American Herbal Pharmacopoeia®
and Therapeutic Compendium

Echinacea purpurea Aerial Parts

Echinacea purpurea (L.) Moench

Standards of Analysis, Quality Control, and Therapeutics

A H P

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Cover Photograph
Echinacea purpurea in cultivation
© 2006 David Bunting

Nomenclature

Botanical Nomenclature

Echinacea purpurea (L.) Moench

Botanical Family

Asteraceae (alt. Compositae)

Pharmaceutical Nomenclature

Herba Echinaceae purpureae

Definition

Echinacea purpurea Aerial Parts consists of the fresh aerial portions of cultivated *Echinacea purpurea* (L.) Moench harvested when flowering and conforming to the methods of identification provided. It contains not less than 1.0% cichoric acid calculated on a dry weight basis and a qualitative confirmation of the presence of the alkamide dodeca-2*E*,4*E*, 8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide.

Common Names

English:	*Echinacea purpurea* (standardized common name), purple coneflower (McGuffin et al. 2000).
French:	*Echinacea*, échinacée.
German:	Sonnenh[illegible].

History

The roots of various *Echinacea* species, especially *E. angustifolia* but also *E. purpurea* and other species, were used medicinally by native peoples throughout the Midwest and Plains regions and later by American Eclectic practitioners. However, there is no recorded historical use of *Echinacea* aerial parts by either group.

The genus name *Echinacea* is derived from the Greek echinus, meaning sea urchin or hedgehog in reference to the prickly bracts covering the floral receptacle. The specific epithet purpurea refers to the color of the flower and comes from the Latin purpurea, meaning a kind of Mediterranean shellfish yielding a purple dye. The common name purple coneflower comes from the cone-like shape of the floral receptacle. What is known as *E. purpurea* today was originally named *Rudbeckia purpurea* by Linnaeus (1753). In 1794, the botanist Conrad Moench changed the name to *Echinacea purpurea*, although it was not until the American botanist Asa Gray used this corrected name in his 1st edition of his *Manual of Botany* (1848) that the new name became common. Due to the complex nomenclatural history of *E. purpurea* (see Botanical Identification), historical references to *E. purpurea* predating 1900 may in fact be referring to the *E. laevigata* of today, while references to *E. serotina* made in the same era may be indicating the *E. purpurea* of today (see Botanical Identification).

E. purpurea aerial parts came into use in the West due to the efforts of Dr. Gerhard Madaus of the German firm Madaus AG. In the early 1900s, homeopathic doctors introduced *Echinacea* to Europe, advocating its use for weakness, wounds that would not heal, and its antiseptic and antiphlogistic activities (Auster and Shafer 1957; Hobbs 1989). During this time frequent reports on the benefits of *Echinacea* began appearing in German medical journals (Stephens 1909). Due to the growing popularity of *E. angustifolia* as a botanical and homeopathic medicine in Europe and the difficulty of getting an adequate supply of the fresh root, Dr. Madaus traveled to the United States (US) in order to obtain *E. angustifolia* seed for cultivation in Europe. The seed obtained turned out to be that of *E. purpurea* and so, due to a simple error and the scarcity of fresh *E. angustifolia* root, studies began in the 1930s on the medicinal uses of *E. purpurea* to determine if it was an effective medicine (Foster 1991). Madaus AG then developed a product named Echinacin® from the alcohol-stabilized expressed juice of fresh *E. purpurea* leaf, stem, and flowers.

Subsequent to research conducted in Germany, the fresh juice of *E. purpurea* flowering aerial parts was popularized here and abroad. This stimulated the widespread use of the fresh or dried aerial parts in domestic preparations, usually in combination with *Echinacea* root. While there is some evidence suggesting a certain level of efficacy for fresh leaf juice there is no evidence for dried aerial material. Today the various commercial species of *Echinacea* are

Figure 1 *Echinacea purpurea*

Source: Eaton ME (1918). Illustration courtesy of Hunt Institute for Botanical Documentation, Carnegie Mellon University, Pittsburgh, PA.

Table 1 Historical timeline of the medical use of *Echinacea purpurea* aerial parts

~1930	Dr. Gerhard Madaus travels to the US and, under the mistaken impression that he has acquired *E. angustifolia* seed, takes *E. purpurea* seed back to Germany for cultivation.
1938	The German company Madaus begins the manufacture of the oldest product form of *E. purpurea*, Echinacin®, which consists of the alcohol-stabilized fresh pressed juice of the flowering aerial parts.
1939 to present	A surge in research, beginning primarily in Germany, occurs on the chemistry, pharmacology, and therapeutic use of *E. purpurea*, especially the expressed juice of the leaves, stems, and flowers.
1984	Echinacin® is first marketed in the United States.
1992	A positive monograph on *E. purpurea* aerial parts is published by the German Commission E.
1996	The injectable form of Echinacin®, available in Germany since 1938, is voluntarily removed from the market.
2002	A draft monograph on *E. purpurea* aerial parts is published by the *European Pharmacopoeia*.
2005	*E. purpurea* aerial parts are included in the *United States Pharmacopeia*.

among the most popular of herbal supplements and medicines used in the US and Europe. Since 1930, *Echinacea* has been the subject of over 800 scientific studies looking at its phytochemistry, pharmacology, and clinical use. *E. purpurea* aerial parts is included in the Dietary Supplements section of the *United States Pharmacopeia* (USP 29-NF 24 2006) and has been proposed for inclusion in the *European Pharmacopoeia* (Pharmeuropa 2004).

IDENTIFICATION

Botanical Identification

Echinacea purpurea (L.) Moench. Herbaceous perennial from a woody caudex. **Root:** Fibrous from a short rhizome. **Stem:** Erect, branched, 52-88 (–180) cm tall; distal portion hirsute (variously dense) with stalked trichomes. **Leaves:** Basal and cauline, alternate, petiolate, blade dark or bright green with 3 (5) major veins arising from the base, variously hirsute to hispid with short hairs on both surfaces or mostly adaxially; margins coarsely serrate to entire; basal blades ovate or lanceolate-ovate, (5–) 15.5-22 (–30) cm long, (1–) 5-10 (–13) cm wide, petiole (0–) 10-17 (–25) cm long; cauline blades linear-lanceolate or lanceolate-ovate, (4–) 11-17 (–24) cm long, (1.5–) 4.5-7 (–11) cm wide, petiole (1–) 3-6 (–18) cm long; leaves may be sessile on upper stem. **Inflorescence:** Heads radiate, (1.4–) 2-3 (–3.5) cm tall, (2–) 2.5-3.1 (–4) cm wide; peduncle less than half the total stem height; receptacle conical, hemispherical, or flat-topped; phyllaries in 4 series, linear-lanceolate, (8–) 10-15 (–20) mm long, (1–) 2-4 (–8) mm wide, recurved or reflexed along stem, margins entire, pubescent abaxially with stalked trichomes, rarely glabrous. **Paleae (receptacular bracts):** Red body with a golden awn having an orange or red blunt tip; body (9–) 11-13 (–15) mm long; awn 0.5 times as long as bract body, straight; keel usually absent, glabrous. **Ray florets:** Sterile; ligule dark pink or purple, rarely pale pink to white, (25–) 35-50 (–60) mm long, (3–) 10-13 (–19) mm wide, perpendicular to stem or spreading to reflexed, pubescent abaxially or on both surfaces. **Disk florets:** Fertile; corolla green and pink or rarely purple, yellow, or orange, tubular, 4.5-5.5 mm long, straight, with 5 short lobes, pubescent; stamens 5, pollen yellow; style branches purple, rarely white or yellow, recurved, mostly shorter than paleae; style base disk-shaped. **Disk fruit (cypselae):** 4-angled, (3–) 3.5-5 (–6) mm long; pappus a crown of short teeth. Chromosome number: $n = 11$.

Distribution: Open woods, thickets, riverbanks, and shaded prairies. Flowers June to July, rarely extending into August. Native from Georgia, the Carolinas, and Virginia in the east; west to Texas, Oklahoma, and Kansas; north to [illegible], Illinois, Michigan, and Ohio; and south to Louisiana, Mississippi, Alabama, and Florida. Although widely distributed, this species is not common anywhere in its range; natural populations tend to be small and are becoming rare and/or extirpated in some areas. All degrees of hairiness may be found within any 1 population (Binns et al. 2002a; McGregor 1968; Moench 1794 [original citation]).

E. purpurea (L.) Moench has a checkered taxonomic and nomenclatural past. *E. purpurea* is the conserved name for the taxon (Binns et al. 2001b). Linnaeus (1753) originally named the taxon *Rudbeckia purpurea*. In 1794, Moench moved it to the genus *Echinacea*. In 1836, De Candolle divided the taxon into 2 species: *E. purpurea* (L.) Moench and *E. serotina* (Nutt.) DC. In 1903, Boynton and Beadle referred to these 2 taxa respectively as Brauneria laevigata C.L. Boynton & Beadle and B. purpurea (L.) Britton (Small 1903), misapplying the species names and using a genus name that was later invalidated. Although the genus name Brauneria was never accepted, the misapplied species designations have been maintained in error. Since that time, the name *E. purpurea* has been mistakenly applied to what was originally *E. serotina* (Nutt.) DC, while the name *E. laevigata* has been applied to the original *E. purpurea* (L.) Moench (Binns et al. 2001a). Binns et al. (2001b) have proposed conserving the name *E. purpurea* in its current usage in order to avoid unnecessary and confusing name changes for such a prominent botanical.

E. purpurea is most easily differentiated from other *Echinacea* species by its unique woodland habitat and fibrous roots. Its range overlaps with that of *E. laevigata*, *E.*

Figure 2 **The three common commercial species of *Echinacea***

Source: Foster (1991). Illustration by Judith Ann Griffith. Permission for use granted by Judith Ann Griffith.

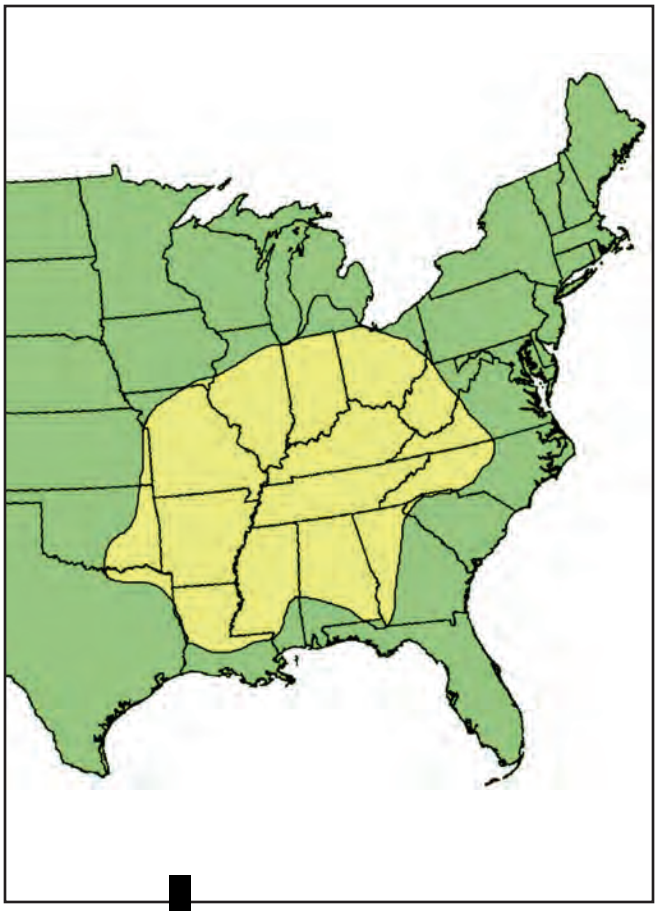


Figure 3 **Geographic range of *Echinacea purpurea***

Source: Kinsch 2005; McGregor 1968.

4a.

4b.

4c.

4d.

4e.

4f.

Figures 4a-f Botanical characteristics of *Echinacea purpurea*

4a. Botanical voucher.
4b. Whole plant.
4c. Flowering heads.
4d. Flower head showing the yellow pollen of the disk florets. The head is young and the receptacle has not yet attained its mature convex structure.
4e. Mature flower head.
4f. Flower head showing the recurv[illegible]ple styles and yellow anthers of the disk florets among the spectacular receptacular bracts (paleae).

Photographs © 2006 courtesy of: (4a-c) Roy Upton, Soquel, CA; (4d) Martin Wall/herbslides.com; (4e) Steven Foster; (4f) Jesse D Grigg.

(4a) Herbarium sheet from the collection of Ronald L McGregor, RL McGregor Herbarium, University of Kansas.

pallida, *E. paradoxa* var. paradoxa, and E. simulata (Binns et al. 2002a; Price 2003, personal communication to AHP, unreferenced), but despite reported hybrids in cultivation, no known hybrid swarms of *E. purpurea* have been reported in nature. Other identifying characteristics include: long, blunt, golden awns on the paleae subtending the disk florets; large, toothed, ovate basal leaves; and a branching habit that supports several inflorescences per plant (Table 2) (Binns et al. 2002a; McGregor 1968; McKeown 1999). The reader should note, however, that many of the identifying morphological characters of *Echinacea* species are variable, especially among wild populations.

