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Original Article

Production of Arbutin by Biotransformation of Hydroquinone Using *Peganum harmala*, *Varthemia persica* and *Pycnocycla spinosa* Cell Suspension Cultures

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Abstract

Cell cultures of *Varthemia persica, Peganum harmala* and *Pycnocycla spinosa* have been studied to evaluate their abilities to bioconvert exogenous hydroquinone. Arbutin is an important substance that has several pharmaceutical applications; therefore, we have established *V. persica* and *P. spinosa* cultures which seem to be able to metabolize hydroquinone. Callus cultures of *V. persica* were established from seedlings, and healthy suspensions were grown using Murashige and Skoog medium supplemented with 2,4-D and kinetin. Exogenous hydroquinone was fed to cell suspension cultures and biotransformation reactions were detected over 24 h of incubation. The cultures then extracted with methanol and extracts subjected to TLC and HPLC analysis. The *V. persica* and *P. spinosa* cultured cells in this study seem to exhibit an ability in the glucosylation of hydroquinone to arbutin. No conversion was observed with *P. harmala* cell suspension cultures. The ability of cultured plant cells for biotransformation of substrates appears to be depended on the culture strains.

Keywords: Arbutin; Biotransformation; Cell culture; *Peganum harmala*; *Pycnocycla spinosa*; *Varthemia persica*.
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1. Introduction

Arbutin, also called hydroquinone-*O*-P-D-glucopyranoside, found in *Arctostaphylos uva-ursi* has attracted much interest for two therapeutical applications. The leaves of *A. uva-ursi* are used as tea preparations for the treatment of infections of the urinary and genital tracts [1]. Furthermore, arbutin is known to inhibit the biosynthesis of the melanin pigment and is used in cosmetics as a skin whitening agent [2]. Various attempts have been made in the past to generate arbutin in a biotechnological way instead of chemical synthesis. Arbutin was enzymatically synthesized from hydroquinone by several researchers using amylase of *Bacillus subtilis* and sucrose phospholylase of *Leuconostoc mesenteroides* and using microbial catalyst *Xanthomonas campestris* [3]. Also, researchers have investigated an alternative process using

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plant cell cultures including biotransformation of hydroquinone [4]. Tabata *et al.* have shown that cultured cells of *Datura innoxia* had a remarkably high capability for glucosylation of hydroquinone to form arbutin [5]. Yokoyama *et al.* selected *Catharanthus roseus* cells as a producer of the enzyme since arbutin was formed efficiently when hydroquinone was added into the suspension culture [6]. Also, Inomata *et al.* reported a high level production of arbutin using cultured cells of *Cataranthus roseus* [7].

Varthemia persica DC. (Asteraceae) is an aromatic plant growing wild in central parts of Iran [8]. The essential oil of *V. persica* has been reported to have several mono- and sesqueterpenes, specially β-eudesmol and spathulenol [9]. There is no report on cell cultures of *V. persica*.

Pycnocycla spinosa Decne. ex. Boiss. (Umbelliferae) is an aromatic plant distributed in central parts of Iran [10]. The essential oil of *P. spinosa* has been analyzed and thirty four components have been identified, of which several major constituents were found to show seasonal variation [11, 12]. There are no data concerning the cell culture of the plant.

Peganums are 30-90 cm high bushy herbs which are widely distributed in the Irano-Turanion region with extensions into the dry Mediterranean regions of Europe and Africa. The seeds and roots of *Peganum harmala* L. (Zygophyllaceae) contain alkaloids such as harmine, harmaline, harmol, and harmalol [13]. Cell cultures of *P. harmala* have been widely studied with a view to investigate their abilities to produce β-carboline alkaloids [14-16]. It was reported that *P. harmala* cultures had great potentials for bioconversion of terpene and non-terpene molecules [17].

Chemical synthesis is not a cost-effective way to produce arbutin. Chemical synthesis methods usually require expensive starting materials, various expensive and toxic solvents and catalysts, significant energy input, and a subsequent purification step to remove impurities. The investment in non-renewable resources is both expensive and environmentally unfriendly. Many higher plants naturally produce arbutin. Members of the Ericaceae, Rosaceae, and Saxifragaceae families have been reported to produce arbutin in amounts up to 20 % of their dry weight. However, these plants suffer from poor agronomic performance [18]. Cost-effective production of arbutin in plants requires a crop plant species with high agronomic performance and an established processing infrastructure. The aim of this study was to examine the capability of *V. persica, P. harmala* and *P. spinosa* cell cultures for bioconversion of exogenously added hydroquinone.

2. Materials and methods

2.1. Cell cultures

The seeds of *V. persica, P. harmala* and *P. spinosa* were obtained from the herbarium of the Faculty of Pharmacy, Isfahan University of Medical Sciences. The seeds were first surface sterilized in 30% w/v hydrogen peroxide containing 1% Tween 80 for 4 min., then germinated on wet filter papers in Petri dishes in the dark at 25 °C. The cotyledons were then transferred onto Murashige and Skoog (MS) media containing 5 ppm ascorbic acid, 2 ppm 2,4-dichlorophenoxyacetic acid and 0.2 ppm kinetin [19]. Calli were maintained by subculturing every 4 weeks, and suspension cultures were formed by agitation of 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 were maintained by subsequent subculturing, using a dilution of 1 to 2, into new fresh liquid media. The callus and suspensions were maintained in the dark at 27± 2 °C and subcultured every 4-6 weeks. Suspension cultures grown over more than twenty generations were used for substrate feeding and bioconversion studies.

