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Full Length Research Paper

Obtaining phenolic acids from cell cultures of various *Artemisia* species

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Plant cell cultures represent a high valuable source for the production of bioactive secondary metabolites which can be used in food industry, medicine and cosmetic industry. In our study, we focused on obtaining phenolic acids from plant cell cultures. We compared cell cultures obtained from nine plant species of two families with a high potential for the production of phenolic substances. The most productive cell cultures for phenolic acids were *Artemisia frigida* and *Silybum marianum*. Isochlorogenic acid and chlorogenic acid were the most abundant phenolic acids identified in the analyzed cell cultures. Cell culture of *Nicotiana tabacum* contained only one hydroxycinnamic acid derivative in low amounts. Altogether *S. marianum* cell culture was verified as the most suitable for production of phenolic acids.

Key words: Plant cell cultures, secondary metabolite, phenolic acids, chlorogenic acid.

INTRODUCTION

Plant cell cultures have recently received much attention as a useful technology for the production of valuable secondary metabolites. They have several advantages over other production technologies since they are not limited by environmental, ecological or climatic conditions and cells can therefore proliferate at higher growth rates than whole plants in cultivation (Zhong, 2001). Under controlled conditions, plant cell cultures are not limited by biotic and abiotic factors. Cell cultures can be used to secure and assure the diversity of most especially endangered plants in the future (Daniel et al., 1999). Cell cultures are also used in studying the biochemical pathway of certain plants to increase the industrial production of secondary metabolites (Ramachandra and Ravishankar, 2002). In cell cultures, bioactive secondary metabolites can be produced under controlled conditions using reproducible protocols (Yang et al., 2001). The accumulation of some metabolites in plant cell cultures is higher than in their parent plants (Han and Zhong, 2002).

Phenolic compounds include signaling molecules, pigments and flavors that can attract or repel enemies. These compounds can protect the plant against insects, fungi, bacteria, and viruses (Vermerris and Nicholson, 2006). Phenolic acids have been proposed to have beneficial effects on human health because of the antioxidant, anti-cardiovascular disease and anticoronary heart disease properties (Petersen and Simmonds, 2003). Phenolic acids in general are phenols with one carboxylic acid group, whereby in hydroxybenzoic acids the carboxylic acid group is directly attached to the ring and hydroxycinnamic acids have a three-carbon side chain.

The metabolism of phenolic substances has been intensively studied in cell suspension cultures derived from different plant species. The results obtained have been used both at the theoretical level (more detailed descriptions of metabolic pathways, their regulation, participation of phenolics in physiological processes, just to name a few) and in practical applications of cell cultures (the production of secondary metabolites or, on the other

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Table 1. Plants used for cell cultures.

Botanical name	Family	Common name
Cynara scolymus	Asteraceae	Artichoke
Silybum marianum	Asteraceae	Milk thistle
Echinacea angustifolia	Asteraceae	Narrow-leaved purple coneflower, blacksamson Echinacea
Calendula officinalis	Asteraceae	Pot marigold, scotch marigold
Artemisia frigida	Asteraceae	Prairie sagewort
Artemisia campestris	Asteraceae	Canadian wormwood, field sagewort
Artemisia vulgaris	Asteraceae	Common wormwood
Nicotiana tabacum	Solanaceae	Common tobacco
Nicotiana paniculata	Solanaceae	Tobacco

hand, the reduction of the inhibitory effect of accumulated phenolic compounds on the viability of cells employed for biotransformation or production functions) (Cvikrová et al., 1988). Even though a variety of plant cell cultures are known to be sources of phenolic compounds, their yields and compositional data are still insufficient.

Hydroxycinnamic acids are in almost every plant and the major representative in food is caffeic acid, which occurs mainly as an ester with quinic acid called chlorogenic acid (Clifford, 1999). Studies on the occurrence of phenolic acids in cell cultures are rare. Therefore, the purpose of this study was to: (1) evaluate a variety of plant cell cultures that have the potential for a high phenolic acid production with respect to their total and individual phenolic acid content; (2) compare the phenolic acid concentration and profile in plant cell cultures from different families and genera. The compounds of interest in the present study were hydroxycinnamic acids such as: vanillic acid, chlorogenic acid, isochlorogenic acid, caffeic acid, *p*-coumaric acid, trans-ferulic acid, and 4-methoxycinnamic acid.

MATERIALS AND METHODS

Plant selection

Based on an extensive literature research, nine plants from two families with a high content of phenolic acids were selected. The studied plants and their common names are shown in Table 1.

