INFLUENCE OF HARVEST TIME ON QUALITY AND MARKETABILITY OF COMMERCIALLY IMPORTANT *Echinacea* spp.

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ABSTRACT

In late 90's echinacea, particularly *Echinacea angustifolia*, has become a lucrative medicinal root crop on the prairies. In the absence of well substantiated and reliable information on optimal time to harvest, roots are harvested in the fall of the 3rd or 4th year of production. The objective of this study was to determine the influence of three harvest times, spring, mid-summer and fall on accumulation of compounds believed to be responsible for the immunostimulatory activity of echinacea. Phenolic glycosides (echinacoside in *E. angustifolia* and chicoric acid in *E. purpurea*) (1), alkylamides (2), and polysaccharides (3), were the three classes of natural compounds used for the phytochemical quality assessment in this study. Results indicated that root of 3-year old *E. angustifolia* plants harvested in the fall contained significantly higher content of polysaccharides, slightly higher content of echinacoside and slightly lower content of total alkylamides than roots collected in spring and summer. Also, roots of 2-year old plants were significantly higher in echinacoside and total alkylamides and significantly lower in polysaccharides than their 3-year counterpart irrespective of harvest time. Roots of 4-year old *E. purpurea* harvested in the fall contained higher amount of chicoric acid than roots harvested in spring and mid-summer. Given that echinacoside content is presently used by herb industry as an indicator of crop quality (and price), and for standardization of botanical preparations (typically 4%), we also determined the distribution of echinacoside and cynarin in fall harvested *E. angustifolia* root system. Both compounds were found to accumulate in greater quantities in small rootlets and root crowns than in main tap roots suggesting that phytomedicinal quality and market value are greatly dependent on the harvesting method. Other aspects of cultivation with implications on marketability of echinacea are also discussed.

Over the last 3 years echinacea has been the best selling medicinal plant and herbal immunostimulant in North America, accounting for 10-12% of total herbal supplement sales (up to a hundred million US $ annually). In response to the growing body of evidence supporting the traditional uses of echinacea as an immunostimulant and the highly publicized testimonials for the effectiveness of echinacea in preventing and

treating colds, there has been an increased interest in cultivation of this plant across Canada. It is estimated that at present there are 150-200 acres planted to *E. angustifolia* in Saskatchewan. This is the only species in the genus *Echinacea* that is also native to southeastern Saskatchewan. While major advances have been made in various aspects of echinacea cultivation (e.g. seed germination, soil type, method of planting, winter protection), disease management and particularly harvesting remain to pose a challenge.

The lack of available information on the effect of harvest time on phytomedicinal quality of echinacea has left many producers approaching harvest of their first crop wondering when to harvest. The issue is further complicated by the fact that phytomedicinal quality evaluation of echinacea has not been addressed adequately to date (due to complexity of chemical composition and even more so the lack of well designed clinical trials) and that no consensus on the definition of quality has been reached yet. While chicoric acid, a caffeic acid derivative, present in *E. purpurea* has been documented to cause stimulation of the immune system, it is uncertain whether echinacoside present in *E. angustifolia* is of any therapeutic relevance. The alkylamides are known to cause a tingling sensation on the tong, which has been considered an indication of quality in folk medicine, and has been widely used as a sensory test by echinacea growers. Polysaccharides isolated from cell cultures of *E. purpurea* with MW 10,000, 25000 and 75,000 were shown to cause stimulation of the immune system. Various echinacea plant parts have been also reported to posses biological activities other than immunostimulatory, e.g. antiinflamatory and antioxidative. Recently, echinacea root, leaves and seed of *E. angustifolia*, *E. pallida* and *E. purpurea* have been shown to posses moderate radical scavenging properties (antixidative) in food muscle systems. Phenolic compounds, such as echinacoside and cynarin, are believed to be responsible for this activity.

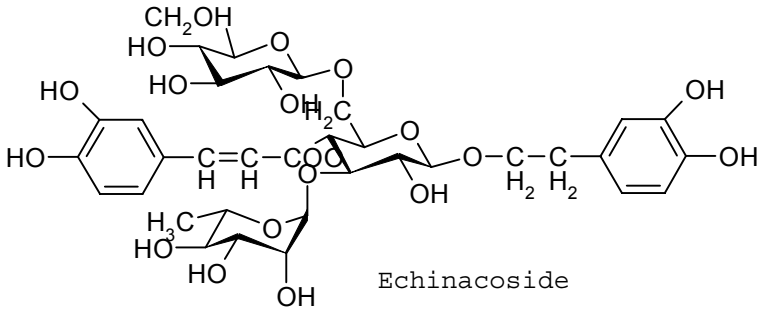
In 1998, in collaboration with echinacea growers and Saskatchewan Herb and Spice Association (SHSA), a first attempt to establish seasonal variation of phenolic marker compounds in echinacea root was undertaken. Analysis of *E. angustifolia* root for the content of echinacoside and cynarin, *E. pallida* root for the content of echinacoside, and *E. purpurea* root for the content of chicoric acid, harvested in spring, summer and fall, gave inconclusive results. Contrary to the expectations, there was no trend in seasonal accumulation of phenolics in any of the three species tested. We came to realize that by sourcing material from growers across Saskatchewan we most likely introduced a number of variables, that could have contributed to the inconclusive outcome of the

