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Original Article

# QUALITATIVE AND QUANTITATIVE ESTIMATION OF β-SITOSTEROL FROM IN VITRO REGENERATED STEM BARK AND CALLUS OF *HELICTERES ISORA* L.

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**ABSTRACT**

**Objective**: Qualitative and quantitative determination of β–sitosterol from nature grown and *in vitro* regenerated plantlets of *Helicteres isora* L. was carried out to assess the presence and relative amount of β–sitosterol in stem bark and callus.

**Methods**: Acetone extract was used for TLC using the mobile phase Toluene: Diethyl ether: Ethyl acetate: Acetic acid (80:10:10:0.2). HPLC detection and quantification was performed at a wavelength 196 nm.

**Results**: Maximum percentage of β–sitosterol was present in the *in vitro* regenerated bark which was found to be 0.18 %.

**Conclusion**: Present findings indicate that *H. isora* plants responded favourably to *in vitro* propagation thus producing higher amount of β–sitosterol as compared to naturally grown plants.

**Keywords:** *Helicteres isora* L., β-sitosterol, *in vitro*, TLC, HPLC

## INTRODUCTION

Plants are also a source of unaccountable number of metabolites whose structure, function and usability have been explored partially. There is considerable interest in the screening of plants and other natural products in modern drug discovery programs, since structurally novel chemotypes with potent and selective biological activity may be obtained [1,2]. Many of these natural products have been used as sources of a large number of pharmaceuticals, agrochemicals, flavours, fragrance ingredients, food additives, and pesticides [3,4]. Our dependence on natural products is expected to continue because some compounds are difficult to synthesize due to their structural complexity. Besides their direct application, the natural plant products serve as model compounds for the chemical synthesis of new and more potent analogues. The search for new plant-derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity [5].

*Helicteres isora* L. commonly known as Indian screw tree or Spiral bush, belonging to family Sterculiaceae is a tall deciduous shrub or tree, 4-5m tall and occurs throughout India, Nepal and Sri Lanka and comprises of about 60 species [6]. Several reports in the past have emphasized the curative property associated with different parts of the plant. The plant is used in the treatment of gastric ailments [7,8] and possess antibacterial activity [9] and hypoglycemic activity [10,11]. The plant also shows inhibitory activity against avian myeloblastosis virus [12] and human immunodeficiency virus [13].

Nature grown stem bark, roots and fruits of this plant has been reported to contain β-sitosterol. β-sitosterol has been shown to possess beneficial effects against a wide variety of human ailments. It is been known to reduce cholesterol levels and is used in treating hypercholesterolemia. Its close resemblance to cholesterol allows it to be incorporated into mammalian cellular membrane thereby blocking the absorption of cholesterol in intestine [14] and plasma [15]. It also improves the liver function activity [16], thus reducing prostate and colon-cancer cell [17,18] and lympholytic leukemia. β-sitosterol also normalizes blood sugar and insulin levels in Type-II diabetics. It slows the rise of blood glucose levels by down regulation of glucose-6-phospahatase by releasing insulin and helps delay age related worsening of glucose tolerance and onset of Type-II diabetes.

However, reports on their yields in *in vitro* cultures of this plant are lacking. The objective of this study was therefore to comparatively quantify the content of β-sitosterol in field grown stem bark and in vitro regenerated stem bark and callus of *Helicteres isora* L. using high performance liquid chromatography (HPLC).

## MATERIALS AND METHODS

### Preparation of extracts

The stock plant of *Helicteres isora* L. (Accession no. 22076) was procured from Jawaharlal Nehru Agricultural University (JNKVV), Jabalpur, Madhya Pradesh, India and planted in kitchen garden. Three different types of plant tissues were used for chemical profiling including natural bark, *in* vitro bark and callus. Nature grown bark (Sample H1) was obtained from three year old plant while *in vitro* bark (Sample H2) was obtained from 1 year old vitro hardened plant and callus (sample H3) was obtained from 1-2 month old cultures. Samples H1-H3 were dried in hot air oven at 50°C in a laboratory oven followed by grinding into fine powder using a pestle and mortar. Thereafter, 1 g each of samples (H1-H3) was prepared according to the standard protocol [19]. The final extract was dissolved in acetone, concentrated, dried and used for TLC and HPLC.

