Studies on the Antioxidant Activity of *Echinacea* Root Extract

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Methanol extracts of freeze-dried *Echinacea (E. angustifolia*, *E. pallida*, and *E. purpurea*) roots were examined for free radical scavenging capacities and antioxidant activities. Root extracts of *E. angustifolia*, *E. pallida*, and *E. purpurea* were capable of scavenging hydroxyl radical. Similar scavenging activities for each variety were found for both 1,1-diphenyl-2-picrylhydrazyl radical and ABTS radical. Meanwhile, antioxidant activities of all three varieties of *Echinacea* were found to delay the formation of conjugated diene hydroperoxide induced by the thermal decomposition of 2,2′-azobis(2-amidinopropane) dihydrochloride and extend the lag phase of peroxidation of soybean liposomes. *Echinacea* root extracts suppressed the oxidation of human low-density lipoprotein, as evaluated by reduced agarose electrophoretic mobility following oxidative modification by Cu^{2+}. The mechanisms of antioxidant activity of extracts derived from *Echinacea* roots included free radical scavenging and transition metal chelating.

Keywords: *Echinacea; free radical; oxidation; antioxidant*

INTRODUCTION

Echinacea is a native herb in both North America and Europe and widely accepted for its immunostimulant medicinal usage. Lipophilic alkamides, polar caffeic acid derivatives (echinacoside), and cichoric acid make a considerable contribution to the immunostimulatory action of alcohol extracts of *Echinacea* (Figure 1). Polysaccharides are also implicated in the activity of expressed *Echinacea* juice and aqueous extract, as well as oral administration of powdered whole drug. The amount of caffeic acid derivatives found in *Echinacea* varies with varieties and plant parts. For example, *E. pallida* root contains 0.4−1.7% echinacoside, compared to 0.3−1.3% in *E. angustifolia* root and undetectable echinacoside in the root of *E. purpurea*. *E. purpurea* root, however, contains 0.6−2.1% cichoric acid, which also exists in trace amount in *E. angustifolia* root (Bauer and Wagner, 1991). Despite the many studies that have shown modification function of the immune system by *Echinacea* species (Melchart et al., 1995; Roesler et al., 1991; See et al., 1997; Tragni et al., 1988), there is little research on the antioxidant activity of *Echinacea*. Echinacoside has been reported to provide dose-dependent protection against hydroxyl radical (•OH) induced degradation of type III collagen, suggesting therefore a protective activity of *Echinacea* polyphenols against photodamage of skin (Facino et al., 1995).

Plant parts used for herbal medicine include the flower, top, aerial, and roots. Glowniak et al. (1996) quantified the phenolic acid content of *Echinacea* species using solid-phase extraction and HPLC and reported a total amount of phenolic acid in the aerial part of *Echinacea* species from 70 to 1400 μg/g of dry herb. Naturally occurring phenolic acids and flavonoids are known to be important antioxidants in herbs and vegetables due to the presence of hydroxyl groups on aromatic rings (Shahidi, 1995). The relatively lower reduction potential of derived phenolic radicals following contact with either alkyl peroxyl radicals or superoxide radicals devotes a potential antioxidant characteristic (Jovanovic et al., 1992; Salah et al., 1995) which makes possible a participation in activity to defense against reactive oxygen species. However, the role, if any, of antioxidant function of *Echinacea* is not known.

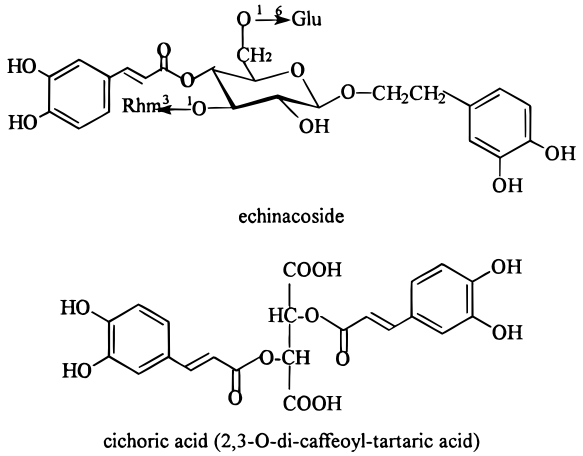

Figure 1. Structures of echinacoside and cichoric acid.

This study was conducted to characterize the antioxidant activities of these *Echinacea* species, namely, *E. angustifolia*, *E. pallida*, and *E. purpurea*, roots extracts using a variety of in vitro model systems.

