Application of *Peganum harmala* L. (Harmal) Plant Cell Culture for the Biotransformation of Different Terpenes

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INTRODUCTION

In vitro protocols of the *Peganum harmala* (Harmal) was established for the development of plant cell culture and biotransformation studies. Biotransformation is a process through which modifications of the functional groups of organic compounds are done by living cells (Veeresham, 2004). In case of plants, mainly enzymes are biocatalysts which increase the speed response rate in the same way like chemical substances. Plant cell cultures reveal enormous biochemical possibilities for secondary metabolite production and such cultures can have a capacity to convert the exogenous substrates into products of importance. The cultured cells can convert particularly inexpensive and abundant substrates into extraordinary and exclusive substances (Ishihara *et al*., 2003). Terpenoids is the biggest group among other natural products; most of them are of plant origin which is currently recognized, making terpenoids the leading group of natural products (Bohlmann and Keeling 2008). Terpenoids shows important biological activities, including anticancer, anti-hyperglycemic, antimicrobial, anti-inflammatory, antifungal, antiviral, and anti-parasitic effects (Kuttan *et al*., 2011, Paduch *et al*., 2007; Yoo & Park 2012;). These compounds are employed in several diverse applications.

***Peganum harmala* L**. is an important therapeutic herbis habitant of North-West India, North-Africa and central Asia (Asghari and Lockwood, 2002). This plant has various pharmaceutical, biochemical (Baytop, 1999) and ornamental importance and generally used as aphrodisiac, abortifacient, galactogogue, emmenagogue and diuretic. It improves the blood and is useful for muscles and brain weakness (Kiritikar, 1995).

Ambrox is a fragrant component of ambergris from sperm whale. Ambergris is oxidatively decayed due to air, sea water and sunlight which yielded several odorous compounds. Among these, ambrox has a amber-like odor and considerable attention has been paid to the total synthesis, as well as the derivatization of ambrox in order to get new fragrances.

Sclareol is a sweet-smelling chemical compound present in clary sage. It is utilized as a scent in cosmetics and perfumes. Sclareol also has ability to abolish human leukemic and colon cancer cells (Dimas *et al*., 1999; Dimas *et al*., 2007).

Cedrol is used in fine fragrances, cosmetics, toilet soaps, shampoos, and other toiletries along with other non-cosmetic products such as detergents and cleaners. Previously, biotransformation studies of cedrol have been reported by using many microorganisms (Fraga *et al*., 1996 and Miyazawa *et al*., 1995). A comprehensive transformation of cedrol has also been conducted by Abraham (1987) and Matooq (1993) to produce several hydroxyl cedrane derivatives

OBJECTIVES

- Production of Plant cell suspension culture of *Peganum harmala* (Harmal).
- Perform biotransformation studies of Terpenes compound.
- Isolation, purification and structure elucidation of biotransformed compound.

METHODOLOGY

Numbers of experiments were designed with different concentrations of auxins and cytokinins ratio to achieve maximum number of in-vitro plants.

Peganum harmala (Harmal) was initiated for establishment of callus induction required for the biotransformation studies. Different combinations of the BAP and NAA Plant Growth Regulators (PGR's) with MS medium were used with multiple replicates.

Various classes of terpenes have been used as substrates for biotransformation studies by using ***Peganum harmala*** plant cell cultures from which three compounds i.e. **ambrox**, **sclareol**, and **cedrol** have transformed different compounds that were isolated

Though most of these known compounds have been produced synthetically or bioconverted from fungus previously, but not through plant cell suspension cultures. Biotransformed products were also subjected to biological activities.

RESULTS / CONCLUSION

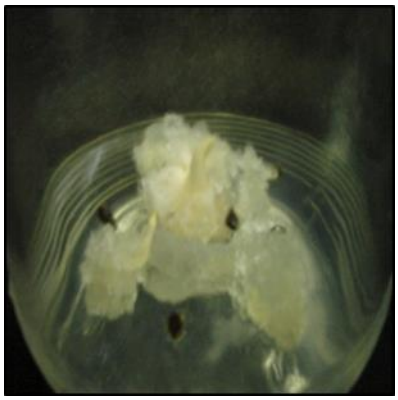
Out of 24 combinations, best combination of the PGR's with MS medium observed for the cell suspension culture was **0.1 mg/L BAP** and **1mg/L NAA**, having callus with friable granular texture in all replicates.

In order to pursue different metabolites of a given compounds (terpenes); cell suspension cultures were used. In this connection, biotransformation of **Ambrox** was achieved first time by plant cell suspension cultures of *Peganum harmala*, which yielded oxygenated products, **1-hydroxy-3oxoambrox, 1,3-dihydroxyambrox,3-hydroxyambrox, 6-hydroxyambrox,3-oxoambrox, 2-hydroxyambrox, 3-hydroxysclareolide, 2,3-dihydroxyambrox and 13,14,15,16-tetranorlabdane-3-oxo-8,12-diol.** 1,3-dihydroxyambrox metabolite was originate as novel compound. These metabolites were structurally categorized on the basis of spectroscopic studies.

When **Sclareol** was incubated with quickly growing cell suspension cultures of *Peganum harmala*, it afforded two known compounds **3β-hyrdroxysclareol** and **3-keto sclareol**, but this is a new path for these biotransformed products. These compounds have not been developed using this plant cell suspension culture.

In a similar manner, **Cedrol** was exposed to biotransformation process retaining cell suspension culture of *Peganum harmala* for the first with each other and yielded a compound **3β hydroxycedrol**.

(a) Callus induction



(b) Callus multiplication

(c, d) Cell suspension culture

Callus induction and cell suspension culture of *Peganum harmala*.

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