and the supernatant was discarded. The residue was dissolved in 5 ml of 0.1 N NaOH. 0.1 ml of this solution was made up to 1.0 ml with distilled water. 5.0 ml of the alkaline copper reagent was added to the dissolved residue and allowed to stand for 10 minutes. 0.5 ml of folin-ciocalteau reagent was added rapidly and mixed immediately. The optical density was measured after 10 minutes at 750 nm in a spectrophotometer (UV-Vis systronic spectrophotometer) against blank. Blank was prepared by adding all reagents except plant material. The amount of protein in the samples was calculated from a standard curve prepared from bovine serum albumin.

Estimation of Peroxidase activity

Peroxidase activity was determined by the method given in Worthington Enzyme Manual (1972).[14]

500 mg (fresh weight) of both *in vivo* and *in vitro* tissues were homogenized in 10.0 ml chilled 0.2 M phosphate buffer (pH = 6.1). The homogenate was centrifuged at 5000 rpm in a refrigerated centrifuge for 15 min at 10°C. Enzyme extract (supernatant) thus prepared was assayed for peroxidase. 2.7 ml of 0.2 M phosphate buffer (pH = 6.1), 0.1 ml of 1mM H_2O_2, 0.5 ml of enzyme solution were added in a cuvette. The absorbance was calibrated to zero. To the mixture 0.1 ml of 2.0 mM o-dianisidine was added and mixed quickly. The absorbance was recorded at 460 nm at 15 second intervals for 10 min. The enzyme activity was expressed in units (change in O.D.)/min/g fresh weight of tissue.

Estimation of Ascorbic Acid

Ascorbic acid was estimated by Jensen's (1962)[15] method.

Both *in vivo* and *in vitro* tissues were homogenized separately with a mortar and pestle in 2% meta phosphoric acid (500 mg sample in 5 ml of meta phosphoric acid) and allowed to macerate for one hour. These were then centrifuged separately at low speed (2500 rpm) for fifteen minutes, the residues were discarded and the supernatants were used.

Standard solutions of ascorbic acid of various concentrations were prepared (0.1 mg/ml to 0.9 mg/ml) in distilled water. Each of the 1 ml of the standard as well as test solutions were mixed with 2 ml of 5% metaphosphoric acid and kept for 30 minutes without stirring at room temperature. To each of these, 5 ml of n-amyl alcohol and 3.2 ml dye (5 mg/100 ml, 2,6-dichlorophenol indo-phenol) were added. Two layers were formed. It was 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 to wave length of 546 nm and set at 100% transmittance using a mixture of 1 ml of extract, 2 ml of 5% metaphosphoric acid, 5 ml of n-amyl alcohol and 3.2 ml distilled water (blank solution) before taking the readings of the standard and the test samples. A regression curve of various concentrations of the standard solutions against their respective absorbances was prepared, which followed the Beer's law. The amount of ascorbic acid in the test samples was calculated by comparing with that of the standard curve.

RESULTS AND DISCUSSION

In the present investigation, *C. colocynthis* was evaluated quantitatively for the analysis of protein, peroxidase activity and ascorbic acid. The results are present in Table 1.

Protein

Among all the samples (*in vivo* and *in vitro*) used, leaf showed higher protein level followed by fruit, root, stem, callus and differentiating callus. (Table 1 and Figure 1) Proteins are the primary components of living things. In similar studies carried out, amongst the plant parts protein content was maximum in leaves of *Jatropha curcas*[3], *Digera muricata*[7], *Withania somnifera*[8], *Nerium indicum*.[9] Contradictory to this, Kumar *et al.* (2007)[16]

reported higher level of proteins in differentiating callus as compared to callus. The presence of higher protein level in the plant points towards their possible increased food value or that a protein base bioactive compound.

Table 1: Estimation of Primary Metabolites in different Plant parts of *Citrullus colocynthis* (Linn.) Schrad.

Plant Parts	Primary Metabolites		
	Protein mg/g fresh weight of tissue	Peroxidase Units/min/g fresh weight of tissue	Ascorbic Acid mg/g fresh weight of tissue
Leaf	3.8	0.64	0.58
Stem	2.32	0.96	0.76
Fruit	2.96	1.12	1.10
Root	2.64	1.44	0.34
Callus	2.16	2.56	1.16
Differentiating Callus	1.84	2.08	2.34

