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Original Article

# Biotransformation of Carvedilol Using Plant Cell Culture of *Decalepis hamiltonii* and *Nicotiana tabacum*

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**ABSTRACT**
The biotransformation reaction of cell suspension cultures produced from *Decalepis hamiltonii and Nicotiana tabacum* leaves were investigated, using Carvedilol drug. Interestingly, the cell suspension cultures showed biotransformation of racemic carvedilol. There is noticeable conversion of S-enantiomer to R-enantiomer of Carvedilol. The bio-conversion was detected and quantified by Chiral HPLC. The ideal biotransformation was achieved with the concentration of 0.1mg/ml Carvedilol at pH 5.8. The optimum incubation time was observed to be 15 days The *Decalepis hamiltonii and Nicotiana tabacum* cell culture have shown the capacity to convert S-isomer to R-isomer, because it has enzymes specific for conversion from S to R form. Among the two plants *Decalepis hamiltonii* have shown better biotransformation when compared to *Nicotiana tabacum*.
**Keywords:** Bio transformation, HPLC, Plant cell cultures, Carvedilol, Nicotiana tabacum.

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## INTRODUCTION

Plants are valuable sources of a number of chemicals including drugs, flavors, pigments and agrochemicals. Some of the biochemical reactions occurring in plant cells are complex and cannot be achieved by synthetic routes. *In vitro* plant cell cultures and plant enzymes act as suitable biocatalysts to perform these complex reactions. A wide variety of chemicals including aromatics, steroids, alkaloids, coumarins and terpenoids can undergo biotransformation using plant cells, organ cultures and enzymes.[1] Biotransformation is a process of altering or modification of functional groups by using the plant cell culture or Microbial culture. The plants are genetically diverse and possess a rich repertory of enzymes.[2] The reactions involved in the biotransformation of organic compounds by cultured plant cells include oxidation, reduction, hydroxylation, esterification, methylation, isomerization, hydrolysis, and glycosylation.[3] The ability of cultured plant cells to metabolize foreign substrates or to convert these substrates to more useful substances is of considerable interest because of the specificity of the transformation which may be affected by cultured cells.

*Decalepis hamiltonii* Wight & Arn. Belonging to Asclepiadaceae is a monogeneric climbing shrub native to the Deccan peninsula and forest areas of Western Ghats of India.[4,5] Tobacco is an agricultural product processed from the leaves of plant *Nicotiana tabacum* belongs to family Solanaceae. It can be consumed, used as a pesticide, in the form of nicotine tartrate.[6]

Carvedilol is an anti hypertensive drug which contain R and S form. Carvedilol is both non-selective beta adrenergic receptor blocker (β1, β2) and an alpha adrenergic receptor blocker (α1). The S(-) enantiomer accounts for the beta blocking activity whereas the S(-) and R(+) enantiomer have alpha blocking activity. The S enantiomer is potent when compare to the R enantiomer after first pass metabolism. R enantiomer exhibit only one tenth of the vasodilating effect of the parent compound. However, S enantiomer is about 13-fold more potent in ß-blocking activity.[7,8] The present study was aimed to convert R enantiomer to S enantiomer of carvedilol by using cell cultures of *Decalepis hamiltonii* and *Nicotiana tabacum*.

## MATERIALS AND METHODS

### Media Preparation

The readymade MS media (Murashige and skoog) for 1 litre were dissolved in sufficient quantity of double distilled water. Then the stock solutions of calcium chloride and required hormones were added. The volume of the media was made up to one litre with double distilled water. The final pH of the media was adjusted to 5.8 with 0.1N NaOH or 0.1N Hcl using pH meter. Then 0.9% w/v Agar-Agar was added and flask was heated to dissolve the agar. Then the media was distributed into culture conical flasks, plugged with non-absorbent cotton, covered with aluminium foil and sterilized by autoclaving at 121°C and 15 lbs/sq. inch for 20min. After sterilization, the flasks were kept aside at room temperature to allow the media to solidify. The culture tubes were kept in inclined position to get slants. The preparation of the media for suspension culture is same as for callus cultures except the addition of agar.

### Collection of Plant Material

Young leaves and stems were collected from the plants of *Decalepis hamiltonii* and *Nicotiana tabacum*. *Decalepis hamiltonii* plant was collected from the medicinal plant garden of Tirupati, Andrhra Pradesh and *Nicotiana tabacum* plant was collected from Raghavapur, Telangana state.