The taxonomy of the genus *Echinacea* has been under recent scrutiny. Based upon a morphometric analysis, Binns et al. (2002a) suggested that the classification of the *Echinacea* genus be altered to reduce many of the species recognized by McGregor (1968) to varieties. According to the evidence supporting their classification, Binns et al. (2002a) consider *E. angustifolia* to be a variety of *E. pallida*. Pending broader acceptance of this new taxonomy, the classification and nomenclature of McGregor (1968) have been maintained. A recent genetic analysis by Kim et al. (2004a) supported McGregor's taxonomy at the species level.

Macroscopic Identification

E. purpurea aerial parts are sold fresh or dried. For a description of fresh leaf, see Botanical Identification. Dried material is available unmilled, cut and sifted, or powdered. Plants are typically harvested while in flower, so bulk supplies usually consist of pieces of stem, leaf, flower, and fruit (seed). However, leaf material and fully matured fruit may each be sold separately. As noted, there is no traditional or modern data supporting the use of dried aerial material.

A. Leaf
Simple, petiolate; blade thick, rough to the touch, linear lanceolate to lanceolate-ovate or ovate, ~11-22 cm long, ~4.5-10 cm wide, with margins coarsely serrate to entire; 3 (5)-nerved from base; upper surface dull medium-green; lower surface lighter than upper surface, major veins prominent, reticulate secondary veins darker than the surrounding lamina; surfaces variously pubescent, with many circular cicatrices from broken trichomes; may blacken when creased or damaged; fracture brittle.

Aroma: Indistinct.

Taste: Bitter.

Powder: Medium-green to yellowish-green; slightly gritty.

B. Stem
Medium green or yellowish-green to light brown or purplish-brown in color, occasionally mottled, with longitudinal ridges; in transverse section 2-5 mm diameter, hollow or solid and filled with a white to creamy yellow pith; fracture flexible and fibrous.

C. Inflorescence
Receptacle conical, hemispherical, or flat-topped, solid when sectioned; phyllaries in 4 series, grayish-green, linear-lanceolate, recurved; paleae yellowish-green to brown, the tip darker than the body, coriaceous, ~12-15 mm long; lig-

Table 2 **Botanical comparison of the aerial parts of *Echinacea purpurea* with those of other commercial *Echinacea* species**

Species	Stem	Stem pubescence*	Ray ligules	Paleae	Pollen	Chromosome number	Habitat	Geographic range
E. purpurea	Highly branched, 5.2-12 dm tall	Distally short-hirsute, variously dense	Dark pink to purple, spreading or reflexed, 5-8x longer than wide	Red body, golden awn with orange tip	Yellow	n = 11	Open woodlands, waterways, shaded prairies	VA to GA; w into ne TX and e OK and KS; n to MI
E. angustifolia	Simple to few branched, 1.5-7.5 dm tall	Hirsute along entire stem	Light pink to medium purple, reflexed, 2-5x longer than wide	Orange body with red awn tip	Yellow	n = 11	Rocky prairies, calcareous soils	s central TX; e into IA, MN; w to MT, WY, CO; n to Sask. and Man.
E. angustifolia var. *strigosa*	Highly branched, 2-6 dm tall	Nearly glabrous below, strigose to strigose-hirsute above	As in *E. angustifolia*, except somewhat shorter and more reflexed	As in *E. angustifolia*	Yellow	n = 11, 22	As in *E. angustifolia*	s central KS to central OK and n central TX
E. pallida	Simple to branched from lower nodes, 6-11 dm tall	Hirsute, usually sparse distally, dense above	Pale pink to white, rarely dark pink, drooping, approx. 10x longer than wide	Body and awn dark purple red, green, or brown	White or pale yellow	n = 22	Prairies, well-drained wooded areas, low plains	ne TX, AR; central and w MO; e NE; w IA, WI, IL, IN MI; e OK, KS; adventive e to OH, PA

* Stem pubescence, although emphasized in the literature, is highly variable within and between species and should not, by itself, serve as a diagnostic character.

ules purple to brown, ~35-[illegible] mm long, 10-13 mm wide; disk florets yellow-green, ~8-10 [illegible] long with ovary attached; inflorescence variously fragmented depending upon processing.

D. Fruit (cypsela)

Pale to medium brown on the exterior, surface smooth to longitudinally wrinkled; 4-angled, ~3.5-5 mm long; apex broader than base, concave, with a dark cicatrice in the center, apex edges elongated into a crown of short teeth that often break off during handling; base acute to obtuse, rounded; quadrangular in transverse-section; seed very dark brown to black.

Aroma: Spicy.

Taste: Bland at first, producing a strong numbing and tingling sensation on the tongue and stimulating salivation; slightly sour or acrid after prolonged mastication. The secondary metabolites responsible for the tingling sensation are alkamides.

Powder: Medium brown; gritty, oily.

E. Leaf, stem, flower

Aroma: Hay-like, indistinct.

Taste: Initially slightly bitter, becoming slightly acrid, causing a tingling or numbing sensation on the tongue after prolonged mastication if fruits are present.

Powder: Dull brownish-green to yellowish-green; slightly gritty.

Microscopic Identification

A. Leaf

Surface view: Upper epidermis consists of polygonal cells with sinuous anticlinal walls that are pitted along the veins; anomocytic stomata frequent, ~35-40 μm long; cuticle striate at the leaf margins and bases of the covering trichomes; covering trichomes up to 550 μm long and ~50 μm across at base, uniseriate, with 3 or 4 thick-walled cells, the apical cell markedly longer than the proximal ones; epidermal cells at the base of the covering trichomes are arranged in a rosette pattern; trichomes are often broken off at the base; glandular trichomes rare, occurring adjacent to veins, up to 100 μm long, 20 μm broad, uniseriate, with very thin-walled cells of equal size and dimension; lower epidermal cells generally more sinuous than upper ones; anomocytic stomata abundant, often a single epidermal cell will be the subsidiary cell for 2 or more stomata; covering and glandular trichomes more frequent on the lower epidermis, resembling those on the upper epidermis; secretory ducts containing yellowish-green oil droplets occur along veins.

Transverse section: Bifacial; epidermis with thick cuticle; palisade cells in 1 or 2 layers; spongy mesophyll somewhat broad; small secretory ducts accompany veins.

B. Stem

Surface view: Epidermal cells axially elongated, with a finely striated cuticle.

Transverse section: Epidermal cells rectangular, radially elongated; cortex consists of angular collenchyma; vascular bundles collateral; fibers cap the phloem bundles; xylem small, with embedded fibers; pith consists primarily of pitted cells, with secretory ducts ~30 μm diameter located near

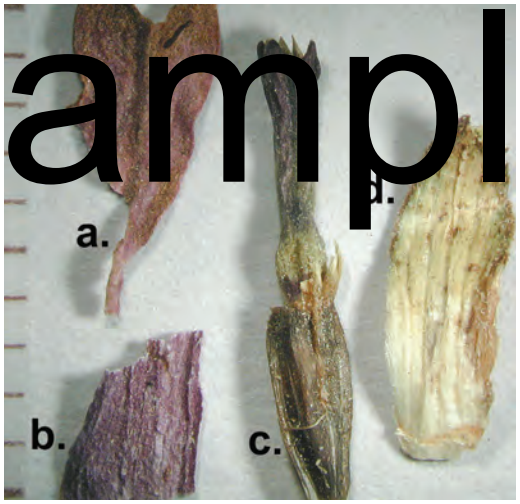
5a.

5b.

5c.

5d.

5e.



5f.

5g.

5h.

Figures 5a-h Macroscopic characteristics of dry *Echinacea purpurea* aerial parts

5a. Fully mature flower head and stem.
5b. Young basal leaves.
5c. Cauline leaves.
5d. Cut and sifted aerial parts.
5e. Aerial parts fragments: (a) leaf fragment, upper surface; b) notched apex of the ligule from a ray floret; c) disk floret with fruit (cypsela) attached; d) disk floret corolla; e) leaf fragment, lower surface; f) leaf fragment, upper surface; g) ligule fragment; h) stem; i) stem transverse section; j) fruit (cypsela); k) phyllary.
5f. Flower fragments (close-up): a-b) notched apex and base of the ligule from a ray floret; c) disk floret with fruit (cypsela) attached; d) phyllary.
5g. Fruit (cypselae).
5h. Fruit (cypsela): a) fruit; b) fruit with wall cut away to reveal the seed; c) seed.

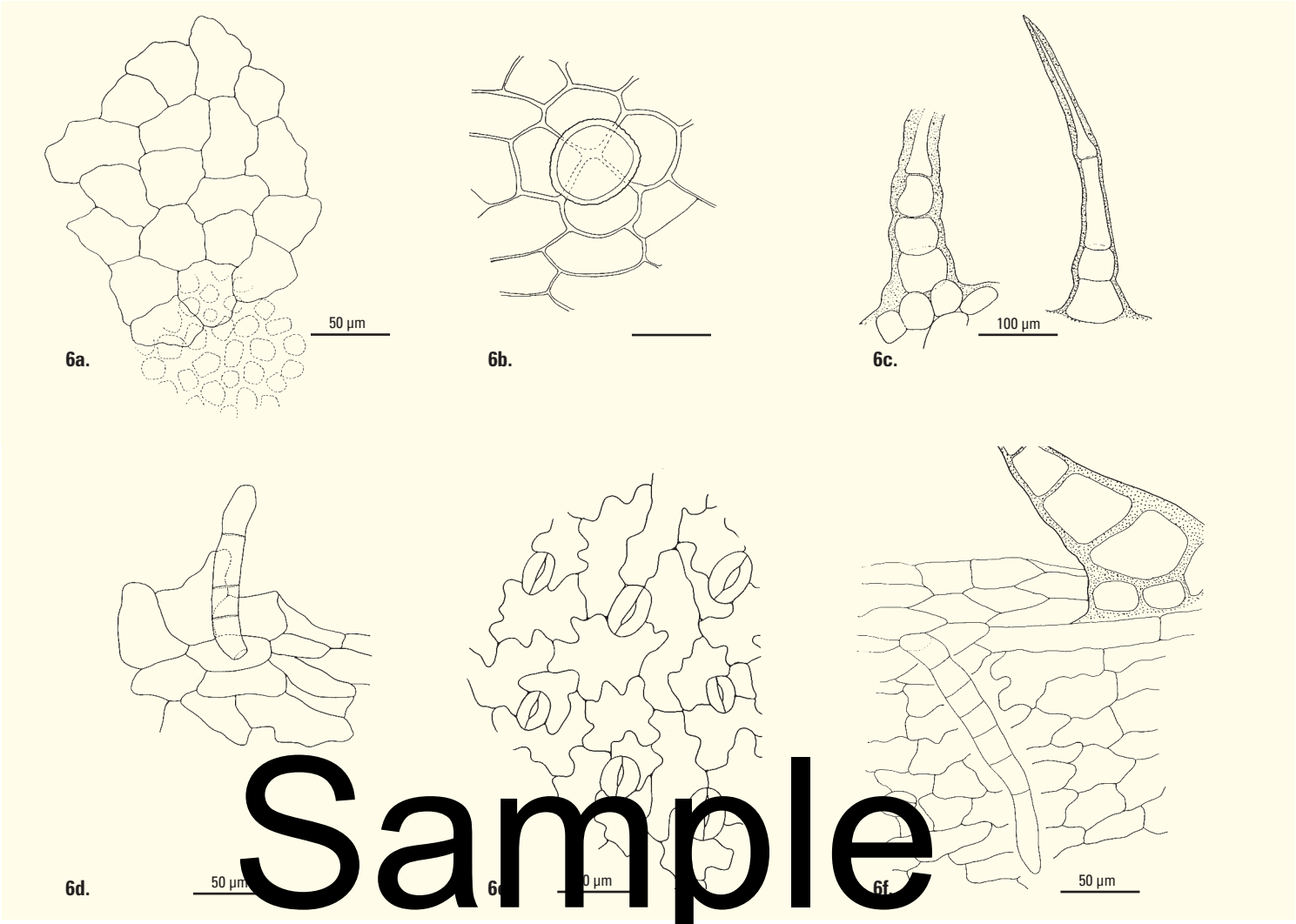


Figure 6 Microscopic characteristics of *Echinacea purpurea* aerial parts

6a. Leaf upper epidermis: polygonal cells with sinuous anticlinal walls. Palisade cells are visible beneath (*sv*).
6b. Basal region of a covering trichome on a leaf (*sv*).
6c. Multicellular covering trichomes from a leaf.
6d. Multicellular glandular trichome on a leaf.
6e. Leaf lower epidermis: cells with sinuous anticlinal walls and anomocytic stomata (*sv*).
6f. Ray floret epidermis: cells with slightly sinuous anticlinal walls, an anomocytic stoma, and a glandular and covering trichome (*sv*).

sv = surface view.

Microscopic drawings courtesy of Reinhard Länger, University of Vienna.

the xylem.

C. Inflorescence and Flower

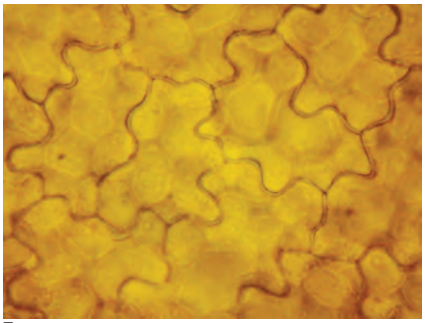
Involucral bract: Stomata and trichomes similar to those found on the leaf.

Ray floret: Covering and glandular trichomes abundant, similar to those found on the leaf; epidermal cells of ligule papillose; secretory ducts occur along veins; multicellular trichomes very short, thick-walled, occurring at the base of the floral tube; pollen grains spheroidal, ~35-42 µm diameter with a spiny exine.

