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Caffeic acid derivatives from a hairy root culture of *Lactuca virosa*

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Abstract In a search for biologically active phenolics, a hydroalcoholic extract from the hairy roots of *Lactuca virosa* was fractionated by chromatographical methods. The procedure led to the isolation of a substantial amount of 3,5-dicaffeoylquinic acid (3,5-DCQA)—a potent free radical scavenger. An analytical RP-HPLC separation of the hydroalcoholic extract from the hairy roots allowed identification of further hydroxycinnamates: caftaric acid (CTA), chlorogenic acid (5-CQA) and cichoric acid (DCTA), as well as small amounts of unbound phenolic acids. A time course of growth and caffeic acid derivatives accumulation in the hairy root culture was also investigated. The highest contents of the compounds in the examined roots were detected at the logarithmic phase of growth. The average content of 3,5-DCQA in the roots (ca. 2.5% DW) was at least one order of magnitude higher than that found in roots of *Lactuca* species and callus culture of *L. virosa*.

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Abbreviations

DW	Dry weight
FW	Fresh weight
5-CQA	Chlorogenic acid
3,5-DCQA	3,5-Dicaffeoylquinic acid
CTA	Caftaric acid
DCTA	Cichoric acid
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
2, 4-D	2, 4-Dichlorophenoxyacetic acid
RP-HPLC	Reversed-phase high-performance liquid chromatography
CC	Conventional column chromatography
MeOH	Methanol
MeCN	Acetonitrile

Introduction

Hydroxycinnamates are a group of phenolic compounds comprising free acids—hydroxyderivatives of cinnamic acid (e.g.: caffeic, ferulic, synapic and *p*-coumaric acids) and their conjugates. One of the commonest conjugates is 5-CQA also known as chlorogenic acid. Dicaffeoylquinic acids (diCQAs) along with tri- and tetra-caffeoylquinic derivatives as well as mono- and di-caffeoyl conjugates with some dicarboxylic aliphatic acids (e.g.: tartaric or succinic acid) are characteristic of Asteraceae plants. The compounds are potent radical scavengers and lipid peroxidation inhibitors (Ohnishi et al. 1994; Kim and Lee 2005; Olmos et al. 2008) and also play a role as infection and insect infestation inhibiting factors in plants (Kodoma et al. 1998; Leiss et al. 2009). Lettuce (*Lactuca sativa* L.) plants are a rich dietary source of hydroxycinnamates

(Degl'Innocenti et al. 2008; Llorach et al. 2008). *Lactuca virosa* L. (Asteraceae, Lactuceae)—poisonous (bitter) lettuce, which due to its multiple virus resistance has been used in breeding programs to introduce the resistance genes into commercial varieties of the garden lettuce (Tamaki et al. 1995), has not been studied before with respect to hydroxycinnamate content. The whole *L. virosa* plant as well as its leaves and latex (known as lettuce opium) reportedly possess medicinal properties and have been mentioned in different Pharmacopoeias (Stojakowska et al. 1999) as antitussic and sedative remedies. Analgesic and sedative activities of lactucin-like guaianolides, sesquiterpene lactones characteristic of *L. virosa* plants, have been recently confirmed by Wesołowska et al. (2006). The lactucin-like guaianolides were absent from undifferentiated tissue cultures of the plant (Stojakowska et al. 1994). Major secondary metabolites accumulated by the callus and suspension cultures were neolignan glycosides (Stojakowska and Kisiel 2000). From a hairy root culture of *L. virosa* sesquiterpene lactones characteristic of roots of the intact plant, sterols and pentacyclic triterpenols were isolated (Kisiel et al. 1995). The presence of terpenoids, however, did not explain an activity of a hydroalcoholic extract from the hairy roots in scavenging of DPPH free radical (data not shown). This observation prompted us to reinvestigate the culture in a search of antiradical agents.

Materials and methods

Plant material

Seeds of botanically verified *L. virosa* plants, collected from natural population were delivered by Botanical Garden in Nantes (France). The seeds were surface sterilized and aseptically germinated on half-strength MS medium (Murashige and Skoog 1962), solidified with 0.8% agar.

Roots and aerial parts from plants of the genus *Lactuca* were collected in the first year of their vegetation periods in the Garden of Medicinal Plants, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

Callus induction and culture

Leaf explants of *L. virosa* aseptic seedlings (ca. 1 cm long) were inoculated onto a solidified MS medium containing 2,4-D (4.52 μM l^{-1}), kinetin (1.39 μM l^{-1}) and 3% sucrose (pH adjusted to 5.8, before autoclaving) and maintained at 28°C, under continuous illumination (40 μmol m^{-2} s^{-1}, cool white fluorescent tubes) to induce calli. The calli were then transferred to a fresh MS agar medium of the same composition as the induction medium, for further growth. Temperature and illumination

conditions of the culture remained unaltered. The tissue was subcultured every 6 weeks by inoculating ca. 2.0 g fresh weight of the callus onto the fresh nutrient medium. For phytochemical investigation the calli were collected at the end of 6 week culture period and dried at room temperature.

