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Comparison of chemical components and antioxidant capacity of different *Echinacea* species

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Abstract

Alcoholic extracts of the roots and leaves of three *Echinacea* species (*E. purpurea*, *E. angustifolia* and *E. pallida*) were analysed for the presence of characteristic chemicals by HPLC directly coupled to ultraviolet absorbance and electrospray mass spectrometric detectors. The method permitted rapid characterization and tentative identification of a large number of caffeoyl conjugates and alkamides in all the samples investigated. The roots of the three species differed markedly in their contents of characteristic compounds. Cichoric acid and verbascoside predominated in extracts of *E. purpurea* root whereas cynarine and dodeca-2E,4E,8Z,10Z/E-tetraenoic acid isobutylamide were the major chemicals characteristic of *E. angustifolia* root extracts. Echinacoside and 6-O-caffeoylechinacoside predominated in extracts of *E. pallida* roots. Characteristic alkamides were also examined by electrospray tandem mass spectrometry (MS/MS) and these compounds provided characteristic fragmentation patterns. Extracts of the roots and leaves of all three species were found to have antioxidant properties in a free radical scavenging assay and in a lipid peroxidation assay.

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Introduction

Preparations of *Echinacea* plants (*E. purpurea*, *E. angustifolia*, *E. pallida* and others) have been traditionally employed to stimulate the immune system (See et al 1997), to treat chronic inflammatory conditions (Bauer et al 1988a), and they are believed to have antiviral activities (Cheminat et al 1988; Thompson 1998). Furthermore, it has been shown that phenylpropanoid glycosides, which are constituents of certain *Echinacea* species, possess antiviral properties (Kernan et al 1998), and are antioxidants (Zheng et al 1993) and free radical scavengers (Wang et al 1996a, b). There are many morphological similarities between the species, especially between *E. angustifolia* and *E. pallida*, and it has been shown that specimens are often confused in the medicinal plant market. This has resulted in confusion over the therapeutic properties of each species (Bauer et al 1987). Certainly, milled materials from different *Echinacea* species are indistinguishable by morphological characteristics.

The chemical composition of each medicinally important *Echinacea* species has been studied (Bauer et al 1988b, 1989; Cheminat et al 1988) and marked differences in the distributions of characteristic chemicals between species have been noted. Thus, a method able to rapidly compare a broad spectrum of the wide variety of characteristic chemicals found in this genus would provide a good tool for distinguishing between raw medicinal materials derived from various *Echinacea*

species. Such a method would also help to identify medicinal extracts and other products derived from various parts of these plants.

The characteristic chemicals of *Echinacea* fall into two general categories: caffeoyl conjugates (phenylpropanoid conjugates of quinic acid, tartaric acid and their glycosides) and alkamides. Previously, these components have been examined separately and to a great extent by thin layer chromatography. Only the alkamides have been investigated by high performance liquid chromatography (HPLC) with electrospray mass spectrometric detection (He et al 1998). In our paper a rapid and simple HPLC method has been described, that permitted separation and identification of caffeoyl conjugates and alkamides as well as some flavonol glycosides present in alcoholic extracts of *Echinacea* roots or leaves, in a single chromatographic run. Identification was based on absorbance and mass characteristics of individual materials as they eluted from the column using ultraviolet absorbance detection followed directly by electrospray mass spectrometry. This method permitted unequivocal identification of chemicals characteristic of *Echinacea* species and allowed discrimination between extracts of the roots and leaves of the three species investigated. After identifying each species by their chemical characteristics, the abilities of root and leaf extracts to prevent lipid peroxidation and to scavenge free radicals were determined.

Materials and Methods

Plant material

Fresh *Echinacea* plants identified as *Echinacea purpurea*, *Echinacea pallida* and *Echinacea angustifolia* were obtained from the Crop Development Centre (CDC) South, Brooks, Alberta, Canada, where these plant lines continue to be cultivated. To provide alcoholic extracts, 30–50 g of clean, air-dried and milled roots and leaves of the three species were separately extracted by 85% ethanol (400–500 mL) at 90°C for 8 h with reflux. The extracts were filtered and the alcohol was removed by evaporation (30°C, under reduced pressure). The remaining aqueous portion was freeze-dried. Product yields by weight from roots of *E. purpurea*, *E. pallida* and *E. angustifolia* were 6.0, 8.6 and 5.4%, respectively. Product yields from leaves of *E. purpurea*, *E. pallida* and *E. angustifolia* were 12.6, 10.4 and 14.8%, respectively. Samples were stored dry at −20°C (for less than three weeks) until analysed. For chromatographic analysis each extract was dissolved/suspended with sonication at a concentration of 10 mg mL^{-1} in 20% acetonitrile. A 100-μL sample of this material was applied directly to the HPLC apparatus.

