Echinacea purpurea

Scientific Name

***Echinacea purpurea* (L.) Moench**

Synonyms

Brauneria purpurea (L.) Britton, *Echinacea intermedia* Lindl. ex Paxton, *Echinacea purpurea* var. *arkansana* Steyerm., *Echinacea purpurea* f. *liggettii* Steyerm., *Echinacea purpurea* var. *serotina* (Nutt.) L.H. Bailey, *Echinacea serotina* (Sweet) D. Don ex G. Don f., *Echinacea speciosa* (Wender.) Paxton, *Helichroa purpurea* (L.) Raf. *Rudbeckia purpurea* L.

Family

Asteraceae

Common/English Names

Black Sampson, Comb Flower, Coneflower, Eastern Purple Coneflower, Echinacea, Indian Head, Missouri Snakeroot, Purple Coneflower, Red Sunflower.

Vernacular Names

Brazil: Equinácea, Equinocea, Flor De Cone (Portuguese)
Chinese: Zi Hua Song Guo Ju, Zi Zhui Ju
Czech: Třapatka Nachová
Danish: Have-Purpursolhat, Purpur Solhat, Solhat
Dutch: Kogelbloemsoort, Purperen Rudbeckia, Rode Zonnehoed
Eastonian: Purpur-Siilkübar
Finnish: Auringonhattu, Kaunopunahattu, Punahattu
French: Echinacée, Echinacée Pourpre, Rudbeckie Pourpre
German: Echinacea, Echinacin, Echter Sonnenhut, Kegelblume, Purpurroter Sonnenhut, roter Scheinsonnenhut, Roter Sonnenhut, Rudbeckie, Sonnenhut
Hungarian: Bíbor Kasvirág, Bíbor Kúpvirág, Lángvörös Kasvirág, Piros Kasvirág
Icelandic: Echinacea, Sólhattur
Italian: Echinacea Purpurea
Norwegian: Echinacea, Purpursolhatt, Rød Solhatt
Polish: Jeżówka Purpurowa, Jeżogłówka Purpurowa, Rudbekia Purpurowa
Portuguese: Equinácea Purpúrea
Russian: Echinacija purpurovaja
Slovaščina: Ameriški Slamnik, Purpurni Ameriški Slamnik, Škrlatni Ameriški Slamnik

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Spanish: Echinacea
Swedish: Purpurrudbeckia, Röd Rudbeckia, Röd Solhatt
Turkish: Ekinazya, Güneş Çiçeği, Kipriciceği, Kipriotu

Origin/Distribution

Eastern Purple Coneflower is native to eastern North America. It is present to some extent in the wild in much of the eastern, southeastern and midwest United States.

Agroecology

In its native temperate range, it occurs in open woodlands, thickets, prairies, near waterways and roadsides. It is extensively grown as ornamentals in gardens and parks and is also cultivated commercially as an herbal remedy.

E. purpurea thrives best in full sun and is shade intolerant. It is not fastidious of soil pH but plant prefers loamy or sandy, well-drained soils. It is also quite drought tolerant.

Edible Plant Parts and Uses

The petals are edible. Some dishes include echinacea Pane bagno (bathed bread) decorated with fresh echinacea petals and a wedge of lemon, American Indian savoury echinacea spread, echinacea and melon fruit salad (Roberts 2000). Petals are fried with watercress, onion and mustard leaves and spread over sweet potatoes.

Botany

A herbaceous perennial 5–120 cm high with erect, branched brownish-green glabrous or hairy stems (Plate 1) and fibrous roots. Leaves alternate, simple and lower leaves broader ovate (Plate 2) and petiolate with weakly winged petioles, lengths decreasing in upper leaves, lamina ovate lanceolate to narrolyl lanceolate, 5–30 by 5–12 cm, margins serrate, apex acute, base rounded to acute. Flowering heads solitary on stout terminal peduncle, 8–25 cm (Plates 1, 3 and 4). Involucral bracts linear to lanceolate. Receptacle with 9–15 mm palea, red orange tipped, slightly curve and pointed. Discs conic to subglobose 1.4–45.5 by 2–4 cm, disc florets corolla greenish to pink or purple, 1.5–4.5×2–4 cm. Ray florets corolla pink to purple pink to purple, laminae spreading to recurved,

Plate 1 Echinacea in flowers and lanceolate upper leaves

Plate 2 Broadly ovate juvenile leaves

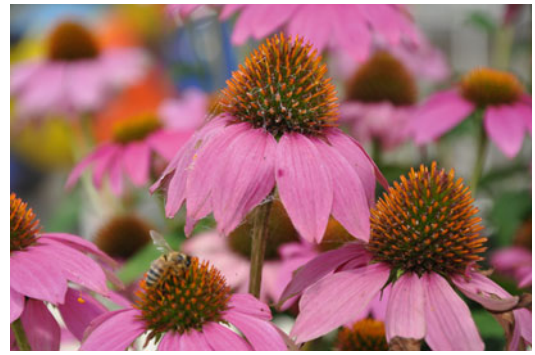

Plate 3 Close view of flowers with conic discs

Plate 4 Flowers with subglobose and flattish round discs

3–8 cm × 0.8–2 cm, two-toothed at apex, sparsely hairy abaxially (Plates 1, 3 and 4). Cypselae, 3–4 angled, glabrous, tan or bicoloured with a dark brown distal band, pappi persistent, 1.2 mm with 0–4 prominent teeth.

Nutritive/Medicinal Properties

Plant/Cell Culture Phytochemicals

Traces of pyrrolizidine alkaloids (0.006 %) tussilagine and isotussilagine were found in dried materials of *Echinacea angustifolia* and *E. purpurea* (Roder et al. 1984).

A 35-kDa water-soluble, acidic polysaccharide, 4-*O*-methyl-glucuronoarabinoxylan, was isolated from hemicellulosic fraction of *E. purpurea* and characterized by Wagner et al. (1984, 1985) and Proksch and Wagner (1987). The polysaccharide contained a (1 → 4)-linked β-D-xylan backbone with branching points at C-2 and C-3. Three homogeneous polysaccharides, two neutral fucogalactoxyloglucans with mean molecular weight of 10,000 and 25,000 and an acidic arabinogalactan with a mean molecular weight of 75,000 were isolated from the medium of *Echinacea purpurea* cell cultures (Wagner et al. 1988). An arabinogalactan protein (AGP) with molecular weight 1.2×10^6 Da, from pressed juice of *Echinacea purpurea*, was isolated from a high molecular weight fraction by precipitation with the β-glucosyl Yariv reagent (Classen et al. 2000). It comprised a high amount of polysaccharide (83 %) with a ratio of galactose to arabinose of 1.8:1, some uronic acids (4–5 %) and a low-protein content (7 %) with high levels of serine, alanine and hydroxyproline. The amino acid profile in the arabinogalactan protein comprised serine 16 %, alanine 12.5 %, hydroxyproline 11.5 %, asparagine/aspartic acid 10.6 %, threonine 10.5 %, glutamine/glutamic acid 9.4 %, arginine 7.5 %, glycine 4.6 %, valine 4.1 %, histidine 3.7 %, lysine 3.3 %, leucine 2.5 %, isoleucine 2.2 % and phenylalanine 1,6 %. The monosaccharide profile in the arabinogalactan protein comprised galactose 59.1, arabinose 33.2, glucosamine 4.0 %, mannose 2.6 % and rhamnose 1.1 %. The molecular weight arabinogalactan protein (AGP) from the pressed juice of *Echinacea purpurea* was found to have hydroxyproline (42.9 % w/w) as the dominant amino acid and the major amino acid responsible for the binding between the protein and the arabinogalactan subunits via an *O*-glycosidic linkage (Volk et al. 2007). Large amounts of glutamine/glutamic acid (24.5 % w/w) and asparagine/aspartic acid (17.3 % w/w) were also found. Another arabinogalactan protein was purified from *E. purpurea* suspension culture (Classen 2007). It comprised high amount of polysaccharide (90 % w/w) with the dominating monosaccharides galactose and arabinose and some glucuronic acid and a small protein moiety (10 % w/w) with the main amino acids alanine, hydroxyproline, serine, glutamine/glutamic acid, asparagines/aspartic acid and threonine. The polysaccharide part was composed of a branched core-polysaccharide of 3-, 6- and 3,6-linked Galp residues with terminal Araf, Arap, Galp and GlcAp residues. Compared to an arabinogalactan protein from pressed juice of the aerial parts of *Echinacea purpurea*, differences particularly in terminal arabinose mono- and oligosaccharides in arabinogalactan (AG) side branches could be detected.

Two groups of minerals (I, Fe, Cu, Mn, Li and, II, Ca, Mg, Zn, Ni) were identified in the aerial parts and roots of *E. purpurea* in Serbia (Razić et al. 2003). The trace element profiles in the roots, stem, leaves and flowers were found to differ significantly.

The aerial parts of *Echinacea purpurea* afforded, in addition to known compounds, five highly unsaturated acetylenic amides and a derivative of linolen (Bohlmann and Hoffmann 1983). The aerial parts of *E. purpurea* were found to contain different structural types of alkamides; the roots yielded similar alkamide pattern (Bauer and Reminger 1989). Alkamide levels differed significantly among roots, rhizomes, stems, leaves and flowers of *E. purpurea* (Perry et al. 1997). Roots were distinguished from other plant parts by higher levels of the C12 diene-diyne alkamides, whereas levels of the C12 tetraene alkamides and C11 diene-diynes were highest in vegetative stems. The ratio of the 2 stereoisomeric C12 tetraene alkamides differed between flowers and all other *E. purpurea* parts. The alkylamides and cichoric acid in dried roots and aerial parts of *E. purpurea* grown in eastern Australia were determined as follows: total alkylamide concentration in root samples was 6.2 mg/g (range 1.2–12.1 mg/g) and in aerial samples was 1.0 mg/g (range 0.2–3.9 mg/g) (Wills and Stuart 1999). The cichoric acid concentration in root samples was 13.2 mg/g (range 1.4–20.5 mg/g) and in aerial samples was 12.9 mg/g (range 4.9–21.4 mg/g). Stuart and Wills (2000) found that total alkamide concentration in *E. purpurea* root, stem and leaf decreased throughout the first growing season while the concentration in flowers increased. In mature plants, the root contained about 70 % of the total plant alkamides with approximately 20 % in flower, 10 % in stem and 1 % in leaf tissue. The relative proportion of individual alkamides in the root did not change during plant growth, but cichoric acid concentration in plant tissues did decrease during plant senescence. Similar concentrations of cichoric acid were measured in root, flower and leaf tissues, but stem levels were lower. In mature plants, the flower and leaf each contained about 35 % of the total plant cichoric acid, while the root and stem contained approximately 20 and 10 %, respectively. Cichoric acid was the main phenolic in *E. purpurea* roots (mean 2.27 % summer, 1.68 % autumn) and tops (2.02 % summer, 0.52 % autumn) followed by caftaric acid was the other main phenolic compound in the roots (0.40 % summer, 0.35 % autumn) and tops (0.82 % summer, 0.18 % autumn) (Perry et al. 2001). Autumn-grown *Echinacea purpurea* plants in Taiwan produced more caffeoyl phenols, particularly cichoric acid and caftaric acid, in leaf and flower tissues than spring-grown plants (Chen et al. 2008). Iranian cultivated *E. purpurea* tops was found to have a high content of cichoric acid (3.5–5.7 %), followed by caftaric acid (3.1–4.5 %) and caffeic acid (0.6–1.1 %) with total polyphenol content of 7.9–10.9 %, (Iranshahi and Amanzadeh 2008). After 2 hours of boiling water extraction, the content of cichoric acid was 5.7 %, whereas the content of this acid in 60:40 ethanol–water extraction did not exceed 3.9 %.

For total alkamides, concentrations (mg/g dry weight basis) among individual *E. purpurea* plants varied from 5.02 to 27.67 (mean = 14.4 %) in roots, from 0.62 to 3.42 (mean = 1.54) in nearly matured seed heads, and from 0.22 to 5.25 (mean = 0.77) in young tops (about ½ flower heads, ¼ leaves, and ¼ stems) (Qu et al. 2005). For cichoric acid, concentrations among individual plants varied from 2.65 to 37.52 (mean = 8.95), from 2.03 to 31.58 (mean = 10.9) and from 4.79 to 38.55 (mean = 18.88) in the roots, the seed heads and the tops, respectively. Dodeca-2*E*, 4*E*, 8*Z*, 10*E*-tetraenoic acid isobutylamide and dodeca-2*E*, 4*E*, 8*Z*, 10*Z*-tetraenoic acid isobutylamide (alkamides 8/9) accounted for only 9.5 % of the total alkamides in roots but comprised 87.9 % in the seed heads and 76.6 % in the young tops. Two cinnamic acids, 2-*O*-caffeoyl-3-*O*-isoferuloyltartaric (3) and 2, 3-di-*O*-isoferuloyltartaric acid (5), along with three known caffeic acids, cichoric acid (1), 2-*O*-caffeoyl-3-*O*-feruloyltartaric acid (2) and 2-*O*-caffeoyl-3-*O*-*p*-coumaroyltartaric acid (4), were isolated and purified from *Echinacea purpurea* (Lu et al. 2012). From 250 mg of crude extracts, 65.1 mg of 1, 8.3 mg of 2, 4.0 mg of 3, 4.5 mg of 4 and 4.3 mg of 5 were isolated, with purities of 98.5, 97.7, 94.6, 94.3 and 98.6 %, respectively.