Hairy roots

A hairy root culture of *L. virosa*, transformed by *Agrobacterium rhizogenes*, was established from aseptic seedlings of the plant obtained from the seeds of known wild origin, delivered by the Botanical Garden in Nantes (France). *A. rhizogenes* strain LBA 9402, containing agropine type Ri plasmid pRi 1855, was used in the experiment. Hairy roots were induced on leaf explants excised from the seedlings, and their transformed nature was proved by opine assay and rol B gene detection in plant genomic DNA, as described elsewhere (Stojakowska and Malarz 2000), except for that the REDTag ReadyMix PCR reaction mix (Sigma-Aldrich Co., St. Louis, MO, USA) replaced the corresponding components of the amplification reaction mixture. The transformed roots were cultivated in a modified liquid MS medium, containing ½ strength macronutrients and 3% sucrose, on a gyrotory shaker (110 r.p.m.), at 25°C, in the dark. The nutrient medium was initially supplemented with 500 mg l^{-1} of cefotaxime to obtain bacteria-free culture. The roots were subcultured every 4 weeks by inoculating 0.5 g of a fresh biomass in 250 ml Erlenmeyer flask containing 30 ml of the fresh medium. A time course experiment was performed by harvesting roots every 5 days during 35 days of culture. The experiment was done in triplicate. A DW of roots, as well as hydroxycinnamate contents were estimated.

Chemicals and solvents

CTA (**1**, purity >97% was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). DCTA (**2**, purity >98% by HPLC), 5-CQA (**3,** purity >97% by HPLC) and cynarin (1, 3-dicaffeoylquinic acid, purity >99% by HPLC) were purchased from Roth (Karlsruhe, Germany). A standard sample of 3,5-DCQA (**4**) was isolated in our laboratory from L. virosa root culture, and identified by comparison of its spectral (^{1}H NMR, 300.08 MHz) and physical ($[\alpha]_D$) data with those found in the literature (Basnet et al. 1996; Pauli et al. 1998; Islam et al. 2002). The compound **4** was of purity 90.0% (by HPLC). MeOH, *n*-hexane, chloroform and *n*-butanol of analytical grade were purchased from POCh S.A. (Gliwice, Poland). Water was purified by a Mili-Q system (Milipore Corp., Bedford, MA, USA). MeOH and MeCN of HPLC grade as well as formic acid

and glacial acetic acid were purchased from Merck (Darmstadt, Germany).

Isolation of 3,5-DCQA from hairy roots

Dried and pulverized roots (26 g) were exhaustively extracted with 80% MeOH at room temperature. The extract was concentrated in vacuo providing an oily residue (10.5 g) which was suspended in water and successively extracted with n-hexane, chloroform and n-butanol. The butanolic fraction (2.2 g) was subjected to CC on Polyamide with MeOH as an eluent and subsequently to CC on Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) eluted with a MeOH/H_2O mixture (4:1, v/v). This procedure led to the isolation of a substantial amount (217 mg) of 3,5-DCQA. Its optical rotation was determined with a PolAAr31 automatic polarimeter (Optical Activity LTD, England) and ^{1}H NMR spectrum was measured in deuterated MeOH on a Varian Mercury-VX 300 spectrophotometer.

Analytical RP-HPLC separation and quantification of hydroxycinnamates

Lyophilised roots (0.05 g) were extracted twice with 12.5 ml of 70% MeOH at room temperature. The combined extracts were evaporated in vacuo and the residue was dissolved in 1 ml of MeOH, left to stand overnight at 4°C, centrifuged (1,1340×g, 5 min) and analysed by RP-HPLC. Analytical RP-HPLC separations of the samples were performed using an Agilent 1200 Series HPLC system (Agilent Technologies, USA) equipped with a Rheodyne manual sample injector, quaternary pump, degasser, column oven and a diode array detector. Chromatographic separations of hydroxycinnamates were carried out at 25°C, on a Zorbax Eclipse XDB-C18 column, 4.6 × 150 mm (Agilent Technologies, USA), with a mobile phase consisting of H_2O/HCOOH/CH_3COOH 99/0.9/0.1 (solvent A) and MeCN/MeOH/HCOOH/CH_3COOH 89/10/0.9/0.1 (solvent B), at a flow rate of 1 ml min^{-1}, using 5 μl injections. The gradient elution conditions described by Spitaler et al. (2006) were applied. The analysis allowed identification of the following hydroxycinnamates of known biological activities present in the plant material [CTA, Rt (6.6 min); 5-CQA, Rt (7.3 min); DCTA, Rt (20.3 min) and 3,5-DCQA, Rt (28.2 min)], by comparison of their retention times and UV spectra with those of authentic samples and co-chromatography with standards. Compounds **1–4** (Fig. 1) were quantified using four point calibration curves, based on peak areas measured at 325 nm, prepared for cynarin, CTA, DCTA and 5-CQA (concentration range 0.02–1.50 mg ml^{-1}).