Chromatography with ultraviolet absorbance and electrospray mass spectrometric detection

Chromatographic separation of constituents of alcoholic *Echinacea* extracts was accomplished by gradient elution on a reverse-phase HPLC column in either a Hewlett Packard 1050 HPLC system or a Gilson HPLC system equipped with model 302 pumps and a model 811 dynamic mixer using a water/acetonitrile gradient. Two mobile phases were employed. Their compositions were: mobile phase A 5% acetonitrile, 0.1% trifluoroacetic acid; mobile phase B 70% acetonitrile, 0.1% trifluoroacetic acid. The gradient started with 100% A and went to 85% A + 15% B over 20 min. Between 20 and 40 min the mobile phase changed from 85% A + 15% B to 100% B. After 40 min the column was washed with mobile phase B for 5 min and then re-equilibrated to mobile phase A over 5 min. The flow rate was 1 mL min^{-1}. The column used for separation was a Zorbax 300SB-C_8 (25 cm × 4.1 mm) and eluting peaks were monitored by ultraviolet (UV) absorbance at 254 and/or 205 nm. For electrospray mass spectrometry the material eluting from the column after UV detection was split so that 50 μL min^{-1} was fed directly into a Fissons VG Quattro electrospray mass spectrometer. Mass signals were monitored between mass 200 and 1000 in positive ion mode using a cone voltage of 30 V. Eluting peaks that did not provide strong electrospray signals (verbascoside for example) were individually collected from the outlet of the flow splitter, freeze-dried and re-evaluated by direct injection into the electrospray apparatus in positive and negative mode. Material used for direct injection was dissolved in a small amount of either a mixture of equal volumes of acetonitrile and 0.1% aqueous solution of trifluoroacetic acid (positive mode) or a mixture of equal volumes of acetonitrile and 10 mM aqueous ammonium hydroxide solution (negative mode).

Tandem electrospray mass spectrometry was used to evaluate several alkamides collected from eluting samples. Eluting alkamide peaks from samples (approximately 2 mg starting material loaded onto column) were collected, freeze-dried, and reconstituted in 100 μL of a mixture of equal volumes of acetonitrile and 0.1% aqueous solution of trifluoroacetic acid, and 10 μL was injected directly into the mass spectrometer. Daughter ions of selected protonated alkamides (e.g. m/z 232 and 252) were recorded in the absence and presence of argon collision gas at cone voltages ranging from 5 to 40 V.

Little or no fragmentation was observed in the absence of argon. In the presence of argon the alkamides provided strong fragmentation spectra.

Estimation of free radical scavenging capacity of *Echinacea* extracts

Hydroxyl free radical scavenging ability was assessed by a modification of the dynamic method developed by Arnao et al (1996). This method is based on the fact that the accumulation of the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical catalysed by peroxidase can be inhibited by the presence of hydroxyl free radical scavengers giving rise to a lag time proportional to the scavenging ability. The method employed was identical to that described by Arnao et al (1996) except that 50 mM Tris-HCl buffer (pH 7.2) was used to represent more closely the physiological conditions and 2 nM horseradish peroxidase was used to offset the reduced enzyme activity at this pH. This dynamic method was chosen over that of the single point method described by Miller et al (1996) after it was determined that a number of plant extracts and flavonoids inhibited the peroxidase reaction. This inhibition could lead to an erroneous estimation of the hydroxyl free radical scavenging capacity of chemicals that are peroxidase inhibitors and/or free radical scavengers.

Prevention of lipid peroxidation by *Echinacea* extracts

The ability of *Echinacea* extracts to inhibit lipid peroxidation was assessed using catecholaminergic neuroblastoma SH-SY5Y cells and a modification of the thiobarbituric acid assay of Ohkawa et al (1979). SH-SY5Y cells (a gift from Dr Peter Yu, University of Saskatchewan) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic mixture (PSN) until they reached confluence and were then seeded at a density of 1×10^6 cells/well in Nunclon 6-well tissue culture plates. Cells were grown for 24 h (subconfluence) and then treated with various concentrations of the extracts in the presence or absence of 0.1 mM ferrous sulfate. After 48 h the cells were harvested, placed in disposable glass culture tubes and centrifuged to provide a pellet. The medium was discarded and the pellet was resuspended in 0.4 mL 1.15% KCl and homogenized with a sonic dismembrator. A portion (0.3 mL) of this mixture was then assayed for lipid peroxides using the method of Ohkawa et al (1979) with tetraethoxypropane as a reference standard. The remaining material was used for the determination of protein using a modification of the method of Lowry et al (1951).

