Patterns of variation in lipophilic and hydrophilic constituents in flower developmental stages of *Echinacea purpurea* (L.) Moench cultivated in Slovakia

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ABSTRACT

The objective of this study was to examine and demonstrate how harvesting age (flower age) contribute to the variations in the quality of *Echinacea purpurea* (L.) Moench. The effects of different flower developmental stages on caffeic acid derivatives and isobutylamide content are described. These phytochemicals were extracted from fresh plants with 60% ethanol and quantified by the HPLC analysis. The results revealed that the quality of *Echinacea* is strongly influenced by the flower developmental stages. The highest content of both hydrophilic and lipophilic components in the anthodium of *Echinacea* plants were found in the third (mature) developmental stage, which is regarded as the optimum one for the harvest to obtain optimum yield levels.

Keywords: *Echinacea purpurea*; anthodium; developmental stages; cichoric acid; echinacoside; chlorogenic acid; isobutylamide; harvest time

The *Echinacea* species are native to the North American prairies. They have been introduced as cultivated medicinal plants in Europe. Nearly all parts of the plants are used for therapeutic preparations. Products may be derived from cultivated or wild stocks. European products rely on cultivated plants, mainly *E. purpurea*. Preparations of *Echinacea* plants have been traditionally employed to stimulate the immune system (See et al. 1997). The immunostimulating properties of *Echinacea* species are mainly attributed to both lipophilic and polar fractions of the extracts, including alkamides, caffeic acid derivatives, glycoproteins, and polysaccharides (Bauer and Wagner 1991).

A variety of *Echinacea* alkamides (mainly isobutylamides) were shown to stimulate immune cell activity (Goel et al. 2002) and to inhibit enzymes (cyclooxygenase and 5-lipoxygenase) involved in the production of inflammatory mediators (Müller-Jakic et al. 1994). Isobutylamides from *E. purpurea* and *E. angustifolia* roots also inhibit arachidonic acid metabolism to inflammatory prostaglandins and may account for some of *Echinacea's* anti-inflammatory effects (Bauer et al. 1988b). When testing the alcoholic extracts from *Echinacea*, the phagocytic enhancement was largely credited to isobutylamides content (Bauer et al. 1988a, Bauer and Wagner 1991). The isobutylamides are thought to cause *Echinacea's* topical effect, which is often described as tingling or tongue numbing (Bauer and Wagner 1991).

Cichoric acid is concentrated in roots and flowers of *E. purpurea*, and in smaller amounts also in other species (Bauer et al. 1988c, Awang and Kindack 1991). It stimulates cell-mediated immunity (Bauer 1999), exhibits antiviral (Cheminat et al. 1988) and antioxidant activity (Sloley et al. 2001), inhibits hyaluronidase (Maffei Facino 1995), an enzyme involved in infection and inflammation, has a stimulant effect on phagocytes and an inhibitive effect on the HIV infection (Reinke et al. 2004). Echinacoside, another caffeic acid derivative, was shown to exhibit potent antioxidant/free-radical scavening (Maffei Facino 1995, Hu and Kitts 2000) and anti-inflammatory activity (Speroni et al. 2002), but does not appear to stimulate immune cells.

The overall immunostimulant activity of the alcoholic and aqueous *Echinacea* extracts appears to depend on the combined effects of several constituents (Bauer and Wagner 1991). Therefore, cichoric acid, echinacoside, and alkamides (iso-

butylamides) are generally used as indices of quality control for standardization of *E. purpurea* row material and finished products (Bauer 1998) and are the most likely candidates for the genus medicinal properties.

Our work is directed to the investigate the pattern of the accumulation of some active compounds during different developmental stages of the *E. purpurea* anthodium. The time of harvesting is one of the most important agronomical factors therefore the optimum yield levels of both hydrophilic and lipophilic components have been studied.

MATERIAL AND METHODS

The anthodia of *Echinacea purpurea* (L.) Moench were used for experiments. Trials were established in 4 replications with the size of individual plots of 1.5 × 3 m, 50 plants were planted per plot. For phytochemical study, the aerial parts of 10 plants were harvested, which was replicated three times. Harvest of the flower heads was performed during the second year of vegetation at least four times, depending on the phase of anthodium development.

The first developmental stage (preflowering) is represented by anthodium with the first appearance of ray florets. In the second developmental stage (flowering) the ray florets are expanded and are of purple colour, the first lines of the disc florets beginning of bloom. In the third developmental stage (mature) the ray florets are fully expanded, disc florets fully opened up to 80–90%. In the fourth developmental stage (overblown) the ray florets start to gradually fall down, the flower heads start to release the fruits.