Quantification of lactucin-like guaianolides

Lyophilised roots (0.1 g) were extracted twice with 10 ml of MeOH at room temperature. The combined extracts were evaporated in vacuo and the residue was dissolved in 1 ml of MeOH, left to stand overnight at 4°C, centrifuged (11,340×g, 5 min) and analysed by RP-HPLC, at 40°C, using the aforementioned equipment. A fractionated sample (5 μl) was injected into a Purospher RP-18e (3 × 125 mm, particle size 5 μm) column (Merck, Darmstadt, Germany) eluted with a mobile phase consisting of water and MeCN, at a flow rate of 1 ml min^{-1}. The gradient elution conditions described by Grass et al. (2006) were applied. The typical retention times of the analysed compounds were as follows: 8-deoxylactucin glycoside (9.0 min), crepidiaside B (10.2 min), 8-deoxylactucin (13.5 min), jacquinelin (15.1 min) and lactucopicrin (26.8 min). Quantification was done by measurement of peak area at 260 nm with reference to the standard curve derived from five concentrations (0.125 to 2.000 mg ml^{-1}) of the guaianolide jacquineline acetate.

Quantification of unbound phenolic acids

Protocatechuic, p-hydroxybenzoic, caffeic, p-coumaric, ferulic and cinnamic acids were quantified, as described elsewhere (Ellnain-Wojtaszek and Zgórka 1999; Ekiert et al. 2009), after extraction of dried biomass of in vitro grown hairy roots with boiling methanol and subsequent RP-HPLC analysis of the obtained extract.

Statistics

All experiments were carried out in duplicate and were repeated at least once. Results are presented as mean values ± standard deviation (Microsoft Excel 2000).

Results and discussions

After 30 days of culture, the biomass of *L. virosa* hairy roots reached 0.57 g per flask (19 g l^{-1}) and 8 g per flask (267 g l^{-1}) measured as DW and FW, respectively (Fig. 2). A growth index (GI) of roots, calculated as a ratio of a final weight of roots at a harvest time to a weight of inoculum, was 15.8 (on a DW basis). The GI of roots was about two times higher than that of calculated for a suspension culture of *L. virosa* (Stojakowska and Kisiel 2000) and a transformed root clone described earlier (Stojakowska et al. 1999). After ca. 5 weeks of culture, a growth arrest could be observed in the roots accompanied by a change in tissue colour from bright yellow to grey. The main phenolic secondary metabolite isolated from the
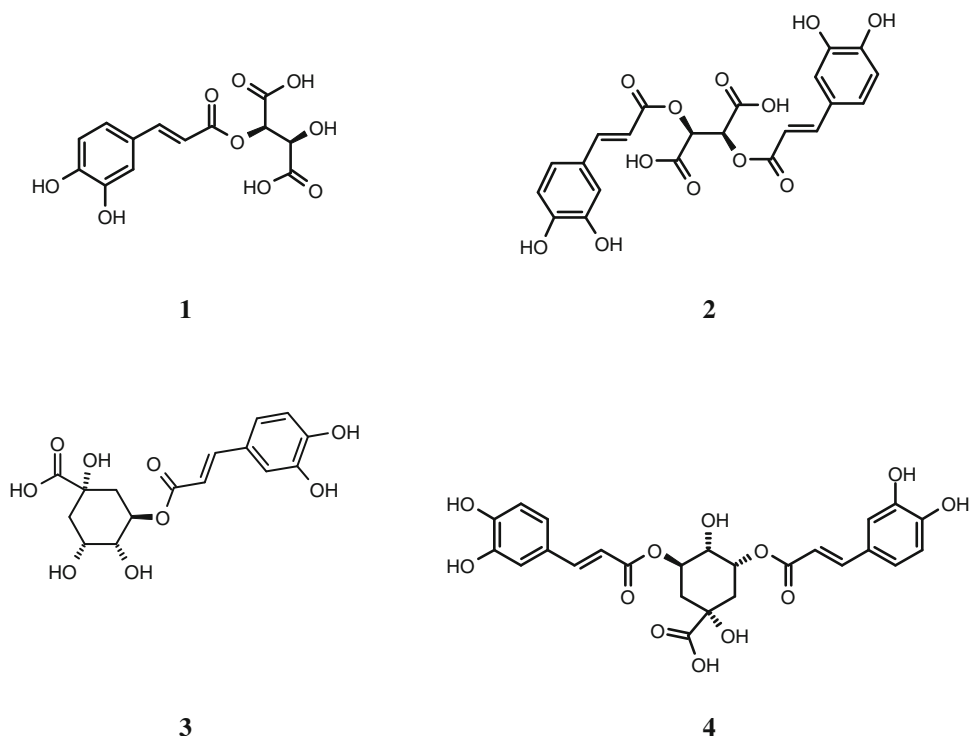
culture was 3,5-DCQA—a potent free radical scavenger (Kim and Lee 2005; Park et al. 2009), an inhibitor of lipid peroxidation and haemolysis (Ohnishi et al. 1994) which exerted protective effect against peroxynitrite and other reactive nitrogen species (Olmos et al. 2008) and protected human cells in vitro against LPS or H_2O_2 induced apoptosis by scavenging intracellular ROS and suppression of caspase-3 activation (Kim et al. 2005; Zha et al. 2007). The compound reportedly possesses inhibitory activities against HIV-1 replication and HIV integrase (Robinson et al. 1996; McDougall et al. 1998). 3,5-DCQA constituted nearly 3% of the DW of the examined roots. A qualitative and quantitative analysis of hydroxycinnamates present in

