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In vivo and *In vitro* biochemical estimation of primary metabolites from *Helicteres isora*: An important medicinal plant

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Abstract
Helicteres isora is an important medicinal herb belonging to family Sterculiaceae. It is commonly known as 'Marorphali'. Several important chemical compounds like Cucurbitacin B, isocucurbitacin B, tetratriacontanol, tetratriaecontanoic, acid, fridelin, lupeol, taraxerone, diosgenin, neolignans, helisterculins A and B, helisorin, tannins, triterpenoids, alkaloids, saponins, flavonoids, phlobatannins, anthaquinones are present in *Helicteres isora*. Plants provide biologically active metabolites and lead structures whose activities can be enhanced by manipulation through combinations with chemicals and synthetic chemistry. These metabolites can be exploited in the field of new drugs research and for the development of modified derivatives with enhanced activity and reduced toxicity.In present investigation, callus raised from leaf explant and different plant parts of *Helicteres isora* were evaluated for their biochemical estimation of primary metabolite quantitatively. Results showed that maximum callus was obtained on MS medium supplemented with a combination of BAP (1.0 mg/l) and NAA (1.0 mg/l). Variation in the content of primary metabolites have been found in different *in vivo* and *in vitro* samples of *Helicteres isora.* It further concludes that *Helicteres isora* serves as a rich source of primary metabolites which can be used as raw material in industries.

Keywords: *Helicteres isora,* callus, MS medium, primary metabolites

Introduction
Plant synthesizes a wide variety of chemical compounds which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolite acts as a precursor for bioactive compounds used as therapeutic drugs [1]. Primary metabolites are directly involved in growth and development of plants. Chlorophyll, amino acids, nucleotides and carbohydrates have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are also used as industrial raw material and food additives.

Primary metabolic routes produce primary metabolites, which are present almost everywhere in nature and are essential for all life forms. These compounds include the common carbohydrates, fats, proteins and nucleic acids that are needed to create and maintain life. Apart from fats, the compounds are polymeric and usually chemically large molecules. In general, primary metabolites obtained from higher plants for commercial use are high-volume, low-value bulk chemicals.

Typically they are involved in the energy regulation of organisms and with growth and development of tissues; in short, they are the building blocks of organisms. The primary synthetic process of nature is photosynthesis by which green plants utilize the energy of the sun for the production of carbohydrates. Further metabolic alterations of carbohydrates lead to the formation of a pool of organic compounds of low molecular weights and simple structures. Among these are the common sugars, low molecular weight carboxylic acids, amino acids, proteins and fats etc., which are the chief components of animal food. Thus, plants of arid and semi-arid regions are good sources of phytochemically important compounds. Hence, the phytochemical investigation of these plants is essential.

The present study was conducted to investigate biochemical changes of various cellular metabolites/enzymes from *in vivo* (leaf, stem bark and root) and *in vitro* (callus and differentiating callus) of *Helicteres isora.*

Material and Methods
Healthy plants of *Helicteres isora* were collected from Jaipur and adjoining areas. Plant was authenticated by the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. Fresh leaves were taken from pot grown plants for callus culture. Callus and all the plant parts

viz. leaf, stem bark and root were dried and powdered with the help of pestle and mortar.

Callus induction

Leaves were surface sterilized with 0.2% Cedepol for 10-15 minutes followed by washing with sterile distilled water to remove the detergent. After that, the explants were treated with 0.1% $HgCl_2$ aqueous solution for 4-5 minutes and then washed thoroughly with sterilized distilled water to remove traces of $HgCl_2$. The leaves were then cut into pieces with sterile scalpel. Leaf discs were inoculated on to the MS medium[2]fortified with different concentrations of various auxin and cytokinin. The pH of the media was adjusted to 5.7±0.1 before autoclaving. All media flasks were sterilized by autoclaving at 121 ºC and 15lbs/psi for 15-20 minutes. The cultures were incubated under carefully regulated temperature and light conditions in an air conditioned room under the ambient conditions (25 ± 2 ºC; 55-60% RH) and 16 hour photoperiod. 20 replicate cultures were established and each experiment was repeated thrice and the cultures were observed at regular intervals.