### Initiation of Callus Culture

#### *Surface sterilization of explants*

The leaves were washed under tap water followed by treating with Bavistin (Pesticide) solution and then were with running tap water. The explants were washed with 20% solution of Tween 20, the excess soap was completely removed with washing under tap water followed

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by rinsing with double distilled water before surface sterilization. Then explants were treated with alcohol (70% v/v) for 1 min, after 1 min the alcohol was decanted and the explants were washed with sterile double distilled water. After were subjected to treatment with Mercuric chloride (Surface sterilizing agent) for 5-6 min. mercuric chloride was discarded and the explants were washed with sterile distilled water for 4-5 times. All the above operations were carried under aseptic conditions.

## Callus Initiation from Explants

The surface sterilized explants (*Decalepis hamiltonii* and *Nicotiana tabacum*) were taken into a sterile Petri dish in laminar air flow cabin near the flame of spirit lamps and cut into small pieces (1 sq.cm) with the help of sterile scalpel. Incisions were made on the explant pieces. Then they were transferred onto the medium slants in culture tubes with the help of sterile forceps aseptically. Then the tubes were kept at 25 ±2°C in incubator.

## Maintenance of Callus Cultures

The cultures were periodically checked for contamination and contaminated tubes discarded. The callus culture was maintained by sub culturing into fresh medium of the same composition at an interval of four weeks (Table 1).

## Initiation of Suspension culture

About 5% w/v of callus after four weeks was used as inoculum for initiation of suspension cultures. The cultures were incubated at 25±2°C in shaker incubator at 120 rpm for two weeks. The cultures were filtered through a pre-sterilized filter under laminar flow to remove the big lumps of the cells. The cultures were then sub cultured into the same medium by 50% v/v as inoculum. The cultures were maintained by sub culturing into the same medium at an interval of two weeks.

## Addition of Carvedilol to the Cell Culture

An accurately weighed quantity 100mg and 200 mg of carvedilol was dissolved in 1ml of methanol. Then 100 mg/L and 200 mg/L drug was added to the cell cultures. The cultures were separated into five groups (Table 2). Each group had triplicates of cell culture. These cultures are kept in a shaker incubator at 25 $^0$C of 120 rpm for 15 days.

## Extraction of Carvedilol and its biotransformed products

After incubation, the culture was taken and extracted with equal volumes of Dichloromethane by shaking with rotary shaker for 1 hr.[9]

**Table 1: Composition of phytohormones.**

| Plant | Concentration of Auxins | Concentration of Cytokinins |
|---|---|---|
| *Decalepis hamiltonii* | 2,4-D (4mg/L) | Kinetin (2mg/L) |
| *Nicotiana tabacum* | IAA (4mg/L)<br>2,4-D (0.5mg/L) | Kinetin (1mg/L) |

**Table 2: Groups and Dose of carvedilol.**

| Group | Name | Dose of carvedilol |
|---|---|---|
| I | Control | -- |
| II | Control + Methanol | -- |
| III | Drug100 mg | 100 mg |
| IV | Drug200 mg | 200 mg |
| V | D+M | 100 mg |

The organic layer was separated and evaporated at room temperature. All the dried samples were reconstituted in 1 ml of HPLC grade methanol. The samples were filtered through 0.22µ filter. The filtered extracts were taken out and used for HPLC analysis.

## Analysis and identification of Biotransformed products of Carvedilol

The HPLC analysis was performed using Shimadzu LC-20AD model by injecting 20µl of sample. Phenomenex Lux Cellulose-4 column (250×4.6 mm; 5µ particle size) Chiral column was used for the analysis. HPLC run with mobile phase Isopropanol: n-Heptane (60:40). The chromatography was performed by gradient method at a flow rate of 1ml/min. The analytes were detected using SPD-2A detector at a wavelength of 254 nm.[10]

# RESULTS

## Induction of Callus

The Leaf explants were transferred into the MS medium, which was maintained at 25 ±1°C. Cream coloured friable callus was induced from *Nicotiana tabacum* and light green coloured friable callus from *Decalepis hamiltonii*, shown in (Figure 1). The callus was maintained in the same medium and subcultured under the similar conditions every 20 days. This was used for further biotransformation investigations.

## Induction of Suspension

The callus tissue was making into small pieces and transferred into MS medium of same composition used for the callus media except Agar. The culture was maintained at 25 ±1°C in incubator rotary shaker at 120 rpm and the suspension of both shown in Figure 2.