MATERIALS AND METHODS

Echinacea (*E. purpurea, E. angustifolia*, and *E. pallida*) root was kindly supplied by Naturally Nova Scotia (Dartmouth, Nova Scotia, Canada). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), L-α-lecithin (from soybean), 2-thiobarbituric acid (TBA), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (diammonium salt, ABTS), human low-density lipoprotein, Chelex-100 ion-

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exchange resin, and 50 mM barbital buffer (pH8.6) were purchased from Sigma Chemical Co. (St. Louis, MO). Agarose was obtained from Bio-Rad Laboratories (Richmond, CA); 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA). Trichloroacetic acid (TCA), hydrogen peroxide (30%), and HPLC-grade acetonitrile were obtained from Fisher Scientific (Fari Lawn, NJ); 2-deoxyribose was obtained from Applied Science Laboratories Inc. (State College, PA). Phosphate buffer was made with distilled deionized water and was eluted through a Chelex-100 column to eliminate the occurrence of transition metals.

Preparation of Echinacea Extracts. Fresh Echinacea root was rinsed with water and freeze-dried immediately. Dry Echinacea root was ground using a coffee grinder at 4 °C in a cold room. Powdered Echinacea root was refluxed with methanol for 6 h, followed by rotary evaporation under 40 °C. Concentrations of Echinacea root extracts were expressed as weight of root/mL.

Echinacea root was also extracted by chloroform for 6 h to compare with methanol extracts in free radical scavenging capacities.

HPLC Profile. Echinacea root metholic extracts used for HPLC analysis were passed through a PTFE syringe filter (0.2 μm) before injection onto a Phenomenex C18 column (250 mm $\times$ 4.6 mm, 5 μm) (Torrance, CA) at ambient temperature. Two Waters M-45 pumps were used with a Lambada-Max LC spectrometer detector (model 481). Solvent A was acetonitrile/water containing 0.1% H_3PO_4 (10:90), and solvent B was acetonitrile/water containing 0.1% H_3PO_4 (25:75). A gradient solvent delivery was used with B increasing from 0% to 100% in 30 min and maintained at 100% for 5 min. The proportion of B decreased to 0% after 5 min, and the column was washed with 100% A for 10 min until the next injection. The flow rate was 1.5 mL/min, and the wavelength of the UV−visible detector was set at 330 nm. Data were collected by a Axxi-Chrom 717 chromatography data system (Axxiom Chromatography Inc. Moorpark, CA).

Reducing Power. The reducing power of *Echinacea* extracts was measured by $K_3Fe(CN)_6$−$FeCl_3$ (Yen et al., 1995). The reducing power was calculated according to a standard curve obtained from ascorbic acid and expressed as ascorbic acid equivalent (AAE) (μg of ascorbic acid/mg of root)

Copper Chelating Capacity of *Echinacea* Root Extracts. A spectrophotometric method was used according to Afanas'en et al. (1989). Briefly, 1 mg/mL of *Echinacea* root extract was incubated with 100 μM $CuCl_2$ at 37 °C for 2 h. The spectra of *Echinacea* root extract as well as $CuCl_2$−*Echinacea* complex were recorded against 10 mM phosphate buffer (pH 7.4) and the corresponding concentration of $CuCl_2$ in a phosphate buffer at 200−600 nm.

Antioxidant Activity Evaluation in Deoxyribose Model. In this assay, a Fenton reaction based model containing 0.1 mM of Fe^{3+} or Cu^{2+} as the catalytic metal was used (Aruoma, 1996). Briefly, 3.6 mM 2-deoxyribose, 0.1 mM EDTA, 0.1 mM metal ion, 0.1 mM ascorbic acid, and 1 mM H_2O_2 were mixed together with or without *Echinacea* extract and incubated at 37 °C for 1 h. Following the incubation, an equal volume of 10% (v/v) trichloroacetic acid (TCA) and 1% 2-thiobarbituric acid (TBA) was added and the reactants were boiled for 15 min. The absorbance at 532 nm was recorded after cooling. Moreover, in the cupric ion mediated model, ascorbic acid was replaced by phosphate buffer (pH 7.4) to evaluate the potential prooxidant activity of *Echinacea* root extract.

Free Radical Scavenging Activity. The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect of *Echinacea* root extracts was measured according to Blois (1958) and Brad-Williams et al. (1995). *Echinacea* root extract was mixed with 0.1 mM DPPH radical in ethanol solution. A 20 min incubation period at room temperature was used before reading the absorbance at 519 nm. The inhibitory percentage of DPPH was calculated according to the following equation:

$$\%\text{Inhibition} = \frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \times 100\%$$

The ABTS radical cation (Pellegrini et al., 1999) model was also used to evaluate the free radical scavenging effect of *Echinacea* root extract. Chloroform and methanol extracts of *Echinacea* root were compared for the relative free radical scavenging capacity in this model. Scavenging effects of *Echinacea* root extracts were compared by determining the percentage of decolorization at 734 nm after 8 min of reaction at room temperature. The effect of *Echinacea* root extract on scavenging ABTS radical was calculated from the following equation:

$$\%\text{Inhibition} = \frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \times 100\%$$