Seeds germination and callus initiation

The seeds were sterilized in 70% ethanol for five minutes, then transferred into 10% sodium hypochlorite for 10 min, and washed three times in sterile water before sowing into petri dishes on Murashige and Skoog medium (MS; Murashige and Skoog, 1962) for germination. To obtain cell cultures, part of sterile plants were placed on MS medium with 2,4-dichlorphenoxyacetic acid (2,4-D, 40 mg/l). After callus formation, cell suspension cultures were established in liquid MS medium in 250 ml Erlenmeyer flasks, supplemented with 3% sucrose and 2,4-D at concentration of 40 mg/l. Cell cultures were transferred to new medium every four weeks and maintained at 25 ± 2°C on a rotary shaker under light irradiation. The inoculum size was about 100 mg fresh weight/l.

Extraction of phenolic acids

The calli of cell suspension were harvested and frozen in liquid nitrogen to prevent phenolic compound volatilization. Afterwards the samples were lyophilized and ground by flint mill (20000 rpm, 2 min). 40 mg freeze dried plant material was used for the extraction of phenolic compounds. Phenolic acids were extracted with 70% methanol (pH 4.0) and placed in ultrasonic water bath with ice for 15 min for better cell wall disintegration. The pellet was re-extracted two times after centrifugation at 4°C for 5 min and 4,500 rpm. The supernatant were collected and concentrated in a vacuum concentrator for about 2 h to a volume of 150 µl and then dissolved in 40% acetonitrile. The samples were filtered and injected into the HPLC.

Instrumentation and chromatographic conditions

The separation of phenolic compounds was performed on a narrow-bore Acclaim PA C16-column (3 µm, 2.1 × 150 mm, Dionex) on a HPLC (Dionex Summit P680A HPLC-System), equipped with a P680 HPLC pump, ASI-100 automated sample injector, and PSA-100 photodiode array detector. The column oven temperature was 35°C. The mobile phase consisted of 0.1% (v/v) phosphoric acid in ultrapure water (eluent A) and of 40% (v/v) acetonitrile in ultrapure water (eluent B). The gradient program is shown in Table 2. The injection volume was 10 µl. Simultaneous monitoring was performed at 290, 330 and 254 nm at a flow-rate of 0.4 ml/min. Cinnamic acid or p-coumaric acid were used as internal standards. Diode array detection was used for the identification of the compounds with the software Chromeleon 6.8 (Dionex, USA). For quantification the wave length of 290 nm was selected. Retention times and UV ± visible spectra of the peaks were compared with those of authentic standards.

RESULTS AND DISCUSSION

Phenolic acids have been repeatedly reported as natural antioxidants in fruits, vegetables, and other plants. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom (Clifford, 1999). In the present study, cell cultures from nine plant species

Table 2. HPLC gradient program.

Minutes	Eluent 0.1% phosphoric acid	Eluent 40% acetonitrile
0 - 1	99.5	0.5
10 - 12	60.0	40.0
18 - 20	20.0	80.0
24	1.0	99.0
30	0.0	100.0
34 - 39	99.5	0.5

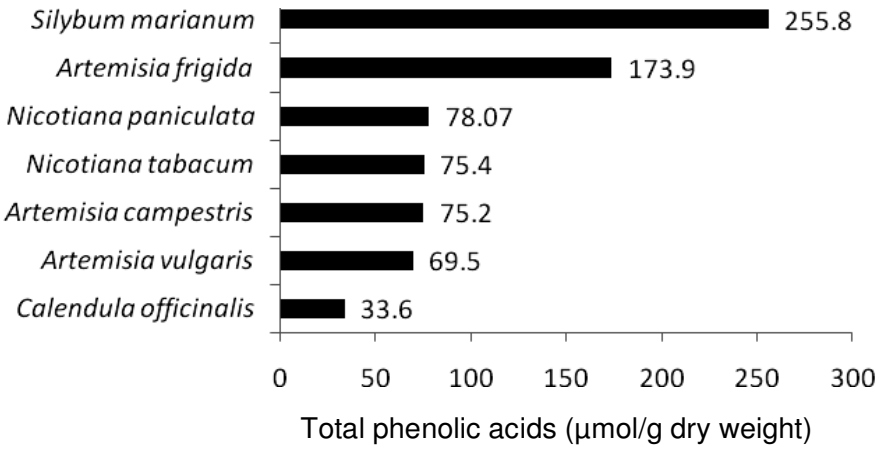

Figure 1. Total phenolic acids content in studied plant cell cultures.

from two families (Asteraceae and Solanaceae) were successfully established. The high genetic potential for phenolic compound accumulation was verified for most of the species (Figure 1).

