

## Biotransformation of Aromatic Aldehydes by Cell Cultures of *Peganum harmala* L. and *Silybum marianum* (L.) Gaertn

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### Abstract

Many aldehydes are important components of natural flavours. They are used in food, cosmetic, and biomedical industries in large amounts. Plant cells or microorganisms carry out their production by biotransformation, which is one of the biotechnological methods that allow them to be defined as 'natural'.

Cell cultures of *Silybum marianum* and *Peganum harmala* have been studied with a view to investigate their abilities to produce flavonolignans and  $\beta$ -carboline alkaloids respectively. However, we have isolated *S. marianum* and *P. harmala* culture strain, which are able to metabolise several aromatic aldehydes. Ten culture strains derived from *S. marianum* and *P. harmala* were examined for their ability to biotransform exogenous aromatic aldehyde compounds, including benzaldehyde, 2-methoxybenzaldehyde, 4-methoxybenzaldehyde, cinnamaldehyde and 3-methoxy, 4-hydroxy benzaldehyde. Callus cultures of *Silybum marianum* and *Peganum harmala* were established from seedlings, and healthy suspensions were grown using the Murashige and Skoog medium. Exogenous aromatic aldehydes were fed to *S. marianum* and *P. harmala* cell suspension cultures. Biotransformation reactions were detected over 24 h of incubation. The cultures then extracted with dichloromethane and extracts subjected to GC and GC-MS analysis. The *S. marianum* cultured cells in this study exhibit greater selectivity in the reduction of aromatic aldehydes than *P. harmala* cultured cells. The ability of cultured plant cells to biotransform substrate appears to be dependent on the culture strains as well as the nature and position of the substituent on the aromatic ring.

**Keywords:** Biotransformation; Aromatic aldehydes; *Silybum marianum*; *Peganum harmala*.

### Introduction

Several aromatic aldehydes are among the most important aromatic flavour compounds used in the food and perfume industries. They are also used in cosmetic and biomedical industries in large amounts.

*Silybum marianum* (L.) Gaertn. (Asteraceae) is an annual or biennial plant, native to the Mediterranean area, which has now spread to other warm and dry regions. Cultivation of *S. marianum* has been carried out for production of flavonolignans silymarin, which is a component

of herbal remedies for the treatment of bile and liver diseases (1).

Cell cultures of *Silybum marianum* have been studied in order to investigate their abilities to produce phenols and flavonolignans (2-4). The growth and flavonolignan production of suspensions were tested using different concentrations of KNO<sub>3</sub>, KH PO<sub>4</sub>, iron and calcium (5). Flavonolignan production from *Silybum marianum* root cultures has also been reported (6).

Peganums are 30-90 cm high bushy herbs which are widely distributed in the Irano-Turanian region with extensions into the dry Mediterranean regions of Europe and Africa.

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The seeds and roots of *Peganum harmala* L. (Zygophyllaceae) contain alkaloids such as harmine, harmaline, harmol, and harmalol (7). The seed extract has antispasmodic, antihistaminic (8), and vasorelaxant effects (9).

Cell cultures of *Peganum harmala* L. have been widely studied with a view to investigate their abilities to produce amines and  $\beta$ -carboline alkaloids (10, 11).

Biotransformations are increasingly being used in the manufacture of specific chemicals, especially flavours and fragrances (12). Several reports on biotransformation of aromatic aldehydes as precursors for the production of aromatic alcohols, using plant cells and microalgae, have been published in the literature (13, 14). So far, there is no report on using *S. marianum* cultures in biotransformation studies for the production of aromatic aldehydes.

## Experimental

### *Cell cultures.*

Seeds of *Silybum marianum* and *Peganum harmala* were surface sterilized in 30% w/v hydrogen peroxide containing 1% Tween 80 for 2 min, then germinated on wet filter paper in Petri dishes in the dark at 25 C. The cotyledons were then transferred onto the Murashige and Skoog media containing 5 ppm ascorbic acid, 2 ppm 2,4-dichlorophenoxyacetic acid and 0.1 ppm kinetin (15). Calli were maintained by subculturing every 4 weeks, and suspension cultures were formed by agitation 5 g callus to liquid medium until a suspension of free cells was formed. The suspensions were then placed on a rotary shaker running at 100 rpm, and maintained by subsequent subculturing, using a dilution of 1 to 2, into new fresh liquid media.

The callus and suspensions were maintained in a 12 h light / dark cycle at 27°C and subcultured every 4 weeks. Suspension cultures grown over more than six generations were used for substrate feeding and bioconversion studies.

### *Substrate feeding and product extraction.*

The compounds chosen for study were a series of aromatic aldehydes including the parent aldehyde benzaldehyde, the 2-methoxybenzaldehyde, the 4-

methoxybenzaldehyde, cinnamaldehyde and 3-methoxy,4-hydroxybenzaldehydes (vanillin).

The substrates were obtained from Sigma. Chemical purity (greater than 98%) was determined by capillary gas chromatography (GC). Substrates were dissolved in a water-miscible solvent (ethanol 70%), which resulted in good mixing of the substrate upon addition to the aqueous medium. The substrates were added to suspension cultures to make a final concentration of 100 ppm cell volume (50% p.v). Control readings were made without the addition of substrate to cultures and with addition of substrate to cell-free medium. The cultures were incubated under the conditions mentioned above. After the incubation period, the flask was swirled to ensure good mixing and two samples were removed with a 10-ml pre-sterilized, glass-tipples pipette. A new pipette was used for each sample.

