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# Cichoric Acid Content and Biomass Production of *Echinacea purpurea*. Plants Cultivated in Slovenia

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# Cichoric Acid Content and Biomass Production of *Echinacea purpurea* Plants Cultivated in Slovenia

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## Abstract

Samples of *Echinacea purpurea* (L.) Moench were taken from 25 plantations at two harvesting times (July and October). Five shoots from each plantation were measured and weighed. The contents of cichoric and caftaric acid were determined in flowers, leaves, and stems of samples harvested in July. All morphological parameters decreased with increasing age of the plantation, but age had no influence on the cichoric and caftaric acid contents. The average weight of leaves and stems in 6-year-old plantations was more than sixfold lower than those from 1-year-old plantations. In flowers, the reduction was fourfold. Cichoric and caftaric acid contents in leaves differed significantly between the regions, but the region had no influence on the morphological parameters. Irrigated plantations yielded more than 50% higher weights of leaves and stems and 25% higher weights of flowers. Irrigation had no influence on cichoric and caftaric acid contents.

**Keywords:** Biomass production, caftaric acid, cichoric acid, *Echinacea purpurea*, purple coneflower.

## Introduction

*Echinace purpurea* (L.) Moench (Asteraceae) is a perennial plant used as a treatment for the common cold and flu because of its immunostimulatory effect. Commercial preparations are all produced from cultivated plants. Two-thirds of the land planted with medicinal or aromatic plants in Slovenia is planted with *E. purpurea*. Germination of seeds of *Echinacea purpurea* has been studied by several groups (Feghahati & Reese, 1994; Pill & Haynes, 1996; Harbage, 2001; Sari et al., 2001), and pests attacking this plant have also been studied (Hwang et al, 1997; Simmons et al., 2000), but the influence of cultivation conditions on active ingredient content is not well characterized. Cichoric acid is one of the bioactive compounds in *E. purpurea* (Bauer, 1996; WHO, 1999); the others are alkamides and polysaccharides. Because there is no routine analytical method developed for analysis of polysaccharides in *E. purpurea*, the recently proposed United States Pharmacopeal monograph on *Echinacea purpurea* aerial parts (Giancaspro, 2004) describes only determination of the content of cichoric and caftaric acids and the content of alkamides (dodecatetraenoic acid isobutylamides) in dried plant material. Cichoric acid content was found to be highly variable (Rogers et al., 1998; Wills & Stuart, 1999; Binns et al., 2002), but the cause of the variation was not identified. Factors influencing the yield of cultivated plants have also not been investigated. Interesting new data are obtained in this study by analyzing the morphology and phytochemistry of the samples from the 25 plantations.

## Materials and Methods

The plants [*Echinacea purpurea* (L.) Moench, Asteraceae] were collected from all 25 existing commercial plantations in Slovenia by cutting the stem 5–10 cm above the ground. They were harvested over two periods: July 11–23, 2002 (18 plantations) and October 4–15, 2002 (18 plantations, including 7 first-year plantations that were not harvested in July), most of the flowers being opened. Five samples were collected from each plantation at each harvest time. The flower heads and leaves were separated from the stems, and the samples were dried at room temperature. Room temperature was

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*Table 1.* Cichoric and caftaric acid contents in three organs of *Echinacea purpurea*.

| | Cichoric acid (mg/g d.w.) | | | | Caftaric acid (mg/g d.w.) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Min. | Max. | Mean | SD | Min. | Max. |
| Flower | 10.76 | 5.61 | 2.17 | 28.88 | 5.05 | 2.52 | 0.74 | 12.56 |
| Leaf | 15.82 | 9.14 | 3.14 | 52.25 | 11.85 | 5.74 | 2.92 | 32.90 |
| Stem | 2.37 | 1.56 | 0.30 | 7.75 | 2.93 | 2.01 | 0.23 | 9.19 |

d.w., dry weight.

chosen because higher temperature increases the loss of cichoric acid (Kim et al., 2000; Stuart & Wills, 2003). Interviews of the farmers collected the data about irrigation and the year when plantation was established.