Nineteen phenolics were identified in the medium of *E. purpurea* cell cultures after elicitation with biotic elicitors (Li and Barz 2005, 2006) . The medium contained lignan, neolignan and acetophenone derivatives as the main elicitor-enhanced products. *E. purpurea* cells mainly contained phenolic glycosides including a new compound α-*O*-β-D-glucopyranosyl-acetovanillone.

Constitutive compounds identified in the cell culture medium of *E. purpurea* included α-hydroxyacetovanillone (1), coniferyl alcohol (4), methyl (*E*)-*p*-coumarate (7), and 1-hydroxypinoresinol (8). After elicitation of *E. purpurea* cell culture with yeast, the compounds found in the medium included α-hydroxyacetosyringone (2); 4-hydroxyacetophenone (3); 4,7,9,8′-tetrahydroxy-3,3′-5′-trimethoxy-8,4′-oxy-9′-norneolignan-7′-one (5); 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-4-[1-(*E*)-propen-3-ol]-phenoxy]-propane-1,3-diol (6a,b); 4,7,9-trihydroxy-3,3′,5′-trimethoxy-8,4′-oxy-9′-norneolignan-7′-one (9); and buddlenol B (10). Phenolics found in *E. purpurea* cells were 4-hydroxyacetophenone (3); scopoletin (11); 4-hydroxybenzoic acid glucose ester (12); 4-*O*-β-glucopyranosylacetophenone (13); α-*O*-β-D-glucopyranosyl-acetovanillone (14); 4-*O*-β-glucopyranosylconferyl alcohol (15); scopoline (16); 1-(4-*O*-B-glucopyranosyl-3-methoxyphenyl)-2-(2-methoxy-4[1-(*E*)-propen-3-ol]-phenoxy]-propane-1,3-diol (17a,b); 4(*O*-β-D-glucopyranosyl)-7,9-dihydroxy-3,3′,5′-trimethoxy-8,4′-oxy-9′-norneolignan-7′-one (18); 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-4-[1-(*E*)-propen-3-ol]-phenoxy]-propane-1,3-diol (6a,b); and longiloroside (19). The optima accumulation of *Echinacea purpurea* suspension culture biomass (73.6 g/l FW and 10.03 g/l DW), phenolics (61.14 mg/g DW) and flavonoids (38.30 mg/g DW) was achieved in 0.5 MS (Murashige and Skoog) medium (Wu et al. 2007b). High adventitious root biomasses (83.1 g/l FW and 15.30 g/l DW) were achieved with feeding of the 0.5 MS medium at the end of 2nd week. This led to slight decreases in the total production of phenolics and flavonoids; however, this feeding was responsible for increases in the accumulation of caftaric acid (5.76 mg/g DW) and cichoric acid (26.12 mg/g DW).

Beside the above, many other studies had isolated and identified alkamides and caffeic acid derivatives in *E. pur*purea plant, various plant parts and products (Bergeron et al. 2000; Laasonen et al. 2002; Mølgaard et al. 2003). Ultrasonic extraction of dried samples of *E. purpurea* roots and aerial parts with methanol–water (7:3) or ethanol–water (7:3) gave good yields of cichoric acid, echinacoside and the alkamides, undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide and a mixture of dodeca-2*E*,4*E*,8*Z*,10*E/Z-tetraenoic* acid isobutylamides (recoveries of 89, 85, 80 and 90 %, respectively) (Bergeron et al. 2000). The HPLC separation of the phenolic compounds cichoric acid, chlorogenic acid and echinacoside was also improved by careful attention to the pH of the mobile phase. The profile of phenolic compounds (mean concentration μg/ml) in *E. purpurea* herb was determined as 0–767 μg caftaric acid, 0–45 μg chlorogenic acid, 0–220 μg caffeic acid, 0 μg cynarin, 0–2,879 μg cichoric acid, 0 μg echinacoside and 0–25 μg tet 8/9 (dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic acid isobutylamide) (Vimalanathan et al. 2005).

Studies showed that the content of caffeic acid derivatives in *E. purpurea* aerial parts and roots reached its highest in the middle stage of full blossoming (Liu et al. 2007). The content of caffeic acid derivatives in fresh raw material was generally higher than that in dried raw material. The developmental pattern of total phenolics in *E. purpurea* was the same as that of caffeic acid derivatives. The stage of mid-bloom was the optimal harvesting period for both caffeic acid derivatives and total phenolics. There was no significant difference in the content of caffeic acid derivatives among three Chinese geographical populations of *E. purpurea*.

E. purpurea extracts stored for 18 months were found to contain caftaric acid, cichoric acid and undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide at concentrations of 0.7, 0.71 and 2.0 mg/ml, respectively (Cech et al. 2006). Using HPLC/electrospray ionization mass spectrometry, isomeric isobutylamides and 2-methylbutylamides could be distinguished. The cichoric acid was the main phenolic compound detected in dried *E. purpurea* materials (flowers, leaves, stems and roots), followed by caftaric acid (Lin et al. 2011). The bioactive constituent contents in different plant parts were in the descending order: flowers > leaves > stems > roots. Both caffeic acid derivatives and total phenolics contents were affected by drying method and storage/packing condition. Cool wind-dried materials retained more bioactive constituents content (>85 %) compared to vacuum freeze-dried materials.

The storability results indicated that the freeze-dried *E. purpurea* materials sealed in polyethylene terephthalate/aluminium foil/polyethylene or nylon/polyethylene bags and stored under 10–20 °C and 40–60 % relative humidity without light conditions retained the highest content of bioactive compounds.

Flower Phytochemicals

Volatiles in the headspace of *E. purpurea* flowers were acetaldehyde, dimethyl sulphide, 2-methylpropanal and acetone, 2-butanone, 2-methylbutanal, 3-methylbutanal, unknown, α-pinene, camphene, hexanal, β-pinene, sabinene/β-thujene, unknown, 2-methyl-4-pentenal, β-myrcene, α-terpinene, limonene, 2-hexenal (*trans*), ocimene, γ-terpinene, *trans-ocimene*, *p*-cymene, α-terpinolene, unknown, 1-hexanol, *allo*-ocimene, 3-hexen-1-ol (*cis*), 2-hexen-1-ol (*trans*) and α-cubebene/α-copaene (Mazza and Cottrell 1999). Major volatiles in headspace of flower tissues were myrcene 43 %, β-pinene 7.4 %, α-pinene 22.6 % and dimethyl sulphide 1.0 %.

The essential oil of *E. purpurea* flower heads was found to contain 89 components of which 63 (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes) were identified comprising 92.8 % of the total oil yield (Diraz et al. 2012). The major components were germacrene D (11.3 %), caryophyllene oxide (8.7 %), β-caryophyllene (7.2 %) and α-cadinol (6.2 %). The other components higher than 1 % included β-pinene (1.3 %), α-phellandrene (2.9 %), *p*-cymene (2.65), β-elemene (2.1 %), a-cadinene (1.0 %), naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-ethylethyl)-,(1*S-cis*)- (3.3 %), α-farnesene (1.0 %), 1,5 epoxysalvia-4(14)ene (3.3 %), naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-1(1-methylethyl) – (1.6 %), α-bisabolene (2.3 %), bicylo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene- (1.8 %), α-cadinene (2.0 %), decatone (1.5 %), ethyl oleate (1.6 %0, vulgarol B (1.9), aromadendrene oxide (1.1 %), 3,4,-difloro-4-methoxybiphenyl (2.7 %), isoaromadendiene epoxide (1.9 %), *trans* (*Z*)-α-bisabolene epoxide (2.3 %), benzenepropanoic acid, octadecyl ester (1.2 %) and diepi-α-cedrene epoxide (1.2 %). Earlier, 36 compounds comprising 70.9 % of sesquiterpenes and 6.4 % monoterpene hydrocarbons were identified in the *E. purpurea* flower-head essential oil in Iran (Mirjalili et al. 2006). Germacrene D (57 %) was the major component.

Rutin and nicotiflorin (3-*O*-rutinoside campherol) were isolated from the flowers, the latter flavonoid being dominant (Kurkin et al. 2011). The major anthocyanins of *Echinacea purpurea* and *E. pallida* were identified as cyanidin 3-*O*-(β-D-glucopyranoside) and cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside) (Cheminat et al. 1989). Cyanidin 3-*O*-(β-D-glucopyranoside) (9.8 mg) and cyanidin 3-*O*-(6″-*O*-β-D-glucopyranoside) (14.3 mg) were obtained from 160 mg crude flower extract with a purity of 95.1 and 98.2 %, respectively (Li et al. 2012).

The profile of phenolic compounds (mean concentration μg/ml) in *E. purpurea* flower was determined as 19–1,212 μg caftaric acid, 0–208 μg chlorogenic acid, 11–179 μg caffeic acid, 0–13 cynarin, 9–734 μg cichoric acid, 0 μg echinacoside, and 0–39 μg tet 8/9 (dodeca-2E,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide) (Vimalanathan et al. 2005). In *Echinacea purpurea* cultivars Magnus and White Swan and line CLS-P2, harvested dry flower heads contained highest cichoric acid content and followed by caftaric acid and chlorogenic acid (Chen et al. 2009). The contents of cynarin and echinacoside in flower heads were relatively low as compared to cichoric acid, caftaric acid or chlorogenic acid. Total caffeoyl derivatives content were 125.3, 116.7 and 145.5 mg/g dry weight for line CLS-P2, Magnus and White Swan, respectively. The alkamides dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (alkamide 8) and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (alkamide 9) were also found in the flower heads, but their content was much lower than in the roots. No statistically significant differences in flower-head alkamides 8 and 9 content were found among the tested *E. purpurea* cultivars and lines.

The extraction yields of phenolics of freeze-dried *E. purpurea* flowers were significantly

affected by the ethanol concentrations at 25 °C with water extraction giving the highest extraction yield (39.8 %) (Tsai et al. 2011). When the ethanol volume percentage in the solvent was increased from 25 % up to 95 %, the extraction yields were decreased from 36.9 to 3.3 %. Water extraction yielded 1,656 mg/g extract of caftaric acid, 0.14 mg chlorogenic acid, 0.53 mg echinoside, 37.23 mg cichoric acid, total caffeic acid derivatives 54.45 mg and total phenolics 97.07 mg. For caftaric acid, ethanol extraction (50 %) yielded the highest 67.07 mg down to 16.35 mg (ethanol 95 %); for chlorogenic acid, ethanol (75 %) gave 2.84 mg down to 0.53 mg at ethanol 95 %; for echinacoside, ethanol (75 %) gave 1.07 mg down to 0.54 mg at ethanol 95 %; for cichoric acid, ethanol extraction (50 %) yielded the highest 180.29 mg down to 42.54 mg (ethanol 95 %). For total caffeic acid derivatives, ethanol extraction (50 %) yielded the highest 250.79 mg down to 59.96 mg (ethanol 95 %). For total phenolics, ethanol extraction (50 %) yielded the highest 441.33 mg down to 104.47 mg (ethanol 95 %). The total phenols, individual and total caffeic acid derivatives contents of extracts were enhanced by elevated extraction temperatures ranged from 25 to 65 °C.

The content of cichoric acid and caftaric acids in dried *E. purpurea* flowers were found to be significantly affected by the drying methods used (Kim et al. 2000b). Although significant loss of cichoric acid was observed when flowers were stored at high moisture, vacuum microwave-dried flowers with a low-moisture content retained the highest levels of cichoric acid and caftaric acid similar to freeze-dried flowers. Flowers that were air-dried at 25 °C retained about 50 %, while those air-dried D at 70 °C had lowest retention of these acids. Although flowers air-dried at 40 °C retained relatively high amounts of cichoric acid and caftaric acid, the time (55 hours) required to reach optimal drying was considerably longer than that (47 minutes) for vacuum microwave drying. They also found that individual alkamide concentrations in roots and leaves were affected by the drying methods used (Kim et al. 2000a). To preserve higher levels of total alkamides, freeze-drying was found to be the best method, vacuum microwave drying was a superior method for drying roots than air-drying at 70 °C, while air-drying at 50 °C was the preferred method for drying leaves of *E. purpurea*.