D. Fruit (cypsela)

From the outside in: Exocarp transparent; mesocarp of slightly thickened, very small, pitted orange-brown cells, elongated in longitudinal section; walls of innermost cells may be coated with phytomelanin; axially elongated cells with fine reticulate wall thickenings occur between the mesocarp and a fibrous layer; axially-oriented fibers form a continuous layer around the fruit, the layer is 1 to several cells thick and fortified by sclereids in the basal and apical regions; fiber cell walls are heavily coated with phytomelanin except at the pits; secretory ducts are located in the fibrous layer, generally at the position of the fruit ribs; small vascular bundles occur interior to the secretory ducts; endocarp and testa inconspicuous; embryo cells thin-walled, containing large amounts of fixed oil; palisade cells in the embryo occur in 1 to several rows.

Powder (leaf, stem, and flower): Pitted parenchyma from the stem pith; fragments of leaves showing the bases of covering trichomes or cicatrices; fragments of covering trichomes; spheroidal pollen grains; bundles of fibers, sometimes with black phytomelanin coating (originating from the fruit present in the flowers).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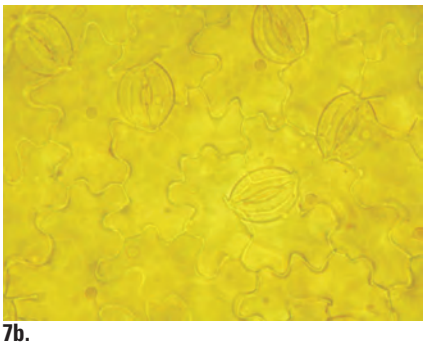
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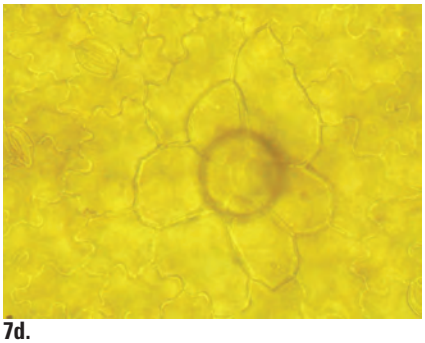
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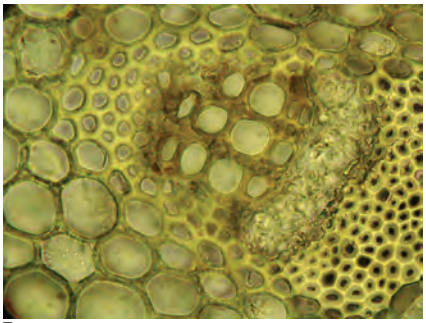
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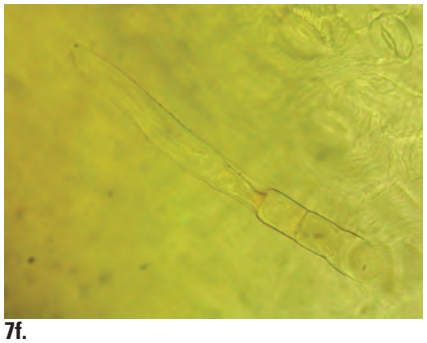
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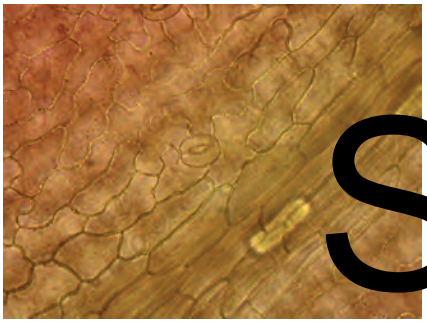

7g.


7h.

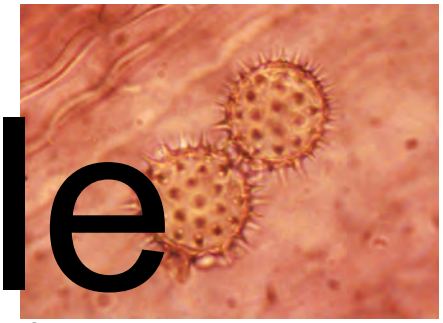

7i.

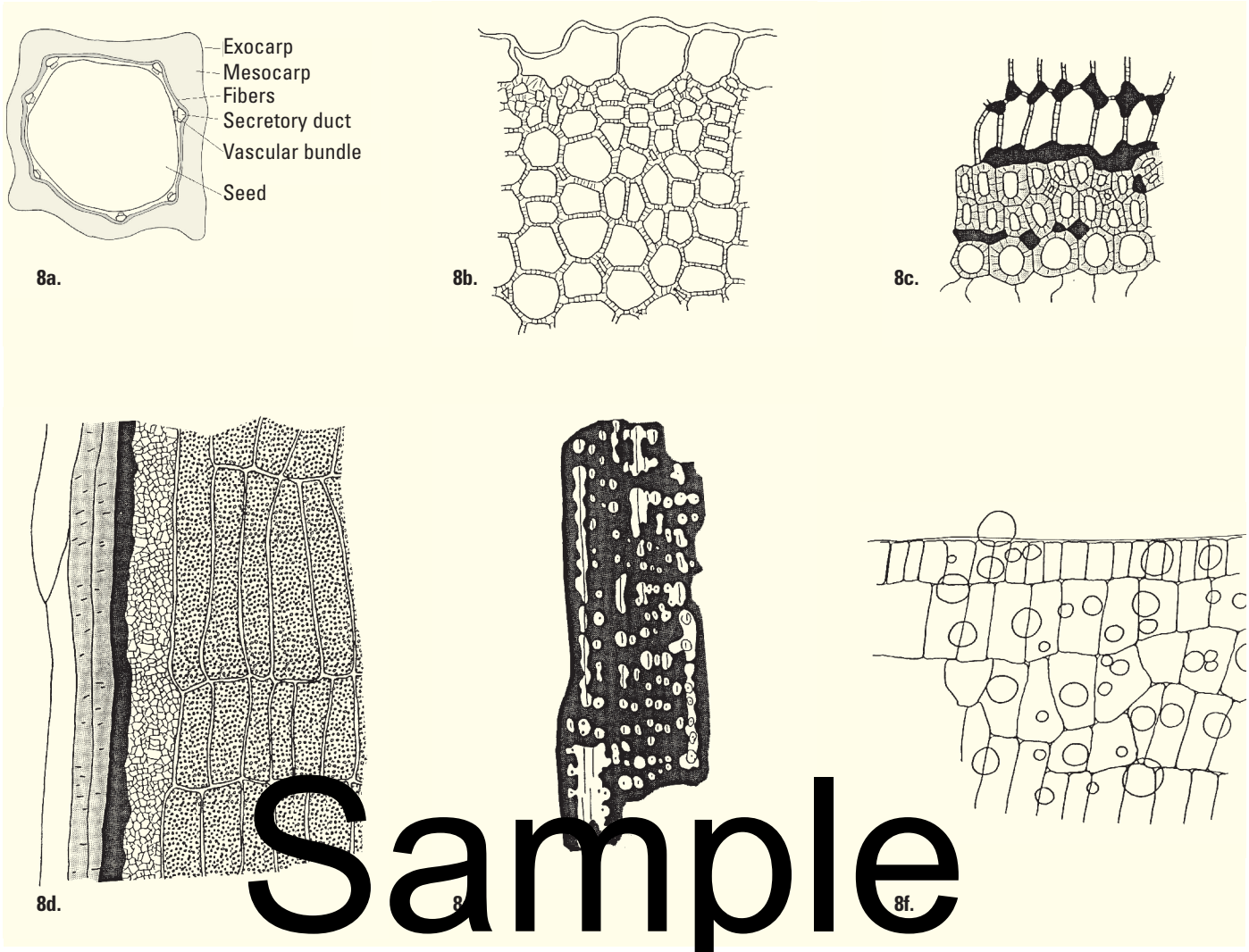
Figure 7a-h Microscopic characteristics of *Echinacea purpurea* aerial parts

7a. Leaf upper epidermis: cells with sinuous anticlinal walls. Palisade cells are visible beneath (*sv*).
7b. Leaf lower epidermis: cells with sinuous anticlinal walls and anomocytic stomata (*sv*).
7c. Multicellular covering trichome on a leaf margin.
7d. Leaf lower epidermis: cells with sinuous anticlinal walls and anomocytic stomata and in the center, the rosette-like arrangement of epidermal cells around the base of a broken covering trichome (*sv*).
7e. Collateral vascular bundle of a stem showing the phloem capped by fibers (*ts*).
7f. Multicellular glandular trichome on a phyllary.
7g. Ray floret epidermis: cells with wavy anticlinal walls, anomocytic stomata, and a light area indicating a secretory duct beneath a vein (*sv*).
7h. Multicellular trichome from the basal region of a ray floret.
7i. Pollen grains with a spiny exine.

sv = surface view; *ts* = transverse section.
Microscopic images courtesy of Reinhard Länger, University of Vienna.

parenchyma with oil droplets; phytomelanin-coated fibers with bright uncoated regions at the pits; heavily pitted elongated cells from the mesocarp; sclereids occasional.

Because commercial supplies of *E. purpurea* are obtained almost exclusively from cultivated sources, the adulteration of *E. purpurea* aerial parts with the aerial parts from other *Echinacea* species is likely to be rare (see Adulterants). Histological differences between the fruits of the 3 commercial species of *Echinacea* may be useful in identifying raw aerial parts material if the fruit is present. In *E. purpurea* and *E. angustifolia*, fibers form a ring around the fruit that is 1 to 2 cells thick in transverse section, whereas in *E. pallida* the ring is 2 to 4 cells thick (Schulthess et al. 1991).



Commercial Sources and Handling

Sourcing

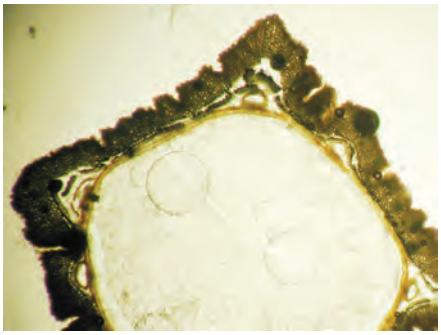
Wild populations of *E. purpurea* are naturally so scattered and often of such low density, that almost all commercial supplies of the plant are and should be cultivated. This species is protected in many of the states to which it is native. Since at least the mid-1990s, the majority of *E. purpurea* aerial parts have come from cultivated sources in North America and abroad (Australia, Germany, Italy, New Zealand, the former Yugoslavia, Switzerland, the Netherlands, and the former Soviet Union). In North America, approximately 259,797 lbs (117,842 kg) of wild and cultivated aerial parts (dried and fresh combined) were harvested in 2003. This represents approximately 10% of the harvest in 1998 and is part of a decreasing trend in harvest and presumably demand from 1998 to 2003 (AHPA 2003; AHPA 2006). In 2003, approximately 122 lbs (55 kg) of the North American aerial parts harvest came from wild sources, representing 0.05% of the total *E. purpurea* aerial parts har-

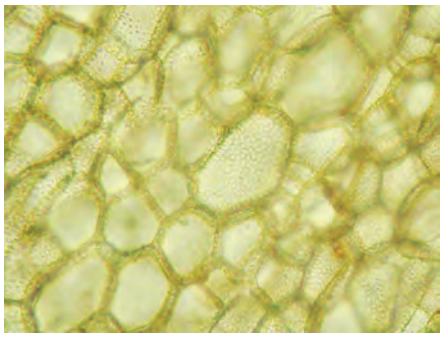
Figure 8a-f Microscopic characteristics of *Echinacea purpurea* fruit (cypsela)

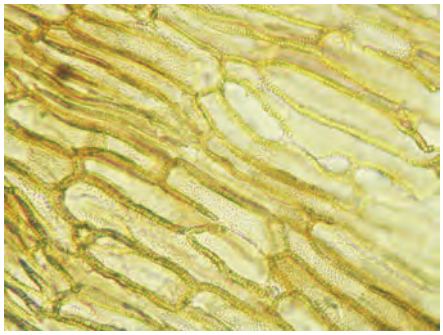
8a. Fruit transverse section.
8b. Exocarp and pitted mesocarp cells (*ts*).
8c. Inner region of the mesocarp and fibrous layer with phytomelanin (*ts*).
8d. Inner region of the mesocarp: pitted cells (right), reticulate cells, and phytomelanin-coated fibers (left) (*ls*).
8e. Fibrous layer showing pits uncoated with phytomelanin (*ls*).
8f. Tissues of the embryo with oil droplets (*ts*).

Key: *ts* = transverse section; *ls* = longitudinal section.

Microscopic drawings courtesy of Reinhard Länger, University of Vienna.


9a.