Results

Extracts of the roots and leaves of the three species of *Echinacea* provided chromatographic profiles characteristic for each preparation. Figure 1 illustrates the UV

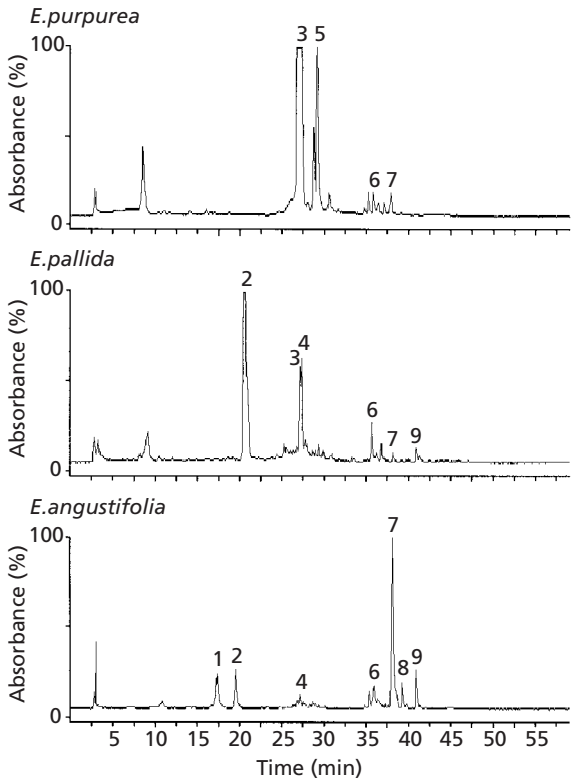

Figure 1 HPLC traces of alcoholic extracts from *E. purpurea*, *E. pallida* and *E. angustifolia* roots. Peaks were detected by UV absorbance at 254 nm. Peaks were identified by comparison of their electrospray mass spectra with expected masses according to previously published studies (Bauer et al 1988a, b, c, 1989; Cheminat et al 1988; Bauer & Remiger 1989). Peak 1: cynarine; peak 2: echinacoside; peak 3: cichoric acid; peak 4: 6-O-caffeoylechinacoside; peak 5: verbascoside; peak 6: undeca-2E-en-8,10-diynoic acid isobutylamide; peak 7: dodeca-2E,4E,8Z,10Z/E-tetraenoic acid isobutylamide; peak 8: dodeca-2E,4E,8Z-trienoic acid isobutylamide; peak 9: dodeca-2E,4E-dienoic acid isobutylamide. Note: peaks were adjusted to 100% of base peak with an absorbance maximum of 3 absorbance units. As a result the signal of cichoric acid in the chromatogram of *E. purpurea* and the signal of echinacoside in the chromatogram of *E. pallida* were truncated at 3 absorbance units permitting visualization of the minor components. In the case of *E. purpurea* the signal from cichoric acid overwhelmed other signals. A number of additional isobutylamides were present in all chromatograms, which provided excellent mass spectral signals. Ketoalkenes and ketoalkynes, characteristic of *E. pallida*, did not provide strong (MH^+) electrospray signals.

Table 1 Representative mass spectral signals and their relative abundances of some characteristic chemicals obtained from reverse-phase chromatographic separations of *Echinacea* root or leaf extracts that were eluted directly into the electrospray mass spectrometer in positive mode.