Primary metabolite estimation

Callus, root, stem bark and leaf parts of *Helicteres isora* were evaluated quantitatively to estimate the total content of soluble sugars, starch, proteins, phenols, ortho dihydroxyphenol, polyphenoloxidase activity, peroxidase activity and ascorbic acid following the established methods for the sugars, starch [3], protein [4], and phenol[5]. All experiments were repeated three times for precision and values were expressed in mean ± standard deviation.

Results and Discussion

Callus induction

MS medium supplemented with different concentrations of various auxins and cytokinins showed different response for callus induction. Leaf showed maximum callus formation on MS medium supplemented with a combination of BAP (1.0 mg/l) and NAA (1.0 mg/l). The callus produced was green, irregularly lobed, compact and friable. It grew profusely and possesses high regenerative potential. The ageing of callus showed green color changing to brownish green. Differentiating callus achieved on MS medium supplemented with BAP (0.5 mg/l) and Kn (0.5 mg/l).

Primary metabolites

In the present set of investigations, biochemical changes of various cellular metabolites/enzymes were observed *in vivo* and *in vitro* tissues of *Helicteres isora*. Carbohydrates are major energy and carbon sources in plants. These play a vital role in the life of plants and animals both as structural elements and in maintenance of functional activity. Alpha-amylase plays a key role in starch degradation in chloroplasts [6]. Several workers have studied carbohydrate contents of various medicinal plant species viz., *Maytenus emarginata*[7]and *Prunella vulgaris* [8].

In the present studies, there were marginal variations in total soluble content in all the samples tested (*in vivo* and *in vitro*). Total soluble sugar was maximum in leaf as compared to *in vivo* stem bark, root, regenerating and non-regenerating callus. Minimum amount of total soluble sugar was observed in non regenerating callus. Results are presented in Fig. A. Khandelwal *et al*. also observed highest sugar contents in leaf as compared to other *in vivo* and *in vitro* tissues of *Mitragyna parvifolia* [9].

Among *in vivo* plant parts, leaf showed maximum reducing sugar level followed by stem bark and root. In *in vitro* samples, non-regenerating had more reducing sugar content as compared to regenerating callus. Results are presented in Fig. B. A declination in level of reducing sugar during shoot bud initiation from differentiating callus, accompanied by low α-amylase activity in the present study is in confirmation with the results by Russel and Morris [10]. This decline in the level of reducing sugar in regenerating callus indicated the probable utilization of sugars in the organ initiation process. Role of sugars in differentiation has been well documented [11].

In *in vivo* plant parts stem bark had higher starch level as compared to root and leaf. *In vitro* regenerating callus showed more starch content as compared to non-regenerating callus. Maximum level of starch was noticed in stem bark and minimum level of starch was observed in non-regenerating callus. Results are presented in Fig. C. Similar results were observed by Sharma and Sarin, in *Sesamum indicum* [12]. According to Sharma [13], the presence of higher levels of starch in intact plant part might be due to more storing capacity to escape the drought conditions. Starch is known to accumulate during shoot formation in callus cultures of *Solanum surattense* [14]. The accumulation of starch may be due to the presence of sucrose in the medium [15] or due to the presence of continuous light [16] (Veirskar and Andersen,1982). Maximum level of starch observed during shoot bud formation from callus was supported by the observation of Jasrai *et al*. [17] as they observed accumulation of starch at specific points from which shoot primordia arose.

α-amylase is the enzyme that is responsible for hydrolysis of starch and represents the best means for mobilization of carbohydrate reserves in the plant. Reduction in α-amylase activity corresponds to the active accumulation of starch in the tissues, as α-amylase acts on α-1-4 glycosidic linkage between starch molecules. In the present investigation maximum α-amylase activity was observed in non regenerating callus as compared to leaf, stem bark, root and regenerating callus. In *in vivo* plant parts, leaf had maximum α-amylase activity as compared to stem bark and root while in *in vitro* samples, non regenerating callus showed more α-amylase activity as compared to regenerating callus. Lowest activity of α-amylase was observed in root. Results are presented in Fig. D. Similarly, Sharma [18] reported minimum α-amylase activity in regenerating callus as compared to non regenerating callus. Maximum α-amylase activity in non regenerating callus tissues of *Helicteres isora* corresponds to the minimum accumulation of starch in callus tissue.