9b.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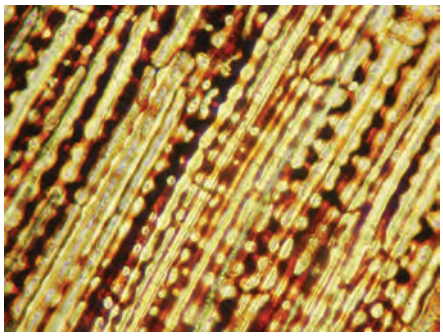
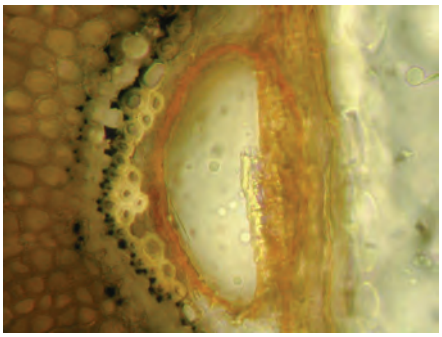

9d.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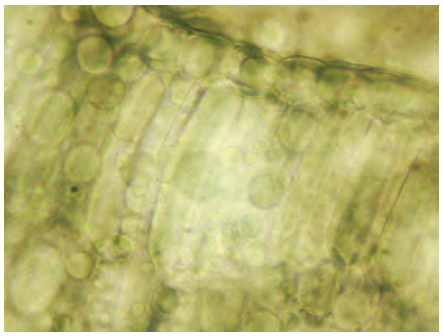

9f.

Figure 9a-f Microscopic characteristics of *Echinacea purpurea* fruit (cypsela)

9a. Fruit transverse section.
9b. Pitted cells of the mesocarp (
9c. Pitted cells of the mesocarp (*ls*).
9d. Phytomelanin-coated fibers (*ls*).
9e. Inner cells of the mesocarp (
and a secretory duct (*ts*).
9f. Palisade cells of the cotyledons with oil droplets (*ts*).

ts = transverse section; *ls* = longitudinal section.

Microscopic images courtesy of Reinhard Länger, University of Vienna.

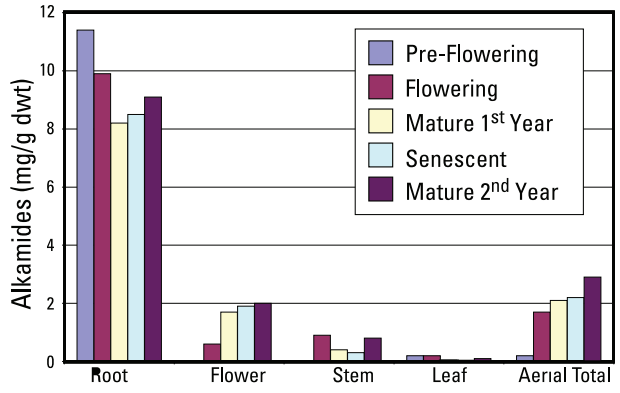
vested in that year (dried and fresh plants combined) (AHPA 2006).

Collection

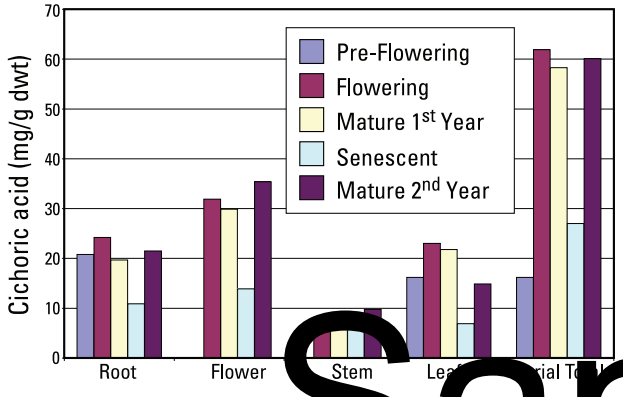
Supplies of *E. purpurea* aerial parts should only come from cultivated sources due to the low density of wild populations. For maximum yields of a broad range of constituents that include alkamides, cichoric acid, and polysaccharides, *E. purpurea* aerial parts should be harvested when flowering.

The most detailed harvesting data come from investigators in Australia (Stuart and Wills 2000a; Stuart et al. 2004) who studied the changing constituent concentrations in cultivated plants harvested at different growth stages (n = 48 for each stage). These data are presented in Figure 10 and provide useful guidelines for optimal harvesting times that may be applicable in other regions of the world. The concentrations of cichoric acid, total alkamides, and polysaccharides in the combined aerial parts (leaf, stem, flower) appeared to be optimized at differing growth stages (statistical analysis of total aerial parts data was not presented). Cichoric acid was fairly constant at flowering (stems and immature flowers) and maturity (mature flowers with seeds), but declined markedly from maturity to senescence. Total alkamides were highest at maturity and senescence, while polysaccharides I and II (Proksch and Wagner 1987) combined were highest during flowering and senescence, decreasing during the mature phase. Optimal harvest time, then, would depend upon which constituents are of interest. If material is to be extracted with a medium to high concentrations of alcohol, a menstruum that does not extract polysaccharides well, then harvesting at maturity makes sense because it optimizes both cichoric acid and total alkamides. If polysaccharides are being targeted, then harvesting at flowering or senescence appears to be best.

A variety of other research groups have documented the effects of harvest date or growth stage on the caffeic acid derivatives and alkamides, in many cases corroborating the data from the Australian research group. Data on the effects of harvest date can be difficult to interpret because the authors do not always correlate date with growth stage. Moreover, comparing constituent concentrations between different studies can be problematic if different analytical methods were employed. Several sources agree that cichoric acid declines over the growing season, beginning either at the onset of flowering or at flower maturation. According to Bauer (1999a), cichoric acid levels in the aerial parts were highest in early July during vegetative growth, declining thereafter. Perry et al. (2001) reported that from the summer (January) to the autumn (April), a 74% and 78% decline in cichoric and caftaric acids, respectively, occurred in tops harvested in New Zealand (cichoric: 2.02% dwt vs 0.52%; caftaric: 0.82% dwt vs 0.18%), and Callan et al. (2005) published similar seasonal trends for cichoric acid in plants grown in North America. In their studies of the cultivar



10a.



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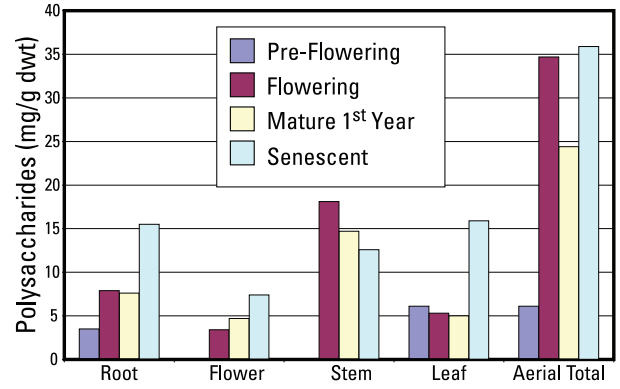

10c.

Figure 10a-c Effect of plant maturity on constituent concentration in *Echinacea purpurea*

Values represent the average of 6 replicate samples comprised of 8 plants each from 2 different growing sites. *Pre-flowering* = basal leaf formation, no stem; *Flowering* = stems and immature flowers; *Mature* = stems and mature flowers; *Senescent* = flowers and small portion of stem discolored, dry, and withered.

Source: Adapted from Stuart and Wills (2000a); Stuart et al. (2004).

"Magical Ruth", Letchamo et al. (2002) found that flowers (n = 60) were highest in cichoric acid when in bud. With regard to the tetraenes, Bauer (1999a) found that they were lowest in July and increased to peak levels in mid-to-late August, declining somewhat over the next month. There is some disagreement between the data from Stuart and Wills (2000a) and data from New Zealand, with Perry et al. (2004) reporting an occasional sharp decrease in total alkamide concentration in the aerial parts starting in the middle-to-late growing season and continuing into senescence. Addressing the effect of plant age on constituent levels in *E. purpurea* aerial parts, Stuart and Wills (2000a) found that total alkamide levels were higher in mature 2nd-year plants compared to 1st-year plants.

Growers appear to be aware of these trends and often set harvest date to optimize a certain class of compounds based upon their own unpublished proprietary information. Those optimizing total phenolics recommend harvesting at 10% bloom (Wheeler 2003, personal communication to AHP, unreferenced) or 40% to 60% bloom (some flowers maturing, others developing) (Fletcher 2002, personal communication to AHP, unreferenced).

In order to avoid collecting senescent leaves, plants can be cut just below the first healthy leaves in order to obtain only high quality leaf material and avoid large quantities of stem in the product (Cech 1995; Sturdivant and Blakley 1999; Wheeler 2004, personal communication to AHP, unreferenced). Aerial parts can be harvested beginning the 2nd year after transplant and in the same year as the root, leaving the roots in for a fall harvest. If starts are put in the ground early, a harvest can be made the same year as transplanting. In some growing regions, 2 harvests a year are possible (Cech 1995). Seeds are typically harvested in the 2nd or 3rd year following transplanting. Leaves and flowers are not harvested in the year in which seeds are to be collected (Sturdivant and Blakley 1999). One source recommends gathering the seeds when fully mature, at which time they separate from the receptacle upon shaking (Cech 1995). However, it is the opinion of another grower that the highest quality product is obtained from seeds that are collected just before they are readily shaken from the head and allowed to after-ripen (Wheeler 2002, personal communication to AHP, unreferenced).

Cultivation

E. purpurea is the easiest of the *Echinacea* species to grow in most climates and soils. Horticultural varieties of it have been cultivated in Europe since the early 1700s (Foster 1991). It can be propagated by seeds or root crown divisions. Divisions can be made in the fall or early spring using mature dormant plants. Most commercial growers of medicinal *Echinacea* propagate from seed. *E. purpurea* seeds generally germinate well in 10 to 20 days without pretreatment (Sturdivant and Blakley 1999). While this finding is supported by research that found that cold moist stratification at 3 to 5 °C for up to 18 weeks did not increase germination of *E. purpurea* seeds (Parmenter et al. 1996), other studies have

11a.

11b.

Figure 11a-b Commercial cultivation of *Echinacea purpurea*

11a. Cultivated field in full flower.

11b. Cultivation in rows.

Photographs © 2006 courtesy of: (a) David Bunting, Williams, OR; (b) Martha Jane Hylton, Trout Lake Farms, Trout Lake, WA.

found that cold stratification (dry or moist) at 2 or 4 °C for 28 to 30 days increased germination rate by approximately 12% to 15% (Romero et al. 2005; Shalaby et al. 1997a). Qu et al. (2005b) noted that *E. purpurea* seeds harvested from cultivated plants exhibited reduced dormancy and increased germination rates compared to seeds from ex situ conserved wild populations. Optimal germination rates are achieved in the greenhouse (Cech 1995; Foster 1991; Sturdivant and Blakley 1999).

E. purpurea typically grows in full sun or partial shade and prefers fertile, well-drained, high-lime loam with a pH ranging from 6 to 8 (Cech 2005; Douglas and Parent 2001; Foster 1991; Sturdivant and Blakley 1999). Typical plant yields 2.5 lbs (1.1 kg) of aerial parts with stem accounting for half of the weight (Sturdivant and Blakley 1999). North American growers recommend that transplants be spaced 12 inches (30.5 cm) apart within rows, with 24 to 30 inches (61 to 76.2 cm) between rows (Cech 1995; Sturdivant and Blakley 1999). The spacing of plants appears to influence plant biomass in *E. purpurea*. A study performed in Egypt reported that plants spaced 20 cm (7.9 inches) apart in a row with 50 cm (19.7 inches) between rows yielded the highest total biomass per unit area, although per plant height and dry weight were optimized at 60 cm (23.6 inches) between plants (Shalaby et al. 1997b). In North America, Callan et al. (2005) found a decreasing trend in aerial parts dry weight as plant density increased.

Fertilizer has been found to affect plant growth, yield, and constituent levels in cultivated *E. purpurea* aerial parts. According to one source, the addition of nitrogen or potassium fertilizer alone increased plant growth and the yield of aerial parts and flowering heads, with potassium having the greatest effect (50 kg [110.2 lbs]/acre); the addition of both nutrients together had a negative effect. The beneficial effect of nitrogen alone on aerial part and flowering head yield increased with application levels (100 to 200 kg [220.5 to 441 lbs]/acre) (Shalaby et al. 1997b). El-Gengaihi et al. (1998) also found that 50 kg/acre potassium had the most beneficial effect on yield of vegetative parts and flowering heads, as well as alkamide content. Gladisheva (1995, cited in Letchamo et al. 2002) reported that cichoric acid content was positively influenced by soil fertility. Additional information regarding soil amendments suitable for the cultivation of *Echinacea* is provided by Galambosi (2004).

In Europe, the use of plastic mulch for weed control is reported to result in up to an 80% decrease in labor costs and a 4% increase in fresh plant weight (Galambosi 2004). Overhead watering during seed maturation may cause molding in the seed heads (Wheeler 2002, personal communication to AHP, unreferenced). For detailed propagation and cultivation methods, see Cech (1995), Foster (1991), Galambosi (2004), and Sturdivant and Blakley (1999).

E. purpurea is susceptible to a variety of diseases, including cucumber mosaic virus, broad bean wilt, other viruses and mycoplasmas, and several fungi (*Cercospora* sp., *Phymatotrichum omnivorum*). One of the most common pests affecting the flower heads is the sunflower moth (*Homoesoma electellum*) (Letchamo et al. 2002). Infection of plants with cucumber mosaic virus has been reported to reduce the alkamides and total lipophilic compounds in *E. purpurea* root extracts (Bellardi et al. 2001; Hudaib et al. 2002). Similarly, flower head pests, root rot, and mycoplasma infection reportedly reduced the cichoric acid content of *E. purpurea* roots (Letchamo et al. 2002). In Europe, it has been reported that chemical pretreatment of seed can aid in the control of various plant diseases (Galambosi 2004).