Samples were powdered and extracted with 50% methanol:water. A solvent to drug ratio of 40 ml/g was found to be insufficient (a further 40% of cichoric acid was extracted with fresh solvent); a ratio of 200 ml/g was shown to be adequate. A similar ratio is used in USP (Giancaspro, 2004). Extraction efficacy increased as the time of extraction varied from 1 to 4 h. To assure complete extraction, 20 h extraction was used for further analyses. The extracts were found to be stable for at least an additional 24 h. Extracts were analyzed by capillary electrophoresis using a modification of the method of Pomponio et al., (2002) as described (Mančet, 2003; Mančet and Kreft, 2005). Briefly, a Hewlett-Packard 3D (HP 3D Capillary Electrophoresis System, Waldbronn, Germany) with a diode array UV-Vis detector (DAD), controlled by HP3DChemStation 6.03, with a glass capillary (57 cm × 50 μm and bubble detection cell) thermostated at 35°C was used. The capillary was rinsed for 1 min with methanol and 1 min with buffer prior to each analysis. The electrophoresis buffer was 75 mM borate, pH 8.8. The sample was injected at 20 mbar for 20 s. Separation was performed at 20 Kv (kilo volts). Detection was at $\lambda = 350$ nm (response time 1 s).

## Results and Discussion

The average contents of cichoric and caftaric acids in the samples (Table 1) was found to be similar to those published by Bauer et al. (1998), Wills and Stuart (1999), Stuart and Wills (2000), and Letchamo et al., (1999), but higher than that reported by Binns et al., (2002). In contrast with a previous report, the content was higher in leaves than in flowers. This may be due to different flower developmental stage at the harvesting time (Letchamo et al., 1999).

The content of cichoric and caftaric acids in individual plants is highly variable. Only a small part of the large variability can be explained by conditions on the cultivation site. Interindividual differences are the main source of variability. Cichoric and caftaric acid contents in leaves differed significantly between the regions ($p < 0.001$) (Fig. 1); 19.4% of the variance of cichoric

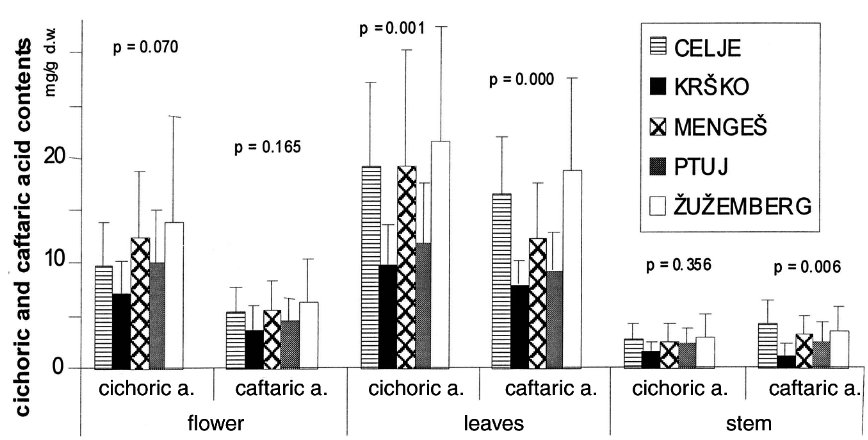


*Figure 1.* Cichoric acid and caftaric acid contents in flowers, leaves, and stems of plants grown in different regions. Region has a significant influence (ANOVA, $p < 0.05$) on the content of both acids in leaves and on caftaric acid in stems. The lowest content of both substances in all organs is found in Krško region, followed by Ptuj region, and the highest content is found in Žužemberg region. The Žužemberg region yields, on average, a twofold higher cichoric acid content in leaf than the Krško region. Standard deviations are represented by the error bars.

