Retention of Alkamides in Dried *Echinacea purpurea*

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Different drying methods, such as freeze-drying (FD), vacuum microwave drying (VMD), and air-drying (AD), were applied to fresh roots and leaves of Canadian-grown *Echinacea purpurea* to determine the optimal method for preserving alkamide levels. Using HPLC, six alkamide fractions (alkamides **1**, **2**, **3**, **6a**/**6**, **7**, **8**/**9**) were quantitated in dried roots, whereas four alkamide fractions (alkamides **1**, **2**, **3**, **8**/**9**) were measured in dried leaves. Different elution conditions used in HPLC for alkamide analysis did not affect the eluted fractions nor the quantitation of different alkamides. Individual alkamide concentrations in roots and leaves were affected by the drying methods used. To preserve higher levels of total alkamides, FD was found to be the best method, VMD was a superior method for drying roots than AD at 70 °C, while AD at 50 °C was the preferred method for drying leaves of *E. purpurea*.

Keywords: *Alkamides; Echinacea purpurea; vacuum microwave drying; air-drying; freeze-drying*

INTRODUCTION

Preparations of *Echinacea* species, primarily *E. purpurea* (L.) Moench., *E. angustifolia* DC., and *E. pallida* (Nutt.) Nutt. are widely used for pharmaceutical purposes, such as treatments of cold, flu, respiratory infections, and inflammations (Bauer and Wagner, 1991). It is believed that *Echinacea* species have antiviral, antibacterial, antifungal, and insecticidal properties due to nonspecific enhancement of the immune system (Bodinet et al., 1993; Skwarek et al., 1996; Burger et al., 1997) and anti-inflammatory properties (Tubaro et al., 1987; Muller-Jakic et al., 1994; Facino et al., 1995). At present, most preparations are derived from the aerial parts of *E. purpurea* and underground parts of *E. purpurea*, *E. angustifolia*, or *E. pallida* (Bauer, 1998).

The immunostimulatory properties of *Echinacea* species have been attributed to both the lipophilic and the polar fractions of the extract, including alkamides, caffeic acid derivatives (especially chicoric acid), glycoproteins, and polysaccharides (Bauer and Wagner, 1991; Bauer, 1996). It is still uncertain as to which constituents primarily contribute to the immunostimulatory action of *Echinacea* species; however, it has been suggested that the lipophilic fraction, e.g., alkamides, is mainly responsible for the immunostimulatory activity of alcoholic *Echinacea* extracts (Bauer and Wagner, 1991). It was reported that the alkamide fractions cause stimulation of phagocyte activity in vitro and in vivo (Bauer et al., 1988a; 1989) and inhibition of cyclooxygenase and 5-lipoxygenase as anti-inflammatory agents (Wagner et al., 1989; Muller-Jakic et al., 1994).

Alkamides in *Echinacea* species are mainly isobutylamides of highly unsaturated carboxylic acids with olefinic and/or acetylenic bonds (Greger, 1984). Using HPLC, alkamides in *Echinacea* have been isolated and identified. Bauer et al. (1988b) identified 10 alkamides from the roots of *E. purpurea*, and subsequently, Bauer and Remiger (1989) identified 11 alkamides from the roots of *E. purpurea* and quantitated tetraene alkamides **8**/**9** levels. Schulthess et al. (1991) analyzed different alkamide levels in achenes of *Echinacea* species and found that during germination tetraene isobutylamides **8**/**9** were mainly formed in *E. purpurea*, *E. angustifolia*, and *E. pallida*. Perry et al. (1997) identified seven alkamides in *E. purpurea* and reported different alkamide levels present in various plant parts. Using standard alkamides identified by Perry et al. (1997), Rogers et al. (1998) also analyzed alkamide levels in Australian-grown *E. purpurea*. He et al. (1998) isolated nine alkamides in the roots of U.S.-grown *E. purpurea* and quantitated tetraene alkamides **8**/**9** levels. El-Gengaihi et al. (1998) examined alkamide levels of *E. purpurea* during plant growth and found that proportional composition of the alkamide mixture was characteristic for the plant part and was essentially stable with plant ontogony.