Fruit (Achene)/Seed Phytochemicals

Two major alkamide peaks were identified in *E. purpurea* achenes as undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide and dodeca-2*E*,4*E*,8*E*,10*E/Z* tetraenoic acid isobutylamide (8/9) (He et al. 1998). The isomer pair, tetraene 8/9, was purified as a standard for quantification of alkamide content in *E. purpurea* achenes and roots.

Seed oil yields of *E. angustifolia*, *E. pallida* and *E. purpurea*, harvested in 1998 and 1999, ranged from 13 to 23 % (Oomah et al. 2006). Vitamin E content of the oils ranged from 29 to 85 mg/100 g oil, with α-tocopherol constituting 83 % of the total tocopherol. The oil was highly polyunsaturated and abundant in linoleic, oleic and palmitic acids, together comprising 95 % of the total fatty acids. *E. purpurea* seed oils contained 66.5 % linoleic, 21.4 %, oleic and 8 % palmitic acids in the 1998 year harvest and 75.6 % linoleic, 12.2 % oleic and 7 % palmitic acids in the 1999 harvest. Fruit (achenes) of *E. purpurea* was found to contain 33.6 % light yellow fatty oil comprising 78.4 % unsaturated fatty acids and 21.6 % saturated fatty acids; the major components were linoleic acid (58.2 %) and oleic (20.2 %) acid, and the equivalent iodine number was 121 (Vandyshev et al. 2009). Eighteen components were identified in *E. purpurea* seed oil, comprising 90.4 % of total area with petroleum ether solvent and 96 % with solvent *n*-hexane solvent (Diraz et al. 2012). The most abundant fatty acids were palmitic acid, stearic acid, oleic acid, and linoleic acid. The fatty acid profile using petroleum ether and *n*-hexane solvents were, respectively, as follows: oleic acid C18:1 (48 %, 29 %), palmitic acid C16:0 (16.6 %, 9.2 %), linoleic acid C18:2 (13.3 %; 51 %) , oxocol C13 (0.1 %, 0 %), myristic acid C14:0 (0.3 %, 0.2 %), pentadecanoic acid C15:0 (0.1 %, 0.1 %), palmitoleic acid C16:1

(1 %, 0.4 %), carbonic acid C17:0 (0.2 %, 0.1 %), heptadecanoic acid C17:1 (0.2 %, 0 %), stearic acid C18:0 (5 %, 2.5 %) elaidic acid C18:1 (0.7 %, 0 %), linolelaidic acid C18:2 (0.8 %, 0 %), arachidic acid C20:0 (0.5 %, 0.6 %), gamma-linolenic acid C18:3 (0 %, 0.5 %), eicosenoic acid C20:1 (0.3 %, 0.5 %), behenic acid C22:0 (1.3 %, 0.7 %), tricosanoic acid C23:0 (0.1 %, 0.1 %), lignoceric acid C24:0 (0.6 %, 0.3 %) and oxiraneoctanoic acid C26 (0.8 %, 0.8 %). Oxocol, heptadecenoic acid, elaidic acid and linolelaidic acid could not be defined with solvent *n*-hexane.

Leaf Phytochemicals

Volatiles in the headspace of *E. purpurea* leaf tissues were acetaldehyde, dimethyl sulphide, propanal, 2-methylbutanal, 3-methylbutanal, 2-ethylfuran, pentanl, α-pinene, α-thujene, camphene, hexanal, β-pinene, sabinene/ β-thujene, pentanal, 2-methyl-4-pentenal, β-myrcene, α-terpinene, limonene, 2-hexanal (*cis*), 2-hexenal (*trans*), ocimene, γ-terpinene, *trans*-ocimene, *p*-cymene, hexyl acetate, α-terpinolene, 3-hexen-1-ol-acetate, 1-hexanol, *allo*-ocimene 3-hexen-1-ol (*cis*), α-ylangene, γ-cadinene, *trans*-caryophyllene, calarene/α-copaene, germacrene D/α-cubebene, 5-ethyl-2(5*H*)-furanone, δ-cadinene, βα-cubebine/γ-cadinene and 2,2,3,3-tetramethyl hexane (Mazza and Cottrell 1999). Major volatiles in headspace of leaf tissues were myrcene 27 %, β-pinene 1.9 %, α-pinene 12.1 %, dimethyl sulphide <1.0 %

Total phenolic contents reported for *E. purpurea* (leaf) were 15.15 mg GAE/100 g DW (Wojdyło et al. 2007). Major phenolic compounds (mg/100 g DW) found were phenolic acids, 620 mg caffeic acid, 115 mg neochlorogenic acid, 19.5 mg *p*-coumaric acid and 17.9 mg ferulic acid, and flavonoids, 12.4 mg quercetin.

In *Echinacea purpurea* cultivars Magnus and White Swan and line CLS-P2, harvested dry leaves contained highest cichoric acid content and followed by caftaric acid and echinacoside (Chen et al. 2009). The contents of chlorogenic acid and cynarin in leaves were relatively low as compared to cichoric acid, caftaric acid and chlorogenic acid. Among the five caffeoyl derivatives examined, CLS-P2 leaves had greater cichoric acid, caftaric acid, cynarin and echinacoside levels than Magnus and White Swan. Total caffeoyl derivatives content were 61.73, 31.75 and 20.02 mg/g dry weight for line CLS-P2, cultivar Magnus and cultivar White Swan, respectively. Line CLS-P2 had the highest dodeca-2E,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (alkamide 8) and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (alkamide 9) content in dry leaves among the tested cultivars and line. The profile of phenolic compounds (mean concentration μg/ml) in *E. purpurea* leaf and stem was determined as 0–1,174 μg caftaric acid, 0–63 μg chlorogenic acid, 0–421 μg caffeic acid, 0–5 cynarin, 0–6,001 μg cichoric acid, 0 μg echinacoside and 0.19 μg tet 8/9 (dodeca-2E,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide) (Vimalanathan et al. 2005).

Stem Phytochemicals

Volatiles in the headspace of *E. purpurea* stem tissues were acetaldehyde, dimethyl sulphide, 2-methylpropanal and acetone, 2-methylbutanal, 3-methylbutanal, α-pinene, geranyl acetate, camphene, hexanal, β-pinene, sabinene/ β-thujene, unknown, 2-methyl-4-pentenal, β-myrcene, α-terpinene, limonene, 2-hexenal (*trans*), ocimene, γ-terpinene, *trans*-ocimene, α-terpinolene, 3-hexen-1-ol-acetate, 1-hexanol, *allo*-ocimene, 3-hexen-1-ol (*cis*), 2-hexen-1-ol (*trans*), α-cubebene/α-copaene, α-ylangene, α-ylangene, germacrene D/α-cubebene, δ-cadinene and βα-cubebine/γ-cadinene (Mazza and Cottrell 1999). Major volatiles in headspace of stem tissues were myrcene 45 %, β-pinene 4.4 %, α-pinene 33.7 % and dimethyl sulphide <1.0 %.

Root Phytochemicals

Volatiles in the headspace of *E. purpurea* roots were acetaldehyde, dimethyl sulphide, 2-methylpropanal and acetone, 2-propenal,

2-methylbutanal, 3-methylbutanal, ethanol, 1-methylpropyl acetate, trichloroacetic acid, α-pinene, camphene, hexanal, β-pinene, 2-methyl-1-propanol, sabinene/ β-thujene, α-phellandrene, α-terpinene, heptanal, limonene, 2-methyl-1-butanol, 3-methyl-1-butanol, ocimene, *p*-cymene, hexyl acetate, unknown, 6-methyl-5-hepten-2-one, 1-hexanol and 1-octen-3-ol, benzaldehyde (Mazza and Cottrell 1999). Major volatiles in headspace of root tissues were 0%, β-pinene 0.2 %, α-pinene 0.6 %, α-phellandrene 16.7 % and dimethyl sulphide 14.7 %. The seventeen lipophilic, volatile to semivolatile components, including the 11 alkamides known to *E. purpurea* roots, were identified (Hudaib et al. 2002). Cucumber mosaic cucumovirus infection was found to be responsible for significant variations in the relative compositions of the major constituents, in particular germacrene D, dodeca-2*E*, 4*E*, 8*Z*, 10*Z*(*E*)-tetraenoic acid isobutylamide *cis*/*trans* isomers, undeca-2*Z*, 4*E*-diene-8, 10-diynoic acid isobutylamide and dodeca-2*E*, 4*Z*-diene-8, 10-diynoic acid isobutylamide.

Root extracts of *E. purpurea* root were found to contain (μg/ml) chlorogenic acid (0.0157 μg), caftaric acid (0.1568 μg), cafeic acid (trace), cichoric acid (1.0147 μg) alkamides 1 undeca-2*E*-4*Z*-diene-8,10-diynoic acid isobutylamide (0.056 μg), undeca-2*Z*-4*E*-diene-8,10-diynoic acid isobutylamide (0.0159 μg), doceca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide (0.0130 μg), undeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (trace), dodeca-2*E*,4*E*,10*E*-trien-8-ynoic acid isobutylamide (0.0156 μg), dodeca-2*E*,4*E*-diene-8,10-diynoic acid 2-methylbutylamide (0.0092 μg), dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (0.2912 μg), dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (0.0396 μg), dodeca-2*E*,4*E*,8*Z*, trienoic acid isobutylamide (0.0102 μg) and undeca-2*E*-ene-8,10-diynoic acid isobutylamide (0.0106 μg) (Bauer et al. 1988; Bauer and Reminger 1989; Binns et al. 2002b; Perry et al. 2001; Hall 2003; Senchina et al. 2006; Pietta et al. 1998; Gotti et al. 2002; Pomponio et al. 2002; Solco 2007). No cynarin and echinacoside were detected in the roots. Five alkylamides, undeca-2*E*,4*Z*-dien-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*Z*-dien-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*E*,10*E*-trien-8-ynoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide and dodeca-2*E*,4*Z*-dien-8,10-diynoic acid 2-methylbutylamide, were isolated from the roots (Bauer et al. 1988). Nine alkamides were identified in the root of *E. purpurea* (He et al. 1998) similar to the fingerprint reported by Bauer and Remiger (1989). Several minor alkamides were also tentatively identified.

The following alkamides were identified in fresh and dry root extracts of *E. purpurea* (Spelman et al. 2009): undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide (A); undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (B); undeca-2*E*-ene-8,10-diynoic acid isobutylamide (C); undeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (D); undeca-2*Z*,4*E*-diene-8,10-diynoic acid 2-methylbutylamide (*E*, new); dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (F); dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide (G); dodeca-2*E*,4*E*,10*E*-triene-8-ynoic acid isobutylamide (H); dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (I); dodeca-2*E*-ene-8,10-diynoic acid isobutylamide (J, used as standard); dodeca-2*E*,4*E*, 8*E*,10*Z*-tetraenoic acid isobutylamide (K); dodeca-2*E*,4*E*, 8*Z*,10*Z*-tetraenoic acid isobutylamide (L, used as standard); dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide (M); dodeca-2*E*,4*E*-dienoic acid isobutylamide (N); dodeca-2*E*,4*E*-dienoic acid isobutylamide (O); trideca-2*E*,7*Z*-diene-8,10-diynoic acid isobutylamide (P); dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (Q); and dodeca-2,4,8,10-tetraenoic acid 2-methylbutylamide (R). Quantities of the isomeric alkylamides K, L and M and alkylamide J in ethanol extracts of *E. purpurea* root were determined in terms of concentrations of the isomeric tetraenes (compounds K, L and M) and dodeca-2*E*-ene-8,10-diynoic acid isobutylamide (compound J) per ml of solvent. The three different *Echinacea* root extracts, fresh 1:2, dry 1:11 and dry 1:5, all contained these alkylamides. However, the dry 1:5 extract contained the greatest amount of these compounds. All three extraction techniques investigated here (fresh 1:2, dry 1:5 and dry 1:11) resulted in very similar

alkylamide profile and gave similar yields of alkylamides and of total dissolved solids. The similarity in alkylamide content in fresh 1:2 and dry 1:11 extracts indicated that drying of root material at 50 °C did not result in a loss of alkylamides. It was concluded that either fresh or dried roots could be used to prepare extracts with high alkylamide content, although the overall yield was slightly lower for fresh extracts.