### Thin layer chromatography (TLC) analysis

β-sitosterol standard was dissolved in chloroform ($0.1mgl^{-1}$) and used for TLC analysis. Using a fine glass capillary tube, three drops of each sample extract and the e β-sitosterol standard were spotted onto the glass plates pre-coated with silica gel $F_{254}$ and run in a solvent system consisting of Toluene : Diethyl ether : Ethyl acetate : Acetic acid (80:10:10:0.2). The solvent front was marked with a pencil before air-drying the plates. The plates were dried at room temperature and visualized in UV light of wavelength 254nm and 365nm.

**HPLC analysis**

β-sitosterol Standard (1mg) and the dried samples were dissolved in 1 ml of methanol and used for the analysis. HPLC analysis of the samples and standard β-sitosterol were carried out in a Shimadzu LC-20AD. A 5 µl of sample was injected (Rheodyne injector, USA) into a C-18 column (Phenomenex, Luna) at a temperature of 25ºC with a flow rate of 1ml/min and a detection wavelength of 196nm (UV-Vis Detector SPD-20A). Isocratic elution was performed with the mobile phase consisting of 85% Acetonitrile and 15% ethanol.

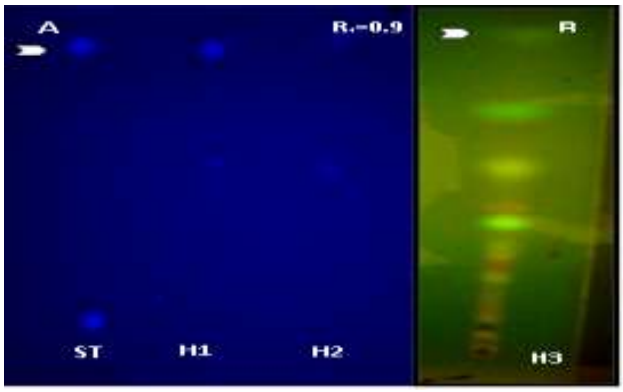


**Fig. 1: TLC analysis of bark and callus of *H. isora*. A. TLC of extracts of nature grown bark (H1), in vitro regenerated bark (H2) & standard β-sitosterol (ST) at 254nm. B. TLC of callus extract (H3) (Arrows show the spot with $R_f$ of 0.9).**

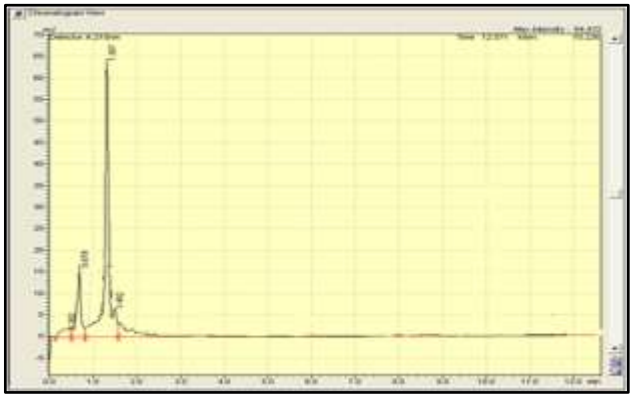

**Fig. 2: HPLC chromatogram of standard β-sitosterol**

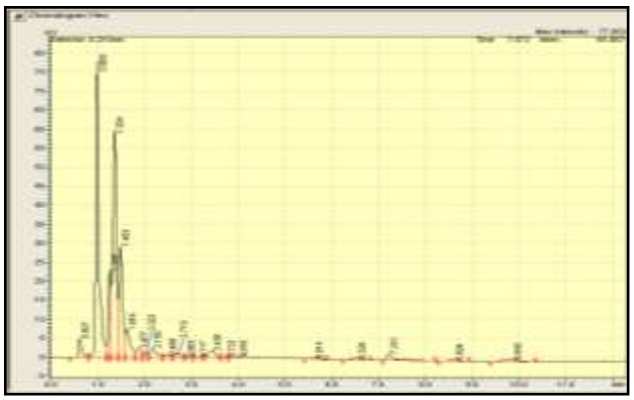

**Fig. 3: HPLC chromatogram of *H. isora* L. nature grown bark extract**

**RESULTS**

TLC analysis of bark (nature grown and in vitro) and callus of *Helicteres isora* L. extracts visualized under UV lamp showed characteristics spots and their $R_f$ value of 0.9 which were comparable to the standard β-sitosterol (Figure 1 A). All the samples also showed various additional spots between an R*f* value of 0.04 to 0.63 with maximum spots being observed in the callus sample thus indicating the presence of several compounds in the sample (Figure 1 B).

