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Alkylamide and cichoric acid levels in *Echinacea purpurea* grown in Australia

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Abstract

Sixty-two commercial dried root and aerial samples of *Echinacea purpurea* grown in eastern Australia were analysed for the medicinally active constituents, the alkylamides and cichoric acid. Total alkylamide concentration in root samples was 6.2 ± 2.4 mg/g (range 1.2–12.1 mg/g), and in aerial samples was 1.0 ± 0.7 mg/g (range 0.2–3.9 mg/g). The proportion of individual alkylamides in root samples was found to be consistent across all samples. The cichoric acid concentration in root samples was 13.2 ± 5.0 mg/g (range 1.4–20.5 mg/g), and in aerial samples was 12.9 ± 4.5 mg/g (range 4.9–21.4 mg/g). The large range in levels of alkylamides and cichoric acid suggests that medicinal quality standards for the marketing of dried raw material should be considered. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Echinacea is a traditional North American perennial medicinal herb that has gained international popularity in recent years through claims that it beneficially stimulates the body immune system. Extensive qualitative research has established that the chemical composition of Echinacea spp. is complex with alkylamides, caffeoyl phenols, and polysaccharides attracting claims of beneficial pharmacological activity (Bauer & Wagner, 1991; Hobbs, 1989). *Echinacea purpurea* (L.) Moench is an important commercial species. It is the major species grown in Australia and is cultivated across a wide region of eastern Australia extending over 2000 km from south-east Queensland to western Victoria. The crop is often marketed as dried, ground root or as aerial material containing stem, leaves and flowers. There have, however, been few studies to quantify the level of active constituents in *E. purpurea* with such studies mostly examining only a limited number of samples and reporting on different plant sections. Bauer and Remiger (1989) reported the first quantification of alkylamides as the two dodeca-tetraenoic acid isobutylamides in German grown plants and found the roots contained 0.04–0.39 mg/g dry weight and the aerial parts 0.01–0.3, mg/g. Perry, van Klink, Burgess and Parmenter (1997) reported levels of total alkylamides in the rhizomes and roots of New Zealand echinacea as 8.0 and 6.2 mg/g, respectively, with the various aerial sections containing 0.24–18.8mg/g. Stuart and Wills (in press) found Australian echinacea to contain total alkylamides of 8.0–9.7 mg/g in mature roots and 1.6–3.5, 0.1, and 0.2–1.3 mg/g in flower, leaf and stem sections of the plant, respectively, while Rogers, Grice, Mitchell, and Griffiths (1998) found the level in Australian aerial samples to range from 0.24 to 1.09 mg/g. Levels of cichoric acid in German grown *E. purpurea* have been reported by Becker and Hsieh (1985) to be 7.6 mg/g in roots with the flower and leaf containing 13 mg/g. Bauer and Remiger reported ranges of 13–30, 4–16 and 2–6 mg/g in the flower leaf and stem, respectively, and Bauer and Wagner (1990) found 6–21 mg/g in the roots. Stuart and Wills (in press) reported ranges of 29.5–38.3, 10.4–23.8, 4.1–15.3, and 38–10.9 mg/g in Australian flower, root, leaf and stem, respectively.

Greater international market competition, at both wholesale and retail level, has led to increasing concern by traders and consumers for the medicinal quality of echinacea. A survey of the levels of alkylamides and caffeoyl phenols in 32 retail echinacea products available to Australian consumers (Wills & Stuart, 1998), found extreme variation in quality with the levels of

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total alkylamides ranging from 0.0 to 1.9 mg/g (or ml), and 0.0 to 8.3 mg/g (or ml) for caffeoyl phenols. It was suggested that the variation in concentration of alkylamides and caffeoyl phenols between products may be due to variation in levels in dried echinacea sold to processors. This paper reports a national survey undertaken to establish levels of total alkylamides and cichoric acid, the major caffeoyl phenol in *E. purpurea* (Stuart & Wills, in press), in dried echinacea offered for sale by Australian growers.

2. Materials and methods

Sixty-two samples of commercial *E. purpurea* material offered for sale from the 1997/98 harvest, were obtained from medicinal herb traders. The samples were received in either a dried whole form or dried crushed form. Material was selected to give an equal number of aerial and root samples ($n = 31$) over a broad geographical spread of growing areas from central Queensland to western Victoria. Each sample was ground to <200 Mm, and the alkylamides and caffeoyl phenols were extracted and analysed by HPLC as previously described by Stuart and Wills (in press). A small modification of the mobile phase used for alkylamide separation utilised acetonitrile/water at 1 ml/min commencing with 40% acetonitrile and a linear gradient ramp to 70% acetonitrile at 20 min.