Effect of *Echinacea* Root Extracts in Protecting Liposome from Peroxyl Radical Induced Peroxidation. Liposomes were made by sonicating soybean lecithin in an ice−water bath for 2 h in 10 mM phosphate buffer (pH 7.4) (Duh, 1998). Peroxyl radical induced liposomal peroxidation was conducted at 37 °C, using a Perkin-Elmer Lambada 6 UV−vis spectrophotometer. Briefly, the reaction was initiated by adding 0.5 mM AAPH to a mixture of 75 μg/mL of liposome in 10 mM phosphate buffer (pH 7.4) which included *Echinacea* root methanol extracts (0, 5, and 25 μg/mL). The absorbance at 234 nm was recorded every 5 min for 2 h. A reaction process was obtained from the generation of conjugated diene hydroperoxide against time based on an absorbance coefficient of $\epsilon = 29\,500\ M^{-1}\,cm^{-1}$ (Puhl et al., 1994). The antioxidant potency of *Echinacea* root was determined from the peroxidation lag phase period determined by the intercept at the *abcissa* in the conjugated diene concentration versus time plot.

Effect of *Echinacea* Root Extract in Protecting Cu^{2+} Induced Human LDL Oxidation. Human LDL containing 0.01% EDTA was dialyzed against 10 mM phosphate-buffered saline (PBS) (pH 7.4, with 0.15 M of NaCl) at 4 °C under N_2 to remove EDTA salt. EDTA-free LDL was used immediately after dialysis. $CuCl_2$ (10 μM) was mixed with 0, 0.5, 1.0, 2.0, 5.0, 10, and 20 mg/mL of *Echinacea* root extract in PBS, and the reaction was started by adding 0.5 mg/mL of dialyzed lipoprotein and incubated at 37 °C for 20 h (Yan et al., 1995). The reaction was stopped by adding 1 μL of EDTA to a final concentration as 20 μM. Agarose electrophoresis in 50 mM barbital buffer was conducted using a submarine electrophoresis system. The gel was visualized by staining with Sudan Black (Noble, 1968). Oxidative modification of LDL exposed to *Echinacea* was compared by the difference of electrophoretic mobility of both native and oxidative LDL.

RESULTS

The HPLC profiles of *E. angustifolia*, *E. pallida*, and *E. purpurea* root extract are shown in Figure 2. Echinacoside and cichoric acid were found in both extracts of *E. pallida* and *E. angustifolia* root, whereas no echinacoside was detected in the *E. purpurea* root extracts. *E. purpurea* root contained a relatively high amount of cichoric acid compared with the other two varieties (Table 1). It is also notable that the reducing power of three *Echinacea* root extracts was higher for *E. pallida* compared to similar lower activities for the other two varieties (Table 2).

The differential spectra of *Echinacea* root extracts following the addition of copper chloride are present in Figure 3A−C for *E. angustifolia*, *E. pallida*, and *E. purpurea*, respectively. All root extracts produced a shoulder peak (see arrow) around 400 nm following incubation with cupric ion. This shift in the absorbance