study. Some of the obvious variables were different seed origin (source of genotypical variability), soil type, soil fertility, harvesting methods and post-harvest handling practices (source of introduced variability due to agronomic management).

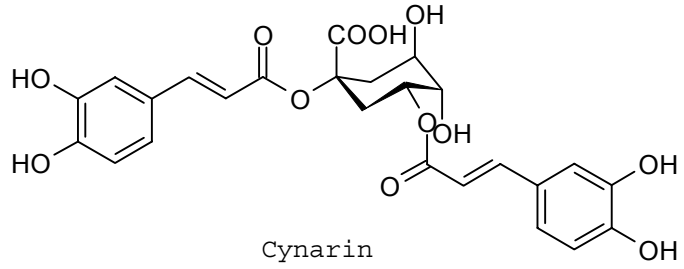
The study was repeated in 1999 using root from a single seed source and production site, namely from SIDC echinacea plots in Outlook. Analysis was expanded to include testing for alkylamides and polysaccharides, which are suspected in the literature to be at least partially responsible for immunostimulatory activity, but have largely been neglected by the industry. The entire roots of three 2-year old and three 3-year old *E. angustifolia* plants, and 3 root samples of 4-year old *E. purpurea* plants, were collected in spring (May 27), summer (July 28) and fall (October 27). Root samples were cleaned after harvest and dried under forced air ventilation at 35^{o}C to moisture content of 9-10%. Samples were stored at 10^{o}C prior to analysis for the content of phenolic markers and alkylamides by high performance liquid chromatograophy (HPLC), and polysaccharides by thin-layer chromatography (TLC). Content of all compounds is reported on dry weight basis.

Root of *E. angustifolia,* and root and flowers of *E. purpurea*, were found to contain the highest content of phenolic marker compounds, echinacoside and chicoric acid, respectively (Figure 1). The content of echinacoside and cynarin in *E. angustifolia* was found to range from 0.18 to 1.75 % and 0.06 to 0.48%, respectively. Echinacoside content was significantly higher in 2-year old plants than 3-year old plants at all three harvest times. Total alkylamides content was higher in 2-year old plants than 3-year old plants and tended to decrease from spring to fall irrespective of plant age. Dominant compound (peak 4) (Table 1) was dodeca-2E, 4E, 8Z, 10E/Z-tetraenoic acid isobutylamide. However, polysaccharide content was significantly higher in 3-year old plants than 2-year old plants and was significantly higher in fall than spring and summer. The results of phytochemical testing revealed great plant to plant variability, which is a major obstacle for successful commercial cultivation of echinacea.

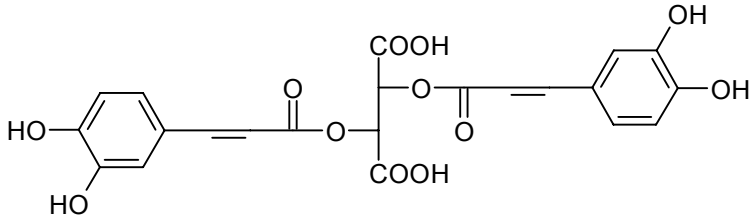
Figure 1. Chemical Structures of Marker Compounds in Commercial Echinacea Species