Herbs are commonly dried using ambient air. However, air-drying depends on weather conditions and is a slow (e.g., several weeks) process (Blose and Cusick, 1993). By using a conventional hot air-drying method, drying temperature can be controlled and drying time can be shortened. However, this method often causes heat damage and adversely affects texture, color, flavor, and nutritional value of products (Yongsawatdigul and Gunasekaran, 1996). Although freeze-drying reduces heat damage and produces products with excellent quality, this method is costly and can cause large losses of volatile components (Flink, 1975). Freeze-dried products are also sensitive to storage conditions such as temperature or oxygen atmosphere (Paakkonen et al., 1989, 1990).

Vacuum microwave drying offers an alternative method to improve the quality of dehydrated products. The low temperature and fast mass transfer conferred by vacuum, combined with rapid energy transfer of microwave heating, generates rapid low-temperature

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drying (Lin et al., 1998). Moreover, the reduced exposure to air during drying may inhibit oxidation. Vacuum microwave drying has been applied to foods to improve food properties such as texture, color, flavor, rehydration potential, and nutritional value (Durance and Liu, 1996; Lin et al., 1998; Yousif et al., 1999).

In this study, different drying methods, namely, hot air-drying (AD), vacuum microwave drying (VMD), and freeze-drying (FD), were used to preserve alkamide levels in leaves and roots of *Echinacea purpurea*. For the analysis of alkamide content present in *Echinacea*, different elution conditions were used to optimize HPLC quantitation.

MATERIALS AND METHODS

Plant Materials. Freshly harvested 3-year-old roots of *Echinacea purpurea* were obtained from Tuscan Farm Gardens (Langley, BC, Canada) and freshly harvested leaves of *Echinacea purpurea* were from Lerra-Flora Botanicals (Cloverdale, BC, Canada).

Preparation of Dried Roots and Leaves. Freshly harvested roots and leaves of *Echinacea purpurea* were washed with water. The roots were sliced to 3-mm thickness with a Hobart slicer (model PD/1-1, Hobart Corp., Troy, OH) and then mixed thoroughly for even distribution of roots parts. Three hundred grams of roots and 420 g of leaves were used for each drying process. Freeze-drying (FD) at 1.6 mmHg with a chamber temperature of 20 °C and a condenser temperature of −55 °C was performed for 5 days for roots and leaves. Using Vers-a-belt dryer (Wal-Dor Industries Ltd., New Hamburg, ON), air-drying (AD) was done at 70 °C for 3.5 h for roots and at 50 °C for 6.5 h for leaves. Air flow rate through 1 m^2 of belt was 0.9 m^3/s, and relative humidity of the hot/inlet air was 10%. Using a 2450-MHz vacuum microwave drier (ENWAVE Corporation, Port Coquitlam, BC), vacuum microwave drying was performed with power of 1.5 kW at full vacuum level of 50 mmHg (VMD) for 17 min for leaves. For vacuum microwave drying of roots, a power of 1 kW with full vacuum of 50 mmHg (VMD) for 25 min and partial vacuum of 200 mmHg (PVMD) was used for 25 min. Air flow rates for VMD and PVMD were 5 and 20 L/min, respectively. The product temperatures for VMD and PVMD at the end of the processings were 38−40 °C and 48−50 °C, respectively, as determined using an infrared thermometer (Cole Parmer, Vernon Hills, IL). VMD samples ($a_w = 0.507$) and PVMD samples ($a_w = 0.598$) were equilibrated over saturated potassium acetate ($a_w = 0.352$).

Dried roots and leaves were ground with Retsch Ultra centrifugal Mill using a 0.5-mm sieve and stored at −18 °C. Moisture contents of ground samples were determined by drying at 84 °C for 22 h using a vacuum oven. Water activities of all the dried samples were measured using the Aqualab (model CX-2, Decagon Devices Inc., Pullman, WA). Moisture contents (and water activities) of FD roots, VMD roots, PVMD roots, and AD roots were 2.5% (a_w= 0.165), 7.3% (a_w= 0.352), 7.3% (a_w= 0.352), and 5.0% (a_w= 0.262), respectively. Moisture contents (and water activities) of FD leaves, VMD leaves, and AD leaves were 3.0% (a_w= 0.313), 7.0% (a_w= 0.415), and 3.3% (a_w= 0.360), respectively.