L. virosa hairy roots also showed a presence of 5-CQA (up to 1.5% DW), DCTA (up to 0.7% DW) and a low amount of CTA (up to 0.027% DW) (Fig. 4). According to Nicolle et al. (2004), 5-CQA and DCTA constituted up to 0.61% DW and 1.12% DW of *L. sativa* aerial parts, respectively. Contribution of DCTA alone to the total antioxidant power (measured as DPPH scavenging activity) of hydroalcoholic extracts from different cultivars of lettuce reached nearly 70%. DiCQAs were also detected as active compounds in *Bidens pilosa* and *Dipsacus asper* extracts which exhibited significant scavenging free radical activity (Chiang et al. 2004; Hung et al. 2006). Thus, hydroxycinnamates seem to
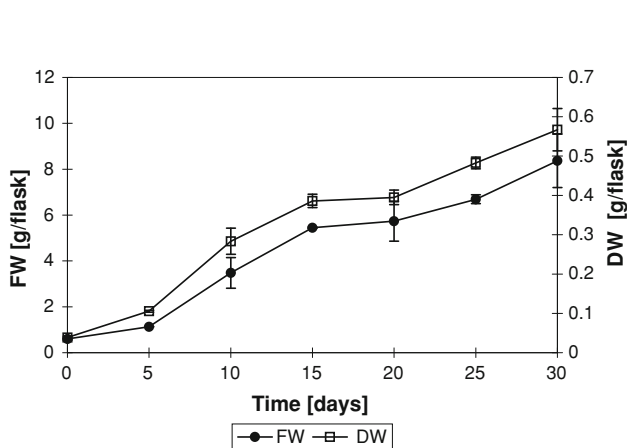

Fig. 2 Time course of biomass accumulation in the transformed roots of *L. virosa* (*open square* dry weight, *black circle* fresh weight). Data are means of four measurements. *Bars* represent standard deviation
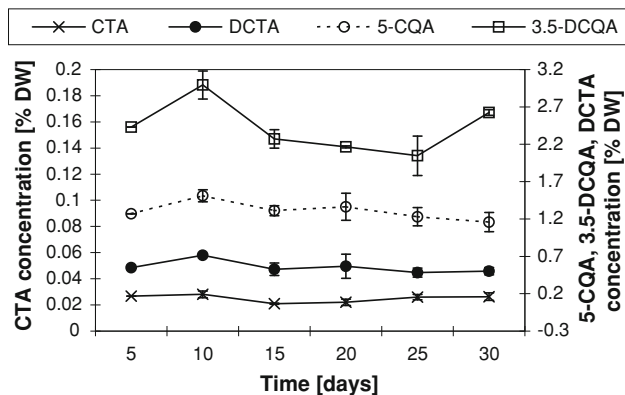

Fig. 3 Time course of hydroxycinnamate accumulation [*times symbol* caftaric acid (CTA), *open circle* chlorogenic acid (5-CQA), *filled circle* cichoric acid (DCTA), *open square* 3,5-dicaffeoylquinic acid (3,5-DCQA]. Data are means of three measurements. *Bars* represent standard deviation