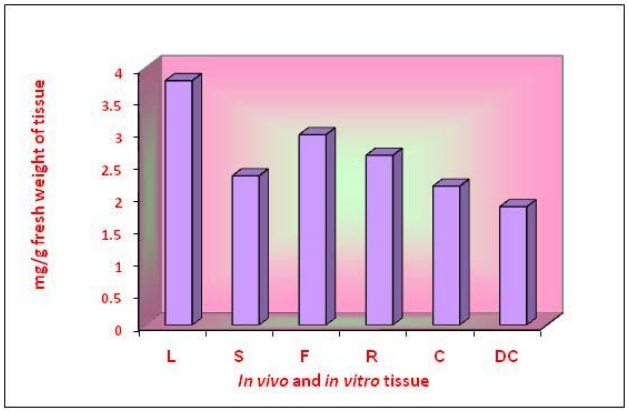

Figure 1: Estimation of Protein

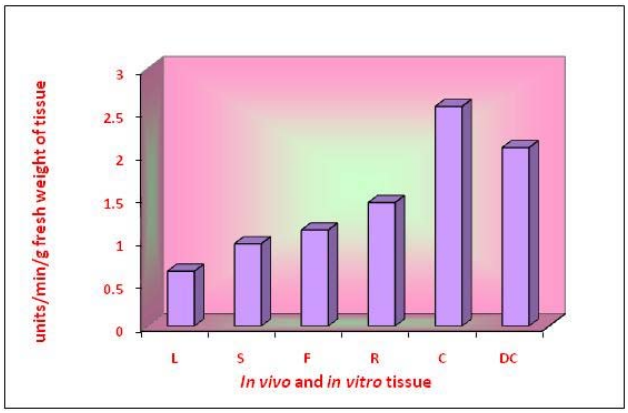

Figure 2: Estimation of Peroxidase activity

Peroxidase activity (PRO)

In *in vitro* samples, callus showed more peroxidase activity than differentiating callus. It was also noticed that *in vitro* samples (callus and differentiating callus) showed higher peroxidase activity as compared to *in vivo* samples (root, fruit, stem and leaf). Maximum activity of peroxidase was observed in callus and minimum activity of peroxidase activity was noticed in leaf. (Table 1 and Figure 2)

Peroxidase has been considered one of the most important enzymes involved in growth and differentiation in higher plants.[17] In the present study, higher peroxidase activity was observed in callus than differentiating callus. It was also noticed that *in vitro* samples (callus and differentiating callus) showed higher peroxidase activity as compared to *in vivo* samples (root, fruit, stem and leaf). Maximum activity of peroxidase was observed in callus and minimum activity of peroxidase was noticed in leaf. Similarly Singh (2004)[18] reported gradual increase in peroxidase activity during callus initiation in *Morus alba*. Aoshima and Takemoto (2006)[19] reported increase in peroxidase activity in tea callus in response to carbohydrates.

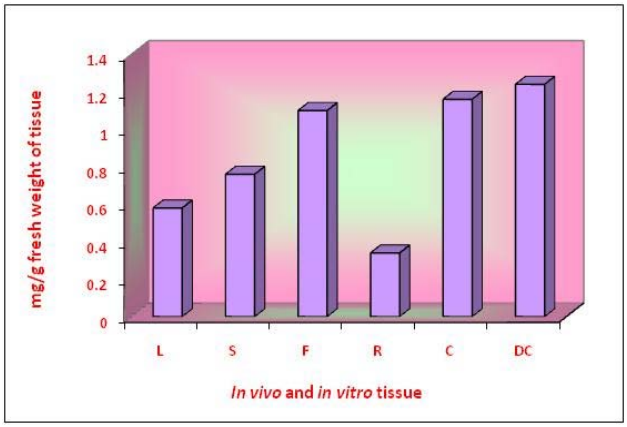

Figure 3: Estimation of Ascorbic Acid

Ascorbic Acid

In the present investigation, for *in vivo* plant parts, ascorbic acid was high in fruits as compared to stem, leaf and root. For *in vitro* tissues, ascorbic acid content was high in differentiating callus as compared to callus. The differentiating callus showed maximum ascorbic acid content and root showed minimum ascorbic acid content among all the samples tested (*in vivo* and *in vitro*). (Table 1 and Figure 3)

Ascorbic acid is also called anti-scorbutic vitamin (Vitamin C). It is an important primary plant product and well known for its property as an electron donor in photosynthetic photophosphorylation. It is an important regulator of oxidation and plays a significant role in germination, growth and metabolism of flowering

plants.[20] The role of ascorbic acid in plant growth and metabolism has been studied by various workers.[21] Ascorbic acid has been reported from many plants like *Terminalia arjuna*[5], *Cucumis sativus*[22], *Boerhaavia diffusa*, *Pedalium murex* and *Tephrosia purpurea.*[23]

Several workers have supported that tissue cultures contain free endogenous ascorbic acid in fair amounts.[24]

CONCLUSION

In the present investigation, maximum amount of protein was observed in leaf samples and minimum amount was observed in differentiating callus. Higher peroxidase activity was observed in callus and minimum in leaf sample. Maximum amount of ascorbic acid was observed in differentiating callus and minimum amount was recorded in root sample. This study suggests that plant parts having rich primary metabolites can be used industrially as raw materials having commercial importance. These primary metabolites could be further used for biosynthesis of secondary metabolites or bioactive compounds. Results obtained show the great interest in plant pharmaceuticals.

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