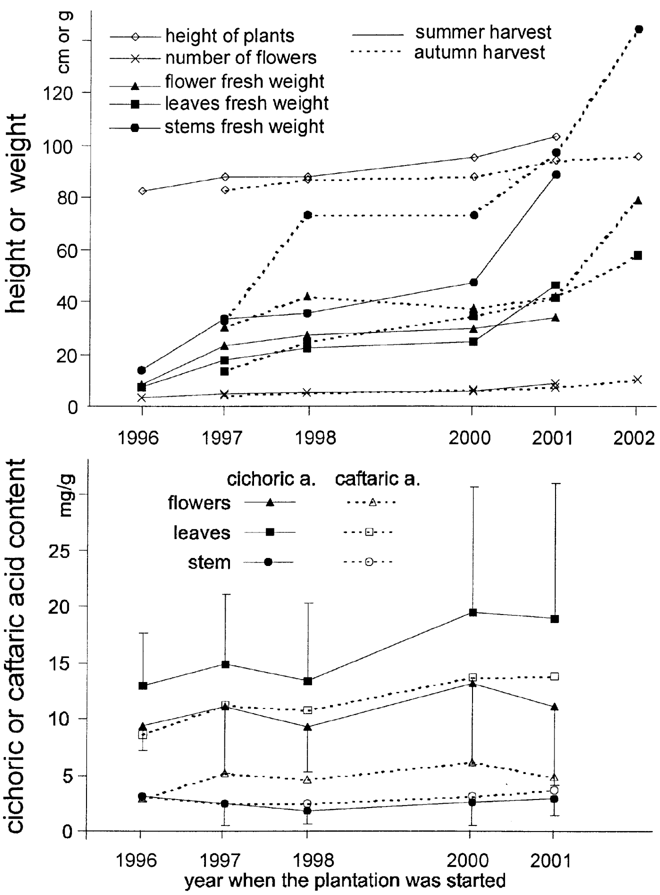


*Figure 2.* Morphological (top panel) and phytochemical (bottom panel) characteristics of plants as a function of the year when the plantation was set out. Because the fields were planted in the spring of the respective year and there was no harvest in the summer of the year, the morphological data for the summer harvest of 2002 is missing (top panel). Morphological parameters decrease markedly with the age of plantation, but the contents of the two acids are not significantly affected. Standard deviations are represented by the error bars.

acid can be attributed to the influence of the region, 3% to the influence of the local field, and 77.6% to interindividual differences between the plants on the same field. The variance of caftaric acid content was 32.7% due to the region, 6.7% to field, and 60.6% to interindividual differences. Our results demonstrate the importance of collecting a sufficient number of plants (>5 plants) to determine the average quality of the harvest. The region with highest cichoric acid contents (Žužemberg region) yielded an average twofold higher content in leaf than the region with lowest contents (Krško region). Žužember region yielded the highest content of both analyzed acids in all three analyzed plant organs (Fig. 1), and Krško region yielded the lowest content of both acids in all organs. The average distance between the regions was 60 km, and the average distance between the plantations within the region was 10 km. The region, on the other hand, did not have such an influence on the morphological parameters. Of the 22 measured parameters (height, number of flowers, fresh and dry weight, water content in three organs at two harvest times), the height of plants at summer harvest was most significantly influenced by the region, although the difference in average height of plants in the two extreme regions was only 22%. This pattern was not reported at the autumn harvest.

All the morphological parameters measured decreased with increasing age of the plantation, but age did not have a significant influence on the cichoric and caftaric acids contents in any organ (Fig. 2). The average weight of leaves and stems in 6-year-old plantations was more than sixfold lower than in a 1-year-old plantations. In flowers, the reduction was fourfold. The highest biomass was obtained in the autumn crop of the first year, the year in which the plants were planted in spring and not harvested in the summer. The total yield of biomass from the two harvests in the second year was only 25% higher

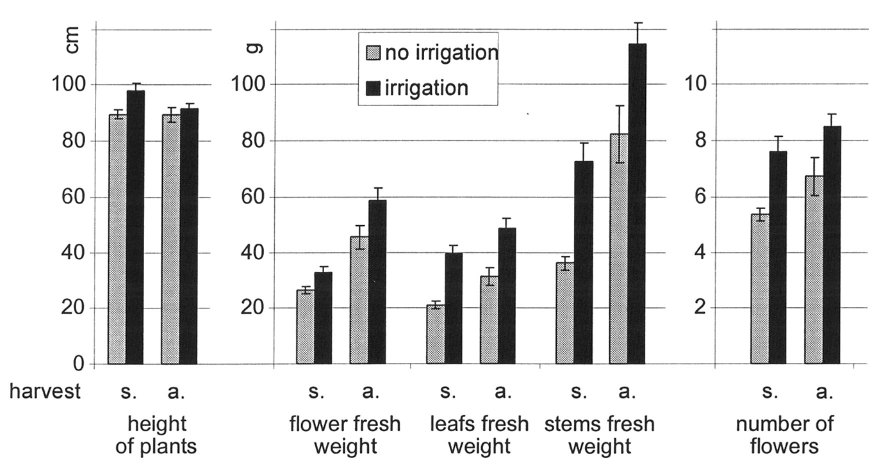


*Figure 3.* The influence of irrigation on plant morphology (s., summer; a., autumn). Standard error of mean is represented by the error bars.

than the biomass of one harvest in the first year. In the 3rd, 5th, and 6th years, the biomass yields were 12%, 20%, and 30% lower, respectively, than in the first year. For optimal production, the plantations should be ploughed up and replanted every 3 years.

Irrigated plantations yielded more than 50% higher amounts of leaves and stems and 25% higher amounts of flowers (Fig. 3). The water content was not significantly higher in plants from irrigated plantations, nor did irrigation have any influence on cichoric and caftaric acid contents. In contrast, Gray et al., (2003) found that drought stress increases the root weight.

The biomass production at the autumn harvest was significantly higher (up to twofold) than at the summer harvest (Fig. 3).

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