Different plant accessions grown under standardized greenhouse conditions show great variability in alkamide levels (Binns et al. 2002c), suggesting genetic control of these constituents. Indeed, selection of *E. purpurea* lines for morphological superiority, high yield, and a stronger, more rapid tongue-numbing effect of the stems has resulted in a 2-fold increase in the average content of caffeic acid

derivatives and isobutylamides (Letchamo et al. 1999). DNA fingerprinting (amplified restricted fragment length polymorphism, AFLP™) has been shown to be useful for predicting cichoric acid and tetraene concentrations in *E. purpurea* whole plant material (Baum et al. 2001). Although such a technique might be useful for identifying particular lines or clones as being high in phytochemical markers, it would not obviate the need to address other factors that influence phytochemical concentrations, including cultivation, processing, and storage techniques. One European research group suggested that the following cultivars be used as sources of medicinal quality aerial parts based upon biomass characteristics and an unspecified measure of quality: Schleisheim, Hybrida, and Vebesserte Leuchstern (Bomme et al. 1992a, 1992b, cited in Galambosi 2004).

Post-harvest Handling

Particular care must be taken in the post-harvest handling of *E. purpurea* aerial parts as alkamides and phenolic compounds are especially sensitive to heat and moisture. moisture (see Drying and Storage). Cichoric acid in particular is relatively unstable (Bauer and Liersch 1993) and has been found to be sensitive to enzymatic degradation in the presence of moisture (Kreis et al. 2000; Nüsslein et al. 2000). Interestingly, in one experiment, physical damage during post-harvest handling of fresh material (cutting, crushing) was shown to have no significant effect on alkamide or cichoric acid levels in *E. p* aerial parts dried within 24 hours of harvest to a moisture content of less than 12% fresh weight at 40 °C (Wil Stuart 00). The authors noted that these results were surp ing in the potential for compound degrad ion and a previous epo that cichoric acid in aerial parts diminished by 20% in less than 2 hours after harvest (Bauer 1999b).

Some companies buy only leaf or leaf and flower with no stem, in which case the leaf and flowers are stripped from the stem. Separation of seeds from the receptacle is best done with a combine; the use of a hammermill for this purpose is too harsh and may damage the seed (Wheeler 2002, personal communication to AHP, unreferenced). Cleaning of small amounts by hand is laborious but can be done using a series of screens and a fan or natural breeze to separate the chaff from the seed (Cech 1995; Sturdivant and Blakley 1999).

Drying

Most *E. purpurea* aerial parts products used in clinical trials are prepared from fresh plant material. Fresh *E. purpurea* aerial parts have been reported to contain 75% (Sturdivant and Blakley 1999) to 90% (Stuart et al. 2004) moisture.

Dried material is widely marketed and used in dietary supplements. The sensitivity of alkamides and cichoric acid to heat (Kim et al. 2000a, 2000b; Stuart and Wills 2003; Tobler et al 1994; Wills and Stuart 2000) and cichoric acid to enzymatic degradation in the presence of moisture (Kreis et al. 2000; Nüsslein et al. 2000), require particular adherence to proper drying conditions. Drying as soon after harvest as possible is recommended in order to minimize constituent degradation. Some growers cut the flower heads into large pieces, typically quarters, in order to facilitate drying, but this is not necessary if forced air is used (Wheeler 2002, personal communication to AHP, unreferenced). There are no published data on optimal final moisture content. Some growers recommend drying down to 12% to 15% moisture.

The most detailed data on optimal drying temperature come from the same Australian researchers mentioned above (Stuart and Wills 2003; Stuart et al. 2004) (Table 3). According to their investigations, the optimal drying temperature varies with the constituent of interest, with alkamides optimized at 40 to 70 °C; cichoric acid at 40 °C; and polysaccharides at either 40 or 70 °C. The optimal drying temperature for the preservation of the 3 primary classes of compounds in *Echinacea* aerial parts appeared to be 40 °C. This is consistent with data from Kim et al. (2000b) and reports from experienced *Echinacea* growers who dry at 30 to 40 °C.

Looking at the available data on drying in more detail, Stuart and Wills (2003) reported that increased drying temperature (40, 50, 60, 70 °C) significantly reduced the amount of cichoric acid found in leaf, stem, and flower ($P = 0.05$). Drying temperature affected alkamide levels in flowers, but not in leaf or stem. Interestingly, the highest drying temperature of 70 °C retained 80% more alkamides in the flowers compared to drying at 40 °C ($P = 0.05$). The authors replicated their experiment using flowers from a different location and y r and found the same significant inverse relationship be een drying temperature and cichoric acid lev , bu no ffec of t perature on alkamide levels. Th autho c ncl drying specifications should be esigne ar nd ichoric acid rather than alkamides. In bo eplicates, increas rying temperature was correlated wit a lower proportional loss of cichoric acid from roots compared to aerial parts, an effect the authors suggested may indicate that cichoric acid is better compartmentalized in the root and hence less vulnerable to the effects of temperature. Hence data on the effects of processing on *E. purpurea* root cannot necessarily be extrapolated to the aerial parts. Stuart et al. (2004) found that the preservation of polysaccharides was best when aerial parts were dried at 40 or 70 °C, with poor results at 50 °C. They postulated that another unknown factor in addition to temperature affected polysaccharide concentrations during drying.

The work of Kim et al. corroborates the findings of Stuart and Wills (2003). In a comparison of freeze drying, vacuum microwave drying (VMD), and air drying at 50 °C applied to *E. purpurea* leaves, Kim et al. (2000a) reported that air drying was the method that retained the highest concentration of total alkamides. The same authors did a similar study looking at the effects of freeze drying, VMD, and air drying at 25, 40, and 70 °C on caffeic acid derivative levels in *E. purpurea* flowers (Kim et al. 2000b). The highest levels of caffeic acid derivatives were retained following freeze drying and VMD. For VMD to be as effective as freeze drying, the flowers needed to be dried to a low moisture content (6.1%); at higher moisture content (9.3%), flowers dried using VMD retained only 80% of the cichoric acid of

freeze-dried samples. Of the air-drying treatments, 40 °C was the best temperature to use for the retention of cichoric and caftaric acids.

One study found that the drying of fresh flowers during storage at 20 °C and 60% relative humidity over a 30-day period resulted in no significant loss of cichoric acid or alkamides. The flowers dried to 10% moisture content and no growth of mold was observed, indicating that such treatment may produce a dried product without loss of active constituents and without the need for energy-intensive air drying (Wills and Stuart 2000).

Storage

Follow general guidelines for storage of dried material by packing in airtight containers protected from light, heat, moisture, and insect infestation. Dried *E. purpurea* aerial parts should not be stored as powder (Pharmeuropa 2004) because alkamides are susceptible to oxidation. Special care should be taken to protect dried material from moisture, as cichoric acid undergoes rapid enzymatic degradation in the presence of water (Bauer 1999b).

Temperature and light have been found to affect the storage stability of alkamides and cichoric acid in dried plant material. Alkamide levels decreased significantly in dried crushed *E. purpurea* aerial parts stored in the dark for 60 days at 30 °C, but only marginally when stored at 5 °C (P = 0.05). When the crushed material was stored for 60 days at 20 °C in the light, alkamide levels decreased especially rapidly, indicating that protection from light is important (Wills and Stuart 2000). The same research group reported no significant loss of polysaccharides I or II from dried aerial part material (spread out in a Petri dish) stored at 5, 20, or 30 °C at a relative humidity of 10% or 90% (Stuart et al. 2004).

Table 3 Effect of drying temperature on constituent concentration in *Echinacea purpurea* (mg/g)

Temp (°C)	Root	Flower	Stem	Leaf	Total aerial
Alkamides					
70	8.8	2.7	0.31	0.07	3.08
60	7.3	2.0	0.35	0.10	2.45
50	7.2	1.7	0.39	0.06	2.15
40	6.8	1.5	0.40	0.07	1.97
Cichoric acid					
70	17.2	11.4	1.4	16.8	29.6
60	19.2	17.3	2.3	24.6	44.2
50	24.6	22.2	4.0	33.4	59.6
40	22.1	26.7	7.6	32.5	66.8
Polysaccharides					
70	29.52	-	-	-	9.79
60	28.05	-	-	-	5.67
50	21.44	-	-	-	5.45
40	31.54	-	-	-	9.73

Values represent the average of 6 pairs of plants.

Source: Modified from Stuart and Wills (2003); Stuart et al. (2004).

Some manufacturers use fresh aerial parts in their products and some growers may store fresh material before drying it. As a rule, fresh aerial parts should be used or dried as soon after harvest as possible. During interim storage and shipping, they should be kept cool. Although Wills and Stuart (2000) reported no significant loss of alkamides or cichoric acid from fresh flowers stored for 30 days at 20 °C and 60% relative humidity, Stuart et al. (2004) reported a 24% loss of polysaccharide I and II combined within the first 10 days of fresh material storage under the same conditions, suggesting the need for more research into optimal storage conditions for fresh material.

Adulterants

Because all or almost all commercial supplies of *E. purpurea* aerial parts are cultivated, adulteration is not common. The tops of *E. angustifolia* and *E. pallida* are used by some manufacturers, making it possible that adulteration could occur, although it has not been reported. *E. purpurea* tops are generally less expensive and more abundant than the tops of other species, and when whole they are easily distinguished from other *Echinacea* species using morphological characters (Table 2), reducing the risk of adulteration. In cut and sifted form, the 3 commercial species of *Echinacea* are difficult to tell apart using physical methods. If seeds are present, microscopic differentiation may be an aid to identification (see Microscopic Identification). Powders would likely be impossible to distinguish using physical methods. Qualitative and quantitative chemical differences between the aerial parts of various *Echinacea* species have been determined for the caffeic acid derivatives (see Analytical; Bauer et al. 1988b; Hahn-Deinstrop and Bauer 1999), although no qualitative difference in alkamide profile has been reported (Bauer 1999a; Bauer et al.1988c; Bauer and Remiger 1989; Perry et al. 2004) (see Constituents). Identification of *E. purpurea* aerial parts may be difficult in cases in which genetic stock on farms has been mixed with other species due to cross-pollination. Contamination of aerial part and seed crops by agricultural weeds and their seeds is likely and should be screened for.

Qualitative Differentiation

Properly handled fresh or dried material should retain the original green color of the leaves and pinkish-purple of the flowers and be free from evidence of disease. When chewed, the seeds should produce a strong numbing sensation on the tongue and stimulate salivation.

Preparations

American manufacturers use different preparations of *E. purpurea* aerial parts in their products. Many include the dried aerial portions in tablets and capsules, although there is no historical use and little clinical evidence of the efficacy of such products (see Lindenmuth and Lindenmuth 2000, 2004). The most widely researched and commonly used *Echinacea* preparation in Europe is the alcohol-stabilized pressed juice from *E. purpurea* flowering aerial parts

(Echinacin) (see Table 4 for characterization). This preparation has been the subject of positive studies regarding efficacy. There are other liquid and dry versions of the freshly expressed leaf, stem, and flower juice also available. Some manufacturers include the fresh aerial portions or expressed leaf juice in their extracts, typically combined with the root.

Extraction

The constituents of *Echinacea* have varying degrees of polarity, ranging from the water-soluble polar polysaccharides and glycoproteins, to the moderately polar caffeic acid derivatives and flavonoids and the non-polar lipophilic polyacetylenes and alkamides. Such variation means that a wide range of solvents may be employed for extraction, including water, alcohol, hexane, or chloroform, depending on the compounds desired (Bauer 1998).

Looking at a range of solvent concentrations, Bergeron et al. (2000) reported that the overall extraction of cichoric acid, chlorogenic acid, and alkamides was optimized in 70% ethanol or methanol, although they also reported that extraction efficiency did not vary greatly at solvent concentrations between 50% and 70%. Stuart and Wills (2000b) found that the extraction of cichoric acid and total alkamides from *E. purpurea* aerial parts was optimized at different solvent concentrations and temperatures. Cichoric acid was optimally extracted in 60% ethanol, whereas the alkamides were highest in 90% ethanol. Increasing temperature stabilized cichoric acid, presumably due to a reduction in enzymatic activity, but caused the alkamides to degrade. Cichoric acid was optimally extracted in 60% ethanol at 60 °C (45% recovery), whereas alkamides were optimally extracted in 90% ethanol at 20 °C (~70% recovery). Overall extraction of both compounds was optimized in 60% ethanol, yielding 73% of the available alkamides and 57% of the available cichoric acid. Optimal temperature at this solvent concentration was not reported. The authors noted that the moisture content of the dried aerial parts in their study was 10% and that as the moisture content of raw material increases, ethanol concentration would need to be increased to maintain maximum levels of extraction. In the same study, it was observed that a high solvent:solute ratio (6:1 or 8:1) optimized both classes of constituents. Particle size also had a strong effect on extraction efficiency, with recovery of both cichoric acid and alkamides doubling with each decrease in size class. The smallest size class with the highest yield of both compounds was 300 to 1200 μm. In work that supports the findings of Stuart and Wills (2000b), Sasagawa et al. (2006) analyzed 95%, 75%, 50%, and 25% ethanolic extracts of *E. purpurea* aerial parts, and found that alkamides were best extracted in 95% ethanol, whereas the individual caffeic acid derivatives were optimized in 50% or 75% ethanol.