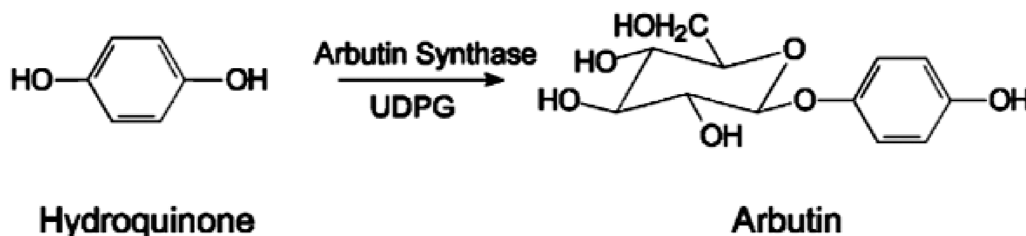

Figure 1. Arbutin bioproduced using *V. persica* and *P. spinosa* cells suspensions.

2.2. Substrate feeding and product extraction

Hydroquinone obtained from Sigma. with a purity greater than 98%. Hydroquinone was dissolved in a water-miscible solvent (ethanol 70%), which resulted in good mixing of the substrate upon addition to the aqueous medium. Hydroquinone was added to suspension cultures to make a final concentration of 100 ppm, and cell volume of the suspension was 50%. Control readings were made without addition of hydroquinone to cultures and with addition of hydroquinone to cell-free medium. The cultures were incubated under the conditions mentioned above. After the incubation period (24 h), the flask was swirled to ensure good mixing and samples were removed. Hydroquinone and arbutin were extracted from the dried cells which had been filtered from suspension cultures. The extract was reduced to a final volume of 1 ml and subjected to thin layer chromatography (TLC). The concentrated methanolic extracts were spotted on precoated silicagel plates(Merck) and chromatographed with arbutin and hydroquinone as reference materials in a saturated chamber containing EtO-AC: formic acid: water (86:6:6) solvent mixture. Visualisation of the separated bands was carried out under UV light (254nm). The glycosides were detected as a fluorescent individual band. The dried plates were then sprayed with Berlin Blue reagent [20]. One individual blue zone was detected on the extract obtained from cultured cells of *V. persica* and *P. spinosa*. Also, the conversion of hydroquinone to arbutin was confirmed by co-chromatography using HPLC- equipped with PDA detector (Waters, Novapac C_{18}, Water and methanol 9:1). The scanning was performed between 200 and 350 nm and their major absorption peaks were compared [21].

3. Results and discussion

The chemical structure and mechanism of biotransformation of hydroquinone by the *V. persica* and *P. spinosa* is presented in Figure 1.

Arbutin was produced by cultured cells of *V. persica* and *P. spinosa*. High level production of arbutin from hydroquinone in suspension cultures of *Catharanthus roseus* plant cell has been reported [7]. No conversion was detected when hydroquinone was introduced to the cultured suspension cells of *P. harmala.* It was reported that *P. harmala* cultures had large capacities for biotransformation and great potentials for the selective structural modification on chiral molecule [22]. This result may indicate that the enzymes involved in glucosylation reactions of hydroquinone are specific to a particular culture strain since hydroquinone cannot be transformed by *P. harmala* culture. Similarly, Skrzypczak-Pietraszek *et al.* have shown that cells from *Echinacea purpurea* (Asteraceae), *Exacum affine* (Gentianaceae), *Melittis melissophyllum* (Lamiaceae), *Ruta graveolens* (Rutaceae) agitating cultures perform a biotransformation reaction on exogenously supplied hydroquinone into arbutin, but *Ammi majus* (Apiaceae) cultures contain trace amounts of the product [23].

Some substrates are toxic to plant cells. Plant systems typically retain secondary metabolites intracellularly, therefore, their

accumulation might interfere with basic physiological functions, especially if the compounds are toxic.

As indicated in Figure 1, *V. persica* cultured cells was able to glycosylate hydroquinone to arbutin. Glucosylation reactions, the transfer of activated glucose to an aglycone substrate, are of special interest because they facilitate the conversion of water-insoluble compounds to water-soluble compounds. The properties of glucosyltransferase from plant cell suspension cultures of the *Rauwolfia serpentina,* which catalyzing the biosynthesis of arbutin was reported [25] The enzyme was characterized and is named arbutin synthase [26]. *P. spinosa* suspension cells were also showed glucosylation ability when fed by exogenous hydroquinone. Biotransformation using plant cell cultures presents the most updated techniques in production of valuable medicinal compounds. The chemical synthesis of the compound requires at least three step reactions and the production cost of arbutin by a plant cell culture process is comparable to the chemical process [27]. In recent years, the yields obtained in plant biotransformation and improved technology of production suggest that economically viable production of many compounds will become possible in the future [28].

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