The total hydroxycinnamic acid content differed between plant species (Figure 1). The most promising cell culture for phenolic acid production was *Silybum marianum* with a mean total phenolic acids concentration of 255.8 µmol/g dry cell weight, followed by *Artemisia frigida* (173.9 µmol/g dry cell weight). The two species from the *Nicotiana* genus showed similar phenolic acid production. Plant cell cultures of *S. marianum*, *Artemisia campestris* and *Nicotiana paniculata* did contain the following phenolic acids: chlorogenic acid, isochlorogenic acid A, B, and C, trans-ferulic acid, 4-methoxy-cinnamic acid and vanillic acid (Figure 2). The three most promising plant species were further quantized for their individual phenolic compounds.

Artemisia species and *S. marianum* were the highest yielders among all tested cultivars. The major phenolic acids have been chlorogenic acid and isochlorogenic acid A, B and C. Especially the genus *Artemisia* has been studied intensively for its phenolic contents and biological acitivities. For example, flavonoids, coumarins, triterpenoids, steroids and phenolics were isolated from different plant parts of *Artemisia annus* as well as from *in vitro* culture as: suspension culture, hairy root culture, and callus tissue (Bhakuni et al., 2001). Phenolic compounds from *Artemisia sphaerocephala* (Zhao et al., 2007), *Artemisia hispanica* (Marco et al., 1988), *Artemisia assoana* (Martínez et al.,1987), *A. campestris* (Teresa et al., 1984; González et al., 1983; Ferchichi et al., 2006), *Artemesia tridentate* (Brown et al., 1975) were studied. But until now, no data are available about the phenolic acids production from *in-vitro* cell culture of *Artemisia* species. We tried the first time to obtain phenolic acids form the cell cultures of various *Artemisia* species.

Most secondary metabolites are isolated from wild or cultivated plants because their chemical synthesis is either extremely difficult or economically infeasible. Biotechnological production in plant cell cultures is an attractive alternative, but to date this has had only limited commercial success because of a lack of understanding of how these metabolites are synthesized. The supply of precursors, the application of elicitors as well as alteration of metabolite composition by special treatments like biotransformation and immobilization, are the strategies to improve the production of secondary meta-