Stuart et al. (2004) found that expressed leaf juice contained a higher concentration of polysaccharides I and II compared to alcoholic extracts. They reported that polysaccharides are not efficiently extracted from dried *Echinacea* aerial parts in any extraction process. A water extract and 10% ethanolic extract of dried aerial parts contained polysaccharide I (3% to 4%) but no polysaccharide II. Higher concentration ethanolic extracts (40%, 60%) contained no polysaccharides. Similarly, preparations made from dried aerial parts yielded only polysaccharide I. In general, aqueous extracts, hydroalcoholic extracts made with low to moderate concentrations of alcohol (25% to 50%), and expressed juice products stabilized with low percentages of ethanol (e.g. 25%), may contain polysaccharides. High concentration alcoholic extracts will contain little or no polysaccharides, for these compounds are poorly extracted and precipitate out with increasing concentrations of alcohol.

Stability

Stuart and Wills (2000b) reported that total alkamides and cichoric acid from *E. purpurea* aerial parts were stable in 40% to 100% ethanol extracts stored for 4 months at 20 °C. Similarly, He et al. (1998) found that the isolated tetraenes were stable in methanol (concentration not specified) stored under refrigeration for 6 months. Cichoric acid degrades enzymatically by the action of polyphenol oxidase during the preparation of *E. purpurea* fresh aerial parts products

Table 4 Polysaccharide content of the pressed juice of *Echinacea purpurea* aerial parts and Echinacin®*

Pressed juice (12% to 13% dwt)	Fresh weight (%)	Dry weight (%)	Sugar combination (mol %)
Fructan	0.4	3.4	Frc (Glc)
Neutral sugar	2.2	18	Rha (2), Fuc (1), Ara (8), Xyl (2), Man (3), Gal (7), Glc (77)
Uronic acid	0.5	4.4	GlcA (GalA)
Echinacin® (6% to 7% dwt)	**Fresh weight (%)**	**Dry weight (%)**	**Sugar combination (mol %)**
Fructan	0.14-0.37	2.16-6.08	Frc (Glc)
Neutral sugar	0.88-0.96	14.2-15.1	Rha (3), Ara (5), Xyl (1), Man (4), Gal (7), Glc (80)
Uronic acid	0.15-0.21	2.70-3.18	GlcA (GalA)

* Echinacin is the alcohol-stabilized pressed juice of flowering *E. purpurea* aerial parts, manufactured by Madaus AG, Germany.

Frc = fructose, Glc = glucose, Rha = rhamnose, Fuc = Fucose , Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, GlcA = glucuronic acid, GalA = galacturonic acid.

Source: Blaschek et al. (1998).

(Kreis et al. 2000; Nüsslein et al. 2000). This enzyme shows the greatest activity at pH 6 and has an affinity for both caffeic and cichoric acids (Kreis et al. 2000). Cichoric acid degradation in raw material and extracts can be minimized by processing as soon after harvest as possible, drying properly if drying is desired, filtering off the plant material after the first day of extract preparation, or by using heat during the manufacturing process to denature the enzymes (Bauer 1999a; Stuart and Wills 2000b; Wills and Stuart 2000). While increased heat during extract manufacturing may preserve cichoric acid, it appears to degrade alkamides based upon the data presented above (Stuart and Wills 2000b), making heat treatments inappropriate if alkamides are to be optimized.

Most of the pressed juice products available are stabilized with approximately 22% ethanol and have poor cichoric acid stability. Nüsslein et al. (2000) found that adding 50 mM of the antioxidant ascorbic acid to fresh *E. purpurea* aerial parts and stabilizing them with 40% ethanol maintained constant levels of cichoric acid over a 4-week period. Ascorbic acid inhibits cichoric acid degradation because it acts as an alternate substrate for polyphenol oxidase. Once the ascorbic acid is completely oxidized, cichoric acid begins to degrade. Heat is also an option for stabilizing the cichoric acid content of aerial parts juice products (Nüsslein et al. 2000). Heat treatment of expressed juice will not compromise alkamide content, since alkamides are absent or occur only in trace amounts in this product.

Bacterial endotoxins (lipopolysaccharides) have been found in both commercial and homemade *Echinacea* products (Morazzoni et al. 2005; Senchina et al. 2005). These compounds can be strong immunostimulants and have been found to have confounding effects in some preclinical studies (see Therapeutics).

Standardization

According to Bauer (1999b), the most appropriate constituents for the standardization of *E. purpurea* aerial parts preparations are cichoric acid and the alkamides because both are associated with pharmacological activity (although the bioavailability of cichoric acid has not been determined); both are labile and sensitive to processing and storage; and validated analytical methods are available. Polysaccharides that would also be good *candida*tes for standardization because they have purported bioactivity and extraction sensitivity. However, their bioavailability is unknown and no validated methods are currently available for their determination. Fresh aerial parts products are generally not chemically characterized. Alkamides are present in only trace amounts in fresh juice products and caffeic acid derivatives are highly variable in concentration due to enzymatic degradation.

A number of studies have analyzed variation in the levels of marker compounds in commercial *E. purpurea* products (Bauer 1999b; Gilroy et al. 2003; Mølgaard et al. 2003; Osowski et al. 2000; Schieffer 2000; Wills and Stuart 1998). Results from at least 2 of these studies found greater variation in alkamide levels compared to caffeic acid derivatives (Bauer 1999b; Wills and Stuart 1998). Bauer (1999b) compared 6 different commercial preparations containing the expressed juice of *E. purpurea* flowering tops and found that alkamide content ranged from 0.01% to 0.18%, while cichoric acid content ranged from 0% to 0.4%, even between batches of the same product line (Bauer 1999b). Wills and Stuart (1998) found similar results upon analysis of 32 commercial preparations of *E. purpurea* root and/or aerial parts, with both alkamides and caffeic acid derivatives entirely absent in some products. The authors suggested that the greater proportion of products with near-zero levels of alkamides (28%) compared to caffeic acid derivatives (16%) might indicate that the alkamides are more sensitive to processing.

Osowski et al. (2000) tested 25 preparations containing *Echinacea* and found great variation in cichoric acid and alkamide concentrations between products and between lots of the same product. This variation was reportedly correlated with type of product (homeopathic mother tincture, spagyric tincture, tablets, pressed juice, combination products), species, and plant part. The data indicated that regardless of the species of *Echinacea*, homeopathic mother tinctures (1:10 drug to extract ratio) yielded the highest concentrations of both compounds, followed by tablets, combination products and pressed juice, and then spagyrics. Tinctures available on the American market typically have a higher drug-to-extract ratio (e.g. 1:5, 1:2, etc.) than do homeopathic mother tinctures. Mølgaard et al. (2003), on the other hand, found that a variety of *Echinacea* tinctures (not fully characterized) had lower, although overall more uniform, concentrations of cichoric acid and alkamides compared to tablets and capsules. According to another report, a 3-times higher concentration of alkamides was found in a 1:10 tincture made from fresh compared to dry material (Tobler et al. 1994).

A contribution to the variability of standardization on any one compound or class of compounds is apparent upon review of the above data on expressed juice products. Bauer (1999b) found low and quite variable concentrations of alkamides and cichoric acid in such products. Osowski et al. (2000) reported that Echinacin, the aerial part juice product manufactured by Madaus, contained very low levels of alkamides, while cichoric acid could not be quantified because it could not be unambiguously identified. Mølgaard et al. (2003) found neither cichoric acid nor alkamides in Echinacin. Low levels of alkamides are expected in expressed juice products because these compounds are lipophilic. Polysaccharides were not tested for in any of these studies. Despite such low levels of alkamides and cichoric acid in Echinacin, this product tested positively in a number of clinical and preclinical trials (see Therapeutics). It is therefore clear that there are large gaps in our knowledge of the constituents contributing to the therapeutic effect of *Echinacea*, making it challenging to interpret data on constituent levels in products and even pharmacokinetic data on the bioavailability of isolated constituents. This highlights the assertion by Bauer (1999a) and the perspective of traditional herbalists that it is the whole extract rather than isolated constituents that contribute to the bioactivity of *Echinacea* products.

Following are specifications for *E. purpurea* aerial parts

products commonly found on the market:

***Echinacea purpurea* Expressed Juice**

The expressed juice of fresh *E. purpurea* flowering aerial parts (leaf, stem, flowers) can be prepared by applying pressure directly to the whole plant (direct pressing) or to chopped material. According to one report, pressing after cutting yielded higher amounts of plant juice (Stuart et al. 2004). After pressing, the juice is stabilized, usually with alcohol. Echinacin is stabilized with 21.95% ethanol v/v to yield a final concentration of 21.6% ethanol. After a storage period (days or weeks), the precipitate is removed by filtering (Blaschek et al. 1998). Precipitates can form over time and may require filtering again. Expressed juice products are also available in a dried encapsulated form.

Color: Light golden brown, translucent.

Taste: Initially bitter, followed by a slight molasses-like sweet taste.

Aroma: Liqueur-like, somewhat like molasses.

Contents of Expressed Juice (unstabilized)

According to the findings of Blaschek et al. (1998), slightly under 25% of the pressed juice dry weight consists of mono-, oligo-, and polysaccharides (Table 4). Tannins, proteins, and pigments accounted for approximately 15% of the dry weight. The remaining 60% consists of largely unknown compounds. No starch was found. Stuart et al. (2004) reported that expressed juice contained approximately 5.1 mg/g of polysaccharides I a[illegible] II.

Contents of Echinacin

Blaschek et al. (1998) repor[illegible] that the dry weight of Echinacin is less than th[illegible] of the [illegible]ess[illegible] juic[illegible] (Ta[illegible]e 4), indicating that some com[illegible]p[illegible]nds [illegible]ese[illegible] in th[illegible] juic[illegible] precipitate out during stabilization with alcohol. Hence the fructans, neutral sugars, and uronic acids occur in lower concentrations in Echinacin compared to the fresh juice. Variation in fructan content is most likely due to variation in raw material. The polysaccharides present in Echinacin do not have the same composition as those found in the aqueous extract of the aerial parts (Bauer and Wagner 1991). Al-Hassan et al. (2000) found no cichoric acid in Echinacin, while alkamides 1/2, 5, 8/9 were identified in the *n*-hexane fraction and p-coumaric acid in the ethyl acetate fraction. Mølgaard et al. (2003) found neither cichoric acid nor alkamides in Echinacin. Glycine betaine has also been found in Echinacin (Soicke et al. 1988).

Storage of Expressed Juice Products

Store in tightly closed containers protected from light. Al-Hassan et al. (2000) reported that alkamide and phenolic levels in Echinacin stored at 25 °C remained relatively stable for an unspecified length of time. Witthohn et al. (2000) reported similar stability in different batches of Echinacin stored at 25 °C for varying periods up to 1 year. This was determined based on the ability of the variously stored preparations to increase the phagocytic activity of PMN granulocytes and monocytes to the same degree, indicating the relative homogeneity of batches and retention of potency over that time period under those storage conditions.

***Echinacea purpurea* Aerial Parts Tincture**

According to one study, the optimal extraction conditions for the manufacture of hydroalcoholic extracts of *E. purpurea* aerial parts that maximize the overall yield of both alkamides and cichoric acid were reported as follows: 60% ethanolic solvent; solvent:solute ratio of 6:1 or 8:1; 300 to 1200 μm particle size; and maceration for 2 weeks (Stuart and Wills 2000b).

Color: Pale to medium amber to greenish amber.

Taste: Mildly aromatic with an earthy vegetal quality; imparting a mild tingling sensation if fruits were present in the raw material.

Aroma: Mildly aromatic; characteristic.

Storage: Store in tightly closed amber glass containers protected from light. Stuart and Wills (2000b) reported that cichoric acid and total alkamides from *E. purpurea* aerial parts extracted in 40% to 100% ethanol were stable for 4 months at 20 °C.

Constituents

E. purpurea aerial parts contain at least 4 major groups of compounds generally considered to be of medicinal interest: alkamides, caffeic acid derivatives (phenylpropanoids), polyacetylenes, and polysaccharides. Recently, melanin has been proposed as a potentially active immunostimulant. Within each gr[illegible]up, there exist few to many related derivati[illegible]ener[illegible] the [illegible]dary metabolic constituents in *E. purpure*[illegible] fit[illegible]e [illegible]ttern [illegible] the genus *Echinacea* and the plan[illegible] famil[illegible] As[illegible]rac[illegible]ae. Unlike the root of this species, the co[illegible]ue[illegible]ts pro[illegible]e o[illegible]erial parts may be useful for species ide[illegible]ification only when combined with other methods. Although an effort is made to provide a phytochemical differentiation between the aerial parts of the 3 commercial species of *Echinacea*, analytical differentiation of the aerial parts is not a practical consideration for routine quality control purposes because *E. angustifolia* and *E. pallida* tops are not frequently traded. More detailed reviews of the phytochemistry of *Echinacea* are provided in Bauer (1998, 1999a), Bauer and Wagner (1990, 1991), and Miller (2004). Studies on the effects of cultivation, handling and processing, drying, and storage on overall phytochemistry have also been reported (see Commercial Sources and Handling).