3. Results and discussion

3.1. Alkylamides

The mean, standard deviation and range of the total alkylamides in the 62 commercial samples of *E. purpurea* are given in Table 1, with the frequency distributions shown in Fig. 1. The data show considerable variation between and within root and aerial products. About 50% of root samples contained alkylamides in the range of 6–9 mg/g and 35% in the range of 3–6 mg/g. There were two samples with a much higher level at about 12 mg/g and two samples with a lower level at about 1.5 mg/g. About 50% of the aerial samples contained alkylamides in the range of 0.5–1.0 mg/g and 90% in the range of 0.2–1.4 mg/g. There were, however, two samples with quite high levels of about 4.0 mg/g. The data support previous findings that the concentration of alkylamides was much higher in dried *E. purpurea* root than in aerial parts of the plant (Bauer & Remiger, 1989; Perry et al, 1997; Stuart & Wills, in press).

There were insufficient samples in the study to fully determine the effect of growing region on alkylamide levels, but a division along latitude 32°S (Port Macquarie, NSW), showed a significantly higher alkylamide concentration in roots grown north of this latitude (7.2 ± 7.24 mg/g) compared to those grown south (4.9 ± 1.98 mg/g) ($P < 0.05$). A similar difference was found in aerial products with a level of 1.12 ± 0.37 mg/g in northern samples and 0.71 ± 0.39 mg/g in southern samples ($P < 0.05$). In addition to differences in daylight between the two regions, latitude 32°S approximates a seasonal change in rainfall with areas to the north having predominantly summer rainfall and areas to the south a winter rainfall, although the pattern applies mainly to inland rather than coastal regions.

The composition of alkylamides was remarkably similar across all samples. The chromatograms of the alkylamides in all root samples showed the same 11 peaks. Table 2 gives the identity of each peak and the relative proportions of seven groups of peaks which were used due to the poor resolution of several pairs of small peaks, and combining the two stereo-isomers of the tetraenoic isobutylamides. Table 2 shows 76% of total alkylamides was contributed by four compounds with 45% from the two tetraenoic isobutylamides. In aerial samples, the tetraenoic isobutylamides comprised 76% of the total alkylamides.

Table 1
Total alkylamides and cichoric acid levels in dried root and aerial samples of *Echinacea purpruea*

Sample		Concentration (mg/g dry weight)			
		Root		Aerial	
Origin	No.	Mean	Range	Mean	Range
Total alkylamides					
North[a]	32	7.2 ± 2.2	3.5–12.1	1.3 ± 0.8	0.6–3.9
South[a]	30	4.9 ± 2.0	1.2–7.5	0.7 ± 0.04	0.2–1.5
All	62	6.2 ± 2.4	1.2-12.1	1.0 ± 0.7	0.2–3.9
Cichoric acid					
North	32	13.8 ± 5.9	1.4–8.0	13.4 ± 3.3	8.1–19.4
South	30	12.5 ± 3.6	4.4–18.0	12.5 ± 5.5	4.9–21.4
All	62	13.2 ± 5.0	1.4–8.0	12.9 ± 4.5	4.921.4

[a] North and South indicate crops grown at latitudes relative to 32°S.

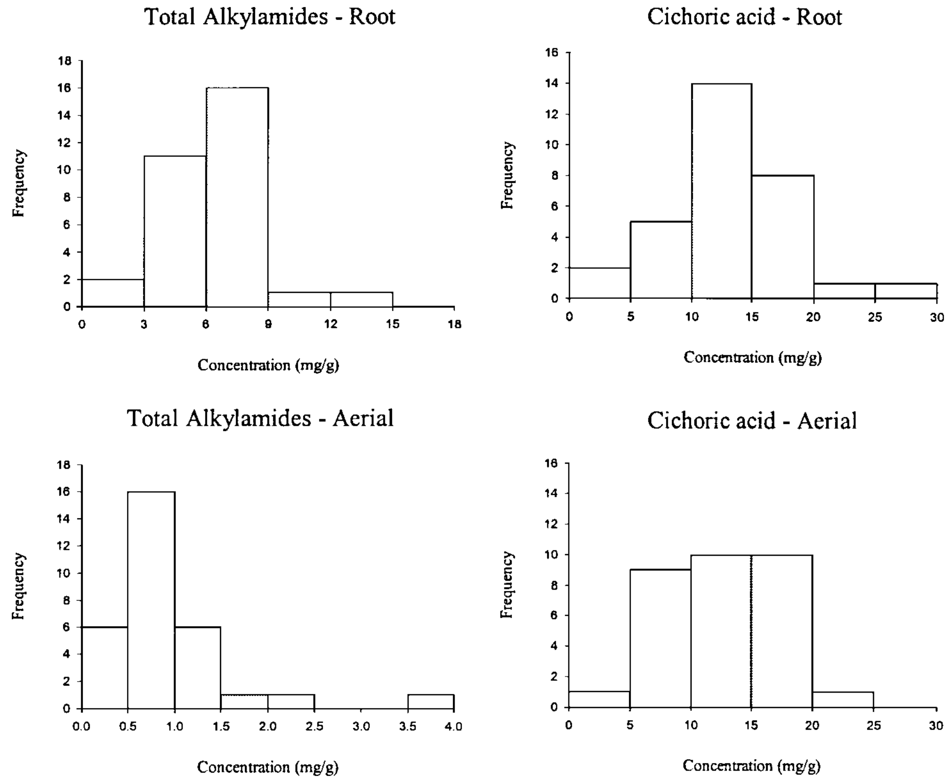

Fig. 1. Frequency distribution of alkylamides and cichoric acid levels in dried *Echinacea purpurea* root and aerial products.