Extraction. Extraction of alkamides from plant material was carried out by the method of Perry et al. (1997) with modification. Ground plant material (1.0 g) was mixed with 10 mL of acetonitrile containing 1.0 mg of *N*-phenylpentamide (RN 10264-18-3) as an internal standard and then homogenized by Ultraturrax for 2 min. The sample was then centrifuged at 500*g* for 10 min or incubated for 1.5 h at room temperature or overnight at 4−6 °C. All three extraction methods gave the same yields of extracted alkamides. One milliliter of supernatant was applied to a Supelclean LC-18 extraction column (Supelco, 1-mL bed volume), which was previously conditioned with 3 mL of acetonitrile/water 9:1. The bound alkamide fraction was eluted with 2 mL of acetonitrile/water 9:1, and the eluates were filtered through a syringe filter (0.45 μm).

Standard Alkamide Solutions. *N*-phenylpentamide as the internal standard and two standard alkamide mixtures were obtained from the Plant Extracts Research Unit, Department of Chemistry, University of Otago, Dunedin, New Zealand. The first standard alkamide mixture consisted of eight purified alkamides. These alkamides were undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide (**1**), undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2**), dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide (**3**), dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**6a**), trideca-2*E*,7*Z*-diene-10,12-diynoic acid isobutylamide (**6**), dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (**7**), dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (**8**), and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (**9**). The second standard was a purified mixture of alkamides **8** and **9**. *N*-phenylpentamide and the standard alkamide mixtures were dissolved in acetonitrile.

Various amounts of alkamides **8** and **9** mixture (0.25−1.5 μg) with internal standard (1 μg) were injected to give the response factor, which was calculated from a regression analysis ($R^2 = 0.995$). The same response factor was used for the other alkamides, since these had similar UV absorbance values at the HPLC detection wavelength of 254 nm (Perry et al., 1997).

High Performance Liquid Chromatography Analysis. Alkamide levels of extracted plant material were determined by HPLC. Analysis equipment consisted of a Hewlett-Packard 1050 series pump control, a Macintosh computer with Dynamax HPLC Method Manager version 1.2 (Rainin Instrument Co. Inc., Woburn, MA), and a Shimadzu SPD-EAV UV detector. Samples were analyzed using a Vydac RP-18 analytical column (250 × 4.6 mm, 5 μm) with a Brownlee RP-18 NewGuard cartridge (15 × 3.2 mm, 7 μm) fitted (Perkin-Elmer, Markham, ON).

To determine the optimal elution conditions for the separation and quantification of alkamides, different flow rates (0.85 or 1.0 mL/min) and linear gradients (40−70%, 40−90%, 50−100% acetonitrile in water) or isocratic (50% acetonitrile in water) solvent systems were tried. Samples of extracted plant materials prepared from different drying methods were analyzed using isocratic (50% acetonitrile in water) elution for 30 min at a flow rate of 0.85 mL/min with UV detection at 254 nm (Rogers et al., 1998).

Alkamides were identified by comparison of retention times and UV profiles at 254 nm of the supplied standard alkamides. The quantities of identified alkamides were calculated using the response factor obtained from a standard alkamides mixture (**8** and **9**) with internal standard. The alkamides levels were expressed as milligrams per gram of dry weight of plant material.