Compound	MNa^+	MH^+	Major fragments
Cichoric acid	497 (14%)	475 (15%)	457 (86%), 295 (100%)
6-O-Caffeoylechinacoside	971 (100%)	949 (49%)	803 (58%), 795 (23%)
Echinacoside	809 (3%)	787 (14%)	625 (14%), 479 (40%), 471 (36%), 325 (100%)
Verbascoside		625 (61%)	479 (18%), 471 (59%), 325 (85%)
Cynarine	539 (83%)	517 (84%)	499 (100%)
Chlorogenic acid	377 (54%)	355 (100%)	
Rutin		611 (55%)	465 (42%), 303 (100%)
Dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide		248 (100%)	
Undeca-2Z,4E-dien-8,10-diynoic acid isobutylamide	252 (10%)	230 (100%)	
Dodeca-2E,4Z-dien-8,10-diynoic acid isobutylamide	266 (6%)	244 (100%)	
Trideca-2E,7Z-dien-10,12-diynoic acid isobutylamide	280 (4%)	258 (100%)	
Pentadeca-2E,9Z-dien-12,14-diynoic acid isobutylamide	308 (6%)	286 (100%)	
Undeca-2E-en-8,10-diynoic acid isobutylamide	254 (23%)	232 (100%)	

Values are m/z (% relative abundance).

absorbance HPLC traces obtained from extracts of *E. purpurea*, *E. pallida* and *E. angustifolia* roots. Compounds eluting between the solvent front and 32 min were generally found to be phenylpropyl conjugates or flavonol glycosides. The electrospray total ion trace (not shown) provided relatively weak, but characteristic signals for the phenylpropyl conjugates and for flavonol glycosides. Representative positive mode mass spectra of seven phenolic chemicals obtained from the traces presented in Figure 1, and equivalent separations of *Echinacea* leaf extracts are given in Table 1. Stronger electrospray mass spectra could be obtained by collecting the eluting peak of interest from the outlet of the flow splitter, concentrating it by freeze-drying and injecting a portion of the concentrated solution directly into the electrospray unit. The phenylpropyl materials provided spectra in positive and negative mode and some fragmentation of these compounds was evident. Compounds eluting after 32 min were generally isobutylamides and related materials, and these provided strong UV absorbance and strong electrospray signals in positive mode. Mass spectral data for six selected isobutylamide-related substances eluting between 35 and 42 min (Figure 1) are tabulated in Table 1. Little or no fragmentation of the alkamides was evident under these conditions. It was evident from the traces in Figure 1 that the major UV absorbing materials in *E. angustifolia* root extracts were isobutylamides (peaks 6–9). In contrast, the major UV absorbing material in *E. purpurea* was cichoric acid (peak 3). The chromatogram for *E. pallida* was dominated by the presence of echinacoside (peak 2) and 6-O-caffeoylechinacoside (peak 4). The ketoalkenes and ketoalkynes characteristic of *E. pallida* did not provide strong electrospray signals under these circumstances.

Extracts of the leaves of the three *Echinacea* species were also chemically distinct (chromatograms not shown). *E. pallida* leaf extracts were distinguished from those of *E. purpurea* and *E. angustifolia* by the absence of dodeca-2E,4E,8Z,10Z/E-tetraenoic acid isobutylamide and by the relative preponderance of cichoric acid in the *E. pallida* extracts. In contrast, *E. angustifolia* leaf extract contained little or no cichoric acid and relatively large quantities of a chemical with a molecular weight of 640. Initially this compound was suspected to be plantamajoside [β-(3,4-dihydroxyphenyl)-ethyl-O-2-L-glucopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl-β-D-glucopyranoside], which is structurally related to verbascoside, since it has a molecular weight of 640. However, when the protonated compound from *E. angustifolia* ($MH^+ = 641$) fragmented it expelled sequentially what was believed to be rhamnose and glucose residues to yield fragments of m/z 495 and 333. This did not support identification as plantamajoside and attempts are being made to identify this compound. *E. purpurea* leaf extract was distinguished by having relatively large amounts of cichoric acid and dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide. The quercetin glycoside, rutin, was also detected in leaf extracts from all three species and was identified by its characteristic mass