Table 2
Alkyamide profile in dried *Echinacea purpurea* root and aerial products

Peak no.	Group no.	Peak identity[a]	% Composition	
			Root	Aerial
1	1	Undeca-2E,4Z-diene-8,10-diynoic acid isobutylamide	6	20
2	2	Undeca-2Z,4E-diene-8,10-diynoic acid isobutylamide	18	
3	3	Dodeca-2E,4Z-diene-8, 10-diynoic acid isobutylamide	15	
4		undeca-2E,4Z-diene-8,10-diynoic acid-2-methylbutylamide		
5	4	Unknown 1	8	
6		dodeca-2Z,4E-diene-8, 10-diynoic acid isobutylamide		
7	5	Unknown 2	8	
8		dodeca-2E,4Z-diene-8, 10-diynoic acid isobutylamide		
9	6	Unknown 3	1	
10	7	dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide	45	76
11		dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide		

[a] Chromatograms matched the peak distribution and UV spectral patterns for *E. purpurea* root described by Bauer and Remiger (1989) and Perry et al. (1997), hence the peaks were ascribed the identifications given by these authors.

The considerable range in concentration of alkylamides across the 31 root and aerial samples is not surprising given that there has not been any attempt to develop the Australian crop from planting material of a narrow genetic base. However, since about half the root samples contained 6–9 mg/g of alkylamides and 35% contained 3–6 mg/g, these levels can be considered to be the quality to be expected from dried Australian *E. purpurea* root. The low values (<3 mg/g) in two samples are probably due to contamination with aerial material while the two high values of 12 mg/g should be of interest to groups attempting to develop a crop with elevated alkylamide levels. No explanation is offered for the higher level of alkylamides in crops grown north of latitude 32°S but it would be worthwhile to ascertain the climactic factors which are causing the effect.

For aerial samples, a level of 0.5–1.0 mg/g would seem to be typical of good quality dried aerial product. Assessing the cause of higher values in aerial samples, which ranged up to 3.9 mg/g, is complicated by the possible inclusion of root material into the product rather than being derived from a higher-yielding plant. The two aerial samples with the highest levels of alkylamides would seem to contain a proportion of root as they showed a lower level of tetraenoic isobutylamides, at about 50%, than other aerial samples, and had all seven alkylamide groups present, which is typical of root material.

3.2. Cichoric acid

The caffeoyl phenol chromatogram showed seven peaks of which cichoric acid constituted 63 and 67% of the relative peak area for the root and areal sections, respectively. Cichoric acid was subsequently used as the indicator of caffeoyl phenols in the commercial products. As with the alkylamides, a large range is seen in the concentration of cichoric acid in root and aerial products (Table 1). The frequency distribution in Fig. 1 shows that the level of cichoric acid in root samples has a gausian-type distribution with about 50% of samples in the concentration range of 10–15 mg/g and 90% of samples in the range of 5–25 mg/g. The aerial samples show a broader range of cichoric acid levels with 90% of samples spread evenly over the range of 5–20 mg/g. There were two outliers in the root samples (1.4 and 4.4 mg/g) and one outlier in the aerial samples (4.9 mg/g); there was no correlation between samples with cichoric acid and alkylamide outliers. There was no significant difference in cichoric acid levels in echinacea grown north and south of latitude 32°S.

The levels of cichoric acid are, overall, similar in root and aerial samples which is consistent with published literature (Bauer & Wagner, 1991; Stuart & Wills, in press). Since the level of cichoric acid in about 50% of root samples was in the range 10–15 mg/g with about 20% of samples containing 15–20 mg/g, these levels can be considered typical of dried Australian *E. purpurea*. The 20% of samples with <10 mg/g could arise from postharvest loss of cichoric acid, which has been found to be readily degraded during handling and drying operations, in contrast to the alkylamides which are quite stable under such conditions (Stuart & Wills, unpublished data). The two high values of >20 mg/g are presumably from a naturally high plant variety. Cichoric acid in the aerial samples shows an even spread of concentration from 5 to 15 mg/g for 29 of the 31 samples. Similar explanations for the variability are offered as for the root samples.

4. Conclusions

The findings of the survey of alkylamides and cichoric acid in Australian-grown dried *E. purpurea* allows the suggestion of industry quality standards to be set and used for international marketing of the crop. A standard of high quality could be for root material to contain >6 mg/g alkylamides and for root and aerial material to contain >15 mg/g cichoric acid. Aerial material cannot be considered as a source of alkylamides. A minimum standard for marketing acceptance could be set at >3 mg/g alkylamides and >5 mg/g cichoric acid.

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