Statistical Analysis. Statistical analysis was performed using Minitab version 12.21 program (Minitab Inc., State College, PA). One-way analysis of variance followed by Tukey test was used to compare means. Significance of difference was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of Elution Conditions on Analysis of Alkamides. Figure 1 shows the HPLC profile of standard alkamides (**1**, **2**, **3**, **6a** + **6**, **7**, **8** + **9**) using isocratic 50% acetonitrile in water at a flow rate of 0.85 mL/min. Alkamides **6a** and **6** as well as alkamides **8** and **9** were eluted as one peak, respectively. This result was in agreement with the reported HPLC profile supplied by Plant Extracts Research Unit (Dunedin, New Zealand) using a linear gradient (50−100% acetonitrile in water) solvent system in 25 min at flow rate of 1.0 mL/min. Elution of stereoisomeric alkamides **8** and **9** as one peak has been reported by other researchers (Bauer and Remiger, 1989; Perry et al., 1997; Rogers et al., 1998; He et al., 1998; El-Gengaihi et al., 1998).

Extracts prepared from freeze-dried roots were used to determine the effects of different elution conditions

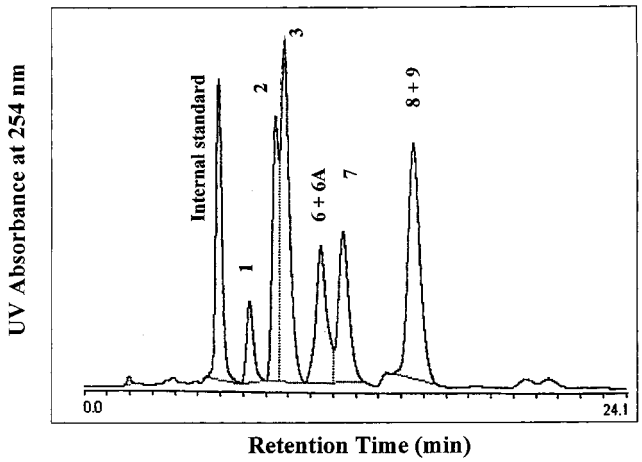

Figure 1. HPLC profile of standard alkamides obtained by isocratic (50% acetonitrile in water) elution at 0.85 mL/min. Numbers indicate alkamide numbers.

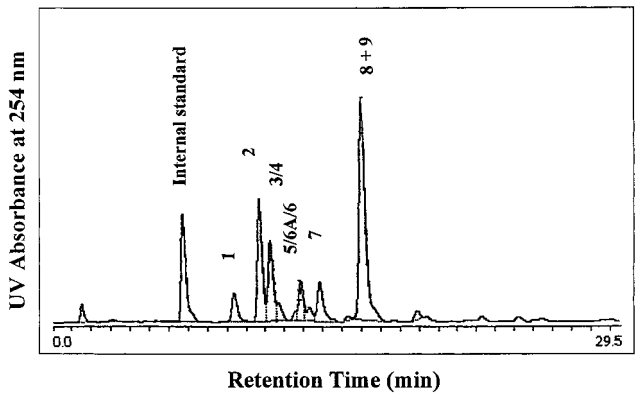

Figure 2. HPLC analysis of alkamides in freeze-dried roots of *E. purpurea* by linear gradient (40−70% acetonitrile in water) elution in 30 min at 1.0 mL/min.

on the HPLC analysis of alkamides. When the root extract was eluted with a linear gradient (40−70% acetonitrile in water) solvent system in 30 min at a flow rate of 1.0 mL/min (Figure 2), alkamides **6a** and **6** were separated. In addition, there appeared two new separated peaks after alkamide **3** and before alkamide **6a**. These additional separated peaks are considered to be alkamides **4** and **5**, respectively, according to the retention times and HPLC profile reported by Bauer and Remiger (1989).

Elution of root extract using isocratic conditions (50% acetonitrile in water) at a flow rate of 0.85 mL/min (Figure 3) resulted in the same separation profile of alkamides as that for the standard alkamide mixture (Figure 1 and Table 1). Alkamides **6a** and **6** were combined as one peak, whereas alkamide **4** was included into the alkamide **3** peak, and alkamide **5** was included into the alkamide **6a**/**6** peak, respectively. Using a flow rate of 1.0 mL/min with isocratic elution or a linear gradient elution (40−90% or 50−100% acetonitrile in water) in 25 min resulted in similar HPLC profiles to those obtained using isocratic elutions (Figures 1 and 3).