Alkamides and Isobutylamides

The alkamides (also known as alkylamides) found in *Echinacea* are often identified by numbers 1 through 25 according to Bauer and Remiger (1989), a numbering system that is often preserved in more recent literature and is followed in this monograph. *E. purpurea* flowering aerial parts contain at least 10 of the alkamides found in the roots (1-3, 5, 6a, 7-11), some in trace amounts (Figure 12) (Bauer and Remiger 1989; Bauer et al. 1988a; Bohlmann and Hoffmann 1983; Perry et al. 1997). Most of these alkamides contain a diene in conjugation with the carbonyl (2,4-diene) (Bauer 1999a). In aerial parts, as in the roots of this species, the major tetraene alkamides occur as a mixture of

the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide isomers 8 and 9.

Alkamide levels from 0.001% to 0.29% have been reported in total or individual *E. purpurea* aerial parts (Table 5). Wills and Stuart (1999) reported that 50% of *E. purpurea* aerial parts cultivated in Australia (n = 31) yielded 0.05% to 0.1% dwt total alkamides, while 90% yielded 0.02% to 0.14%. Variation between studies in phytochemical concentrations reported for *E. purpurea* aerial parts may have to do with varietal differences, growth conditions, drying and storage conditions, as well as analytical methods.

The tetraenes reportedly comprise 76% of the total alkamides in the aerial parts (Qu et al. 2005a; Wills and Stuart 1999). The disk florets and their seeds were reported by Bauer et al. (1988a) to contain the highest levels of the tetraenes of all the individual aerial parts. However, Perry et al. (1997), found the highest concentration of tetraenes in the vegetative stem (1.41% dwt) followed by the flowers (0.27%), reproductive stem (0.13%), and leaves (0.02%). According to Stuart and Wills (2000a), leaf and stem contained only the tetraenes in quantifiable amounts, while the flowers also contained alkamide 1. These same authors found that 70% of the total alkamides were concentrated in the roots and rhizomes, while 20% were in the flowers, 10% in the stem, and 1% in the leaves of mature plants (Stuart and Wills 2000a).

There is no qualitative difference in alkamide profile between the aerial parts of the 3 commercial species of *Echinacea* (Bauer 1999a; Bauer and Remiger 1989; Perry et al. 2004). According to Bauer et al. (1988b), *E. purpurea* aerial parts contain the highest concentration of alkamides, followed by *E. pallida*, and then *E. angustifolia*.

The alkamides have reported immunostimulant and anti-inflammatory properties (see Therapeutics) and are responsible for the tongue tingling or numbing analgesic sensation experienced when chewing on *Echinacea* roots and seeds. The alkamides are lipophilic compounds and as such may be found in alcoholic preparations, with only trace amounts in water extracts and expressed juice (Bauer 1999a).

Caffeic Acid Derivatives (Phenylpropanoids, Phenolics)

The principle caffeic acid derivatives in *E. purpurea* aerial parts are cichoric acid (2,3-*O*-dicaffeoyltartaric acid, also called chicoric acid), first isolated from leaf material of *Echinacea* by Becker and Hsieh (1985), and caftaric acid (2-*O*-caffeoyltartaric acid) (Bauer 1998; Perry et al. 2001) (Figure 13). Bauer (1999a) reported on the presence of 2-*O*-caffeoyl-3-*O* feruloyl tartaric acid, cichoric acid methyl ester, 2,3-*O*-diferuloyl tartaric acid, 2-*O*-feruloyl tartaric acid and 2-*O*-caffeoyl-3-*O* cumaroyl tartaric acid in *E. purpurea* leaves.

The concentrations of the caffeic acid derivatives found in *E. purpurea* aerial parts by various research groups are given in Table 5. Cichoric acid has been found in somewhat variable amounts in dry *E. purpurea* aerial parts and it appears to occur in decreasing amounts in the flowers, leaves, and stems, with content depending upon the time of harvest and plant growth stage (see Commercial Sources and Handling; Figure 10). According to Bauer (1999a), cichoric acid is most abundant in the ray florets (1.2% to 3.1% dwt). Stuart and Wills (2000a) reported that in mature plants, the flowers and leaves each contained approximately 35% of the total plant cichoric acid, while the underground organs contained 20% and the stem 10%. Other researchers have also found generally lower amounts of cichoric acid in the roots (0.5% to 2.8% dwt) compared to the aerial parts (0.4% to 4% dwt) (Bergeron et al. 2000; Binns et al. 2002c).

Significant quantitative and qualitative differences in caffeic acid derivatives have been found between the 3 commercial species of *Echinacea*. *E. purpurea* aerial parts generally contain the highest concentration of cichoric acid (up to 4% dwt) compared to either the aerial parts or roots of *E. angustifolia* and *E. pallida*. According to 2 sources, the aerial parts of *E. angustifolia* contain 0.02% to 0.34% dwt cichoric acid while those of *E. pallida* contain 0.12% to 1.6% dwt (Bauer et al. 1988b; Binns et al. 2002c). *E. purpurea* aerial parts generally do not contain chlorogenic acid, isochlorogenic acid, echinacoside, or verbascoside, which are typically found in *E. angustifolia* and *E. pallida* aerial parts. Nor do they contain cynarin, which is typical of *E. angustifolia*. Therefore, the absence of these compounds may be used as a general identifying marker for *E. purpurea* aerial parts, recognizing that echinacoside, cynarin, and chlorogenic acid have been shown to be vulnerable to degradation during processing and may therefore be misleadingly absent in *E. angustifolia* and *E. pallida* products (Perry et al. 2001; Turner et al. 2005; Wölkart et al. 2004). Also compiling the literature, low concentrations of echinacoside (up to 0.08%), chlorogenic acid (< 0.01% to 0.08%), and cynarin (< 0.01%) have been reported in *E. purpurea* aerial parts by various investigators (Binns et al. 2002c; Letchamo et al. 1999, 2002; Perry et al. 2001). The phenolics profile of the various *Echinacea* species may vary with growing conditions (Binns et al. 2002c) or genetic selection (Letchamo et al. 1999). Such intraspecific variation, combined with the fact that caffeic acid derivatives are present in other species of *Echinacea* and other plant genera, precludes the use of these compounds as definitive identification markers for *Echinacea* species.

Cichoric acid has in vitro immunostimulant and anti-hyaluronidase activity (Bauer 1998, Facino et al. 1993) and several caffeic acid derivatives have been shown to have antioxidant activity (Hu et al. 2004) and in vitro HIV antiviral effects due to integrase-inhibitory activity (Robinson et al. 1996). The caffeic acid derivatives are hydrophilic polar compounds that may be found in aqueous and hydroalcoholic preparations, as well as expressed juice products (Bauer 1999a).

Glycoproteins and Polysaccharide-glycoprotein Complexes

A number of high molecular weight polysaccharides and glycoproteins have been identified in *E. purpurea* flowering tops, including: 4-*O*-methyl-glucuronoarabinoxylan (35 kDa; polysaccharide I) and an acidic highly branched arabinorhamnogalactan (450 kDa; polysaccharide II). Polysaccharide I consists of xylose, 4.9 mol; arabinose,

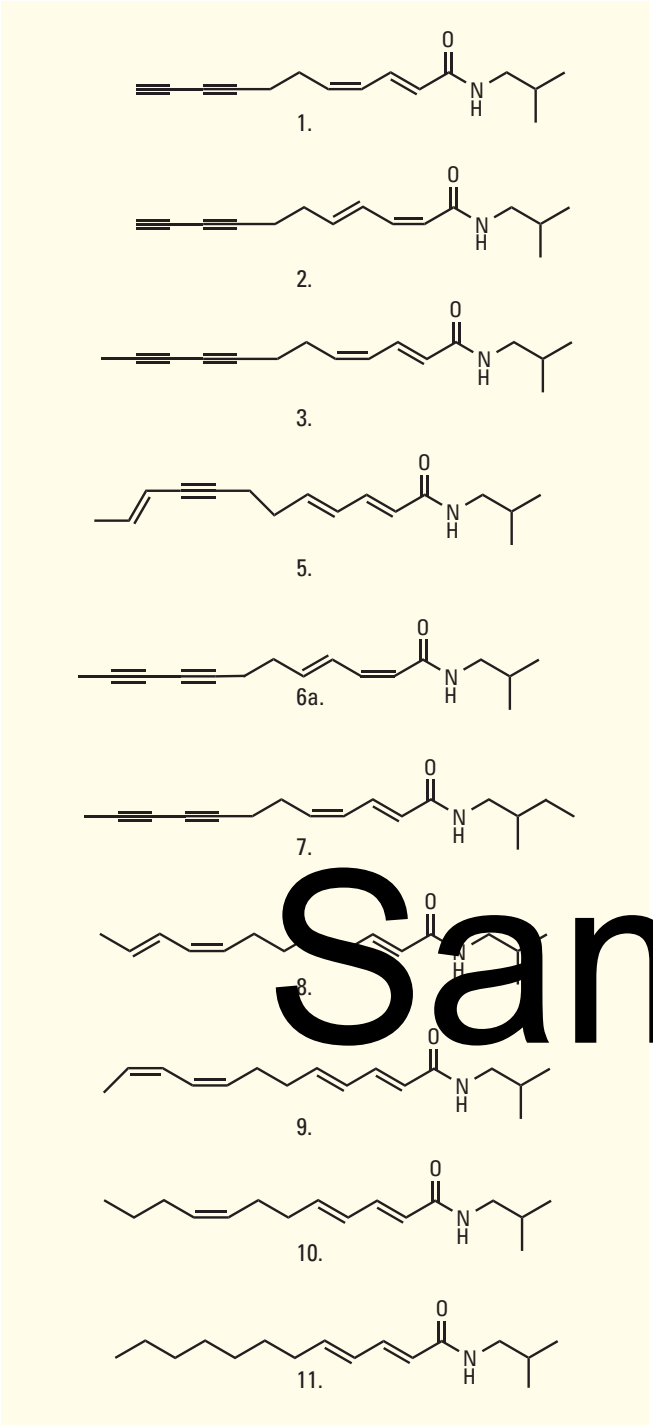

Figure 12 Alkamides in *Echinacea purpurea* aerial parts

1. undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide
2. undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide
3. dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide
5. dodeca-2*E*,4*E*,10*E*-trien-8-ynoic acid isobutylamide
6a. dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide*
7. dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide
8. dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide
9. dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide
10. dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide
11. dodeca-2*E*,4*E*-dienoic acid isobutylamide

* All compounds except 6a are numbered according to Bauer and Remiger (1989); 6a is numbered according to Perry et al. (1997).

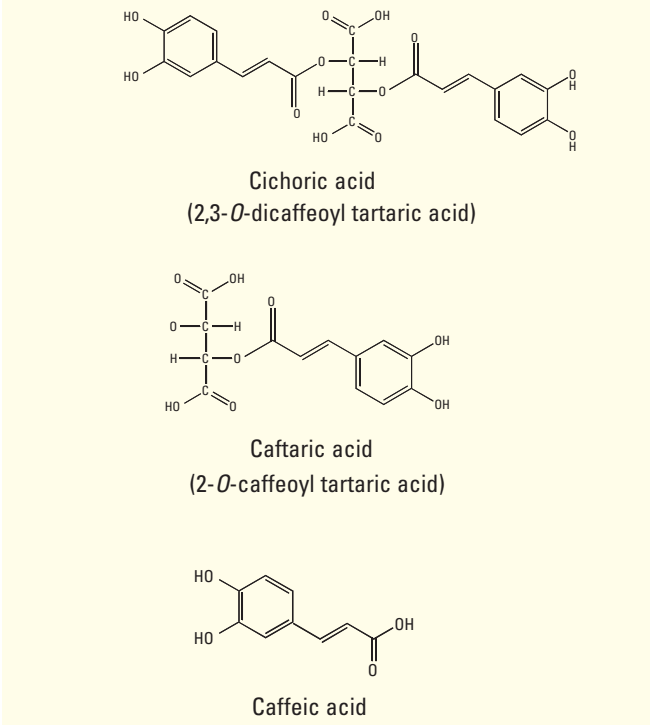

Figure 13 Major caffeic acid derivatives found in *Echinacea purpurea* aerial parts

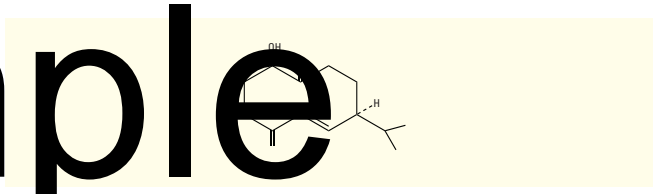

Figure 14 Germacra-4(15),5*E*,10(14)-trien-1-ol, a compound of the essential oil of *E. purpurea*

1.0 mol; glucuronic acid 0.9 mol; 4-O-methlyglucuronic acid, 0.9 mol; glucose, 0.4 mol; rhamnose, 0.3 mol. Polysaccharide II consists of arabinose, 1.0 mol; rhamnose, 0.8 mol; galactose, 0.6 mol; glucuronic acid, 0.6 mol; and a xyloglucan (79 kDa) (Bauer 1999a; Proksch and Wagner 1987). Classen et al. (2000) characterized a high-molecular weight arabinogalactan-protein (1200 kDa) with a protein content of 7% and high levels of serine, alanine, and hydroxyproline from fresh pressed juice of *E. purpurea* aerial parts.

Primary cell wall polysaccharides were isolated from cell suspension cultures: 2 neutral fucogalactoxyloglucans (10 and 25 kDa) and an acidic arabinogalactan (75 kDa) (Emmendörffer et al. 1999; Wagner et al. 1988). Polysaccharides derived from tissue cultures differ from those found in the whole plant because cultured cells do not possess secondary cell walls. Fructans have been reported to occur in *E. purpurea* aerial parts at levels 10 times lower than in the root (Giger et al. 1989).