Caffeic acid derivatives, caftaric acid, chlorogenic acid, caffeic acid, cichoric acid and alkamides: undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide; undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide; dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide; undeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide; dodeca-2*E*,4*E*,10*E*-triene-8-ynoic acid isobutylamide; trideca-2*E*,7*Z*-diene-10,12-diynoic acid isobutylamide; dodeca-2*Z*, 4*E*-diene-8,10-diynoic acid isobutylamide; dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide; dodeca-2*E*,4*E*, 8*E*,10*Z*-tetraenoic acid isobutylamide, dodeca-2*E*, 4*E*,8*Z*-trienoic acid isobutylamide; and dodeca-2*E*,4*E*-dienoic acid isobutylamide, were detected in the roots and extracts (Luo et al. 2003). Three alkamides and nitidanin diisovalerianate were identified, together with 14 known alkamides and one sesquiterpene from the roots of *Echinacea purpurea* (Hohmann et al. 2011). Cichoric acid and verbascoside predominated in extracts of *E. purpurea* roots (Sloley et al. 2001). A total of 16 alkamides, three ketoalkenes, two ketoalkynes and four phenolic acids (echinacoside, cichoric acid, caftaric acid and chlorogenic acid) were identified in aqueous ethanol (70 %) root extracts of *Echinacea purpurea* and *Echinacea pallida* (Thomsen et al. 2012). The major alkamides in the roots of *E. purpurea* were at their lowest concentration in the middle of autumn and early winter, while all of the major phenolic acids were at their highest concentrations in spring. In *E. purpurea* root extract, the major alkamide, dodeca-2*E*, 4*E*, 8*Z*, 10*E*/*Z*-*tetraenoic* acid isobutyl amide, was not significantly affected by storage at any of the temperatures (−20, 25 and 40 °C), but cichoric acid content declined significantly at both 25 and 40 °C as compared to low-temperature storage (Livesey et al. 1999). In the root powder, the major alkamide showed a significantly reduced level at 25 and 40 °C, while cichoric acid did not decline significantly.

Biomass accumulation and production of caffeic acid derivatives (caftaric acid, chlorogenic acid and cichoric acid) in *Echinacea purpurea* adventitious root cultures was optimal under incubation temperature of 20 °C among the different incubation temperatures tested (10, 15, 20, 25 and 30 °C) (Wu et al. 2007a). Biomass of adventitious roots was highest in cultures grown under dark, while accumulation of caffeic acid derivatives was optimum in the cultures grown under 3/21 hours light and dark cultural regimes. Studies found that 15-day-old hairy root culture stimulated every 5 days by ultrasound for 6 minutes produced the highest amount of caffeic acid derivatives (CADs) after 30 days of culture among all ultrasound treatment experiments (Liu et al. 2012). The obvious increase of CADs production in *E. purpurea* hairy roots stimulated by ultrasound was related to the increase of both rolB-regulated endogenous indole-3-acetic acid biosynthesis and phenylalanine ammonium lyase (PAL) activity.

In *Echinacea purpurea* cultivars Magnus and White Swan and line CLS-P2, harvested dry roots contained highest cichoric acid content and then followed by caftaric acid, cynarin, echinacoside and chlorogenic acid (Chen et al. 2009). The caffeoyl derivatives were 16.53, 19.71 and 27.40 mg/g dry weight for CLS-P2, Magnus and White Swan, respectively. Compared to flowers and leaves, roots contained the highest content of alkamides, dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (alkamide 8) and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (alkamide 9). White Swan accumulated considerably higher alkamides 8 and 9 content in dry roots than Magnum and line CLS-P2.

Highly water-soluble fructans were isolated from *E. purpurea* roots (Wack and Blaschek 2006). The fructans represented linear inulin-type fructans with almost exclusively β-(2 → 1)-linked fructosyl units, terminal glucose

and terminal fructose. Small proportions of β-(2→1,2→6)-linked branch point residues were detected. 80 % ethanol-insoluble fructan from *E. purpurea* showed an average mean degree of polymerization of 35, 60 % ethanol-insoluble fructan of 44, and 40 % ethanol-insoluble fructan of 55.

Commercial *Echinacea* extracts are manufactured primarily from three *Echinacea* species, namely, *Echinacea purpurea* (herb, roots or seeds), *E. angustifolia* (roots) and *E. pallida* (roots) (Mahady et al. 2001). Current recommendations for use of these products include oral administration for the prophylaxis and treatment of the common cold, bronchitis, influenza and bacterial and viral infections of the respiratory tract. However, based on existing data products containing pressed juice or hydroalcoholic extracts, *Echinacea purpurea* (leaf juice and roots) and *E. pallida* (roots) had the most convincing data supporting their use. According to Spelman et al. (2009), *Echinacea purpurea*, a top-selling botanical medicine, is currently of considerable interest due to immunomodulatory, antiinflammatory, antiviral and cannabinoid receptor 2 (CB_2)-binding activities of its alkylamide constituents. It is an immunostimulating drug, containing multiple bioactive substances such as polysaccharides, caffeic acid derivatives (caffeic acid, cichoric acid, caftaric acid, chlorogenic acid), alkamides and glycoproteins (Manček and Kreft 2005). Among the many pharmacological properties reported for *E. purpurea* extracts, immunomodulation of macrophages had been demonstrated most convincingly (Barrett 2003). Several dozen clinical studies—including a number of blind randomized trials—had reported health benefits. The most robust data were from studies testing *E. purpurea* extracts in the treatment for acute upper respiratory infection. Although indicative of modest benefit, these studies were limited both in size and in methodological quality. Although a great deal of moderately good-quality scientific data regarding *E. purpurea*, effectiveness in treating illness or in enhancing human health exist, much has not yet been proven beyond a reasonable doubt. Numerous studies had shown that *E. purpurea* exhibited immunostimulating, antimicrobial, antiinflammatory, antioxidant, cytochrome enzyme inhibitory, antiandrogenic, cannabinoidomimetic, radioprotective and antitumorous activities (Gupta et al. 2012).

Antioxidant Activity

The mechanisms of antioxidant activity of extracts derived from *E. angustifolia*, *E. pallida* and *E. purpurea* roots included free radical scavenging and transition metal chelating (Hu and Kitts 2000). Root extracts of these *Echinacea* spp. were capable of scavenging hydroxyl, DPPH and ABTS radicals. These root extracts delayed the formation of conjugated diene hydroperoxide induced by the thermal decomposition of 2, 2′-azobis(2-amidinopropane) dihydrochloride and protracted the lag phase of peroxidation of soybean liposomes. These root extracts also suppressed the oxidation of human low-density lipoprotein, as evaluated by reduced agarose electrophoretic mobility following oxidative modification by Cu^{2+}.

Studies showed that *E. purpurea* extracts had antioxidant activity similar to that of ascorbic acid but had no serious effect on inhibiting chicken's peripheral blood mononuclear cells viability (Lee et al. 2009), In 2,2-diphenyl-1-picril hydrazyl radical (DPPH) scavenging capacity, the ED_{50} for the extract was measured at 0.23 mg/ml. The superoxide anions scavenging capacity of the extract was nearly equivalent to ascorbic acid (91.1 % vs. 93.0 %) at the same concentration of 1.6 mg/ml, and ED_{50} was 0.32 and 0.13 mg/ml, respectively. Reducing power of the extract increased linearly with its concentration, and the concentration at 2.0 mg/ml reached about 65 % of ascorbic acid at 0.3 mg/ml. The chelating capacity of ferrous iron (Fe^{2+}) was 70 % as good as that of the synthetic metal chelater EDTA when added to 5.0 mg/ml of *E. purpurea* extract. The polysaccharides content of the extract was 159.8 mg/g dry weight (DW), and total phenolic compound was 11.0 mg gallic acid equivalent/g

DW. Microculture tetrazolium assays showed extracts had 92 % cell viability at 1.6 mg/ml for chicken's peripheral blood mononuclear cells (PBMCs) and 84 % for RAW 264.7 macrophages, neither reaching the IC_{50} level.

Extracts of the roots and leaves of *E. purpurea* were found to have antioxidant properties in a free-radical scavenging assay and in a lipid peroxidation assay (Sloley et al. 2001). The methanol root extract of *E. purpurea*, *E. pallida* and *E. angustifolia* exhibited DPPH antioxidant activity with EC_{50} values of 134, 167 and 231 μg/ml, respectively (Pellati et al. 2004). The radical scavenging activity of *Echinacea* root extracts reflected their phenolic content. The total phenolic content was 23.23 mg/g for *E. purpurea*, 17.83 mg/g for *E. pallida* and 10.49 mg/g for *E. angustifolia*. Caftaric acid, chlorogenic acid, caffeic acid, cynarin, echinacoside and cichoric acid were identified and quantified in *Echinacea* roots and derivatives. Pure echinacoside had the highest capacity to quench DPPH radicals (EC_{50} = 6.6 μM), while caftaric acid had the lowest (EC_{50} = 20.5 μM).

The antioxidant activity of three extracts, one alkamide fraction, four polysaccharide-containing fractions, and three caffeic acid derivatives from *Echinacea purpurea* root was evaluated by measuring their inhibition of in-vitro Cu(II)-catalyzed oxidation of human low-density lipoprotein (LDL) (Dalby-Brown et al. 2005). Among the extracts the 80 % aqueous ethanol extract exhibited ten times longer lag phase prolongation (LPP) than the 50 % ethanol extract, which in turn exhibited a longer LPP than the water extract. The antioxidant activity of the tested *Echinacea* extracts, fractions and isolated compounds was dose dependent. Synergistic antioxidant effects of *Echinacea* constituents were found when cichoric acid (major caffeic acid derivative in *E. purpurea*) or echinacoside (major caffeic acid derivative *in Echinacea pallida* and *Echinacea angustifolia*) was combined with a natural mixture of alkamides and/or a water extract containing the high molecular weight compounds.

The extracts of the stems, leaves and roots of *Echinacea purpure*a and their constituent cichoric acid were found to be efficient scavengers of DPPH radicals with an activity comparable to that of rosmarinic acid, a well-characterized antioxidant (Thygesen et al. 2007). The efficacy of the extracts in the reaction with DPPH correlated well with the amount of cichoric acid present in the various extracts. The alkamides alone showed no antioxidant activity in any of the tests. Alkamides present in the extract increased, however, the antioxidative effect of cichoric acid in the peroxidation lipid emulsion assay. Antioxidant activity in terms of TEAC (μM trolox/100 g DW) of *E. purpurea* leaves reported was 12.3 μM for ABTS, 75 μM for DPPH and 94.6 μM for FRAP (ferric reducing antioxidant power) assays (Wojdyło et al. 2007). Total phenolic contents reported were 15.15 mg GAE/100 g DW. The DPPH scavenging reached 93.6 %, and the values of EC_{50} were (34.16) μg/ml and (65.48) μg/ml for the extracts obtained by the classical and ultrasound extractions, respectively (Stanisavljević et al. 2009).

At level of 10 μg/ml, the scavenging abilities of tested samples on DPPH radicals were in the descending order of ascorbic acid > BHA (butylated hydroxyanisole) > flower extract > α-tocopherol (Tsai et al. 2011). At level of 30 μg/ml, the flower extract and α-tocopherol showed 90.82 and 93.10 % scavenging abilities, respectively. The radical scavenging ability of *E. purpurea* flower extract could be attributable to the caffeic acid derivatives, especially cichoric acid with two adjacent hydroxyl groups of its phenolic rings showed the highest radical scavenging ability. The order of potency against DPPH radicals was the following: echinacoside > cichoric acid > chlorogenic acid > caffeic acid > caftaric acid. At the level of 100 μg/ml, the reducing power of samples was in the descending order of ascorbicacid > BHA > flowerextract > α-tocopherol. The ascorbic acid, BHA, and flower extract attained the same maximum reducing power (2.3 AU) at 100, 200 and 400 μg/ml, respectively. The flower extracts exerted an 81.88 % ferrous ions chelating effect at 3 mg/ml concentration when compared with the EDTA (ethylenediaminetetraacetic acid) concentration of 10 μg/ml.

Immunomodulatory Activity

Studies found that the main immunostimulatory activity of *Echinacea* resided in the water-soluble materials rather than the lipoidal small molecules (Pillai et al. 2007). The use of flow cytometry demonstrated a link between the polysaccharides in *Echinacea* and the biologic immunostimulatory effect. *E. purpurea*, *E. pallida* and *E. angustifolia* leaves, stems, flowering tops and roots all produced substantial immunostimulatory activity. In-vitro and in-vivo studies indicated that the therapeutic effects of *Echinacea* were due to a stimulation of cellular immune response (Mahady et al. 2001).

In-Vitro Studies

Purified polysaccharides (EPS) prepared from *Echinacea purpurea* were shown to strongly activate macrophages that developed pronounced extracellular cytotoxicity against tumour targets (Stimpel et al. 1984). The splenic lymphocytes from mice treated with *E. purpurea* and *Hypericum perforatum* at the two dose levels used (30 and 100 mg/kg/day) were shown to be significantly more resistant to apoptosis than those from mice treated only with the vehicle (Di Carlo et al. 2003). Further, mice treated with these natural substances showed a decrease in Fas-Ag expression and an increase in Bcl-2 expression.