When alkamide levels of root extract were calculated using the response factor, concentrations of individual alkamides peaks **1**, **2**, **3**/**4**, **5**/**6a**/**6**, **7**, **8**/**9** and total alkamides (alkamides **1** + **2** + **3**/**4** + **5**/**6a**/**6** + **7** + **8**/**9**) were similar for all the samples analyzed using different elution conditions (Figure 4). Considering the ratio of alkamide retention times to internal standard retention times (Table 1), the gradient elution of 40−70% acetonitrile in water in 30 min or isocratic elution at 0.85 mL/min gave better separations of alkamides, especially **7** and **8**/**9**. In this study, isocratic (50% acetonitrile in water) elution, at a flow rate of 0.85 mL/min was chosen for the analysis of alkamides levels in dried plant materials.

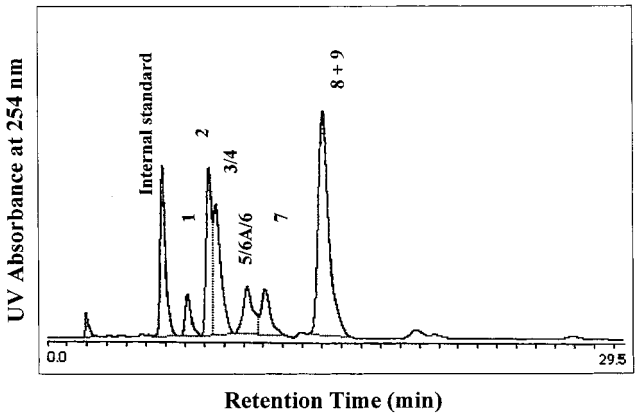

Figure 3. HPLC analysis of alkamides in freeze-dried roots of *E. purpurea* by isocratic (50% acetonitrile in water) elution at 0.85 mL/min.

Effects of Drying Methods on Alkamide Levels in Dried Roots of *Echinacea purpurea*. Figure 5A shows alkamide levels in dried roots of *Echinacea purpurea* by different drying methods. In all the dried roots of Canadian-grown *Echinacea purpurea*, tetraene alkamides **8** and **9** were predominant (43−49% of the total alkamides), confirming earlier reports (Bauer et al., 1988b; Bauer and Remiger, 1989; El-Gengaihi et al., 1998; Wills and Stuart, 1999). In AD at 70 °C roots, levels of alkamides **8**/**9** and total alkamides (alkamides **1** + **2** + **3**/**4** + **5**/**6a**/**6** + **7** + **8**/**9**) were 1.34−1.46 and 2.78−2.92 mg/g, dry weight, respectively. For alkamides **8**/**9**, Perry et al. (1997) found 0.8−3.6 mg/g in New Zealand-grown AD roots (at 40 °C for 7 days) of *E. purpurea*, while Bauer and Remiger (1989) reported 0.04−0.39 mg/g in *E. purpurea* roots from U.S.A. and Germany. Wills and Stuart (1999) reported 6.2 ± 2.4 mg/g for the total alkamide concentration in commercially dried roots of *E. purpurea* grown in eastern Australia. These differences in alkamide content might be due to different analytical methodologies used for quantification or different alkamides levels present in *E. purpurea* roots grown in different areas (Perry et al., 1997; Rogers et al., 1998).

For alkamides **1** and **8**/**9**, FD roots, VMD roots, and AD at 70 °C roots contained the same concentration of these alkamides, whereas PVMD roots contained lower concentration as compared to other samples. For alkamides **2** and **3**/**4**, PVMD roots and AD at 70 °C roots contained similar levels of these alkamides, which were lower than those in FD roots and VMD roots. For alkamides **5**/**6a**/**6** and **7**, FD roots contained the highest level of these alkamides, whereas AD at 70 °C roots contained the lowest level of these alkamides. When the alkamides containing diynoic acid (**1**, **2**, **3**/**4**, **5**/**6a**/**6**, and **7**) were accumulated and compared according to drying method (Figure 5B), the concentration from highest to lowest was FD roots > VMD roots > PVMD roots = AD at 70 °C roots. When all alkamides (**1**, **2**, **3**/**4**, **5**/**6a**/**6**, **7**, and **8**/**9**) were accumulated, the concentration from highest to lowest was FD roots > VMD roots > AD at 70 °C roots > PVMD roots. It seems that the higher