Proteins are biochemical compounds consisting of one or more polypeptides typically folded into a globular or fibrous form, facilitating a biological function. Proteins are the chief actors within the cell, said to be carrying out the duties specified by the information encoded in genes [19]. Protein contents have been estimated in several medicinal plant species by various workers such as *Cassia grandis* [20]; *Ventilago calyculata* [21]. Among all the samples (*in vivo* and *in vitro*) tested, leaf showed higher protein level followed by regenerating callus, non-regenerating callus, root and stem bark. In the present study, total proteins were found to be higher in regenerating callus than non-regenerating callus. Results are presented in Fig. E. Since, during differentiation the cells are quantitatively changing their activities, new proteins have to be synthesized, thus the protein concentration was high before differentiation. Similar observation was also recorded by Singh *et al*. [22].

Phenolic compounds are main constituents of many plant species and play an important role in the regulation of plant growth and development. These compounds have a variety of functions in plants like defence mechanism against microbial attack through phytoalexins, control of enzyme, lignification, auxin-activity and cell-wall synthesis. During the present studies, it was observed that among all samples (*in vivo* and *in vitro*) tested; leaf had maximum total phenolic contents as compared to stem bark, root, non regenerating callus and regenerating callus. Regenerating callus showed minimum amount of total phenolic contents. The results are presented in Fig. F. Among all the samples (*in vivo* and *in vitro*) regenerating callus showed maximum amount of o-dihydroxy phenolic contents and leaf showed minimum amount of o-dihydroxy phenolic contents. Results are presented in Fig. F. Phenolic compounds have been studied in different plant species like *Tylophora indica* [23]. Singh *et al.* [24]. reported higher amount of total phenols in non-differentiating callus than in differentiating callus of *Commiphora wightii*.

Polyphenol oxidase activity was found high in *in vitro* tissue as compared to *in vivo* tissue. Maximum activity of polyphenol oxidase was noticed in regenerating callus while minimum was observed in leaf. Results are presented in Fig. G. In the present study high accumulation of phenolic compound with low polyphenol oxidase activity has been recorded in leaf. Low amount of total phenols with high activity of polyphenol oxidase could be recorded in regenerating callus. This could be attributed to the absence of enzyme inhibitor like substances in *Helicteres isora*.

Peroxidase has been considered one of the most important enzymes involved in growth and differentiation in higher plants [25]. In the present study, In *in vitro* samples, regenerating callus showed more peroxidase activity than non-regenerating callus. It was also noticed that *in vitro* samples (non- differentiating callus and differentiating callus) showed higher peroxidase activity as compared to *in vivo* samples (root, stem bark and leaf). Maximum activity of peroxidase was observed in regenerating callus and minimum activity of peroxidase activity was noticed in leaf. Results are presented in Fig. H. The increase in peroxidase activity can be correlated with the fact that when plants are grown under *in vitro* conditions and exogenous growth regulators (auxins and cytokinins) are also present in growth medium, calli exhibit high ethylene production [26]. As a result of ethylene production, defense mechanisms at a transcriptional level and generation of active oxygen species including H_2O_2 are activated, which result in increased peroxidase activities [27].

Ascorbic acid is an important regulator of oxidation and plays a significant role in germination, growth and metabolism of flowering plants [28]. The role of ascorbic acid in plant growth and metabolism has been studied by various workers [29]. Ascorbic acid has been reported from many plants like *Withania somnifera,* [30]; *Terminalia* sp., and *Microcitrus* sp. [31]. In the present investigation, for *in vivo* plant parts, ascorbic acid was high in stem bark as compared to leaf and root. For *in vitro* tissues, ascorbic acid content was high in regenerating callus as compared to non-regenerating callus. The regenerating callus showed maximum ascorbic acid content and root showed minimum ascorbic acid content among all the samples tested (*in vivo* and *in vitro*) Results shown in Fig. I. Several workers have supported that tissue cultures contain free endogenous ascorbic acid in fair amounts [32, 33].