The 4-*O*-methyl-glucuronoarabinoxylan, isolated from *E. purpurea* showed immune-stimulating activity in several in-vitro immunological test systems (Wagner et al. 1984, 1985; Proksch and Wagner 1987). The fucogalactoxyloglucan of mean molecular weight 25,000 isolated from *E. purpurea* cell culture enhanced phagocytosis in vitro and in-vivo (Wagner et al. 1988). The arabinogalactan specifically stimulated macrophages to excrete the tumour necrosis factor (TNF). The arabinogalactan protein (AGP) from pressed juice of the aerial parts of *Echinacea purpurea* demonstrated binding to lymphocytes, monocytes and granulocytes of different donors (Thude et al. 2006). A high molecular weight arabinogalactan protein (AGP) from the pressed juice of *Echinacea purpurea*, known to exhibit immunomodulatory properties in vitro, was characterized (Volk et al. 2007). Normal human peripheral blood macrophages cultured in concentrations of *Echinacea purpurea* fresh pressed juice as low as 0.012 μg/ml produced significantly higher levels of IL-1, TNF-α, IL-6 and IL-10 than unstimulated cells (Burger et al. 1997). The high levels of IL-1, TNF-α and IL-10 induced by very low levels of echinacea were consistent with an immune-activated antiviral effect. Echinacea induced lower levels of IL-6 in comparison to the other cytokines measured. Echinacea herb and root powders were found to stimulate murine macrophage cytokine secretion as well as to significantly enhance the viability and/or proliferation of human peripheral blood mononuclear cells in vitro (Rininger et al. 2000). In contrast, echinacea extracts chemically standardized to phenolic acid or echinacoside content and fresh pressed juice preparations were found to be inactive as immunostimulatory agents but did display, to varying degrees, antiinflammatory and antioxidant properties.

Studies showed that *E. purpurea* root and leaf–stem extract exhibited opposite (enhancing vs. inhibitory) modulatory effects on the expression of the CD83 marker in human dendritic cells (DCs) (Wang et al. 2006) Downregulation of mRNA expression of specific chemokines (e.g., CCL3 and CCL8) and their receptors (e.g., CCR1 and CCR9) was observed in stem- and leaf-treated DCs. Other chemokines and regulatory molecules (e.g., CCL4 and CCL2) involved in the c-Jun pathway were found to be upregulated in root-treated DCs. In another study, following 48 hours exposure of dendritic cells from C57Bl/6 mice to *E. purpurea* root and leaf extracts, it was found that the polysaccharide-rich root extract increased the expression of MHC class II, CD86 and CD54 surface biomarkers whereas the alkylamide-rich leaf extract inhibited expression of these molecules (Benson et al. 2010). Production of IL-6 and TNF-α increased in a concentration-dependent manner with exposure to the root, but not leaf, extract. In contrast, the leaf but not root extract inhibited the enzymatic activity of cyclooxygenase-2. The leaf but not

root extract inhibited the antigen-specific activation of naïve CD4+ T cells from OT II/Thy1.1 mice. These results suggested that *E. purpurea* could be immunostimulatory, immunosuppressive and/or antiinflammatory depending on the plant part and extraction method.

E. purpurea extracted in a solvent mixture of 95:5 ethanol/water dose-dependently inhibited interleukin IL-2 production in human Jurkat T cells (Sasagawa et al. 2006). This IL-2 inhibitory activity correlated with the presence of alkylamides but not caffeic acid derivatives. *E. purpurea* extract was both IL-2 suppressive and cytotoxic at 50 and 100 μg/ml. Lower concentrations from 6.25 to 25 μg/ml significantly decreased IL-2 production, but not cell viability. Alkylamides at concentrations found in a 50 μg/ml extract decreased IL-2 production by approximately 50 %, but not cell viability in a dose-dependent manner. *Echinacea* and several of its phytochemical components were found to have opposing effects on NFκB expression by Jurkat cells (a human T-cell line) (Matthias et al. 2008). In the absence of stimulation, *Echinacea* and its components exerted no significant effect on basal NFκB expression levels. In the presence of endotoxin (LPS), NFκB expression was decreased. However, this decrease was significantly reversed by treatment with cichoric acid, an *Echinacea* root extract (prepared from both *Echinacea angustifolia* and *Echinacea purpurea*) and the alkylamide fraction derived from this combination. For the phorbol myristate acetate stimulation of Jurkat cells, effects on NFκB expression were mixed. Depending on the concentration, cichoric acid and a 2,4-diene alkylamide significantly induced NFκB levels, whereas a 2-ene alkylamide caused a significant inhibition. In contrast, both the *Echinacea* and the mixed alkylamide fraction exerted no effect.

Pugh et al. (2012) found that differences in total bacterial load within *Echinacea purpurea* samples were strongly correlated with in-vitro macrophage activity (NF-κB activation in THP-1 cells) and content of bacterial lipopolysaccharides in the extracts. The results added to the growing body of evidence that bacteria within *Echinacea* were the main source of components responsible for enhancing innate immune function.

Animal Studies

Polysaccharides purified from large-scale *Echinacea purpurea* plant cell cultures were found to activate human phagocytes in vitro and in vivo and to induce acute-phase C reactions (Roesler et al. 1991b). These substances enhanced the spontaneous motility of polymorphonuclear leukocytes (PMN) under soft agar and increased the ability of these cells to kill staphylococci. Monocytes were activated to secrete tumour necrosis factor alpha (TNF-α) and interleukins IL-6 and IL-1, whereas class II expression was unaffected. Intravenous application of the polysaccharides to test subjects immediately induced a fall in the number of PMN in the peripheral blood, indicating activation of adherence to endothelial cells. This decline was followed by a leukocytosis due to an increase in the number of PMN and a lesser increase of monocytes. The acute-phase C-reactive protein (CRP) was induced, probably due to activation of monocytes and macrophages to produce IL-6. Subsequent in-vivo studies found that the purified polysaccharides (EP) from *Echinacea purpurea* plant cell cultures enhanced mice phagocytes' activities thus protecting against systemic infections with *Listeria monocytogenes* and *Candida albicans* (Roesler et al. 1991a). They confirmed their hypothesis that macrophages (Mφ) from different organ origin could be activated to produce IL-1, TNFα and IL-6 to produce elevated amounts of reactive oxygen intermediates and to inhibit growth of *Candida albicans* in-vitro and that in-vivo the substances could induce increased proliferation of phagocytes in spleen and bone marrow and migration of granulocytes to the peripheral blood. In a subsequent study, EP was found effective in activating peritoneal macrophages isolated from animals after administration of cyclophosphamide (CP) or cyclosporin A (CsA) (Steinmüller et al. 1993). EP-treated macrophages exhibited increased production of tumour necrosis factor-alpha (TNF) and enhanced cytotoxicity against tumour target WEHI 164 as well as against the intracellular parasite

Leishmania enrietti. After a CP-mediated reduction of leukocytes in the peripheral blood, the polysaccharides induced an earlier influx of neutrophil granulocytes as compared to PBS (phosphate buffer saline)-treated controls. EP treatment of mice, immunosuppressed with CP or CsA, restored their resistance against lethal infections with the predominantly macrophage-dependent pathogen *Listeria monocytogenes* and predominantly granulocyte-dependent *Candida albicans*.

The results of studies suggested that oral gavage with *Echinacea* preparations containing optimal concentrations of cichoric acid, polysaccharides and alkylamides were potentially effective in stimulating an in-vivo, nonspecific immune response in normal male Sprague–Dawley rats (Goel et al. 2002a, b). Among the components the alkylamides at the dose level of 12 μg/kg body weight/day significantly increased the phagocytic activity as well as phagocytic index of the alveolar macrophages. The alveolar macrophages obtained from this alkylamide-administered group also produced significantly more TNF-α and nitric oxide after an in-vitro stimulation with LPS than any other active component or the control. The immunomodulatory effects of alkylamides appeared to be more pronounced in lungs than in spleen. Studies demonstrated *Echinacea purpurea* extracts to be potent activators of natural killer (NK) cells cytotoxicity (Gan et al. 2003). NK cytotoxicity was augmented 100 % at the concentration of 0.1 μg/ml of *Echinacea* in a short time (4-hour) assay. *Echinacea* augmented the frequency of NK target conjugates and activated the programming for lysis of NK cells. In another study, dietary administration of *E. purpurea* (14 days) to aging, normal mice or thyroxin injection revealed that *E. purpurea*, but not thyroxin, had the capacity to increase natural killer (NK) cell numbers, in aging mice, reflecting increased new NK cell production in their bone marrow generation site, leading to an increase in the absolute numbers of NK cells in the spleen, their primary destiny (Currier and Miller 2009). The *E. purpurea*-mediated increase in NK cell numbers was also paralleled by an increase in their antitumour, cytolytic functional capacity. In a recent study levamisole and *Echinacea purpurea* separately and together exerted a stimulant effect on the immune system in rats (Sadigh-Eteghad et al. 2011). The gamma globulin level, white blood cells, neutrophil and monocyte counts and phagocyte activity increased significantly in comparison with normal saline group during the study. In the group that received *Echinacea* and levamisole simultaneously, these effects were synergistically increased.

Male rats were orally treated with two different doses (30 and 100 mg/kg) of extract of *Echinacea purpurea* (EP), *Hypericum perforatum* (HP) and *Eleutherococcus senticosus* (ES) drugs for 3 or 15 days (Di Carlo et al. 2005). A 3-day treatment was not able to modify prolactin serum levels, whereas a 15-day treatment with EP and HP at the higher dose significantly inhibited prolactin production; prolactin had been reported to play an important role in immune system regulation. The treatment with ES was ineffective. They suggested that a possible mechanism for this effect could be that both *Echinacea purpurea* and *Hypericum perforatum* extracts displayed a direct dopaminergic activity, although an involvement of the GABAergic system could not be excluded.

Clinical Studies

Year-and-a-half old, dried *Echinacea* roots were found to retain cytokine-modulating capabilities in an in-vitro human older adult model of influenza vaccination (Senchina et al. 2006). In this model, peripheral blood mononuclear cells were collected from subjects 6 months postvaccination and stimulated in vitro with the two type A influenza viruses contained in the trivalent 2004–2005 vaccine with a 50 % alcohol tincture prepared from the roots of one of seven *Echinacea* species: *E. angustifolia*, *E. pallida*, *E. paradoxa*, *E. purpurea*, *E. sanguinea*, *E. simulata* and *E. tennesseensis*. Four species (*E. angustifolia*, *E. purpurea*, *E. simulata*, *E. tennesseensis*) augmented interleukin IL-10 production, diminished IL-2 production, and had no effect on interferon IFN-gamma production. Parnham (1996) reported squeezed sap of *E. purpurea*, widely used in self-medication, to be well tolerated on long-term oral administration with no adverse

event in healthy adults. In contrast, parenteral administration of the squeezed sap of *E. purpurea* (Echinacin®) may be associated with symptoms of immunostimulation (shivering, fever, muscle weakness). Echinacin® had little or no effect on lymphocyte responses but had been reported to cause transient lymphopenia in some patients with infections of various etiologies. In a double-blind placebo-controlled crossover design study with two treatment periods of 14 days involving 40 healthy male volunteers (age range 20–40 years), oral administration of *Echinacea purpurea* pressed juice for 1 and 2 weeks had only minor effects on two out of 12 lymphocyte subpopulations determined in the study (Schwarz et al. 2005). The small differences observed in the number of CD8+-T lymphocytes and natural killer cells were only of questionable physiological relevance.

Echinacea purpurea was found to have immunomodulatory property. Ritchie et al. (2011) found that a subgroup of volunteers who showed low pretreatment levels of the cytokines MCP-1, IL-8, IL-10 or IFN-γ ($n=8$) showed significant stimulation of these factors upon Echinaforce® treatment (*E. purpurea*) (30–49 % increases), whereas the levels in subjects with higher pretreatment levels remained unaffected. Volunteers who reported high stress levels ($n=7$) and more than 2 colds per year experienced a significant transient increase in interferon IFN-γ upon Echinaforce® treatment (>50 %). Subjects with low cortisol levels ($n=11$) showed significant downregulation of the acute-phase proteins interleukin IL1-β, IL-6, IL-12 and TNF-α by Echinaforce® (range, 13–25 %), while subjects with higher cortisol levels showed no such downregulation. The authors concluded that Echinaforce® thus regulated the production of chemokines and cytokines according to current immune status, such as responsiveness to exogenous stimuli, susceptibility to viral infection and exposure to stress.