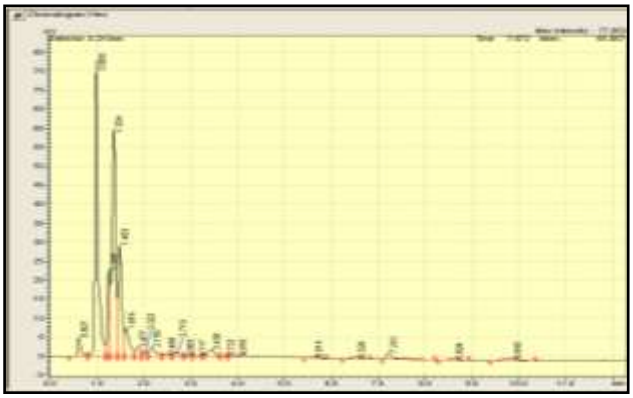

**Fig. 4: HPLC chromatogram of *H. isora* L. *in vitro* regenerated bark extract**

β-sitosterol was quantitatively evaluated in different samples by HPLC analysis. Standard β-sitosterol showed a peak at 1.304 min retention time (Figure 2). HPLC of the nature grown and in vitro regenerant's bark and callus showed multiple peaks, indicating the presence of several compounds.

**Fig. 5 : HPLC chromatogram of *H. isora* L. callus extract**

**Table 1: β-sitosterol content in different extracts of *H. isora*. L.**

| S.No | Sample | Retention time (min) | Area under the curve | % Concentration |
|---|---|---|---|---|
| 1 | Standard | 1.304 | 1806359 | 100 |
| 2 | Nature grown Bark | 1.341 | 127796 | 0.07 |
| 3 | *In vitro* regenerants' bark | 1.354 | 321710 | 0.18 |
| 4 | Callus | 1.349 | 271460 | 0.15 |

Nature grown and in vitro regenerant's bark as well as callus samples showed β-sitosterol peak at 1.341 min, 1.354 min and 1.349 min retention time respectively (Figs. 3, 4, 5). In vitro regenerated plant's bark showed higher concentration (0.18% area) of β-sitosterol as compared to the natural bark (0.07% area). Callus also showed a higher content of β-sitosterol (0.15% area) as compared to the nature grown bark (Table 1).

**DISCUSSION**

The present study has concentrated on the assessment of relative amounts of the secondary metabolite β-sitosterol in the *in vitro* regenerated plant parts (bark and roots) and callus and also to compare the possible increment of the secondary metabolite β-

sitosterol in the tissue cultured plants compared to the nature grown plant parts.

In addition to qualitative detection, TLC also provides semi-quantitative information on the major active constituents of a crude drug and is therefore suitable for monitoring the identity and purity of crude drugs [20]. In the present study, beta sitosterol was present in bark and callus. Various spots indicated the presence of several other unknown compounds in the sample.

Quantification by HPLC revealed that maximum content of β-sitosterol was in *in vitro* derived stem bark followed by callus as compared to the nature grown *H. isora* plants. A higher amount of β-sitosterol in *in vitro* regenerated plantlets and callus suggests that the tissue culture protocols involving the use of different PGRs influence the enhancement of the β-sitosterol. Beta-sitosterol has been isolated from nature grown plants of *Ampelocissus latifolia* [21] as well as from *in vitro* grown plants of *Terminalia chebula* [22], as well as from callus of several plants species [23-26].

## CONCLUSION

This study is the first report of the qualitative and quantitative estimation of β-sitosterol in *in vitro* plant parts of *Helicteres isora* L. Higher percentage of β-sitosterol in tissue culture regenerated bark and callus underlines the fact that tissue culture plays an important role in the enhancement of the secondary metabolite in the plants. A higher amount of the metabolite in callus also presents an alternate method for isolation of metabolite in cultures through different biotechnological strategies like elicitation, hairy root culture and large scale cultivation in bioreactor system.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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