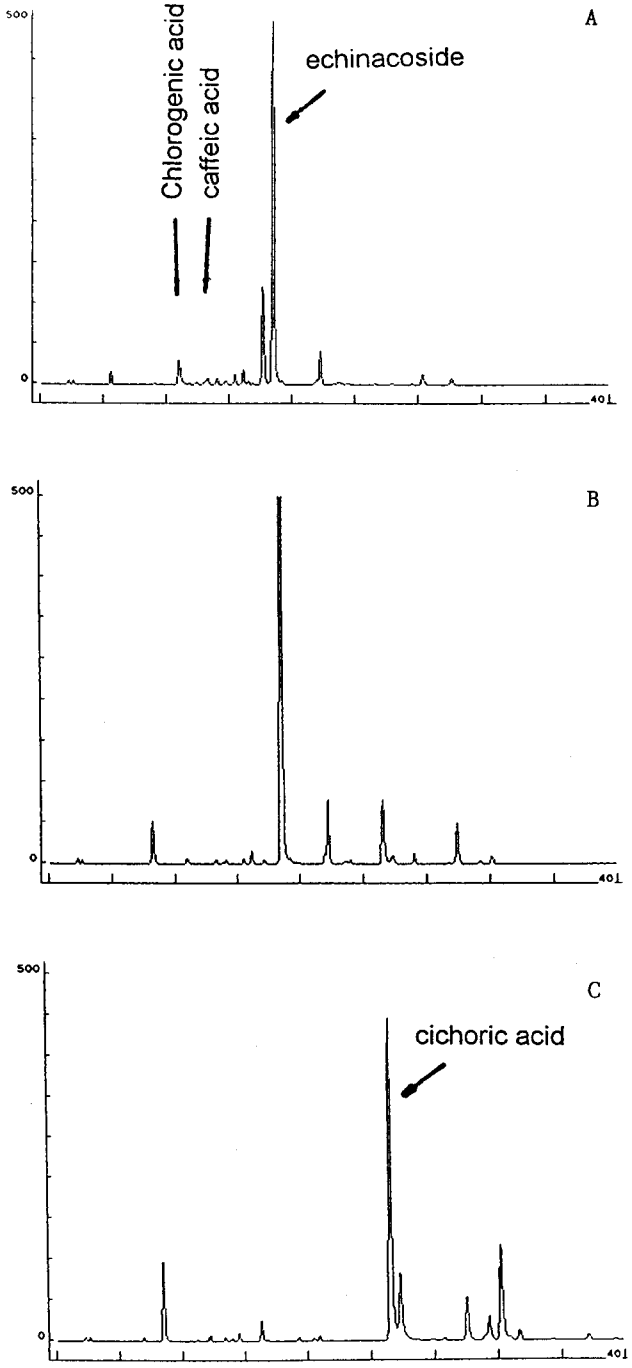

Figure 2. HPLC chromatograms of the phenolic acids in *Echinacea* root extracts, separation parameter as shown in text: (A) *E.angustifolia*, (B) *E. pallida*, (C) *E. purpurea* (operating parameters in text).

Table 1. Concentration of Chlorogenic Acid, Caffeic Acid, Echinacoside, and Cichoric Acid in *Echinacea* Root Samples (%)[a]

	chlorogenic acid	caffeic acid	echinacoside	cichoric acid
E. angustifolia	0.03%	0.035	1.0	0.04
E. pallida	NA	NA	2.5	0.105
E. purpurea	NA	NA	NA	0.49

[a] NA: not detectable or lower than 0.01%.

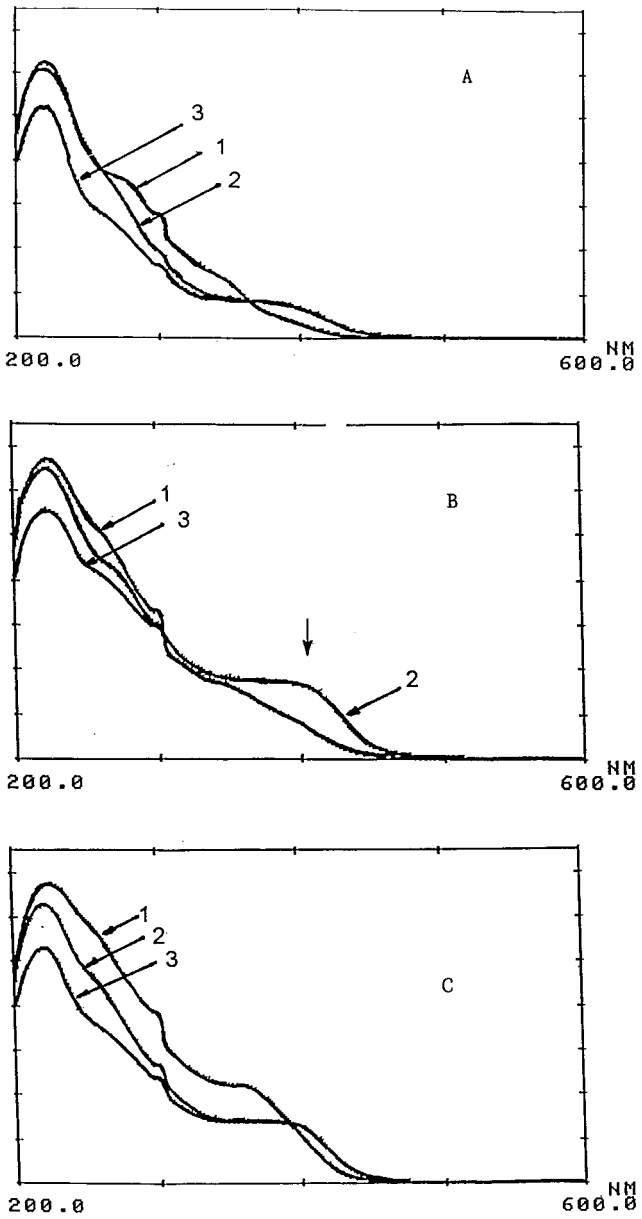

Figure 3. Differential spectra of *Echinacea* root extracts with copper: (A) *E. angustifolia*, (B) *E. pallida*, (C) *E. purpurea*. Spectrum 1 represents the spectrum of *Echinacea* against phosphate buffer (pH 7.4); spectrum 2 represents the spectrum of a mixture of *Echinacea* and Cu^{2+} against phosphate buffer (pH 7.4); spectrum 3 represents the spectrum of a mixture of *Echinacea* and Cu^{2+} against $CuCl_2$.