Review Studies

In a review of immunomodulatory efficacy of preparations containing extracts of *Echinacea*, 26 controlled clinical trials (18 randomized, 11 double blind) were identified by Melchart et al. (1994). Their study indicated that preparations containing extracts of *Echinacea* could be efficacious immunomodulators. However, present evidence was still insufficient for clear therapeutic recommendations as to which preparation to use and which dose to employ for a specific indication.

Antiviral Activity

The roots of *E. purpurea* were found to induce a substance showing interferon-like activity (Skwarek et al. 1996). Biological activity studies showed that the protective titre (the largest dilution which protected cells by 50 % against virus infection) of the interferon-like materials was 1:6–1:15. *Echinacea purpurea* and *Panax ginseng* extracts at concentrations ≥0.1 or 10 μg/kg, respectively, significantly enhanced natural killer cell function against human erythromyeloblastoid leukaemia cell line, K562 cells in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome (AIDS) patients (See et al. 1997). Similarly, the addition of either herb significantly increased antibody-dependent cellular cytotoxicity (ADCC) against human herpesvirus 6 infected H9 cells of peripheral blood mononuclear cells (PBMC) from all subject groups. Thus, extracts of *Echinacea purpurea* and *Panax ginseng* enhanced cellular immune function of PBMC both from normal individuals and patients with depressed cellular immunity. Extracts of 8 taxa of the genus *Echinacea* were found to have antiviral activity against herpes simplex (HSV) virus type I in vitro when exposed to visible and UVA light (Binns et al. 2002a). *n*-Hexane extracts of roots containing alkenes and amides were more active in general than ethyl acetate extracts containing caffeic acids. *Echinacea purpurea n*-hexane root extract (MIC = 0.12 mg/ml) was one of the most potent inhibitors of HSV.

Aqueous extracts of *E. purpurea* root contained a relatively potent activity against herpes simplex virus (HSV) and influenza virus (FV) but not against rhinovirus (RV) (Hudson et al. 2005).

These fractions had low amounts of caffeic acids and alkamides. The ethyl acetate fraction contained significant but weak activity against both HSV and FV and contained significant levels of cichoric acid. All aqueous fractions of *E. purpurea* aerial parts contained potent activity against herpes simplex virus and influenza virus (Vimalanathan et al. 2005). The antiviral activity could partly be attributed to polysaccharide and cichoric acid components; their individual contributions could not account for the total antiviral activity. Additionally, the ethanol- and ethyl acetate-soluble fractions from leaves and stem contained an uncharacterized but potent antiviral photosensitizer, which was absent from the flower extract. None of the fractions, however, contained anti-rhinovirus.

Studies showed that exposure of a cultured line of human bronchial epithelial cells to rhinovirus 14 infection stimulated the release of at least 31 cytokine-related molecules, including several important chemokines known to attract inflammatory cells. Most of these effects were reversed by simultaneous exposure to either of the two *Echinacea* extracts (Sharma et al. 2006a). Rhinovirus infection of BEAS-2B cell line resulted in a more dramatic increase in multiple transcription factors including proinflammatory factors examined, such as NFkB, AP-1, AP-2 and STATs 1–6 (Sharma et al. 2006b). However, when rhinovirus-infected cells were treated with either of the two *Echinacea* extracts, transcription factors levels were reduced to low levels, although the pattern of the reductions was different for the two extracts. The results could help to explain the beneficial effects of *Echinacea* consumption. All the viruses tested, rhinoviruses 1A and 14, influenza virus, respiratory syncytial virus, adenovirus types 3 and 11, and herpes simplex virus type 1, induced substantial secretion of IL-6 and IL-8 (CXCL8), in addition to several other chemokines, in a line of human bronchial epithelial cells (BEAS-2B) (Sharma et al. 2009). In every case, however, *Echinacea* (Echinaforce, an ethanol extract of herb and roots of *E. purpurea*) inhibited this induction. The *Echinacea* preparation also showed potent virucidal activity against viruses with membranes, indicating the multifunctional potential of the herb. The results supported the concept that certain *Echinacea* preparations could alleviate 'cold and flu' symptoms, and possibly other respiratory disorders, by inhibiting viral growth and the secretion of proinflammatory cytokines.

In a separate in-vitro study, human H1N1-type IV, highly pathogenic avian IV (HPAIV) of the H5- and H7-types, as well as swine origin IV (S-OIV, H1N1) were all inactivated in cell culture assays by the *Echinacea purpurea* (Echinaforce®, EF) preparation at concentrations ranging from the recommended dose for oral consumption to several orders of lower magnitude (Pleschka et al. 2009). Detailed studies with the H5N1 HPAIV strain indicated that direct contact between EF and virus was required, prior to infection, in order to obtain maximum inhibition in virus replication. Hemagglutination assays showed that the extract inhibited the receptor-binding activity of the virus, suggesting that the extract interferes with the viral entry into cells. Recent studies reported that Echinaforce® the standardized extract of *Echinacea purpurea* exhibited immunomodulation and broad antiviral effects against respiratory tract viruses (Schapowal 2012). Haemagglutinin and neuraminidase were blocked. In contrast to Oseltamivir no resistance was caused by Echinaforce®. A randomized, double-blind, placebo-controlled study over 4 months confirmed that Echinaforce® supported the immune resistance and acted directly against a series of viruses. He reported Echinaforce® to be efficacious and safe in respiratory tract infections for long-term and short-term prevention as well as for acute treatment.

An open-label, fixed-sequence study of 15 HIV-infected patients showed that co-administration of *E. purpurea* root extract containing capsules with etravirine (a nonnucleoside reverse transcriptase inhibitor of HIV) was safe and well tolerated in HIV-infected patients (Moltó et al. 2012). The geometric mean ratio for etravirine coadministered with *E. purpurea* relative to etravirine alone was 1.07 for the maximum concentration, 1.04 for the area under the concentration-time curve from 0 to 24 hours

and 1.04 for the concentration at the end of the dosing interval. The data suggested that no dose adjustment for etravirine was necessary.

Rhinoviruses, influenza viruses, herpes viruses and calcivrus were found to be susceptible to *E. purpurea* ethanol extract and aqueous extract; respiratory syncytial virus, coronavirus (mouse) were susceptible only to *E. purpurea* ethanol extract, and polio virus was not susceptible to both extracts (Hudson et al. 2005; Vimalanathan et al. 2005; Pleschka et al. 2009; Hudson 2012).

Echinacea and Respiratory Tract Infection

In-Vitro Studies

A standardized *Echinacea* extract (Echinaforce) was found to have dual action against several important respiratory bacteria, a killing effect and an antiinflammatory effect (Sharma et al. 2010). It readily inactivated *Streptococcus pyogenes*, often associated with sore throat and more severe pulmonary infections, *Haemophilus influenzae* and *Legionella pneumophila*, and reversed their proinflammatory responses. *Staphylococcus aureus* (methicillin-resistant and sensitive strains) and *Mycobacterium smegmatis* were less sensitive to the bactericidal effects of *Echinacea* however, but their proinflammatory responses were still completely reversed. In contrast some other pathogens tested, including *Candida albicans*, were relatively resistant. The results supported the concept of using a standardized *Echinacea* preparation to control symptoms associated with bacterial respiratory infections.

The alkylamides undeca-2*Z*,4*E*-diene-8,10-diynic acid isobutylamide, dodeca-2E,4*E*,8*Z*, 10*E*/*Z*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*-dienoic acid isobutylamide, and undeca-2*E*-ene-8,10-diynoic acid isobutylamide from *E. purpurea* suppressed production of TNF-α and PGE2 from RAW 264.7 macrophage-like cells infected with the H1N1 influenza A strain PR/8/34 (Cech et al. 2010). Dodeca-2*E*,4*E*-dienoic acid isobutylamide was especially effective at inhibiting production of these mediators and also strongly inhibited production of G-CSF, CCL2/MCP-1, CCL3/MIP-1α and CCL5/RANTES. In contrast, the ethanol extracts (75 %), prepared from *E. purpurea* dormant roots, displayed a range of effects from suppression to stimulation of mediator production. Analysis of the extracts revealed slight variations in concentration of alkylamides, caftaric acid and cichoric acid, but the activity of the extracts did not strongly correlate with concentrations of these compounds. The in-vitro studies suggested *E. purpurea* extracts to have the potential for use in alleviating the symptoms and pathology associated with infections with influenza A.

Clinical/Human Studies

Some herbal preparations, including *Echinacea purpurea*, had been reported to improve cold symptoms in adults but ineffective in children (Fashner et al. 2012). Randolph et al. (2003) found that the overall gene expression pattern at 48 hours to 12 days after taking *Echinacea* product for cold and flu by six healthy nonsmoking subjects (18–65 years of age)was consistent with an antiinflammatory response. The expression of interleukin-1 beta, tumour necrosis factor-alpha, intracellular adhesion molecule and interleukin-8 was modestly decreased up through day 5, returning to baseline by day 12. The expression of interferon-alpha steadily rose through day 12, consistent with an antiviral response. The data presented a gene expression response pattern that was consistent with *Echinacea*'s reported ability to reduce both the duration and intensity of cold and flu symptoms.

Mixed results have been obtained in clinical studies on the efficacy of *E. purpurea* in combating respiratory tract infections. In a randomized, double-blind, placebo-controlled study, 246 of 559 recruited healthy, adult volunteers contracted a common cold and took 3 times daily 2 tablets of either Echinaforce® (*Echinacea purpurea* preparation from 95 % herba and 5 % radix), *Echinacea purpurea* concentrate (same preparation at 7 times higher concentration), special *Echinacea purpurea* radix preparation (totally different from that of Echinaforce®) or placebo until they felt healthy again but not longer than 7 days

(Brinkeborn et al. 1999). It was found that Echinaforce® and its concentrated preparation were significantly more effective than the special *Echinacea* extract or placebo. All treatments were well tolerated. Among the *Echinacea* groups the frequency of adverse events was not significantly higher than in the placebo group. They concluded that *Echinacea* concentrate as well as Echinaforce® represented a low-risk and effective alternative to the standard symptomatic medicines in the acute treatment of common cold.

In a randomized, double-blind, placebo-controlled trial, 282 subjects aged 18–65 years, a total of 128 subjects contracted a common cold (59 echinacea, 69 placebo) (Goel et al. 2004). The total daily symptom scores were found to be 23.1 % lower in the echinacea group than in placebo in those who followed all elements of the study protocol. Throughout the treatment period, the response rate to treatments was greater in the echinacea group. A few adverse event profiles were observed in both groups. The researchers concluded that early intervention with a standardized formulation of echinacea resulted in reduced symptom severity in subjects with naturally acquired upper respiratory tract infection. In a clinical study, Echinilin (a formulation prepared from freshly harvested *Echinacea purpurea* plants and standardized on the basis of three known active components: alkamides, cichoric acid and polysaccharides) or placebo was administered to volunteers at the onset of their cold for a period of 7 days, with eight doses (5 ml/dose) on day 1 and three doses on subsequent days (Goel et al. 2005). The decrease in total daily symptomatic score was more evident in the echinacea group than in the placebo group. These effects of echinacea were associated with a significant and sustained increase in the number of circulating total white blood cells, monocytes, neutrophils and natural killer cells. In the later part of the cold, the echinacea treatment suppressed the cold-related increase in superoxide production by the neutrophils. The results suggested that Echinilin, by enhancing the nonspecific immune response and eliciting free radical scavenging properties, may have led to a faster resolution of the cold symptoms.