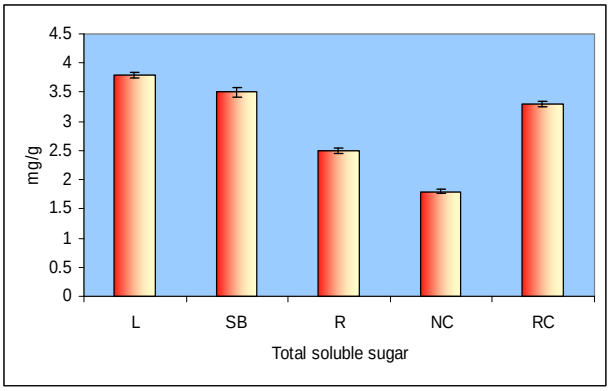

Fig A: Estimation of Total Soluble Sugar

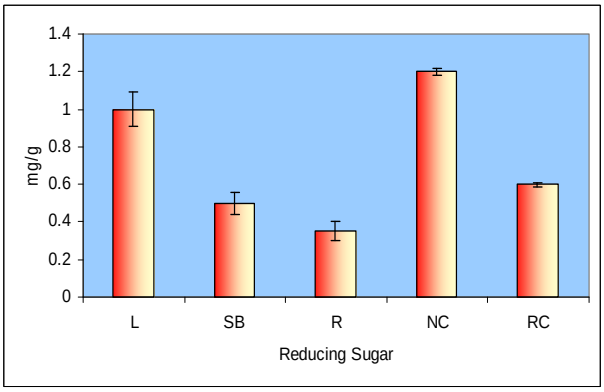

Fig B: Estimation of Reducing Sugar

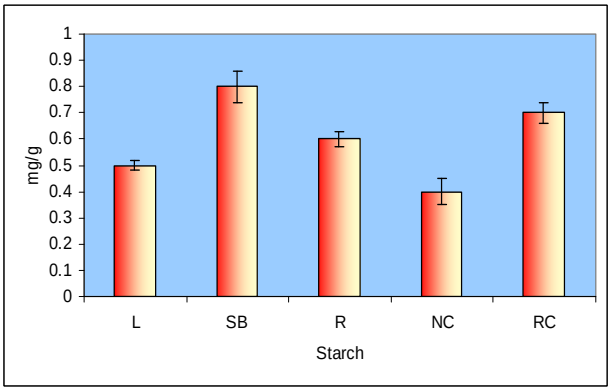

Fig C: Estimation of Starch

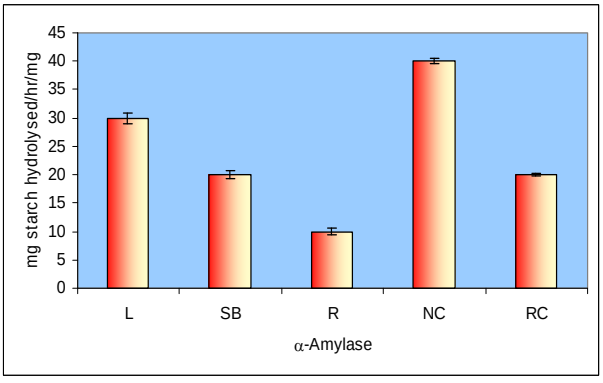

Fig D: Estimation of alpha-amylase activity

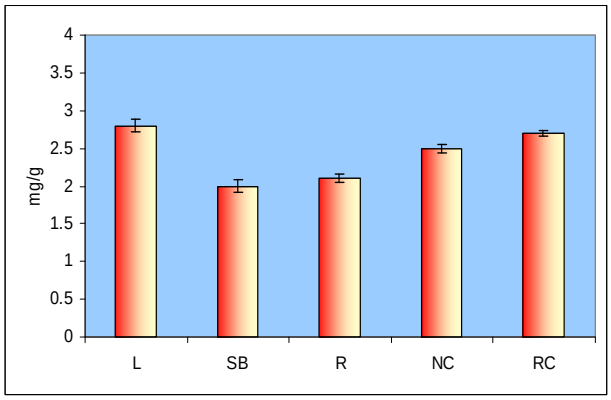

Fig E: Estimation of Total protein

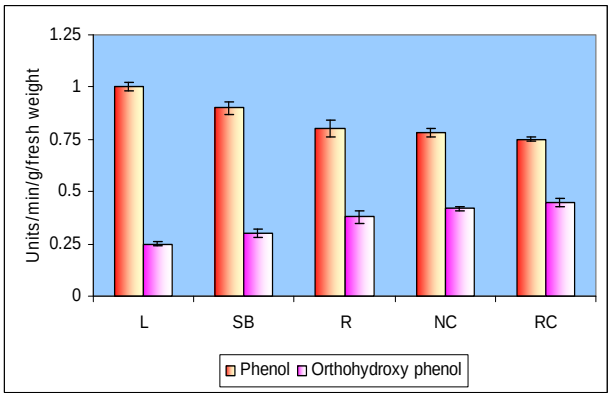

Fig F: Estimation of Phenol and Orthohydroxy phenol

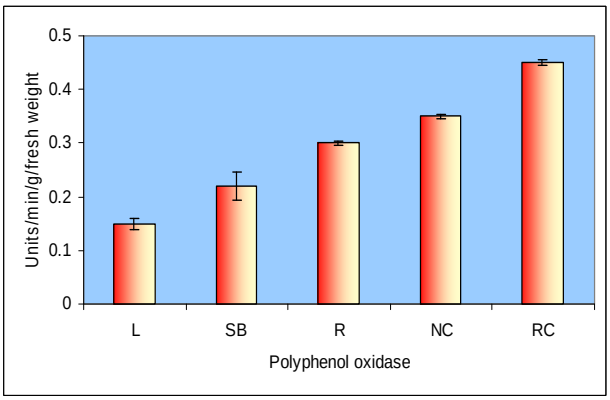

Fig G: Estimation of Polyphenol oxidase activity