Table 2. Reducing Power (AAE)[a] and ABTS Radical Scavenging Activity[b] of *Echinacea* Root Extract

sample	AAE	ABTS scavenging (CH_3OH extract)[c]	ABTS scavenging ($CHCl_3$ extract)[d]
E. angustifolia	3.84 ± 0.03	20.6 ± 1.5	1.8 ± 1.4
E. pallida	5.07 ± 0.00	63.8 ± 7.5	3.2 ± 0.5
E. purpurea	3.48 ± 0.07	11.0 ± 0.1	3.2 ± 0.2

[a] AAE = ascorbic acid equivalent amount (μg of ascorbic acid/mg of *Echinacea* root). [b] ABTS scavenging activities are equal to percentage inhibition of ABTS radical. [c] Concentrations were 0.8 mg/mL. [d] Concentrations were 10 mg/mL.

spectra indicated chelation of the cupric ion and was more pronounced in the *E. pallida* variety (Figure 3B).

All varieties of *Echinacea* displayed inhibitory activities against metal ions catalyzed •OH generation in the Fenton reaction using the deoxyribose assay (Figure 4a,b). Although the maximum inhibition of •OH in this assay ranged from only 20% to 34% for all three varieties, there was a concentration-dependent effect

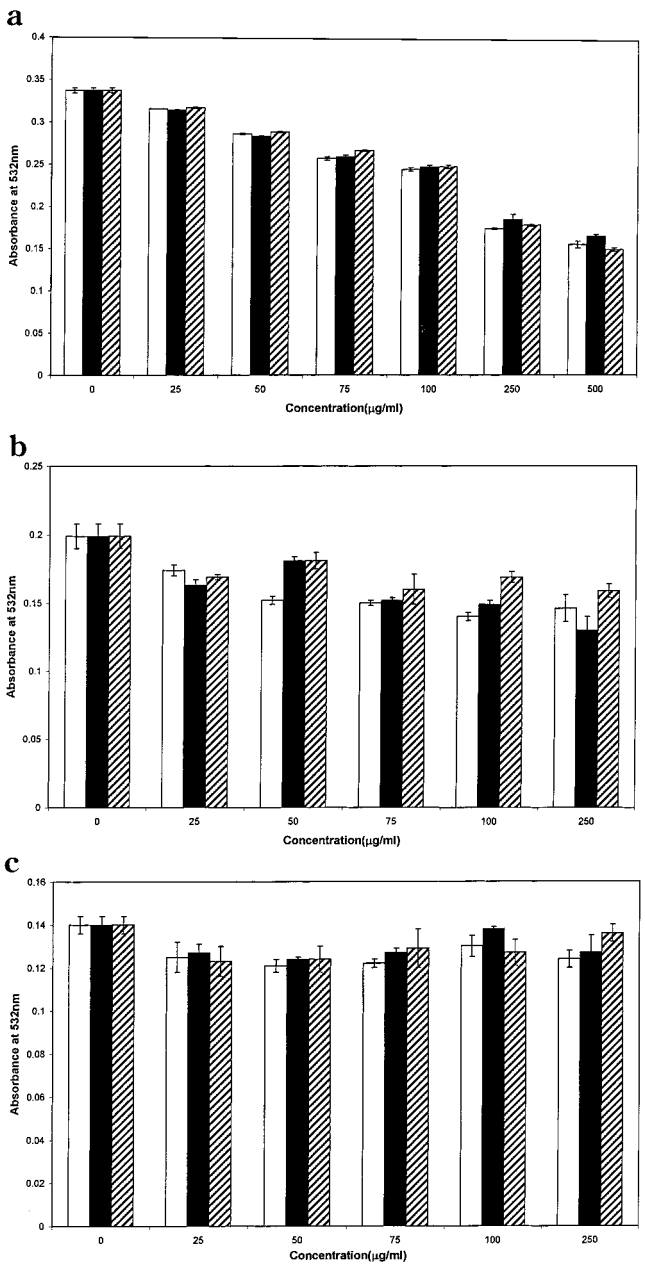

Figure 4. *Echinacea* root extracts scavenge hydroxyl radical generated in ascorbic acid mediated Fenton reaction monitored degradation of deoxyribose: (a) Fe^{3+} mediated reaction with ascorbic acid, (b) Cu^{2+} mediated reaction with ascorbic acid, (c) Cu^{2+} mediated reaction without ascorbic acid. (white box) *E. angustifolia*, (black box) *E. pallida*, (striped box) *E. purpurea*.

over a range of 0−100 μg/mL of *Echinacea* present in the reaction mixture. The potential for prooxidant activity of *Echinacea* components was also evaluated by testing the effect of the reducing activity on the basis of initiating prooxidant activity (Figure 4c). In the control sample, the presence of 0.1 mM ascorbic acid was sufficient to generate Fenton •OH, as evidenced by the high absorbance obtained from the TBA reagent, which in turn reacts with oxidized fragments of deoxyribose. Replacing ascorbic acid with *Echinacea* in this assay did not result in the same prooxidant activity as that observed with ascorbic acid. This finding suggests that the reducing activity noted from *Echinacea* is either insufficient to generate •OH in the cupric ion mediated Fenton reaction or alternatively the free radical scavenging activity of *Echinacea* root extracts exceeds the prooxidant activity.