Fig. 4 HPLC separation of hydroxycinnamates from hairy roots of *L. virosa* ($\lambda = 325$ nm): *1* CTA, *2* DCTA, *3* 5-CQA, *4* 3,5-DCQA. **a** Separation of a crude extract from the cultured roots; **b** cochromatography of the crude extract from the roots with CTA standard sample; **c** cochromatography of the crude extract from the roots with DCTA standard sample
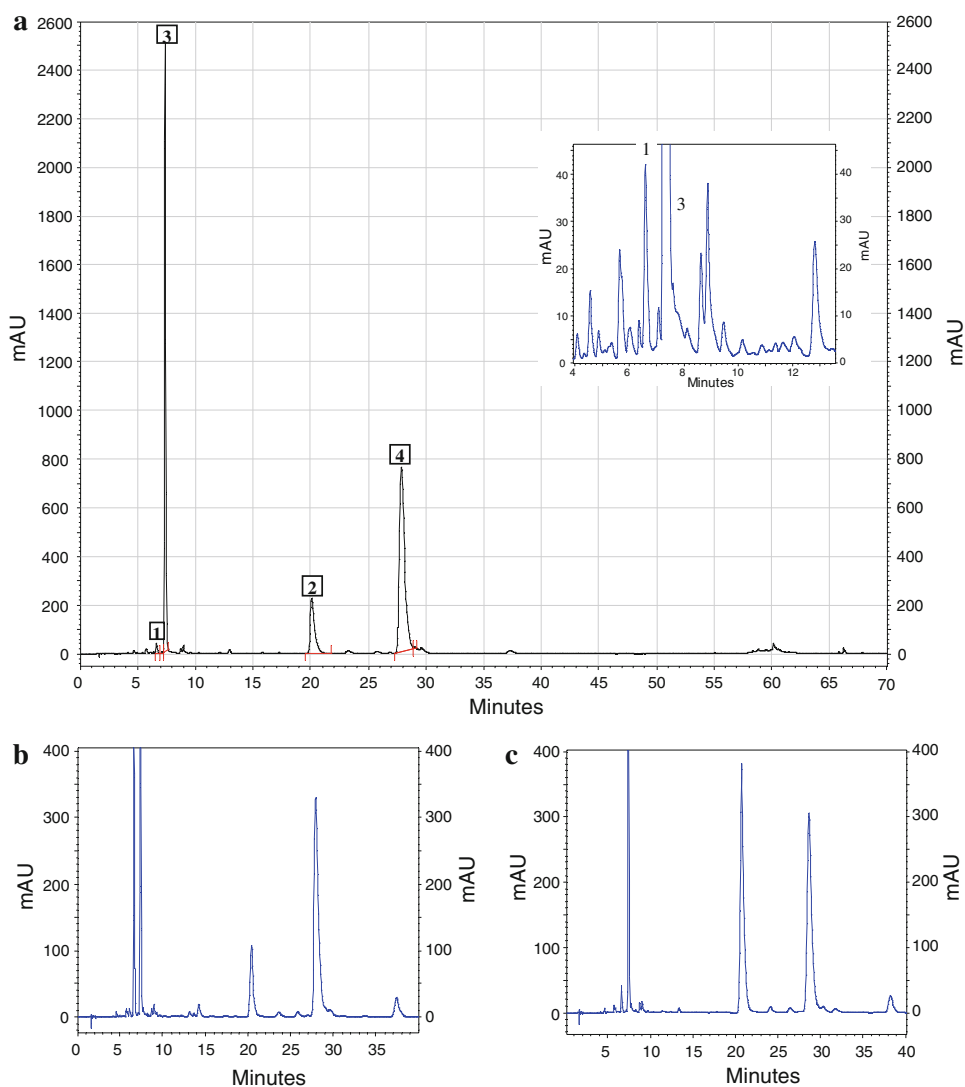
be responsible for high activity of extracts from *L.virosa* hairy roots in DPPH scavenging. Although, the contents of hydroxycinnamates in the roots remained relatively stable throughout the culture period (Fig. 3), their maxima could be detected at the day 10th of the culture (at the logarithmic phase of growth). The hydroxycinnamate contents were higher than those found in roots and aerial parts of *L. sativa* and *L. virosa* plants grown in the garden (Table 1). A callus culture of *L. virosa* produced a similar set of hydroxycinnamates but the estimated contents of the compounds were significantly lower (Table 1). In our previous study (Kisiel et al. 1995), we described an isolation of sesquiterpene lactones from hairy roots of *L. virosa*, however, the data concerning the culture growth and time course of lactucin-like guaianolide accumulation in the roots were not published previously (Fig. 2; Table 2). In the cultured roots, unlike in roots of the intact plant, the sesquiterpene lactones are accumulated mainly as their glucosides. In the

beginning of the growth cycle, the ratio of guaianolides which possess highly reactive egzomethylene group, responsible for their numerous biological activities (8-deoxylactucine and its glucoside) to their dihydroderivatives of low activity (jacquinelin and crepidiaside B) was ca. 5:1. At the stationary phase of the culture, the ratio dropped to 2:1. The high ratio in the beginning of the culture growth might be related to the stress-induced defence response of the tissue caused by the damage done during the transfer of inoculum to the fresh medium. The analysed sesquiterpene lactones reached their maximum contents in roots at the different phases of the culture growth and except for that of 8-deoxylactucin glucoside the maxima did not coincide with maximum accumulation of hydroxycinnamates. Low amounts of unbound phenolic acids were also detected in the roots harvested after four-week culture (Table 3).