Table 1. Retention Times (min) for Alkamides of *Echinacea purpurea* Roots[a] by Different Elution Conditions in HPLC Analysis

peak	std alkamides isocratic: 50% ACN,[b] 0.85 mL/min	gradient: 40−70% ACN in 30 min, 1.0 mL/min	isocratic: 50% ACN, 0.85 mL/min	isocratic: 50% ACN, 1.0 mL/min	gradient: 40−90% ACN in 25 min, 1.0 mL/min	gradient: 50−100% ACN in 25 min, 1.0 mL/min
internal standard	5.901	6.743	5.831	4.841	6.491	4.755
1	7.276 (1.23)[c]	9.351 (1.39)	7.126 (1.22)	5.770 (1.19)	8.371 (1.29)	5.523 (1.16)
2	8.426 (1.43)	10.700 (1.59)	8.226 (1.41)	6.608 (1.37)	9.305 (1.43)	6.125 (1.29)
3/**4**	8.806 (1.49)	11.256 (1.67)	8.580 (1.47)	6.846 (1.41)	9.650 (1.49)	6.280 (1.32)
		11.668 (1.73)				
5/**6a**/**6**	10.446 (1.77)	12.635 (1.87)	10.165 (1.74)	8.033 (1.66)	10.680 (1.65)	7.026 (1.48)
		12.860 (1.91)			10.911 (1.68)	
		13.303 (1.97)				
7	11.425 (1.94)	13.850 (2.05)	11.071 (1.90)	8.676 (1.79)	11.245 (1.73)	7.366 (1.55)
8/**9**	14.508 (2.46)	16.040 (2.38)	14.008 (2.40)	10.845 (2.24)	12.610 (1.94)	8.486 (1.78)

[a] Freeze-dried. [b] 50% ACN: 50% acetonitrile in water. [c] Values for the ratio of alkamides retention time to internal standard retention time are in parentheses.

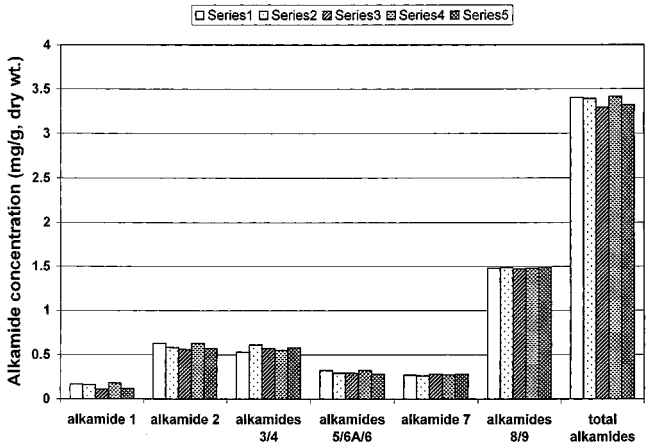

Figure 4. HPLC analysis of alkamide levels in freeze-dried roots of *E. purpurea* by different elution conditions. (ACN = acetonitrile). Series 1: linear gradient, 40−70% ACN in 30 min at 1.0 mL/min. Series 2: isocratic, 50% ACN in 30 min at 0.85 mL/min. Series 3: isocratic, 50% ACN in 30 min at 1.0 mL/min. Series 4: linear gradient, 40−90% ACN in 25 min at 1.0 mL/min. Series 5: linear gradient, 50−100% ACN in 25 min at 1.0 mL/min.