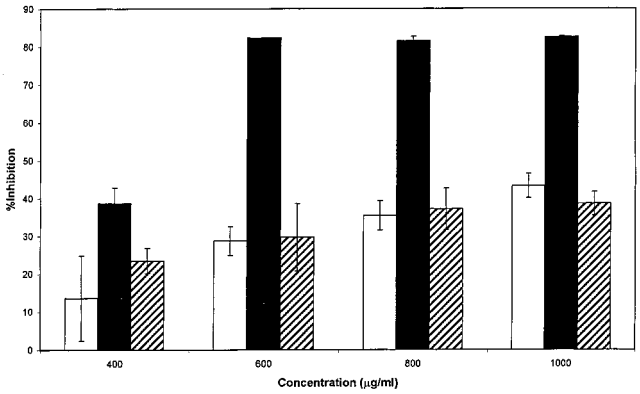

Figure 5. DPPH radical scavenging activity of *Echinacea* root extracts. (white box) *E. angustifolia*, (black box) *E. pallida*, (striped box) *E. purpurea*.

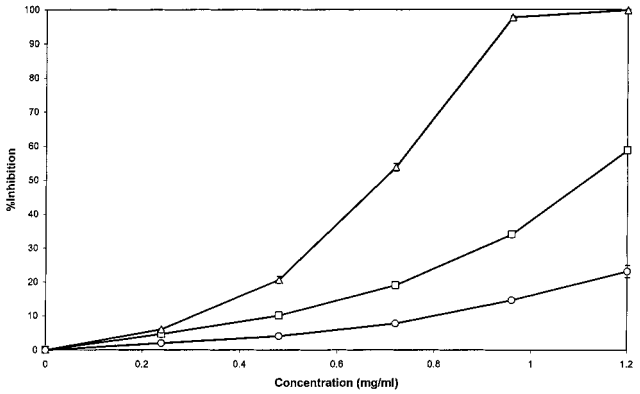

Figure 6. ABTS radical scavenging activity of *Echinacea* root extracts: (□) *E. angustifolia*, (△) *E. pallida*, (○) *E. purpurea*.

The free radical scavenging activity of *Echinacea* was examined using stable (DPPH and ABTS) radicals and peroxyl radical generated by the thermolysis of AAPH. In the DPPH model, all three *Echinacea* root extracts exhibited radical scavenging activity. *E. pallida* produced a relatively greater sequestering activity of the DPPH radical compared with *E. angustifolia* and *E. purpurea*. These two varieties of *Echinacea* showed similar scavenging effects in the DPPH model reaction (Figure 5).

In the ABTS model reaction, the methanol extract of *E. pallida* exhibited the highest inhibitory activity in the ABTS radical, followed by *E. angustifolia* and *E. purpurea*, respectively (Figure 6). Chloroform extracts from all three *Echinacea* root varieties did not produce an ABTS radical scavenging effect up to concentrations as high as 10 mg of root/mL (Table 2).

Figure 7 shows liposome peroxidation initiated by a peroxyl radical generated by thermal decomposition of APPH. *Echinacea* roots exhibited a strong protective effect against AAPH induced liposome peroxidation, compared to control samples without *Echinacea*. The addition of *Echinacea* root extracts to the reaction mixture at 5 μg/mL resulted in a relatively greater scavenging activity of the *E. pallida* compared to both *E. angustifolia* and *E. purpurea*. Lag phases for generation of conjugated diene hydroperoxides from oxidized liposome materials were 26 min for control and 51, 58, and 82 min, respectively for *E. purpurea, E. angustifolia*, and *E. pallida* roots (Figure 7a). Increasing the