## HPLC Analysis of Bio-transformed product of *Decalepis hamiltonii*

The HPLC chromatogram of Figure 3 represents the R-enantiomer and S-enantiomer respectively. The S-enantiomer is converted into

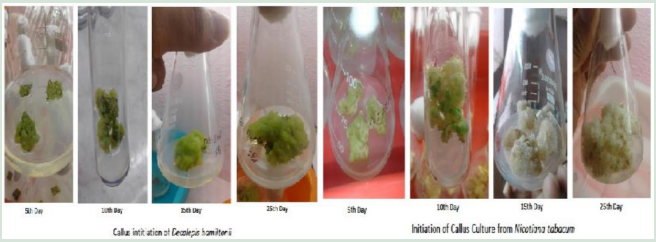

**Figure 1:** Callus initiation of *Decalepis hamiltonii*, and *Nicotiana tabacum*.

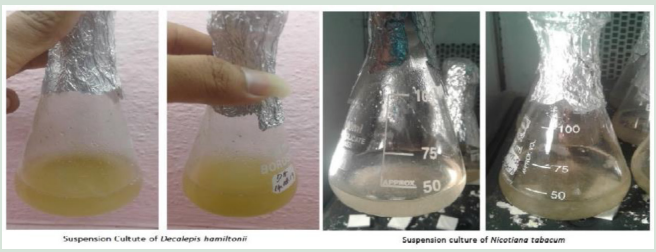

**Figure 2:** Suspension culture of *Decalepis hamiltonii*, and *Nicotiana tabacum*.

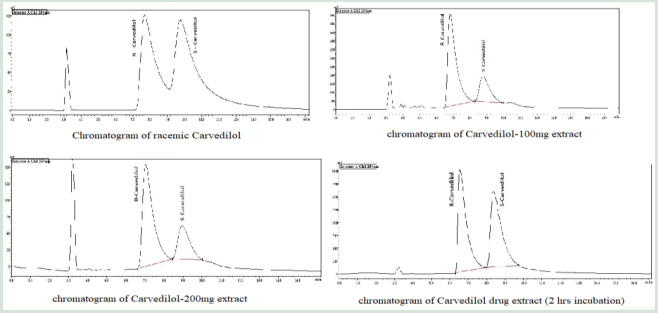


**Figure 3:** HPLC Analysis of Bio transformed product of *Decalepis hamiltonii*.

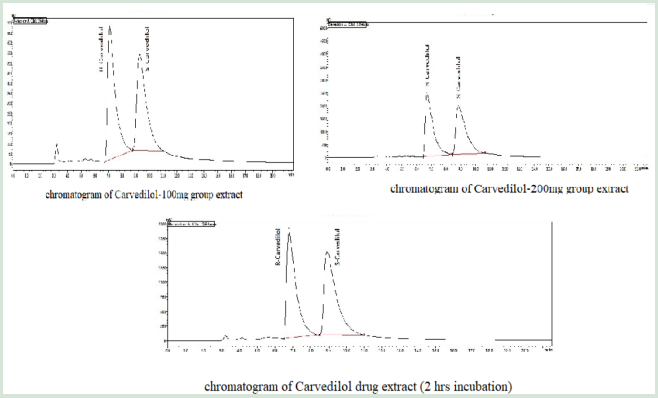


**Figure 4:** HPLC Analysis of Bio transformed product of *Nicotiana tabacum*.

**Table 3: Percentage conversion of S-Enantiomer to R-Enantiomer in the presence of *Decalepis hamiltonii* and *Nicotiana tabacum* cell culture.**

| Decalepis hamiltonii | | Nicotiana tabacum | |
|---|---|---|---|
| Group | % Conversion | Group | % Conversion |
| DH-100mg | 23.053 | NT-100mg | 10.17 |
| DH-200mg | 18.37 | NT-200mg | 6.14 |
| DH- D+M (100mg) | 1.6 | NT- D+M (100mg) | 1.45 |

R-enantiomer in the presence of *Decalepis hamiltonii* cell culture after 15 days incubation. The S-enantiomer of carvedilol is converted into R-enantiomer. Table 3, the percentage conversion of S to R enantiomer are less for Carvedilol extract (2 hrs incubation) when compare to Carvedilol-100mg and Carvedilol-200mg groups (15 days incubation).

### HPLC Analysis of Bio-transformed product of *Nicotiana tabacum*

The HPLC chromatogram of Figure 4 represents, the S-enantiomer is converted into R-enantiomer in the presence of *Nicotiana tabacum* cell culture after 15 days incubation.

The Table 3 shows that the S-enantiomer of carvedilol is converted into R-enantiomer in the presence of *Nicotiana tabacum* plant cell culture. But, the percentage conversion of S to R enantiomer is less for Carvedilol extract (2 hrs incubation) when compare to Carvedilol-100mg and Carvedilol-200mg group (15 days incubation) extracts.