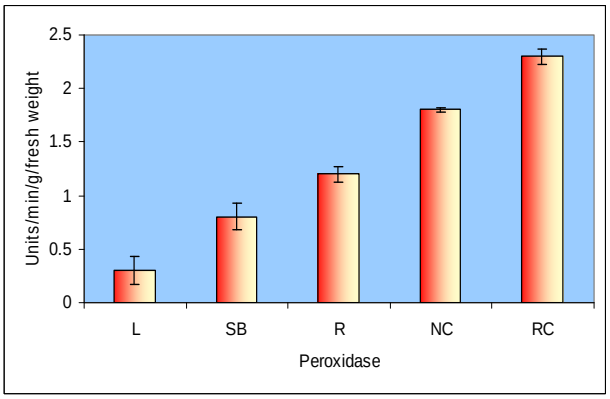

Fig H: Estimation of Peroxidase activity

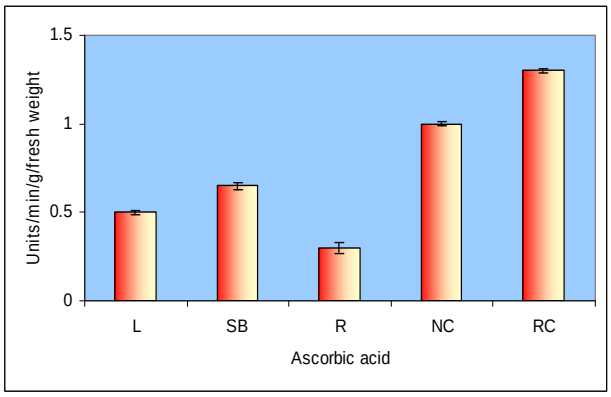

Fig I: Estimation of Ascorbic acid

Conclusion

Economic use of plants depends partially on the quantitative and qualitative aspects of these organic reserves, especially carbohydrates, proteins, phenols and ascorbic acid. In the present investigation comparison of various primary metabolites from *in vitro* and *in vivo* samples of *Helicteres isora* had been carried out. 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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