Jawad et al. (2012) conducted a 4-month randomized, double-blind, placebo-controlled trial to investigate the safety (risk) and efficacy (benefit) of *Echinacea purpurea* (95 % herba and 5 % root) extract in the prevention of common cold episodes with 755 healthy subjects. *Echinacea* extract found to reduce the total number of cold episodes, cumulated episode days within the group, and painkiller-medicated episodes. *Echinacea* inhibited virally confirmed colds and especially prevented enveloped virus infections. *Echinacea* showed maximal effects on recurrent infections, and preventive effects increased with therapy compliance and adherence to the protocol. A total of 293 adverse events occurred with echinacea and 306 with placebo treatment. Nine and 10 % of participants experienced adverse events, which were at least possibly related to the study drug (adverse drug reactions). Thus, the safety of *Echinacea* was noninferior to placebo. The study concluded that compliant prophylactic intake of *E. purpurea* over a 4-month period appeared to provide a positive risk to benefit ratio

In a randomized, placebo-controlled, double-blind study, treatment with fluid extract of *Echinacea purpurea* (widely used for the prevention and treatment of colds and respiratory infections), did not significantly decrease the incidence, duration or severity of colds and respiratory infections compared to placebo (Grimm and Müller 1999). During the 8-week treatment period, 35 (65 %) of 54 patients in the echinacea group and 40 (74 %) of 54 patients in the placebo group had at least one cold or respiratory infection. The average number of colds and respiratory infections per patient was 0.78 in the echinacea group, and 0.93 in the placebo group. There were no significant differences between treatment groups in the number of infections in each category of severity. Side effects were observed in 11 patients (20 %) of the echinacea group and in seven patients (13 %) of the placebo group. In a randomized, double-blind placebo-controlled trial of 175 adults travelling back from Australia to America, Europe or Africa for a period of 1–5 weeks on commercial flights via

economy class, supplementation with standardized *Echinacea* tablets, taken before and during travel, may have preventive effects against the development of respiratory symptoms during travel involving long-haul flights (Tiralongo et al. 2012). In another study, 128 patients were enrolled within 24 hours of cold symptom onset in a randomized, double-blind, placebo-controlled design wherein patients received either 100 mg of *E. purpurea* (freeze-dried pressed juice from the aerial portion of the plant) or a lactose placebo 3 times daily until cold symptoms were relieved or until the end of 14 days, whichever came first (Yale and Liu 2004). No statistically significant difference was observed between treatment groups for either total symptom scores or mean individual symptom scores. The time to resolution of symptoms was not statistically different. The efficacy of *E. purpurea* in reducing common cold was not confirmed.

In a multicenter randomized placebo controlled trial, *Echinacea purpurea* was found ineffective for treating upper respiratory tract infections in children aged 2–11 years (Mainous 2004). There was no significant difference in duration or severity of symptoms with *Echinacea purpurea* compared with placebo in children with upper respiratory tract infection. In another randomized, double-blind, placebo-controlled trial of 407 children aged 2–11 years including 337 upper respiratory tract infections (URIs) treated with echinacea and 370 with placebo, *Echinacea purpurea* was found not effective in treating URI symptoms in these children, and its use was associated with an increased risk of rash (Taylor et al. 2003).

Review Studies

Scoop et al. (2006) conducted a meta-analysis on the therapeutic effectiveness of *Echinacea* in the treatment and the prevention of colds, wherein 3 suitable studies were selected for pooling of data, and 231 were excluded from the analysis because they related to studies of spontaneous common colds. The meta-analysis suggested that standardized extracts of *Echinacea* were effective in the prevention of symptoms of the common cold after clinical inoculation, compared with placebo. However, more prospective, appropriately structured clinical studies were required to confirm. Although *Echinacea* preparations tested in clinical trials differed greatly, there was some evidence that preparations based on the aerial parts of *Echinacea purpurea* might be effective for the early treatment of colds in adults but results were not fully consistent (Linde et al. 2006). Beneficial effects of other *Echinacea* preparations and for preventative purposes might exist but had not been shown in independently replicated, rigorous, randomized trials. A meta-analysis of 14 studies provided published evidence supporting *Echinacea*'s benefit in decreasing the incidence and duration of the common cold (Shah et al. 2007). Woelkart et al. (2008) stated that *Echinacea* preparations were more effective than no treatment, more effective than placebo or similarly effective to other treatments in the prevention and the treatment of the common cold. In a more recent review on the efficacy of *Echinacea* in cold prevention, Hart and Dey (2009) reiterated that in view of the residual uncertainty and the gaps between the evidence and the ways that this was summarized on webpages, it may prove difficult for consumers to assimilate the evidence. As well as undertaking high-quality trials in complementary medicine, there was a need to ensure precision in the reporting of uncertainty.

Antimicrobial Activity

Echinacea (including *E. purpurea*) were found to have phototoxic antimicrobial activity against fungi, including clinically relevant pathogenic fungi. Results showed that hexane extracts of *Echinacea* variably inhibited growth of yeast strains of *Saccharomyces cerevisiae*, *Candida shehata*, *C. kefyr*, *C. albicans*, *C. steatulytica* and *C. tropicalis* under near UV irradiation (phototoxicity) and to a lower extent without irradiation (conventional antifungal activity) (Binns et al. 2000). The presence of polyacetylenes and alkylamides in extracts of different organs was confirmed in *Echinacea purpurea* and was related to phototoxic activity. Significant phototoxicity was demonstrated by pure

trideca-1-ene-3,5,7,9,10-pentayne from *E. purpurea* roots, while only minor phototoxicity was induced by the other two *E. purpurea* acetylenic compounds undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide. Phototoxic activity of *Echinacea* spp. was primarily attributed to the ketoalkenes and ketoalkynes abundantly present in the roots. Root extracts of the eight *Echinacea* species including *E. purpurea* showed antifungal activity against most of the pathogenic fungi (*Trichophyton tonsurans*, *T. mentagrophytes*, *Microsporum gypseum*, *Pseudallescheria boydii*, *Cryptococcus neoformans* and two *Candida albicans* isolates) (Merali et al. 2003). Recent studies on *Echinacea purpurea* had revealed that certain standardized preparations contain potent and selective antiviral and antimicrobial activities (Hudson 2012). In addition, they displayed multiple immunomodulatory activities, comprising stimulation of certain immune functions such as phagocytic activity of macrophages and suppression of the proinflammatory responses of epithelial cells to viruses and bacteria, which are manifested as alterations in secretion of various cytokines and chemokines. *E. purpurea* dried aerial part ethanol extract showed a considerable growth inhibition on *Candida albicans* and *Saccharomyces cerevisiae*, while no growth inhibition zones were observed for *Aspergillus niger* (Stanisavljević et al. 2009). In-vitro studies showed a standardized preparation of *Echinacea purpurea* (Echinaforce®) could provide a safe twofold benefit to acne individuals by inhibiting proliferation of the Gram-positive bacterium *Propionibacterium acnes* and reversing the bacterial-induced inflammation (Sharma et al. 2011). *E. purpurea* completely reversed the bacterial increase in secretion of substantial amounts of several proinflammatory cytokines, including IL-6 and IL-8 (CXCL8), and brought the cytokine levels back to normal.

Antiinflammatory Activity

Root extracts of the three commercial species of *Echinacea* (*E. purpurea*, *E. pallida* var. *angustifolia*, *E. pallida* var. *pallida*) inhibited the 5-lipoxygenase (5-LOX) enzyme (Merali et al. 2003). The results show that *Echinacea* spp. had significant antiinflammatory activity. Alcohol extracts of *Echinacea angustifolia*, *Echinacea pallida* and *Echinacea purpurea* significantly inhibited NO production by lipopolysaccharide (LPS)-activated the RAW 264.7 macrophage cell line (Zhai et al. 2009). Arginase activity of RAW 264.7 cells stimulated with 8-bromo-cAMP was significantly increased by alcohol extracts of all three *Echinacea* species. The polar fraction containing caffeic acid derivatives enhanced arginase activity, while the lipophilic fraction containing alkamides exhibited a potential of inhibiting NO production and iNOS expression. The results suggested that the antiinflammatory activity of *Echinacea* might be due to multiple active metabolites, working together to switch macrophage activation from classical activation towards alternative activation.

At 100 g/ml, several *E. purpurea* alkamides, isolated from *E. purpurea* roots, inhibited cyclooxygenase COX-I and COX-II enzymes in the range of 36–60 % and 15–46 %, respectively, as compared to controls (Clifford et al. 2002). Chicca et al. (2009) demonstrated that ethanol *E. purpurea* radix and herba extracts elicited synergistic pharmacological effects on the endocannabinoid system in vitro. Supra-additive action of *N*-alkylamide combinations was observed at the level of intracellular calcium release as a function of cannabinoid receptor type-2 (CB_2) activation. Likewise, synergism of the radix and herba tinctures was observed in LPS-stimulated cytokine expression from human PBMCs. While the expression of the antiinflammatory cytokine IL-10 was significantly superstimulated, the expression of the proinflammatory TNF-α protein was inhibited more strongly upon combination of the extracts. They concluded that *N*-alkylamides in the extracts acted in concert to exert pleiotropic effects modulating the endocannabinoid system by simultaneously targeting the CB_2 receptor, endocannabinoid transport and degradation. Cannabinoid type-1 (CB_1) and CB_2 receptors belong to the family of G protein-coupled receptors and are the primary targets of

the endogenous cannabinoids *N*-arachidonoyl ethanolamine and 2-arachidonoyl glycerol (Gertsch et al. 2006). CB_2 receptors are believed to play an important role in distinct pathophysiological processes, including metabolic dysregulation, inflammation, pain and bone loss.

A mitogen-induced murine skin inflammation study suggested that alkamides were the active antiinflammatory components present in *Echinacea* plants (Hou et al. 2010). Mixed alkamides and the major component, dodeca-2*E*,4*E*,8*Z*,10*Z*(*E*)-tetraenoic acid isobutylamides (8/9), were then isolated from *E. purpurea* root extracts for further bioactivity elucidation. In macrophages, the alkamides significantly inhibited cyclooxygenase 2 (COX-2) activity and the lipopolysaccharide-induced expression of COX-2, inducible nitric oxide synthase and specific cytokines or chemokines [i.e., TNF-α, interleukin (IL)-1α, IL-6, MCP-1, MIP-1β] but elevated heme oxygenase-1 protein expression. Cichoric acid, however, exhibited little or no effect. In another study, three alkamides and nitidanin diisovalerianate were identified, together with 14 known alkamides and one sesquiterpene from the roots of *Echinacea purpurea* (Hohmann et al. 2011). Their interaction with G-protein-coupled cannabinoid receptors was examined on rat brain membrane preparations. Both partial and inverse agonist compounds for cannabinoid (CB1) receptors were identified among the metabolites, characterized by weak to moderate interactions with the G-protein signalling mechanisms. Upon co-administration with arachidonyl-2′-chloroethylamide, a number of them proved capable of inhibiting the stimulation of the pure agonist, thereby demonstrating cannabinoid receptor antagonist properties. In an earlier study, alkylamides, anandamide and SR144528 were found to potently inhibit lipopolysaccharide-induced inflammation in human whole blood and exerted modulatory effects on cytokine expression, but these effects were not exclusively related to CB_2 binding (Raduner et al. 2006). The alkylamides dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (A1) and dodeca-2*E*,4*E*-dienoic acid isobutylamide (A2) bound to the CB_2 receptor more strongly than the endogenous cannabinoids. A1, A2, anandamide, the CB2 antagonist SR144528 ($Ki < 10$ nM), and also the non-CB_2-binding alkylamide undeca-2*E*-ene,8,10-diynoic acid isobutylamide all significantly inhibited lipopolysaccharide-induced tumour necrosis factor alpha, IL-1beta and IL-12p70 expression (5–500 nM) in a CB2-independent manner.

Anticancer Activity

Animal studies found that dietary administration with *E. purpurea* preparations significantly decreased prostate weight of rats and increased lymphocyte numbers after 8 weeks (Skaudickas et al. 2003). *Echinacea purpurea* root hexane extract reduced cell viability of human pancreatic cancer MIA PaCa-2 and colon cancer COLO320 cell lines in a concentration- and time-dependent in vitro (Chicca et al. 2007). *E. purpurea* flower extract and cichoric acid significantly inhibited proliferation in a dose- and time-dependent manner human colon cancer cells Caco-2 and HCT-116 (Tsai et al. 2012). Cichoric acid treatment decreased telomerase activity in HCT-116 cells. Further, cichoric acid effectively induced apoptosis in colon cancer cells, which were characterized by DNA fragmentation, activation of caspase-9, cleavage of PARP and downregulation of β-catenin.

Anti-teratogenic Activity

Studies found that *E. purpurea* could stimulate immune system of mice more than levamisole and had better prophylactic effect against the teratogenic effect of phenytoin as evidenced by lower incidence of phenytoin-induced cleft palate, but it was not significant (Mahabady et al. 2006). Cleft palate incidence was 16, 5.3 and 3.2 % in fetuses of mice that received only phenytoin, phenytoin with levamisole, and phenytoin with *Echinacea* extract, respectively. Mean weight and length of fetuses of animals that received levamisole and *Echinacea* extract were significantly greater than those that received only phenytoin.

Hepatoprotective Activity

Studies showed that dodeca-2*E*,4*E*,8*Z*,10*Z*(*E*)-tetraenoic acid isobutylamides (Alk-8/9), isolated from *Echinacea purpurea* roots, dose-dependently induced heme oxygenase (HO)-1 protein expression in lipopolysaccharide-stimulated murine macrophages that was likely regulated by the JNK-mediated pathway through increasing SAPK/JNK phosphorylation, c-Jun protein expression, and phosphorylation and transcription factor AP-1 binding consensus DNA activity (Hou et al. 2011). Further, Alk-8/9 markedly induced c-Jun and HO-1 protein expression and suppressed serum aminotransferase activities, TNF-α expression, and hepatocyte damage in liver tissues of lipopolysaccharide/D-galactosamine-treated mice. The results suggested a potential application of *Echinacea*, a top-selling herbal supplement, as a hepatoprotective agent.