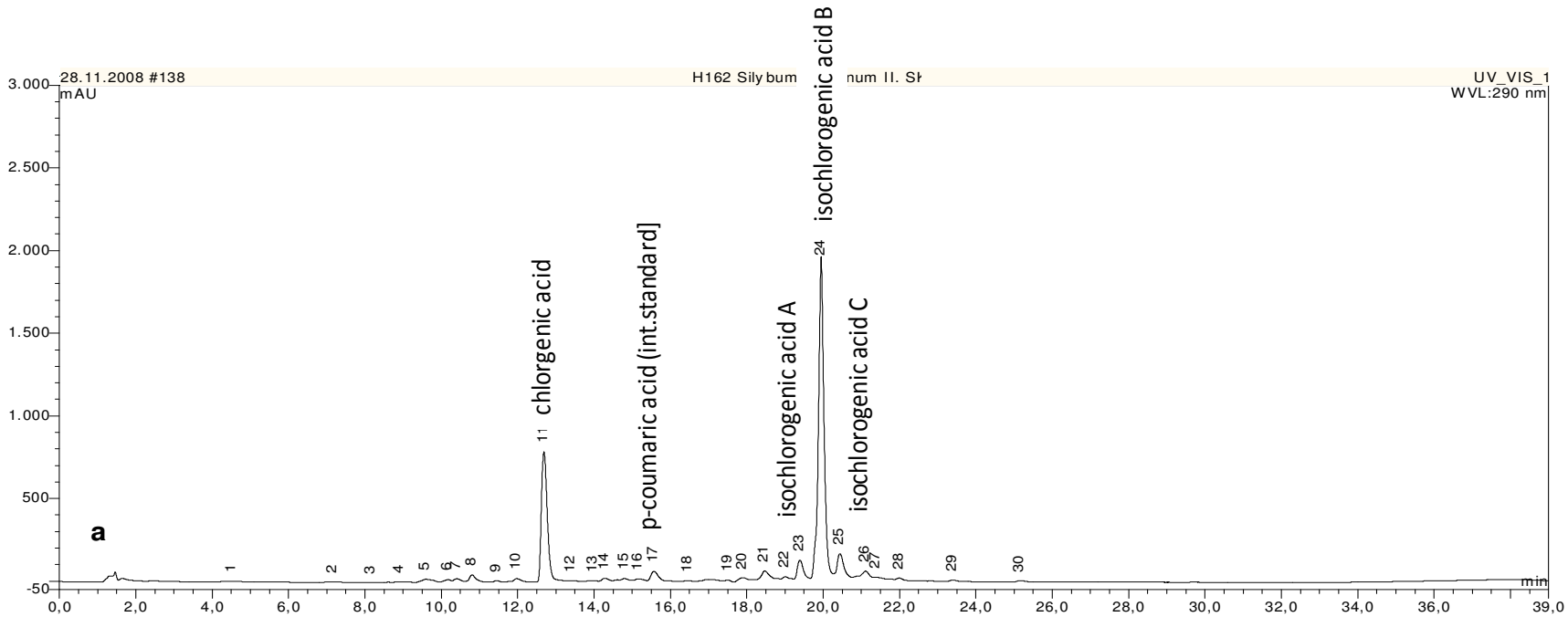

Figure 2. HPLC chromatograms of phenolic acid extracts from cell cultures, (a). *Silybum marianum*, (b). *Artemisia campestris*, (c). *Nicotiana paniculata*.

bolites (Doernenburg and Knorr, 1995; Smetanska, 2008), which we will apply in our further research.

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REFERENCES

Bhakuni RS, Jain DC, Sharma RP, Kumar S (2001). Secondary metabolites of *Artemisia annus* and their biological activity. Curr. Sci. 80: 35-48.

Brown D, Aspl RO, Vern A., McMahon VA (1975). Phenolic constituents of *Artemisia tridentata spp. Vaseyana*. Phytochemistry, 14(14): 1083-1084.

Clifford MN (1999). Chlorogenic acids and other cinnamates - nature, occurrence and dietary burden. J. Sci. Food Agric. 79: 362-372.

Cvikrová M, Hrubcová M, Meravý L, Pospíšil F (1988). Changes in the content of phenolic substances during the growth of *Nicotiana tabacum* cell suspension culture. Biologia Plantarum (Praha). 30: 185-192.

Daniel O, Meier MS, Schlatter J, Frischknecht P (1999). Selected phenolic compounds in cultivated plants: ecologic functions, health implications and modulation by pesticides. Environ. Health Perspect. 107: 109-114.

Doernenburg H, Knorr D (1995). Strategies for the improvement of secondary metabolite production in plant cell cultures. Enzyme Microb. Technol. 17: 674-684.

Ferchichi L, Merza J, Landreau A, Ray AML, Legseir B, Seraphin D, Richomme P (2006). Occurrence of isocoumarinic and phenolic derivatives in *Artemisia campestris L. subsp. campestris*. Biochem. Syst. Ecol. 34: 829-832.

González AG, Bermejo J, Estévez F, Velázquez R (1983). Phenolic derivatives from *Artemisia glutinosa*. Phytochemistry, 22: 1515-1516.

Han J, Zhong JJ (2002). High density cell culture of *Panaxnotoginseng* for production of ginseng saponin and polysaccharide in an airlift bioreactor.

Biotechnol. Lett. 24: 1927-1930.
Marco JA, Barberá O, Rodríguez S, Domingo, Adell J (1988). Flavonoids and other phenolics from *Artemisia hispanica*. Phytochemistry, 27: 3155-3159.
Martínez V, Barberá O, Sánchez-Parareda J, Marco JA (1987). Phenolic and acetylenic metabolites from *Artemisia assoana*. Phytochemistry, 26: 2619-2624.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
Petersen M, Simmonds MSJ (2003). Rosmarinic acid. Phytochemistry, 62: 121-125.
Ramachandra RS, Ravishankar GA (2002). Plant cell cultures: chemical factories of secondary metabolites. Biotechnol. Adv. 20: 101-153.
Smetanska I (2008). Production of secondary metabolites using plant cell cultures. Adv. Biochem. Eng. Biotechnol. 111: 187-228.
Teresa JDP, González MS, Muriel MR, Bellido IS (1984). Phenolic derivatives from *Artemisia campestris* subsp. Glutinosa. Phytochemistry, 23: 1819-1821.
Vermerris W, Nicholson R (2006). Phenolic compound biochemistry. Springer. The Netherlands.
Yang SW, Ubillas R, McAlpine J, Stafford A, Ecker DM, Talbot MK, Rogers B (2001). Three new phenolic compounds from a manipulated plant cell culture, Mirabilis jalapa. J. Nat. Prod. 64: 313-317.
Zhao DB, Li LX, Liu XH, Li MJ, Wang WL (2007). Two new phenolic compounds from *Artemisia sphaerocephala*. Chin. Chem. Lett. 18: 551-553.
Zhong JJ (2001). Biochemical engineering of the production of plant-specific secondary metabolites by cell suspension cultures. Adv. in Biochemical Engineering/Biotechnology. Springer Berlin / Heidelberg.