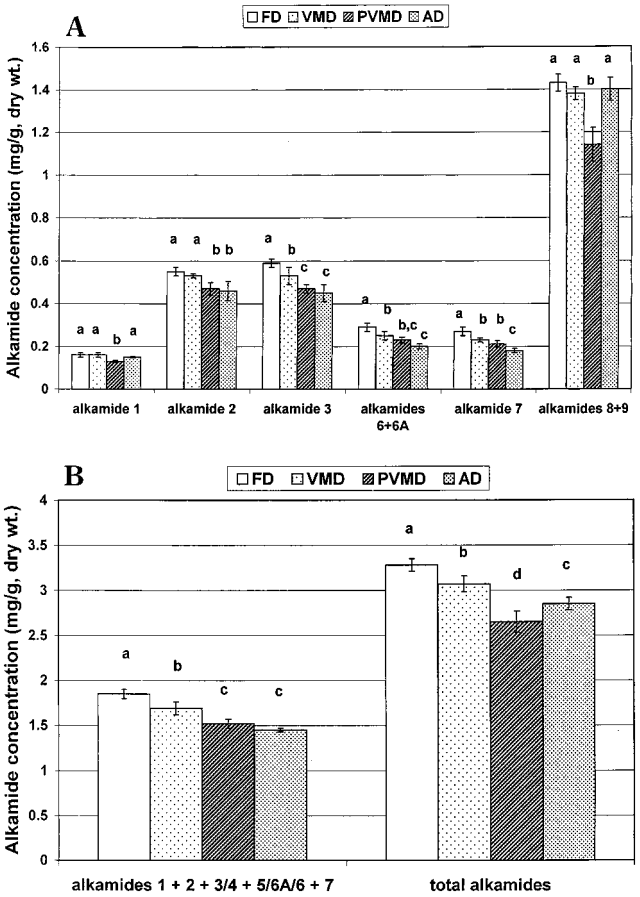

Figure 5. (Panels A and B) Alkamide levels in dried roots of *E. purpurea*. FD: freeze-dried, VMD: vacuum microwave dried, PVMD: VMD with partial vacuum, AD: air-dried at 70 °C. Different letters in the same group of bars indicate a significant difference ($p < 0.05$). Averages of three replicated extracts with duplicate determinations ($n = 6$).

oxygen exposure and temperature produced during PVMD induced greater degradation of alkamides in roots of *E. purpurea*. Being highly unsaturated substrates, alkamides are prone to oxidation, although they are probably somewhat protected in the natural plant (Bauer, 1998). It seems that alkamides containing diynoic acid were more susceptible to degradation at higher temperatures and oxygen levels. It was reported that *Echinacea* alkamides are susceptible to degradation, since alkamides levels decreased by about 13% after powdered roots of *E. angustifolia* were stored at room temperature for 2 months (Rogers et al., 1998).

Effects of Drying Methods on Alkamide Levels in Dried Leaves of *Echinacea purpurea*. Figure 6 shows alkamide levels extracted from leaves of *E. purpurea* after different drying methods. A typical HPLC profile of alkamides from dried leaves prepared by AD at 50 °C is shown in Figure 7. In all the dried leaves, alkamides **1**, **2**, **3**, and **8**/**9** were found as reported by Perry et al. (1997). Also, there was an unknown peak (retention time at 6.6 min) eluted before alkamide **1** in all the dried leaves. The levels of alkamides were much lower in dried leaves than in dried roots of *E. purpurea*, as reported by others (Bauer and Remiger, 1989; Perry et al., 1997; Wills and Stuart, 1999). In AD at 50 °C leaves, alkamides **8**/**9** were predominant (55% of the total alkamides), which is in agreement with previous results (Bauer and Remiger, 1989; Perry et al., 1997). In AD at 50 °C leaves of Canadian-grown *Echinacea purpurea*, the levels of alkamides **8**/**9** and total alkamides (alkamides **1** + **2** + **3** + **8**/**9**) were 0.10 and 0.18 mg/g, dry weight, respectively. Perry et al. (1997) reported 0.2 mg/g for alkamides **8**/**9** and 0.24 mg/g for total alkamides in AD leaves (at 40 °C for 7 days) of New Zealand-grown *E. purpurea*.