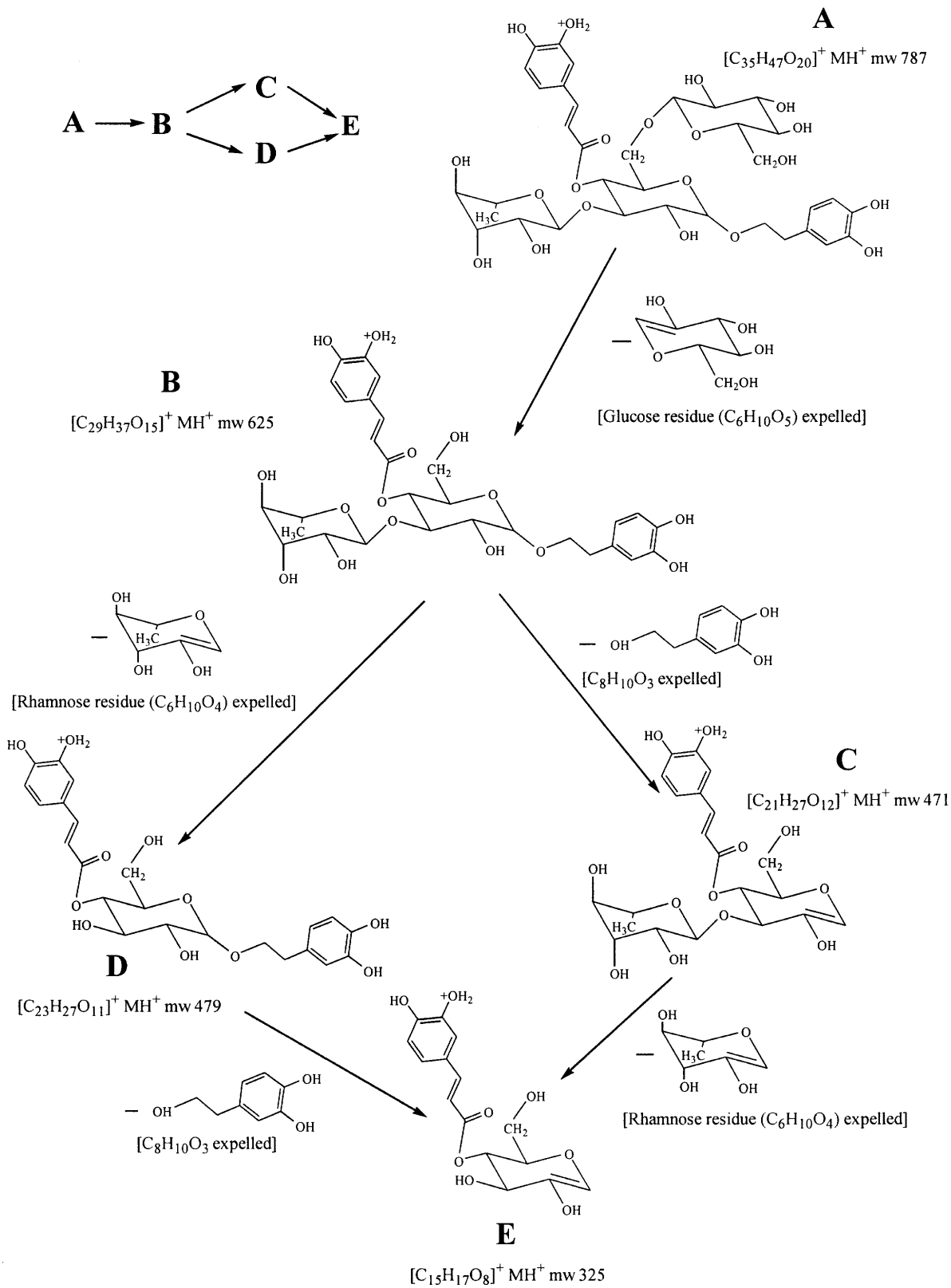

Figure 2 Proposed positive mode fragmentation patterns for echinacoside and verbascoside under the described electrospray conditions.

spectrum ($MH^+ = 611$, fragments at m/z 465, m/z 303 and m/z 265).

The phenylpropyl conjugates provided characteristic fragmentation patterns under these electrospray conditions, so tandem mass spectrometry (MS/MS) evaluations were not required. Echinacoside was readily characterized by positive ion electrospray MS. An initial loss of a glucose residue resulted in the production of verbascoside (Figure 2). This was followed by the expulsion of either a rhamnose residue or a 2-(3,4-dihydroxyphenyl)-ethanol molecule to give two products (structures C and D in Figure 2) which further fragmented to produce ion E.

Cichoric acid was readily characterized by its electrospray MS, which depicted sequential loss of water (m/z 475 → m/z 457) followed by caffeic acid (m/z 457 → m/z 295) or caffeic acid (m/z 475 → m/z 313) followed by water (m/z 313 → m/z 295).