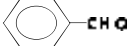
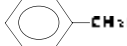
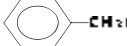
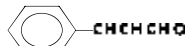
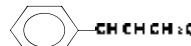
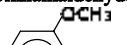

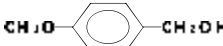
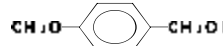

After 24 h both the cells and the media were extracted using dichloromethane, followed by centrifugation (1000 g for 5 min). The extract was reduced to a volume of 100  $\mu$ l under nitrogen, then 0.1  $\mu$ l was analyzed by gas liquid chromatography (GLC).

### *Analysis.*

Gas chromatography analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a BP-1 capillary column (39 m x 0.25 mm; film thickness 0.25  $\mu$ m). The carrier gas was helium with a flow rate of 2 ml/min. The oven temperature for the first 4 min was kept at 60°C and then increased at a rate of 4°C /min until reached a temperature of 280°C. Injector and detector temperatures were set at 280°C.

Confirmation of peak identity was effected by co-chromatography with standards and GC-MS. The mass spectra were recorded on a Hewlett Packard 6890 MS detector coupled with Hewlett Packard 6890 gas chromatograph equipped with a HP-5MS capillary column (30 m x 0.25 mm; film thickness 0.25  $\mu$ m). The gas chromatography condition was as mentioned previously. Mass spectrometer condition was as follows: ionized potential 70 eV, source temperature 200 C (16, 17).

**Table 1.** Aromatic aldehydes biotransformation using *P. harmala* and *S. marianum* cell suspensions

Substrates (Aromatic aldehydes)	Product ( <i>P. harmala</i> cell culture)	Product ( <i>S. marianum</i> cell culture)
 Benzaldehyde	 Benzyl alcohol	 Benzyl alcohol
 Cinnamaldehyde	 Cinnamyl alcohol	No conversion
 2-methoxybenzaldehyde	No conversion	No conversion
 4-methoxybenzaldehyde	 4-methoxy benzyl alcohol	 4-methoxy benzyl alcohol
 3-methoxy,4-hydroxybenzaldehydes	No conversion	No conversion

## Results and Discussion

The biotransformation of aromatic aldehydes by *S. marianum* and *P. harmala* is summarised in Table 1. Using aromatic aldehydes the relationship between structure and biotransformation ability of the test cultured plant cells was studied.

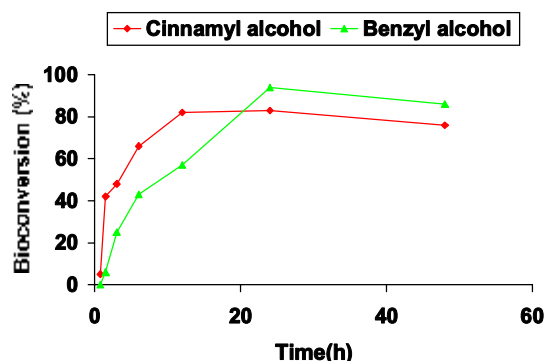
Benzaldehyde was readily reduced to benzyl alcohol by both culture strains derived from different plant species. This transformation was completed within a 24 h period.

A selective reduction of methoxybenzaldehyde was catalysed by both culture strains. Only 4-methoxybenzaldehyde was transformed to its corresponding alcohol. In fact no conversion was observed after four days when 2-methoxybenzaldehyde was fed to both culture strains. The *S. marianum* reduced the 4-

methoxybenzaldehyde as effectively (>95%) as the *P. harmala*. Almost a total biotransformation of 4-methoxybenzaldehyde occurred within the one day period. However, Lappin et al. demonstrated that suspension cultures of *Lavandula angustifolia* reduced monoterpenoid aldehydes to their corresponding primary alcohols, but octanal was not reduced (18).

Of the cultures under investigation, none exhibited the ability to biotransform 3-methoxy,4-hydroxybenzaldehyde. Compounds such as vanillin and ethylvanillin which have hydroxy groups at the ortho or para position were similarly reported to be difficult to transform or were not transformed at all by *Dunaliella tertiolecta* cultures (19). These results give further evidence regarding the importance of the nature of functional group in the substrate administered, and the structural moieties in the vicinity of the functional group.

The interesting point to note is that when cinnamaldehyde was added to both suspension cultures, a reduction was only observed in the cultured cells of *P. harmala*. The biotransformation of cinnamaldehyde in *P. harmala* was more rapid than that of benzaldehyde (Figure 1). It was reported that *P. harmala* cultures had large capacities for biotransformation and great potentials for the selective structural modification on chiral molecule (20). No conversion detected when cinnamaldehyde was introduced into the



**Figure 1.** Time course of the conversion of benzaldehyde and cinnamaldehyde by *P. harmala* cell suspension to their corresponding alcohols, showing duplicate results at each data point.

cultured suspension cells of *S. marianum*. This result may indicate that the enzymes involved in reduction reactions of cinnamaldehyde are specific to a particular culture strain, since cinnamaldehyde cannot be transformed by the *S. marianum* culture. Similarly, Tabata et al have shown that from cell suspension cultures of ten different plant species, only six were able to glucosylate salicyl alcohol (21).

In conclusion, it appears that the aromatic aldehydes reduction reaction may vary with culture strains as well as the chemical structure of substrates.

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