The best known in vitro culture systems for production of hydroxycinnamates are root cultures of *Echinacea*

Table 1 Hydroxycinnamate contents in roots and aerial parts of *Lactuca* sp. plants grown in the open field and in in vitro cultures of *L. virosa*, harvested after four week culture (means of four measurements ± SD)

Plant material	Species	Hydroxycinnamate contents [% dry weight]			
		CTA	5-CQA	DCTA	3,5-DCQA
Roots	*Lactuca sativa* L. cv. British Hilde	0.012 ± 0.006	0.046 ± 0.011	0.096 ± 0.040	0.078 ± 0.013
	Lactuca virosa L.	0.029 ± 0.004	0.054 ± 0.008	0.179 ± 0.033	0.162 ± 0.001
Aerial parts	*Lactuca sativa* L. cv. British Hilde	0.149 ± 0.006	0.333 ± 0.002	1.501 ± 0.030	0.123 ± 0.003
	Lactuca virosa L.	0.203 ± 0.018	0.389 ± 0.024	1.499 ± 0.101	0.197 ± 0.018
	Lactuca serriola L. f. *serriola*	0.219 ± 0.003	0.313 ± 0.013	2.503 ± 0.145	0.111 ± 0.001
Callus	*Lactuca virosa* L.	0.012 ± 0.005	0.152 ± 0.015	0.061 ± 0.012	0.488 ± 0.060
Hairy roots	*Lactuca virosa* L.	0.052 ± 0.001	1.127 ± 0.030	0.785 ± 0.059	2.578 ± 0.063

CTA caftaric acid, *5-CQA* chlorogenic acid, *DCTA* cichoric acid, *3,5-DCQA* 3,5-dicaffeoylquinic acid

Table 2 Time course of lactucin-like guaianolides accumulation in hairy roots of *L. virosa*

Culture age [days]	Contents of lactucin-like guaianolides [% DW][a]				
	8-Deoxylactucin glucoside	Crepidiaside B (jacquinelin glucoside)	8-Deoxylactucin	Jacquinelin	Lactucopicrin
5	0.696 ± 0.019	0.135 ± 0.008	0.059 ± 0.008	0.021 ± 0.007	0.030 ± 0.008
10	0.721 ± 0.078	0.121 ± 0.020	0.049 ± 0.013	0.015 ± 0.001	0.078 ± 0.007
15	0.606 ± 0.058	0.121 ± 0.019	0.060 ± 0.021	0.013 ± 0.002	0.153 ± 0.012
20	0.545 ± 0.063	0.157 ± 0.019	0.054 ± 0.016	0.018 ± 0.001	0.113 ± 0.008
25	0.556 ± 0.043	0.185 ± 0.006	0.054 ± 0.010	0.014 ± 0.002	0.123 ± 0.037
30	0.464 ± 0.009	0.220 ± 0.003	0.033 ± 0.005	0.013 ± 0.002	0.130 ± 0.012

[a] means of four measurements ± SD

Table 3 Unbound phenolic acids in hairy roots of *L. virosa*

Name of the compound	Content in hairy roots [mg g^{-1} DW][a]
Protokatechuic acid	0.107 ± 0.012
p-Hydroxybenzoic acid	0.059 ± 0.008
Caffeic acid	0.044 ± 0.007
p-Coumaric acid	0.104 ± 0.008
Ferulic acid	0.017 ± 0.001

[a] means of four measurements ± SD

purpurea (L.) Moench. (Liu et al. 2006; Abbasi et al. 2007; Wu et al. 2007; Jeong et al. 2009). According to Pellati et al. (2004), total phenolics content in roots of *E. purpurea* plants was 2.3% DW. Adventitious root cultures of *E. purpurea* investigated by Jeong et al. (2009) accumulated 0.49% DW of CTA, 0.60% DW of 5-CQA and 2.81% DW of DCTA in the optimized culture conditions (GI 10.9). Hairy roots of the plant produced 12.9 g l^{-1} DW of biomass after 35-day culture. Yields of hydroxycinnamates were similar to those from adventitious roots (Abbasi et al. 2007). Elicited cell cultures of *E. purpurea* produced wide array of phenolic compounds (lignans, neolignans, acetophenone derivatives and phenolic glycosides) partly accumulated in cells and partly liberated to the culture medium. Caffeoyltartaric and caffeoylquinic acids, however, were not found in the cultures (Li and Barz 2006). Although *E. purpurea* root cultures were better CTA and DCTA producers than *L. virosa* hairy roots, their capability to accumulate caffeoylquinic derivatives was limited. Hairy roots of *L. virosa* accumulated nearly three times more of 5-CQA than root cultures of *E. purpurea* and up to 3% DW of 3,5-DCQA which was absent from *E. purpurea* roots. Undifferentiated cultures of the both plant species were poor sources of hydroxycinnamates. Aerial parts of *Lactuca sativa* L. intact plants accumulated DCTA as major caffeic acid derivative and only minor amounts of 3,5-DCQA (Degl'Innocenti et al. 2008; Ribas-Agustí et al. 2011). In the roots of *L. sativa* and *L. virosa* plants cultivated in our garden, contents of DCTA were similar to those of 3,5-DCQA (Table 1). Moreover, the DCTA contents in roots were much lower than those found in the aerial parts of the plants. In a callus culture of *L. sativa* var. *crispa* L. (Tamura et al. 2006) 3,5-DCQA predominated (leaves of the parent plant contained 3.8 times less of 3,5-DCQA than the cultured cells). Similar change in

hydroxycinnamate accumulation pattern (domination of 3,5-DCQA) was observed in our callus and root cultures (Table 1).