Little or no fragmentation of the isobutylamides was demonstrated under the present electrospray conditions and individual compounds were identified by their masses [(MH^+) and (MNa^+) values]. However, MS/MS provided informative characteristic fragmentation patterns for several alkamides. Figure 3 illustrates representative fragmentation patterns obtained from undeca-2Z/E-ene-8,10-diynoic acid isobutylamide (daughters of m/z 232) and dodeca-2E,4E-dienoic acid isobutylamide (daughters of m/z 252) after fragmentation in the presence of argon with a collision energy of 30 V.

Extracts of roots or leaves of all three *Echinacea* species demonstrated an ability to scavenge free radicals (Table 2). In this antioxidant model, extracts of *E. purpurea* roots had significantly greater free radical scavenging capacity than those of either *E. pallida* or *E. angustifolia*. Extracts of *Echinacea* leaf had less free radical scavenging capacity than those of the roots. *E. angustifolia* leaf extract had significantly less free radical scavenging capacity than either *E. purpurea* or *E. pallida* leaf extracts. While these crude extracts demonstrated an ability to scavenge free radicals that was the equivalent of only 1% to 4% that of pure ascorbic acid, pure cichoric acid, which was collected from eluting samples of *E. purpurea* root, provided 3.18 ± 0.11-times the free radical scavenging capacity of ascorbic acid on a molar basis.

Extracts of roots or leaves of all three *Echinacea* species inhibited Fe^{2+}-induced lipid peroxidation. In this antioxidant model the root extract (Figure 4) had a lesser antioxidant capacity than the leaf extract (Figure 5). No significant differences were determined for the three *Echinacea* species in the lipid peroxidation model.

Discussion

HPLC coupled to UV absorbance and electrospray mass spectrometric detection provided a rapid and simple method for chemically distinguishing extracts obtained from the roots and leaves of three *Echinacea* species. Alcoholic extracts of the roots of *E. purpurea*, *E. pallida* and *E. angustifolia* provided distinct chemical profiles characterized by the presence of a number of caffeoyl conjugates and isobutylamides which were eluted by means of a single chromatographic separation. The distinct distribution of the characteristic chemicals among species reflected observations previously determined for these plants using a multiplicity of separation methods. These have included TLC and HPLC separations designed for either caffeoyl conjugates (Cheminat et al 1988) or isobutylamides (Bauer et al 1988c; Bauer & Remiger 1989; He et al 1998), but not for both. For example, the presence of isobutylamides in these three species has been examined (Bauer et al 1987, 1988c, 1989; Bauer & Remiger 1989; He et al 1998). Those studies demonstrated a characteristic distribution of isobutylamides that was readily confirmed in this study. The distribution of caffeoyl conjugates has also been characterized in these three *Echinacea* species. Again, however, all three species have not been compared simultaneously with each other for caffeoyl conjugate distribution or for the distribution of the isobutylamides that are also present in these extracts (Bauer et al 1988b; Cheminat et al 1988; Facino et al 1995). In our method it was demonstrated that cichoric acid was by far the most predominant caffeoyl conjugate present in extracts of *E. purpurea* root. In contrast, echinacoside and 6-caffeoylechinacoside were the predominant caffeoyl conjugates present in extracts of *E. pallida* root. Extracts of *E. angustifolia* roots could be distinguished from those of *E. purpurea* and *E. pallida* by the presence of both cynarine and echinacoside and by the absence of cichoric acid.

Leaf extracts also provided distinct HPLC UV and electrospray mass spectral profiles that indicated the presence of cichoric acid in *E. purpurea* and *E. pallida* but not in *E. angustifolia*. Echinacoside was found in extracts of leaves of *E. pallida* but not in extracts of *E. purpurea* or *E. angustifolia* leaves.

MS/MS provided extensive characteristic fragmentation patterns for a number of isobutylamides that had been collected as relatively pure peaks eluting from the HPLC column. Characteristic molecular expulsions included the loss of 2-methylpropylene and 2-methylpropylamine. The loss of 2-methylpropylamine was often followed by the loss of a carbon monoxide molecule