FD leaves and AD at 50 °C leaves contained the same level of alkamide 1, which was higher than that present in VMD leaves. However, for alkamides **2**, **3**, and **8**/**9**

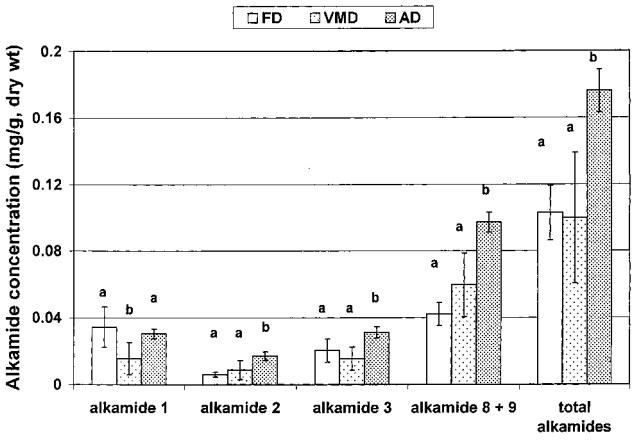

Figure 6. Alkamide levels in dried leaves of *E. purpurea*. FD: freeze-dried, VMD: vacuum microwave dried, AD: air-dried at 50 °C. Different letters in the same group of bars indicate a significant difference ($p < 0.05$). Averages of three replicated extracts with duplicate determinations ($n = 6$).

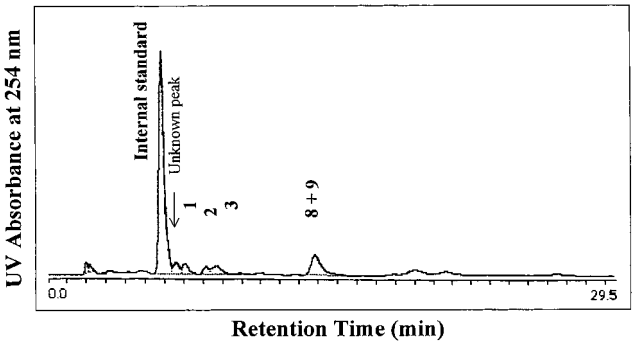

Figure 7. Typical HPLC profile of alkamides in air-dried leaves of *E. purpurea* by isocratic (50% acetonitrile in water) elution at 0.85 mL/min.

and total alkamides (**1** + **2** + **3** + **8**/**9**), AD at 50 °C leaves contained higher levels of these alkamides than FD or VMD leaves. There were no differences in these alkamide levels in FD and VMD leaves. These results were different from those obtained for dried roots processed by similar drying methods involving AD at 70 °C, FD, and VMD. It is not certain why different drying methods have such uniquely different effects on alkamide levels in roots and leaves of *E. purpurea*. It may be plausible that differences between the structures of cell walls in roots and leaves lead to different susceptibility to the degradation caused by different drying methods.

At present, it is not known which of the different alkamides have the most active immunostimulatory properties in *E. purpurea*. Since the main constituent, alkamides **8**/**9**, exhibited less bioactivity than the total lipophilic alkamides extract, the most effective constituents remain to be found (Muller-Jakic et al., 1994; Bauer, 1998). It has been suggested that the particular structure (Muller-Jakic et al., 1994), the position of double bonds as well as E/Z isomerism (Greger, 1984), and the abundance of double bonds or acetylenic moieties (Stohr et al., 1999) of alkamides are involved in bioactive properties.

For the immunostimulatory activity of *Echinacea* extracts, not a single, but several plant constituents including alkamides, chicoric acid, glycoproteins, and polysaccharides may be involved (Bauer, 1998). In this study, the effects of different drying methods on alkamide levels were specifically examined. Considering our results with total alkamides levels, *Echinacea* roots contained higher concentration of alkamides (2.65−3.28 mg/g) than leaves (0.10−0.18 mg/g), thereby implying that roots are a better source for alkamides than leaves. To preserve higher levels of alkamides, it was shown that FD was the best drying method, with VMD with full vacuum being superior to AD at 70 °C for drying roots. In contrast, AD at 50 °C was the best method for drying leaves to preserve the alkamide levels in *E. purpurea*.

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