Antimutagenic Activity

The 50 % ethanol flower extract did not show toxicity and mutagenicity towards *Salmonella typhimurium* TA98 and TA100 with or without S9 mix (Tsai et al. 2011). The ethanol extract at 0.25–5 mg/plate exhibited a dose-dependent inhibitory effect against the mutagenicity of 2-aminoanthracene. They concluded that freeze-dried *E. purpurea* flower ethanol extract exhibited good antioxidant and antimutagenic activities.

Radioprotective Activity

E. purpurea administration significantly ameliorated the detrimental reduction effects of gamma rays on peripheral blood haemoglobin and the levels of red blood cells, differential white blood cells and bone marrow cells and antioxidant activity in mice (Abouelella et al. 2007). The radioprotection effectiveness was similar to the radio recovery curativeness in comparison to the control group in most of the tested parameters. The radioprotection efficiency was greater than the radio recovery in haemoglobin level during the first 2 weeks, in lymphoid cell count and thiobarbituric acid-reactive substances (TBARs) level at the fourth week and in superoxide dismutase (SOD) activity during the first 2 weeks, as compared to the levels of these parameters in the control group.

Actoprotective/Adaptogenic Activity

Purple coneflower tincture was found to improve both the work capacity and the endurance characteristics of white male mice in the conventional forced swim test (Kurkin et al. 2006). The actoprotective effect was attributed to a phenylpropanoid, echinacoside.

In a double-blind design placebo-controlled and self-administered study of 24 men (24.9 ± 4.2 years) for 4 weeks, oral *Echinacea* supplementation resulted in significant increases in erythropoietin (EPO), VO2max (maximal oxygen uptake) and running economy compared to placebo (Whitehead et al. 2012).

Anti-Tyrosinase Activity

Cichoric acid extracted from *E. purpurea* flowers was found to have significant tyrosinase inhibition activity in a broad range of concentration (10–20 mg/ml) (Jiang et al. 2012).

Trypanocidal Activity

Various *Echinacea* extracts could inhibit the proliferation of three species of trypanosomatids: *Leishmania donovani*, *Leishmania major* and *Trypanosoma brucei* (Canlas et al. 2010). The standardized ethanol extract of *E. purpurea* (L.) Moench reversed the proinflammatory activity (production of cytokines IL-6 and IL-8) of *Leishmania donovani* in human bronchial epithelial cells and in human skin fibroblasts.

Larvicidal Activity

Several alkamides isolated from *E. purpurea* roots exhibited mosquitocidal activity causing 100 % mortality of *Aedes aegypti* larvae (Clifford et al. 2002).

Herb–Drug/Herb–Herb Interaction Activity

Studies showed *Echinacea* alkylamides were degraded in a time- and NADPH-dependent manner in microsomal fractions suggesting they were metabolized by cytochrome P450 (P450) enzymes in human liver (Matthias et al. 2005b). There was a difference in the susceptibility of 2-ene and 2,4-diene pure synthetic alkylamides to microsomal degradation with (2*E*)-*N*-*isobutylundeca*-2-ene-8,10-diynamide metabolized to only a tenth the extent of (2*E*,4*E*,8*Z*,10*Z*)-*N*-isobutyldodeca-2,4,8,10-tetraenamide under identical incubation conditions. Alkylamide metabolites were detected and found to be the predicted epoxidation, hydroxylation and dealkylation products. These findings suggested that *Echinacea* may affect the P450-mediated metabolism of other concurrently ingested pharmaceuticals.

Cytochrome P450 enzymes (P450s) appeared to be the principal system responsible for the metabolism of *Echinacea* components and most of the main hepatic and some extrahepatic isoforms appeared to be involved (Toselli et al. 2009). Epoxide formation, N-dealkylation and hydroxylation were reported as the main metabolic pathways mediated by P450s and interactions with P450s determined the circulating concentrations and duration of action of these phytochemicals as well as any potential interactions with other chemicals. In-vivo studies in rats showed that *E. purpurea* may interact cytochrome P450 enzymes and induce significant herb–drug interactions which may alter pharmacotherapy (Mrozikiewicz, et al. 2010). The *Echinacea* ethanol extract could potently inhibit the expression of CYP3A1 (41 %) and CYP3A2 (25 %) mRNAs. A weaker inhibitory effect was observed for CYP2D2 by 15 % and CYP2C6 by 18 % after long application of the *Echinacea* ethanol extract. CYP2D2 and CYP2C6 activities were also inhibited by extract but in a lesser degree than CYP3A1 activity. The findings suggested that *Echinacea* extract may influence the P450-mediated metabolism of different drugs and may initiate chemical carcinogenesis by activation of some compounds to their carcinogenic metabolites.

The multiherbal product Sambucus Force containing *Echinacea purpurea* and *Sambucus nigra* as its main constituents was found to inhibit CYP3A4 activity with IC_{50} value of 1,192 (1,091–1,302) μg/ml (Schrøder-Aasen et al. 2012). The inhibitory potency appeared exclusively to be exerted by *E. purpurea*, implicating an insignificant inhibition by *S. nigra*. *Echinacea purpurea* acted differently in the multiherbal product, which showed a dual inhibition profile with both an uncompetitive (substrate-dependent) inhibition and a time-dependent (substrate-independent) inhibitory mechanism. These mechanistic differences were suggested to be caused by herb–herb interactions in the multiherbal product.

Allergy Problems

A woman with atopy experienced anaphylaxis after taking a commercial extract of *Echinacea*; hypersensitivity was confirmed by skin prick and RAST testing (Mullins 1998). Regular ingestion of *Echinacea* by up to 5 % of surveyed patients with atopy, combined with detection of *Echinacea*-binding IgE in atopic subjects (19 % by skin testing; 20 % with moderate to strong reactivity by RAST testing), indicated the possibility of severe allergic reactions, even with first-time use, due to cross-reactivity with other structurally similar allergens.

Toxicity and Safety Studies

According to the review of Huntley et al. (2005), despite the voluminous data availability on the efficacy of *Echinacea* (*Echinacea* spp. namely

E. angustifolia, *E. pallida* and *E. purpurea*), safety issues and the monitoring of adverse events had not been focused on. Short-term use of *Echinacea* was reported to be associated with a relatively good safety profile, with a slight risk of transient, reversible, adverse events. The major adverse events reported with *Echinacea* products are allergic reactions, ranging from contact dermatitis to anaphylaxis (Mahady et al. 2001). Patients with an allergy to plants in the daisy family (Asteraceae) should be instructed not to use products containing *Echinacea.* The use of *Echinacea* products during pregnancy and lactation would appear to be ill-advised in light of the paucity of data in this area. According to the review by Perri et al. (2006) there was good scientific evidence from a prospective cohort study that oral consumption of *Echinacea* during the first trimester did not increase the risk for major malformations. Low-level evidence based on expert opinion showed oral consumption of *Echinacea* in recommended doses to be safe for use during pregnancy and lactation. *Echinacea* was non-teratogenic when used during pregnancy. However more quality studies were needed to determine its safety.

After 4 weeks of oral administration of expressed juice of *E. purpurea* in doses amounting to many times, the human therapeutic dose laboratory tests and necropsy findings presented no evidence of any toxic effects in rats (Mengs et al. 1991). Tests for mutagenicity carried out in microorganisms and mammalian cells in-vitro and in mice all gave negative results. In an in-vitro carcinogenicity study, *E. purpurea* did not produce malignant transformation in hamster embryo cells.

Pharmacokinetic Studies

Studies showed that alkylamides from *Echinacea* species can readily be transported across Caco-2 monolayers indicating that they can be transported across the intestinal barrier and may contribute to the in-vivo effects of *Echinacea preparations* (Jager et al. 2002; Matthias et al. 2004) but not caffeic acid conjugates (Matthias et al. 2004).

Studies of nine healthy volunteers found that alkamides were rapidly absorbed and were measurable in plasma 20 minutes after *Echinacea* (*E. purpurea* and *E. angustifolia*) tablet ingestion after standard high-fat breakfast and remained detectable for up to 12 hours (Matthias et al. 2005a). The maximal concentrations for the sum of alkamides in human plasma were reached within 2.3 hours post ingestion and averaged 336 ng eq/ml plasma. No obvious differences were observed in the pharmacokinetics of individual or total alkamides. Caffeic acid conjugates could not be identified in any plasma sample at any time after tablet ingestion.

Alkamides dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-*tetraenoic* acid isobutylamides were found to be rapidly absorbed and measurable in plasma 10 minutes after administration of 0.21 and 0.9 mg *Echinacea purpurea* phytotherapeutic lozenges and remained detectable for 3 hours for the 0.21 mg lozenges and >3 hours for the 0.9 mg lozenges; 0.07 mg lozenges were measurable 20 minutes after administration and remained detectable for only 2 hours after the administration (Guiotto et al. 2008). A significant dose-independent downregulation of the proinflammatory cytokines IL-12p70, IL-8, IL-6, IL-10 and TNF was observed after 24 hours. The results demonstrated that pharmacokinetics of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z* -tetraenoic isobutylamides were linear and that absorption was very rapid ($t_{1/2}$=6 minutes) with apparently no lag time, thus indicating the possibility that a fraction of the drug was absorbed through the oral mucosa. In an earlier study, after oral ingestion, the arithmetic mean *C*(max) of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z* -tetraenoic acid isobutylamides absorbed for *Echinacea purpurea* tincture was 0.40 ng/ml serum with 30 minutes *t*(max), while for the *Echinacea* tablet the *t*(max) of tablets was 45 minutes with a *C*(max) of 0.12 ng/ml (Woelkart et al. 2006). Both *E. purpurea* preparations led to the same effects on the immune system according to the concentration of proinflammatory cytokines TNF-alpha and IL-8. Twenty-three hours after oral application a significant downregulation of TNF-alpha and IL-8 in LPS pre-stimulated whole blood was found. However, no significant changes in the concentration of IL-6 were observed.

The alkylamide, undeca-2-ene-8,10-diynoic acid isobutylamide, a constituent of *E. purpurea* (Goel et al. 2011) and dodeca-2*E*,4*E*,8*Z*,10*E*/*Z* - tetraenoic acid isobutylamides (DTAI), the most abundant alkylamide in *E. purpurea*, were successfully quantified in the patients plasma after ingestion of *E. purpurea* extract by LC–MS/MS assay (Goey et al. 2012).

Traditional Medicinal Uses

Echinacea spp. are native to North America and were traditionally used by the Indian tribes for a variety of ailments, including mouth sores, colds and snakebites (Kindscher 1989). Traditional uses of *E. purpurea*, *E. angustifolia* and *E. pallida* include the following: respiratory infections, colds and flu, bronchitis, strep throat, toothache; urinary tract infections, herpes sores and gonorrhoea; skin disorders, staph infections, cold sores, ulcers, wounds, burns, insect bites, eczema, allergies and others; and rheumatoid arthritis (Hudson 2012).

Other Uses

Dried *Echinacea purpurea* (EP) can be used as a feed additive to improve the meat quality and oxidative status in Arbor Acres broilers (Lee et al. 2013). The addition of 0.5 and 1.0 % EP significantly increased water-holding capacity and decreased storage loss of breast and thigh fillets at 35 days old. Results for Trolox equivalent antioxidant capacity, catalase and superoxide dismutase were significantly higher for the 0.5, 1.0 and 2.0 % EP supplemental groups than control group in serum. Liver and spleen tissues results showed that the antioxidative enzyme activities were higher with EP powder at 35 days of age.

Studies showed *E. purpurea* to have potential as immunostimulatory feed additive against Newcastle disease virus in laying hens and in fattening pigs by intermittent application (Böhmer et al. 2009). The performance of laying hens was not impacted with feed additive application of pressed *E. purpurea* (aerial parts) juice in ethanol or fermented juice. Significant changes were found in the number of lymphocytes, phagocytosis rate and Newcastle Disease Virus (NDV) antibody titre. The number of lymphocytes was highest in the group receiving ethanol juice for five consecutive days. Phagocytosis was reduced in both groups provided with ethanol juice (2 or 5 days). Highest NDV antibody titres were seen in the groups receiving fermented juice for 2 days. Additionally, phagocytosis of granulocytes was determined in fattening pigs (80–100 kg) after 5 days of *Echinacea* application with ethanol or fermented juice. A significant increase was found with both *Echinacea* formulations. The number of lymphocytes was also increased significantly in the groups provided with *Echinacea*.

Comments

Purple coneflower is easily propagated either from seeds or vegetatively by division, root cuttings and basal cuttings. It blooms throughout spring and summer and is pollinated by butterflies and bees.

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