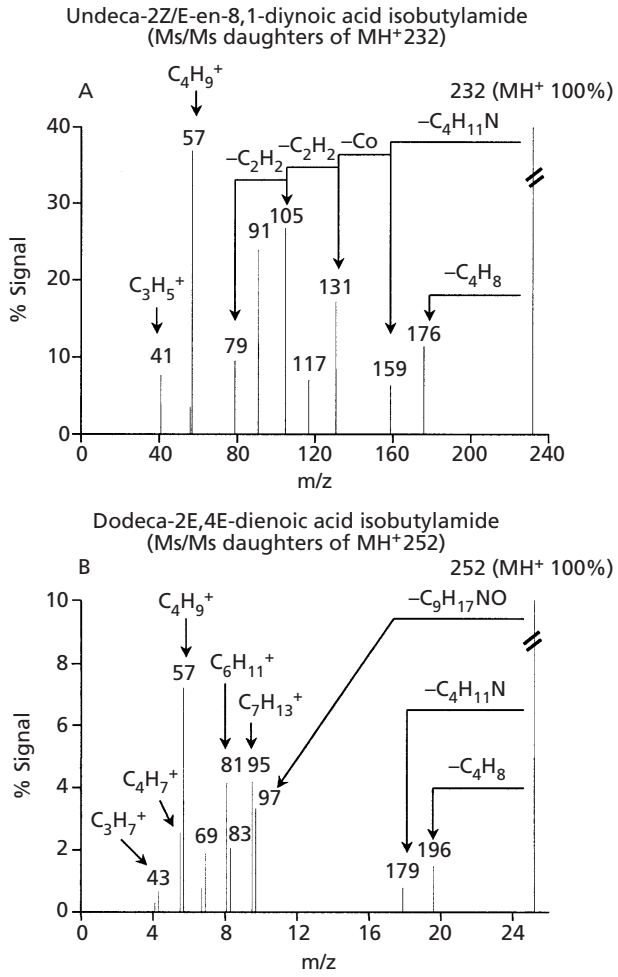

Figure 3 Representative mass spectra resulting from fragmentation of undeca-2Z/E-en-8,10-diynoic acid isobutylamide (daughters of MH^+ 232) (A) and dodeca-2E,4E-dienoic acid isobutylamide (daughters of MH^+ 252) (B). To illustrate the fragments properly the base peak (MH^+ 232) in A was arbitrarily reduced to 40% and the base peak (MH^+ 252) in B was arbitrarily reduced to 10%. Fragments contributing less than 0.5% signal were removed. Several positively charged fragments and molecular expulsions have been identified on the Figure.

Table 2 Free radical scavenging capacity of alcoholic extracts of three *Echinacea* species.

Species	Plant part	Scavenging capacity (% ascorbic acid by weight)
E. purpurea	Root	4.45 ± 0.04*
E. pallida	Root	2.79 ± 0.23
E. angustifolia	Root	2.83 ± 0.04
E. purpurea	Leaf	1.55 ± 0.02
E. pallida	Leaf	1.33 ± 0.01
E. angustifolia	Leaf	1.13 ± 0.02*

Values are the means ± s.e.m. based on three determinations. $*P < 0.05$ compared with other species samples of the same plant structures.

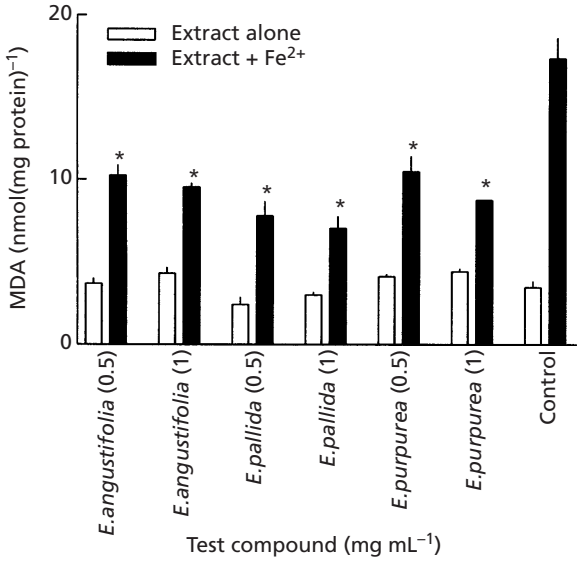

Figure 4 Effect of root extracts of *E. purpurea*, *E. pallida* and *E. angustifolia* on lipid peroxidation of SH-SY5Y cells. Values are the means ± s.e. based on three determinations. $*P < 0.05$ compared with Fe^{2+}-exposed controls.

leaving a long alkyl chain as a major ion. Characteristic positively-charged ions included those of propylene (m/z 41), propane (m/z 43), 2-methylpropylene (m/z 55), 2-methylpropane (m/z 57) and larger fragments derived from the alkyl chain. It is preferable to perform MS/MS studies on isolated peaks eluting from the HPLC column since it is possible to distinguish sodium adducts of certain isobutylamides (e.g. $C_{15}H_{19}NO\,MNa^+ = 252$) from protonated species having the same molecular weight (e.g. $C_{16}H_{21}NO\,MH^+ = 252$). If the initial chromatographic separation is not performed interpretation of MS/MS spectra produced by mixtures of isobutylamides derived from extracts of *Echinacea* species becomes difficult.