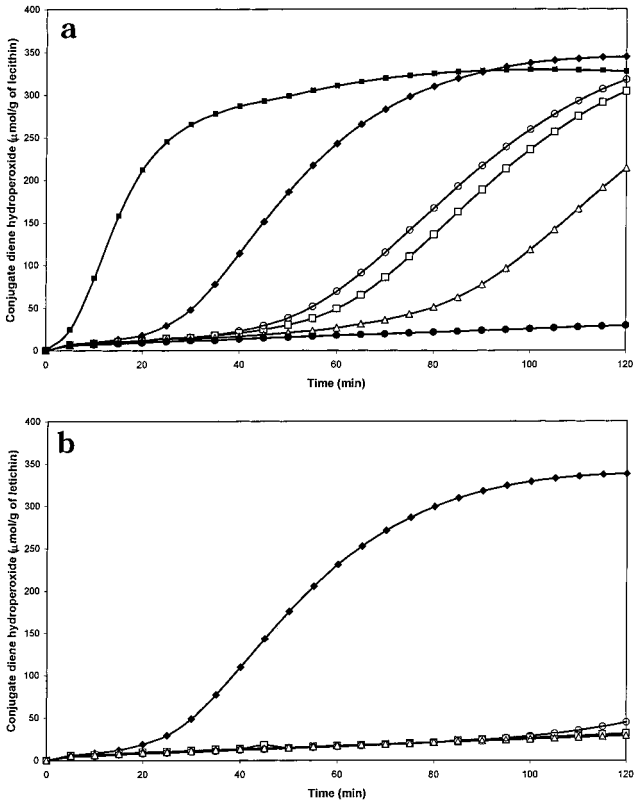

Figure 7. Effect of *Echinacea* root extracts in protecting liposome from AAPH induced peroxidation at 37 °C: (a) concentration of *Echinacea* is 5 μg/mL, (b) concentration of *Echinacea* is 25 μg/mL. Concentrations of ascorbic acid and Trolox are 2.5 and 2.5 μg/mL, respectively: (◆) control, (□) *E. angustifolia*, (△) *E. pallida*, (○) *E. purpurea*, (■) ascorbic acid, (●) Trolox.

Echinacea root concentration to 25 μg/mL resulted in almost complete inhibition of peroxyl radical induced liposome peroxidation over a time period of 100 min (Figure 7b).

The inhibitory effect of *Echinacea* root extract on copper induced LDL oxidation was studied within a concentration range of 0−20 mg/mL. Oxidation of LDL resulted in a greater migration on agarose gel electrophoresis compared to the native LDL standard. All three varieties of *Echinacea* produced a similar protection against cupric induced LDL oxidation (Figure 8).

DISCUSSION

Components of the *Echinacea* root extracts were effective at suppressing the generation of hydroxyl radical generated by the Fenton reaction containing H_2O_2, EDTA, and ascorbic acid in a test system where the generation of $^{\bullet}OH$ could be measured by the degradation of deoxyribose into remnants that react with thiobarbituric acid under acidic condition (Halliwell et al., 1987). This indirect evidence of free radical scavenging potential by *Echinacea* constituents was confirmed using a more direct approach obtained with the DPPH radical. DPPH is a stable radical which has been used to evaluate the antioxidant activity of plant and microbial extracts (Ko et al., 1998; Larrauri et al., 1998). In this assay, root extracts derived from the *E. pallida* variety exhibited the greatest DPPH radical scavenging activity. This result corresponded to a greater reducing power present in *E. pallida* extract relative to *E. angustifolia* and *E. purpurea* varieties.

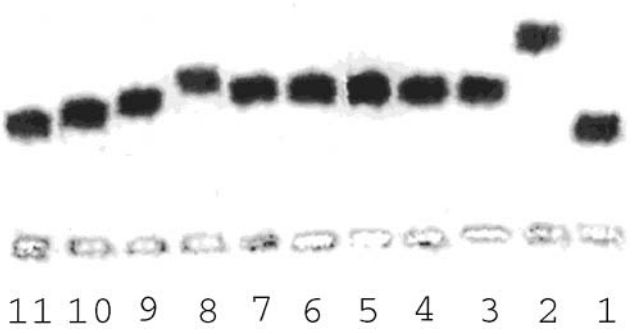

Figure 8. Agarose gel electrophoresis of LDL incubated with Cu^{2+} with/without *Echinacea* root extracts: lane 1 = native LDL; lane 2 = LDL incubated with cupric ion; lane 3 = LDL incubated with cupric ion and 5 mg/mL of *E. angustifolia*; lane 4 = LDL incubated with cupric ion and 10 mg/mL of *E. angustifolia*; lane 5 = LDL incubated with cupric ion and 20 mg/mL of *E. angustifolia*; lane 6 = LDL incubated with cupric ion and 5 mg/mL of *E. pallida*; lane 7 = LDL incubated with cupric ion and 10 mg/mL of *E. pallida*; lane 8 = LDL incubated with cupric ion and 20 mg/mL of *E. pallida*; lane 9 = LDL incubated with cupric ion and 5 mg/mL of *E. purpurpea*; lane 10 = LDL incubated with cupric ion and 10 mg/mL of *E. purpurpea*; lane 11 = LDL incubated with cupric ion and 20 mg/mL of *E. purpurpea*.

It is interesting to note that the chloroform extract from *Echinacea* roots had no ABTS radical scavenging activity, whereas the methanol fraction contained antioxidant activity. Chloroform has been used as an extraction solvent for recovery of alkamides in various *Echinacea* species (Bauer and Remiger, 1989) due to the fact that alkamides are highly lipophilic compounds. The polarity of methanol being higher than that of chloroform suggests that more polar compounds, such as phenolic components, were extracted by methanol. Thus, alkamides present in *Echinacea*, attributed in part to the noted bioactivity involving the immune system (Bauer and Wagner, 1991), are not likely to be the principle components contributing to free radical scavenging activity.