Echinacoside



Cynarin



Chicoric Acid

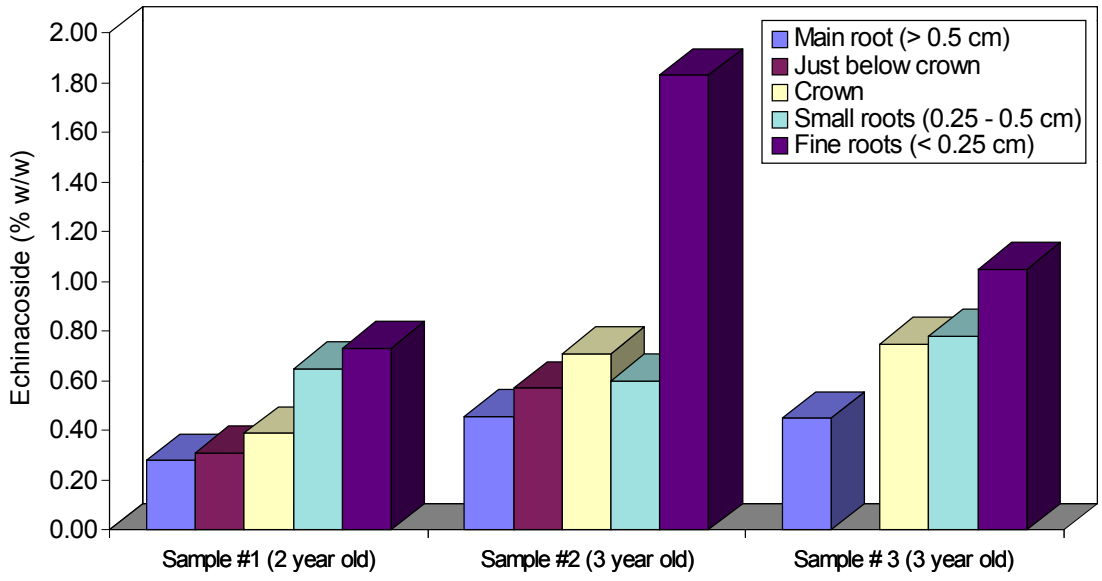
Table 1. Effect of Harvest Time and Age on Relative Content of Alkylamides in *Echinacea angustifolia* Root[1] (1999 Harvest)

	Peak 1 (9.3 min)	Peak 2 (10.9 min)	Peak 3 (12.0 min)	Peak 4 (16.0 min)[2]	Peak 5 (19.0 min)	Peak 6 (21.6 min)	Peak 7 (23.9 min)	Total Area
2 year								
May	10.7	14.5	6.6	30.2	12.6	1.1	1.2	76.9
July	3.2	4.8	3.7	17.5	5.0	0.5	0.6	35.3
October	5.1	8.3	5.6	15.2	6.2	0.6	0.7	41.7
3 year								
May	4.4	7.4	3.0	10.0	3.4	0.1	0.0	28.3
July	3.6	7.2	3.5	13.2	3.4	0.4	0.0	31.3
October	3.3	5.7	2.7	9.8	2.5	0.1	0.0	24.1

[1] Content of alkylamides (1-7) expressed as peak area units x 10^6 @ 210 nm
[2] Isomers dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide
and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (Bauer and Remiger, 1989)

The second objective of the study was to establish the distribution of phenolic marker compounds in 3-year old *E. angustifolia* root harvested in the fall. The fine rootlets with diameter <2.5 mm and root crowns were found to contain the highest content of phenolic markers, while the main body of tap roots contained the lowest content of the same markers (Figure 2).

Figure 2. Distribution of Echinacoside in Echinacea Root Harvested in 1998



The data obtained up to date indicate that in order to ensure high quality of echinacea root, it is extremely important to optimize harvest time. Considering that root yield of 3-year old plants is considerably higher than of 2-year old plants, and providing that polysaccharides are the most contributing class of natural compounds to the immunostimulatory properties of echinacea, this study confirms that *E. angustifolia* root should indeed be harvested in late fall after frost. Also, an obvious problem from the quality perspective, is great plant to plant variability, which is believed to be primarily responsible for the inconsistency in quality of crude echinacea drug. More efforts need to be directed toward advancing knowledge required for the commercial production of echinacea of consistent high quality.

REFERENCES

Barl, B. and Dunlop, D. 1998. Species authentication and content of marker compounds in three *Echinacea* species of commercial importance. In: Proceedings of the Prairie Medicinal & Aromatic Plants Conference 1998, March 5-7, Saskatoon, SK, p.2

Barl, B., Amarowicz, R. and Pegg, R. 1999. Antioxidant activity of extracts obtained from selected species in the genus Echinacea. In: Abstracts of the Annual Meeting of Canadian Institute of Food Science and Technology (CIFST), June 5-9, 1999, Kelowna B.C., p. 75.

Bauer, R., Khan, I. A. and Wagner, H. 1988. TLC and HPLC Analysis of Echinacea pallida and E. angustifolia Roots. Planta Medica 54, 426-430.

Bauer, R. and Remiger, P. 1989. TLC and HPLC Analysis of Alkamides in Echinacea Drugs. Planta Medica 55:367-371.

Bauer, R. 1998. Echinacea: Biological Effects and Active Pronciples. In: Phytomedicines of Europe, ACS Symposium Series 691, Oxford University Press, p. 140-157.

Melchart D, Walther E, Linde K, Brandmaier R, Lersch C. 1998. Echinacea root extracts for the prevention of upper respiratory tract infections. Archives of Family Medicine 7: 541-545.

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