Electrospray mass spectra, while contributing greatly to the identification of a compound, may not always unequivocally identify a particular chemical. Many isomers would be expected to provide almost identical spectra (e.g. verbascoside and forsythiaside and E and Z isomers of isobutylamides). In some cases use of authentic standards in conjunction with elution profiles from chromatographic columns and mass spectra are still required for unequivocal chemical identification.

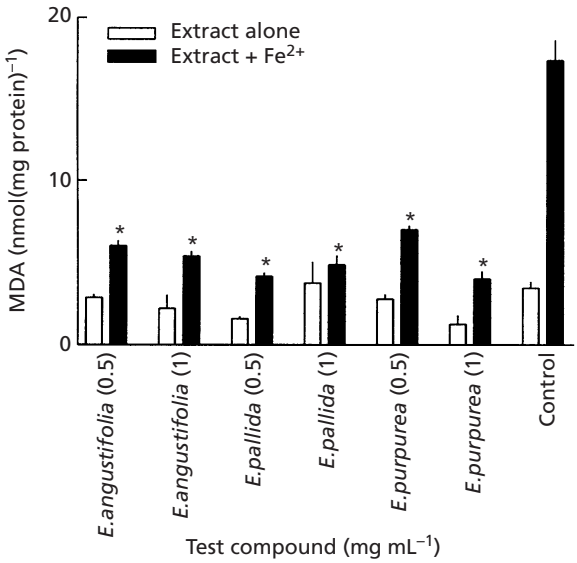

Figure 5 Effect of leaf extracts of *E. purpurea*, *E. pallida* and *E. angustifolia* on lipid peroxidation of SH-SY5Y cells. Values are the means ± s.e. based on three determinations. $*P < 0.05$ compared with Fe^{2+}-exposed controls.

The root and leaf extracts of the three *Echinacea* species examined demonstrated an ability to free radical scavenge and to inhibit Fe^{2+}-induced lipid peroxidation. Caffeoyl conjugates are known free radical scavengers, metal chelators and antioxidants (Facino et al 1995; Wang et al 1996a, b; Xiong et al 1996; Sestili et al 1998). The demonstration that cichoric acid was a potent free radical scavenger with a molar capacity for hydroxyl free radicals approximately three times that of ascorbic acid supported the idea that the numerous caffeoyl conjugates found in *Echinacea* species could contribute to beneficial pharmacological effects by preventing unwanted oxidative reactions. The lipid peroxidation assay also indicated that extracts of *Echinacea* roots and leaves had antioxidant properties. Unfortunately, this assay failed to discriminate between antioxidant effects resulting from the scavenging of free radicals or those resulting from Fe^{2+} chelation. The relative contribution of either process to the observed antioxidant effect remains to be determined.

The antioxidant capacity of each of the three *Echinacea* species did not appear to differ greatly although their chemical profiles were very distinct. It would seem that the various caffeoyl conjugates all contributed to varying degrees to the total antioxidant effect and that an approximately equivalent antioxidant capacity was found in each species despite their varied chemical constituents. Furthermore, the antioxidant effect was not expected to be completely confined to the caffeoyl conjugates as flavones also present in extracts of *Echinacea* leaves are potent free radical scavengers and metal chelators (Metodiewa et al 1997; Kostyuk & Potapovich 1998; Sestili et al 1998).

This method provided a simple and rapid approach for the simultaneous analysis of caffeoyl conjugates and isobutylamides in plant extracts. This permitted the unequivocal identification of extracts belonging to the genus *Echinacea*. Furthermore, this method easily distinguished the three most commonly used *Echinacea* species and will permit the standardization of raw materials destined for the nutraceutical market. This contrasts with the method described on the internet website of the Institute for Nutraceutical Advancement (www.nutraceuticalinstitute.com/methods/echinaceaset.html and www.nutraceuticalinstitute.com/methods/ech_chroma.html) which provides no information concerning chemical differentiation and identification of *Echinacea* species.

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