Hairy root cultures of *L. virosa* are efficient producers of caffeic acid derivatives of high antioxidant and chemo-preventive activity. Their unique features are capability to synthesize 3,5-DCQA as a sole diCQA which makes an isolation of the pure compound easy, and simultaneous accumulation of other chemopreventive hydroxycinna-mates as well as anti-inflammatory active (Cavin et al. 2005) sesquiterpene lactones.

References

Abbasi BH, Tian C-L, Murch SJ, Saxena PK, Liu C-Z (2007) Light-enhanced caffeic acid derivatives biosynthesis in hairy root cultures of *Echinacea purpurea*. Plant Cell Rep 26:1367–1372

Basnet P, Matsushige K, Hase K, Kadota S, Namba T (1996) Four di-*O*-caffeoyl quinic acid derivatives from propolis. Potent hepatoprotective activity in experimental liver injury models. Biol Pharm Bull 19:1479–1484

Cavin C, Delannoy M, Malnoe A, Debefve E, Touche A, Courtois D, Schilter B (2005) Inhibition of the expression and activity of cyclooxygenase-2 by chicory extract. Biochem Biophys Res Commun 327:742–749

Chiang YM, Chuang D-Y, Wang S-Y, Kuo Y-H, Tsai P-W, Shyur L-F (2004) Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. J Ethnopharm 95:409–419

Degl'Innocenti E, Pardossi A, Tattini M, Guidi L (2008) Phenolic compounds and antioxidant power in minimally processed salad. J Food Biochem 32:642–653

Ekiert H, Szewczyk A, Kuś A (2009) Free phenolic acids in *Ruta graveolens* L. in vitro culture. Pharmazie 64:694–696

Ellnain-Wojtaszek M, Zgórka G (1999) High performance liquid chromatography and thin-layer chromatography of phenolic acids from *Ginkgo biloba* L. leaves collected within vegetative period. J Liq Chrom Tech 22:1457–1471

Grass S, Zidorn C, Blattner FR, Stuppner H (2006) Comparative molecular and phytochemical investigation of *Leontodon autumnalis* (Asteraceae, Lactuceae) populations from Central Europe. Phytochemistry 67:122–131

Hung TM, Na MK, Thuong PT, Su ND, Sok DE, Song KS, Seong YH, Bae KH (2006) Antioxidant activity of caffeoyl quinic acid derivatives from the roots of *Dipsacus asper* Wall. J Ethnopharm 108:188–192

Islam MS, Yoshimoto M, Yahara S, Okuno S, Ishiguro K, Yamakawa O (2002) Identification and characterization of foliar polyphenolic composition in sweetpotato (*Ipomea batatas* L.) genotypes. J Agric Food Chem 50:3718–3722

Jeong J-A, Wu C-H, Murthy HN, Hahn E-J, Paek K-Y (2009) Application of an airlift bioreactor system for the production of adventitious root biomass and caffeic acid derivatives of *Echinacea purpurea*. Biotechnol Bioprocess Eng 14:91–98

Kim HJ, Lee YS (2005) Identification of new dicaffeoylquinic acids from *Chrysanthemum morifolium* and their antioxidant activities. Planta Med 71:871–876

Kim S–S, Park R-Y, Jeon H-J, Kwon Y-S, Chun W (2005) Neuroprotective effects of 3,5-dicaffeoylquinic acid on hydrogen peroxide-induced cell death in SH-SY5Y cells. Phytother Res 19:243–245

Kisiel W, Stojakowska A, Malarz J, Kohlmünzer S (1995) Sesquiterpene lactones in *Agrobacterium rhizogenes*-transformed hairy root culture of *Lactuca virosa*. Phytochemistry 40:1139–1140

Kodoma M, Wada H, Otani H, Kohmoto K, Kimura Y (1998) 3,5-Di-*O*-caffeoylquinic acid, an infection-inhibiting factor from *Pyrus pyrifolia* induced by infection with *Alternaria alternata*. Phytochemistry 47:371–373

Leiss KA, Maltese F, Choi YH, Verpoorte R, Klinkhamer PGL (2009) Identification of chlorogenic acid as a resistance factor for thrips in *Chrysanthemum*. Plant Physiol 150:1567–1575