Immediately after harvest, the flower heads were separated by hand from the stems. The plant material was dried in an air-ventilated oven at 36°C. After drying, the samples were ground to powder. 50 mg of the powdered plant material were extracted three times in 8 ml 70% ethanol using 10min sonication, centrifugation, collection of the supernatant and adjustment of the volume to 25 ml. All extracts were filtered (0.2 µm PTFE membrane) prior to the HPLC separations. HPLC chromatografic methods were based on Bauer et al. (1988c) and Bauer and Remiger (1989) with slight modification. The liquid extracts were centrifuged, the oil sample was diluted with ethanol and the supernates were filtered as above prior to injection. In both systems, 5 µl of sample was injected on 75 × 4.6 mm reversed-phase C-18 column, 3 µm particle size. Lipophilic compounds were detected at 210 and 260 nm and hydrophilic compounds at 326 nm.

The results were obtained from 10 plants replicated three times. The results of analysis were recalculated to the dry matter.

RESULTS AND DISCUSSION

As a member of Asteraceae family, each anthodium of *E. purpurea* is a conglomeration of many tiny florets. The disc florets end in spines, and are surrounded by outer ray florets with teeth at their ends. The quality of the anthodium with regard to the incidence of individual florets has been changing.

Results of our experiments show that the content of cichoric acid changes significantly during senescence of the plant. The anthodium of *E. purpurea* plant contains the greatest amount of cichoric acid at the first developmental stage and during other developmental stages it gradually declines to overblown stage. The maximum content of chlorogenic acid and echinacoside occurred at preflowering and mature stages, respectively (Table 1). The concentration of isobutylamide was maximal at mature flower developmental stage (Table 2).

Recent phytochemical investigation shows that there is a tremendous variation in the quality of *Echinacea* products (Letchamo et al. 1999).

The effect of growing site and harvest time on levels of phenolics and alkamides in *Echinacea* were studied previously by many authors. Wills and Stuart (1999) observed that cichoric acid concentration in plant tissues of *E. purpurea* did not change during plant growth, but did decrease during plant senescence. Stuart and Wills (2000)

Table 1. Cichoric acid, chlorogenic acid and echinacoside content in flower developmental stages of *E. purpurea* (L.) Moench grown in Slovakia

Flower developmental stage	Hydrophilic compounds (% of dry matter)		
	cichoric acid	chlorogenic acid	echinacoside
I. Preflowering	3.55	0.048	0.005
II. Flowering	2.49	0.022	0.020
III. Mature	2.22	0.020	0.048
IV. Overblown	0.92	0.016	0.029

Table 2. Isobutylamide content in flower developmental stages of *E. purpurea* (L.) Moench grown in Slovakia

Flower stage	Lipophilic compounds (% of dry matter) isobutylamide
I. Preflowering	0.010
II. Flowering	0.012
III. Mature	0.018
IV. Overblown	0.012

analyzed *E. purpurea* roots and various aerial parts grown at two sites in Australia and harvested at four growth stages for alkamides and cichoric acid. The level of cichoric acid showed no significant change during the flowering and mature stage. The alkamide level increased in the flower tissues to the senescence. On the contrary, Perry et al. (2004) found the late season drop in flower alkamide levels in *E. purpurea* grown in New Zealand. El-Gengaihi et al. (1998) observed that the alkamide content varies over *E. purpurea's* life cycle, gradually decreasing in the aerial parts and increasing in the roots as the plant matures.

Letchamo et al. (1999) studied the accumulation of active ingredients during development of flower heads of the American grown *E. purpurea*. They found the highest content of cichoric acid at stage 1 and highest content of isobutylamide at stages 3 and 4. This is in accordance with our results, with difference in amounts of mentioned constituents. Letchamo et al. (1999) observed the maximum content of echinacoside at stage 2, whereas we found it at stage 3. Canada grown *E. purpurea* lacked echinacoside in flower heads at all ages and growth conditions (Binns et al. 2002). Kreft (2005) observed *E. purpurea* plants cultivated in Slovenia from 23 plantations and two harvesting times, observed no influence of age on the cichoric acid content.

In conclusion, caffeic acid derivatives and alkamides of *E. purpurea* represent important constituents for pharmaceutical industry. Our analysis revealed the highest content of both hydrophilic and lipophilic constituents at the mature developmental stage of anthodium. To obtain optimum yield levels of the above mentioned components, *E. purpurea* flower heads should be harvested at the third developmental stage. According to the presented results it is evident that the quality of *Echinacea* is strongly influenced by the developmental stages of anthodium.

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Received on October 30, 2008

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