The comparative protective effects of root extracts derived from three different species of *Echinacea* on liposomal peroxidation can be explained on the basis of compositional difference. The similar reducing power of *E. angustifolia* and *E. purpurea*, both of which were lower than that of *E. pallida*, did not reflect the relative polyphenol content since all three methanolic extracts contained only trace amounts of chlorogenic acid and caffeic acid. In fact, *E. pallida*, which exhibited the highest reducing power and greatest antioxidant, contained less chlorogenic acid and caffeic acid than *E. angustifolia*. The fact that the cichoric acid content also varied between species, with the highest concentration obtained in *E. purpurea*, also signifies that this constituent of *Echinacea* was not likely responsible for the relatively greater antioxidant activity observed for *E. pallida* in the peroxyl radical induced liposomal peroxidation reaction. The plant constituent that best represented the high reducing power and antioxidant activity of the three *Echinacea* species was the total echinacoside content, present in the highest concentration as *E. pallida* in root. The extended conjugation and greater number of hydroxyl groups on the echinacoside ring structure could be one of the reasons for the higher reducing power and greater potential to donate hydrogen atoms to lipid peroxyl radicals. The net result of this action is the generation of low reactivity oxidized

species, shown in eq 1

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet} \quad (1)$$

where, $LOO^{\bullet}$ represents lipid peroxyl radical, AH represents antioxidant, LOOH represents lipid peroxide, and $A^{\bullet}$ represent intermediate radical of antioxidant.

It is important to emphasize that trace amounts of transition metal ions were eliminated by ion-exchange chromatography prior to the exposure to AAPH in this particular assay. Thus, this procedure removed the possibility that decomposition of LOOH was in part attributed to a prooxidant effect derived from transition metal ion present in the reaction medium. It can therefore be concluded that the antioxidant activity of *Echinacea* root components present in the methanol extract was attributed to direct scavenging of aqueous free radical rather than an indirect effect caused by sequestering metal ions required for catalysis of lipid oxidation. This conclusion is also supported by the extended lag time when in the presence of *Echinacea* root extract. This is a notable characteristic of antioxidant activity occurring in the initiation phase of lipid autoxidation reactions. Scavenging the aqueous peroxyl radical in the initiation phase of the liposome autoxidation reaction by constituents in the *Echinacea* root extract also reduced the oxidation rate in the propagation phase as evidenced by the typically lower rate of hydroperoxide formation.

There is substantial evidence for the oxidative modification of low-density lipoprotein in the pathogenesis of atherosclerosis (Steinberg et al., 1989). Although the physiological significance of in vitro Cu^{2+} induced LDL oxidation remains controversial (Kontush and Beisiegel, 1999), this method has been a useful model for evaluating naturally occurring antioxidant compounds (Yan et al., 1995; Frankel et al., 1998; Heinonen et al., 1998). The binding of redox-active Cu^{2+} to LDL at both low- and high-affinity binding sites is critical for the initiation of LDL oxidation (Ziouzenkova et al., 1998) and subsequent modification of amino acid apo-B protein residue (Stadtman, 1994; Retsky et al., 1998). In this study, the protective affinity for chelating free Cu^{2+} and thus reducing metal-catalyzed oxidation of LDL was shown. The sensitivity of this mechanism of antioxidant activity for the different varieties of *Echinacea* was not significantly different, despite the noted relatively higher Cu^{2+} sequestering activity of *E. pallida*.

Of further importance in the interpretation of our findings with the LDL oxidation model was the fact that although we added *Echinacea* before the initiation of Cu^{2+} induced LDL oxidation, there was no evidence that suggested potential prooxidant activity. Abuja et al. (1998), on the other hand, have reported that the addition of elderberry extract 10 min after Cu^{2+} initiated LDL oxidation actually resulted in prooxidant activity. This result could be attributed to the fact that the phenolic antioxidants possibly reduce Cu^{2+} to Cu^{+} bound to LDL, which alone could account for their prooxidant potential (Abuja et al., 1998). Similar observations have been made with some flavonoids, such as catechins from tea, which accelerate DNA damage in the presence of transition metal ion (Scott et al., 1993).

In conclusion, *Echinacea* root constituent(s), most notably the echinacoside, exhibited in vitro antioxidant activity depending on the method used to evaluate peroxidation reactions. Notwithstanding this, however, we show that *Echinacea* root contains antioxidant components which have distinct affinities for both scavenging free radicals as well as sequestering transition metal ions. Finally, the sequestering properties of *Echinacea* components would appear to have a larger impact in reducing lipid peroxidation than the reducing power, as evidenced by the absence of a prooxidant activity in the presence of transition metal. The methanolic extract derived from *E. pallida* root exhibited the greatest relative antioxidant activity of the three varieties studied.

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