Li W–W, Barz W (2006) Structure and accumulation of phenolics in elicited *Echinacea purpurea* cell cultures. Planta Med 72:248–254

Liu C-Z, Abbasi BH, Gao M, Murch SJ, Saxena PK (2006) Caffeic acid derivatives production by hairy root cultures of *Echinacea purpurea*. J Agric Food Chem 54:8456–8460

Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, Gil MI, Ferreres F (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 108:1028–1038

McDougall B, King PJ, Wu B-W, Hostomsky Z, Reinecke MG, Robinson WE Jr (1998) Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type I integrase. Antimicrob Agents Chemother 42:140–146

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nicolle C, Carnat A, Fraisse D, Lamaison J-L, Rock E, Michel H, Amouroux P, Remesy C (2004) Characterisation and variation of antioxidant micronutrients in lettuce (*Lactuca sativa folium*). J Sci Food Agric 84:2061–2069

Ohnishi M, Morishita H, Iwahashi H, Toda S, Shirataki Y, Kimura M, Kido R (1994) Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. Phytochemistry 36:579–583

Olmos A, Giner RM, Recio MC, Ríos JL, Gil-Benso R, Máñez S (2008) Interaction of dicaffeoylquinic derivatives with peroxynitrite and other reactive nitrogen species. Arch Biochem Biophys 475:66–71

Park KH, Park M, Choi SE, Jeong MS, Kwon JH, Oh MH, Choi HK, Seo SJ, Lee MW (2009) The anti-oxidative and anti-inflammatory effects of caffeoyl derivatives from the roots of *Aconitum koreanum* R. Raymond Biol Pharm Bull 32:2029–2033

Pauli GF, Poetsch F, Nahrstedt A (1998) Structure assignment of natural quinic acid derivatives using proton nuclear magnetic resonance techniques. Phytochem Anal 9:177–185

Pellati F, Benvenuti S, Magro L, Melegari M, Soragni F (2004) Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. J Pharm Biomed Anal 35:289–301

Ribas-Agustí A, Gratacós-Cubarsí M, Sárraga C, García-Regueiro J -A, Castellari M (2011). Analysis of eleven phenolic compounds including novel *p*-coumaroyl derivatives in lettuce (*Lactuca sativa* L.) by ultra-high-performance liquid chromatography with photodiode array and mass spectrometry detection. Phytochem Anal. doi:10.1002/pca.1318

Robinson WE Jr, Reinecke MG, Abdel-Malek S, Jia Q, Chow SA (1996) Inhibitors of HIV-1 replication that inhibit HIV integrase. Proc Natl Acad Sci 93:6326–6331

Spitaler R, Schlorhaufer PD, Ellmerer EP, Merfort I, Bortenschleger S, Stuppner H, Zidorn C (2006) Altitudinal variation of secondary metabolite profiles in flowering heads of *Arnica montana* cv. ARBO Phytochem 67:409–417

Stojakowska A, Kisiel W (2000) Neolignan glycosides from a cell suspension culture of *Lactuca virosa*. Pol J Chem 74:153–155

Stojakowska A, Malarz J (2000) Flavonoid production in transformed root cultures of *Scutellaria baicalensis*. J Plant Physiol 156:121–125

Stojakowska A, Malarz J, Kisiel W (1994) Sesquiterpene lactones in tissue culture of *Lactuca virosa*. Planta Med 60:93–94

Stojakowska A, Malarz J, Kisiel W (1999) *Lactuca virosa* L. (bitter lettuce): In vitro culture and production of sesquiterpene lactones. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Medicinal and aromatic plants XI, vol 43. Springer-Verlag, Heidelberg, pp 261–273

Tamaki H, Robinson RW, Anderson JL, Stoewsand GS (1995) Sesquiterpene lactones in virus-resistant lettuce. J Agric Food Chem 43:6–8

Tamura H, Akioka T, Ueno K, Chujyo T, Okazaki K-I, King PJ, Robinson WE Jr (2006) Anti-human immunodeficiency virus activity of 3, 4, 5-tricaffeoylquinic acid in cultured cells of lettuce leaves. Mol Nutr Food Res 50:396–400

Wesołowska A, Nikiforuk A, Michalska K, Kisiel W, Chojnacka-Wójcik E (2006) Analgesic and sedative activities of lactucin and some lactucin-like guaianolides in mice. J Ethnopharmacol 107:254–258

Wu C-H, Murthy HN, Hahn E-J, Paek KY (2007) Enhanced production of caftaric acid, chlorogenic acid and cichoric acid in suspension cultures of *Echinacea purpurea* by the manipulation of incubation temperature and photoperiod. Biochem Eng J 36:301–303

Zha R-P, Xu W, Wang W-Y, Dong L, Wang Y-P (2007) Prevention of lipopolisaccharide-induced injury by 3,5-dicaffeoylquinic acid in endothelial cells. Acta Pharmacol Sin 28:1143–1148