## DISCUSSION

The callus culture of *Nicotiana tabacum* and *Decalepis hamiltonii* were produced from leaves of the plants and suspension culture was sub-cultured from the callus. Some cell cultures could not produce valuable secondary metabolites. Both types of producing and non-producing cells were utilized for biotransformation which gave the same pattern of bioconversion, but quantitatively their amount of bio-converted products and utilization of substrate were different.[11] Selected suspension culture was tested for the biotransformation of carvedilol. The carvedilol drug did not show any toxic effect on the cell cultures of *Nicotiana tabacum* and *Decalepis hamiltonii*. So, the present study was aimed at conversion of racemic carvedilol to S-carvedilol in the *Decalepis hamiltonii* and *Nicotiana tabacum* cell culture.

The plants are genetically very diverse and possess a rich repository of enzymes. Enzil *et al*(1995) investigated on the biotransformation of (z)-Abienol using plant cell culture of *Nicotiana sylvestris*. They have reported the role of peroxidase in the biotransformation.[12] Hamada (1988) investigated on the Enantioselectivity in the biotransformation of mono and bicyclic terpenes with the cultured cells of *Nicotiana tabacum*. Cultured cells of *Nicotiana tabacum* reduces stereo selectively the C-C double bond of the carvone and discriminate the enantiomers of monoterpene.[4] Suga *et al.* (1983) reported that cultured cells of *Nicotiana tabacum* have ability to discriminate between the enantiomers of the bicyclic terpene alcohols.[13] From the above investigations, it was found that *Nicotiana tabacum* contains different enzymes like peroxidase, Hydrolase and also the presence of isomerase. So, the conversion of isomer by plant cell cultures may be due to the presence of isomerase enzyme in the plant cell cultures. Till now, no research work was reported on *Decalepis hamiltonii* cell culture related to biotransformation.

The Chiral HPLC analysis of the plant cell cultures of *Nicotiana tabacum* and *Decalepis hamiltonii* have shown biotransformation of carvedilol but against the expectation the carvedilol drug was biotransformed to R-isomer from the racemic carvedilol.

The present study was evidenced that *Nicotiana tabacum* and *Decalepis hamiltonii* cell cultures have shown isomerization property to convert S to R isomer of carvedilol drug.

## CONCLUSION

The plants, *Decalepis hamiltonii* and *Nicotiana tabacum* cell cultures have shown Biotransformation with the Carvedilol drug. The 100 mg concentration of Carvedilol shows good percentage of biotransformation when compared with 200 mg of Carvedilol. The carvedilol of 2hrs incubation was shown very less biotransformation with cell culture of both plants. The carvedilol requires more time for incubation with cell culture for biotransformation.

*Nicotiana tabacum* and *Decalepis hamiltonii* cell cultures have shown isomerization property to convert S to R isomer of carvedilol drug. It was concluded that Carvedilol drug has shown good biotransformation with *Decalepis hamiltonii* cell culture when compared with *Nicotiana tabacum* cell culture.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**2,4D:** 2,4 Dichlorophenoxy acetic acid; **IAA:** Indole acetic acid; **HPLC:** High performance liquid chromatography; **DH:** Decalepis hamiltonii; **NT:** Nicotiana tabacum.

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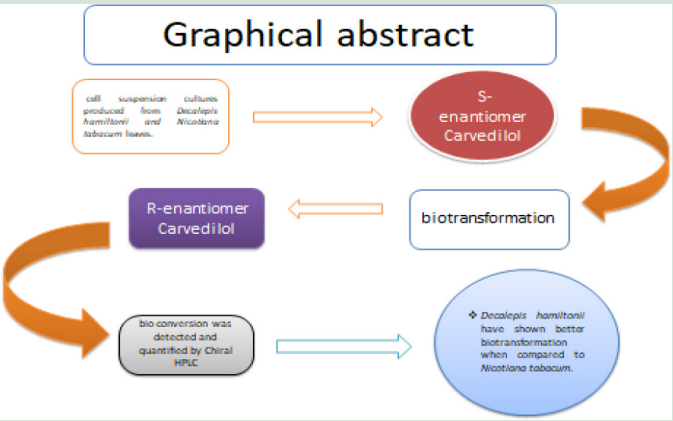
## GRAPHICAL ABSTRACT




## SUMMARY

Decalpis hamiltonii and Nicotiana tabacum cell cultures has shown the biotransformation of carvidelol. This is due to isomerisation property of cell cultures to convert to S-Carvedilol to R-Carvedilol.

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