The lack of exhaustive extraction and precise analytical methods to measure polysaccharides has made their quan-

Table 5 Concentrations of primary compounds found in *Echinacea purpurea* aerial parts (means and/or range)

Compound (% dwt)	Plant part	Source
Total alkamides		
0.001-0.20	different aerial parts; n = 5	Perry et al. 1997
0.077 (0.02-0.1)	aerial parts	Rogers et al. 1998
0.1 (0.02-0.39)	aerial parts; n = 31	Wills and Stuart 1999
0.38	aerial parts; n = 48	Stuart and Wills 2000a
0.066	aerial parts	Stuart and Wills 2000b
0.08 (0.02-0.53)	aerial parts	Qu et al. 2005a
0.15 (0.06-0.34)	nearly matured seed heads	Qu et al. 2005a
0.018	leaf	Kim et al. 2000a
Tetraenes 8/9		
0.001-0.03	aerial parts	Bauer and Remiger 1989
0.42	aerial parts	Bergeron et al. 2000
0.03 (0.01-0.05)	aerial parts; n = 9	Rogers et al. 1998
0.02-1.41	different aerial parts; n = 5	Perry et al. 1997
0.3	inflorescences; n = 46	Binns et al. 2002b
Cichoric acid		
1.3	aerial parts	Becker and Hsieh 1985
3.0	aerial parts	Bergeron et al. 2000
1.12-4.93	aerial parts; n = 9	Letchamo et al. 2002
0.52-2.02	aerial parts; n = 8	Perry et al. 2001
1.29 (0.49-2.14)	aerial parts; n = 31	Wills and Stuart 1999
1.89 (0.48-3.86)	aerial parts	Qu et al. 2005a
2.2, 1.0, 0.4	flowers, leaves, stem, respectively	Bauer et al. 1988b
up to 3.8, 2.9, 1.1	flowers, leaves, stem, respectively	Stuart and Wills 2000a
0.89	inflorescences	Binns et al. 2002b
1.1	inflorescences	Kim et al. 2000a
0.43-3.97	inflorescences	Letchamo et al. 2002
1.09 (0.20-3.16)	[illegible]matured seed heads	Qu et al. 2005a
Caftaric acid		
0.18-0.82	[illegible]erial parts	Pe[illegible]y et[illegible]2001
Cynarin		
< 0.01	aerial [illegible]rts	Pe[illegible]y et[illegible]. 2001
Chlorogenic acid		
< 0.01	aerial parts	Perry et[illegible]. 2001
Echinacoside		
< 0.01	aerial parts	Perry et al. 2001
0.01-0.08	inflorescences	Letchamo et al. 2002
Polysaccharides		
1.26-1.81	stem	Stuart et al. 2004
0.50-1.59	leaf	Stuart et al. 2004
0.34-0.74	inflorescences	Stuart et al. 2004
0.35-1.55	root	Stuart et al. 2004

Values represent multiple commercial samples or material grown under different growing conditions and subject to different forms of handling and analytical methods.

tification in *Echinacea* tissues a challenge. Hence values presented in the literature must be taken as relative rather than absolute values. The most recent data come from researchers in Australia who developed an HPLC method for quantifying polysaccharides in *E. purpurea* (Stuart et al. 2004). According to their findings, the aerial parts contain polysaccharides at concentrations ranging from 5.2 mg/g to 13 mg/g dwt over the growing season, with the highest concentrations generally occurring after the plant senesces (Figure 10). Total polysaccharide concentration was higher in 2nd-year compared to 1st-year plants. The concentration of polysaccharides varies with plant part. The stem (12.2 mg/g) and root (12.1 mg/g) were reported to yield higher concentrations than the leaf (7.7 mg/g) and flower (5.1 mg/g). Polysaccharide II appears to be absent in leaf or flower, while polysaccharide I is present in all aerial parts once flowering has begun (Stuart et al. 2004).

Polysaccharides and glycoproteins have been reported to have immunostimulant activity in vitro and in vivo in laboratory animals, although the oral bioavailability of these compounds remains uncertain (Bauer 1998). According to Bauer (1999a), the fructans have no pharmacological relevance. Classen et al. (2004, 2005) report the arabinogalactans possess immunomodulatory activity. Polysaccharides are poorly extracted and are more likely to be present in aqueous or low-alcohol preparations than those made with

other solvents (see Preparations).

Volatile Constituents and Essential Oils

Limonene, α- and β-pinene, and myrcene are the main naturally occurring volatiles from the *E. purpurea* aerial parts (Mazza and Cottrell 1999). Borneol, bornylacetate, pentadeca-8-*en*-2-one, caryophyllene, caryophyllene epoxide, germacrene D, and palmitic acid are also present in the oil (Bauer 1999a). Both germacrene D and germacra-4(15),5E,10(14)-trien-1β-ol have been found in fresh but not dried aerial parts (Bauer et al. 1988b). Germacrene alcohol is a characteristic compound in fresh plant extracts and is present as a major constituent in homeopathic mother tinctures (Bauer 2000). According to one review, the aerial parts contain less than 0.1% essential oil (Bauer 2000). An earlier review by the same author reported that the essential oil content in the various aerial parts ranged from 0.005% to 0.64%, with the highest quantities in the leaves and flower heads (Bauer and Wagner 1991).

The aerial parts of *E. pallida* and *E. angustifolia* reportedly contain the same essential oil profile as *E. purpurea*, making differentiation of the 3 species using their volatile compounds difficult (Bauer 2000). However, Schulthess et al. (1991) and later Oomah et al. (2006) reported that the oil profile of the fruits differs between the 3 species. Schulthess et al. (1991) found that each species contained compounds that occur only in trace amounts in the other 2 species. *E. purpurea* fruits are characterized by caryophyllene and germacrene D, as well as a high concentration of carvomenthene compared to the fruits of the other 2 species. *E. pallida* fruits contain 1,8-pentadecadiyne and a germacrene D derivative, while *E. angustifolia* fruits contain epithiodihydrol, β-farnesene, and one unidentified compound. Oomah et al. (2006) reported 39 to 47 mg/100 g total tocopherols and 37 to 44 mg/100 g vitamin E in *E. purpurea* fruit. These authors claim to have distinguished the fruits of the 3 species using a variety of methods, including UV/Vis, fluorescence spectra, differential scanning calorimetry, and TLC.

Alkaloids and Labdane Derivatives

E. purpurea whole dried plants were reported to contain glycine betaine at 0.565%, 1.120%, 1.645% in flowers, leaves, and stems, respectively (Soicke et al. 1988). Two pyrrolizidine alkaloids, tussilagine (up to 0.006%) and isotussilagine, were also identified (Röder et al. 1984) and found to lack the 1,2 unsaturated necine ring system required for hepatotoxicity (Mattocks 1986; Röder 1995).

Polyacetylenes

These highly unsaturated lipid soluble compounds are typical of the family *Asteraceae* and are found mainly in the roots, but also to a very small extent in the flower heads. The most abundant of these compounds is pentayne-ene, which is present in buds at 0.5% (Schulte et al. 1967). Several diyne, triyne, and tetrayne compounds have also been isolated in lower amounts. These compounds are unstable and prone to oxidation in dried plant material. *Echinacea* polyacetylenes were neglected in pharmacological studies until it was found that hexane extracts of *E. purpurea* roots and flowers containing polyacetylenes and diacetyleneic amides had near UV-mediated inhibitory activity towards several strains of *Candida* isolated from immunocompromised patients (Binns et al. 2000). This points to the potential of these compounds as antifungal agents.

Flavonoids

Several quercetin and kaempferol glycosides have been reported in *E. purpurea* aerial parts. Leaves reportedly contain 0.48% flavonoids, of which quercetin derivatives and rutin (mean of 0.038% to 0.060%) (Lin et al. 2002) are the major constituents (Bauer 1999a; Bauer and Wagner 1991). Other flavonoids include patuletin-3-*O*-rutinoside (0.015% to 0.021% dwt). In 1989, Cheminat and colleagues reported the occurrence of the following anthocyanins in *E. purpurea* dried flowers: cyanidin 3-*O*-(β-D-glucopyranoside) and cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside). Antioxidant activities have been reported for many of the flavonoids in *Echinacea* (Hu et al. 2004).

Miscellaneous Compounds

E. purpurea, *E. angustifolia*, and *E. pallida* all contain melanin. Concentrations vary between the species and different plant parts (approximately 5% to 10% dwt). Results from animal studies suggest that melanin extracted from different plant parts and species can have highly variable in vitro immunostimulatory activity (see Therapeutics). Because melanin is reportedly poorly soluble except in 90% aqueous phenol, it is unlikely to be present in commercial extracts but may be relevant in crude preparations (Pugh et al. 2005).

ANALYTICAL

The primary analytes of interest for *Echinacea* are total phenolics (caffeic acid derivatives), alkamides, and polysaccharides. The phenolics and alkamides are of quantitative analytical interest because they have been correlated with biological activity in laboratory animals and both are subject to degradation during harvesting, processing, and storage if proper procedures are not followed (see Commercial Sources and Handling). A method for the quantification of polysaccharides would be useful for these same reasons, but a well-validated method for this class of compounds does not currently exist.

For the analysis of *E. purpurea* aerial parts, high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) methods for the quantification of phenolics and alkamides in raw material and finished products are provided. The HPTLC and HPLC phenolics methods provide characteristic fingerprints for the identification of *E. purpurea* aerial parts. The HPLC methods allow for the quantification of phenolics and alkamides.

The caffeic acid derivatives are useful for differentiating *E. purpurea* aerial parts from the aerial parts and roots of *E. angustifolia* and *E. pallida*, both qualitatively and quantitatively. Cichoric and caftaric acids are the predominant caffeic acid derivatives found in *E. purpurea* aerial parts, whereas cynarin is absent and echinacoside and chlorogenic

acid are generally lacking or only occur in trace amounts. *E. purpurea* aerial parts contain much higher levels of cichoric acid (up to 4% dwt) than do the aerial parts of *E. angustifolia* (0.02% to 0.34% dwt) and *E. pallida* (0.12% to 1.6% dwt) or the roots of *E. purpurea* (0.5% to 2.8% dwt), *E. pallida* (0.05% to 0.119% dwt), and *E. angustifolia* (up to 0.1% dwt) (see Constituents). The aerial parts of the 3 commercial species of *Echinacea* do not appear to differ markedly in their alkamide profile, although *E. purpurea* reportedly contains higher levels of these compounds compared to the other 2 species (Bauer et al. 1988b). The aerial parts of the 3 commercial *Echinacea* species are weaker in alkamides compared to the roots. According to 1 report, *E. purpurea* root contains 70% of total plant alkamides, compared to the 30% found in the aerial parts, primarily the flowers (Stuart and Wills 2000a).

Given the potential difficulty in differentiating the commercial species of *Echinacea*, it is best to use a botanical reference standard (e.g., AHP-Verified™) in addition to the chemical reference standards when testing for the identity and quality of *E. purpurea* aerial parts. In addition to being positively identified, the botanical reference standard should be harvested less than 1 year prior to analysis, properly stored, and freshly ground.

High Performance Thin Layer Chromatography (HPTLC) for the Identification of Alkamides and Phenolics in *Echinacea purpurea* Aerial Parts

The HPTLC methods adopted were developed by CAMAG (Muttenz, Switzerland) and Flachsmann AG (Zurich, Switzerland). *E. purpurea* and *E. angustifolia* aerial parts both contain 2,4 diene alkamides and are therefore difficult to differentiate using HPTLC alkamide profiles alone. The phenolics method has been proposed for inclusion in the *European Pharmacopoeia* (Pharmeuropa 2004). This method allows for differentiation between the aerial parts of *E. purpurea* and *E. angustifolia* and between the seeds of these species and *E. pallida*. Of the phenolic standards used, caftaric acid and cichoric acid are characteristic of *E. purpurea* aerial parts, while echinacoside, chlorogenic acid, and cynarin are typically absent or present only in trace amounts. All of the phenolic standards are included in the method so that adulteration can be screened for and the 3 commercial species of *Echinacea* compared. However, while caftaric and cichoric acid standards are required for analysis of *E. purpurea* aerial parts, the other standards are optional.

1) Analysis of Alkamides

Sample Preparation

Place 1 g of freshly powdered herb and/or seeds (or crushed tablets) in a flask, add 10 mL of dichloromethane, and sonicate for 5 minutes. Filter the extract through a 0.2 µm syringe filter. This is the test solution. Hydroalcoholic extracts and juice preparations can be applied directly to the plate.

Standard Preparation

Individually dissolve 2 mg of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide (available from Chromadex, US and Phytolab, Germany) and β-sitosterol (AHP-Verified™) in 10 mL methanol each. These are the standard solutions.

Note: The identity and purity of AHP-Verified™ reference standards has been confirmed. AHP-Verified™ standards are available from Chromadex, Santa Ana, CA.

Reagent Preparation

Anisaldehyde reagent: 1 mL of anisaldehyde, 20 mL of acetic acid (100%), and 170 mL of cold methanol are mixed in this order. While cooling with ice, 10 mL of sulfuric acid are carefully added.

Chromatographic Conditions

Stationary Phase:

HPTLC plates 10 x 10 cm or 20 x 10 cm silica gel 60 F254 (Merck or equivalent).

Relative Humidity:

30% to 35%.

Mobile Phase:

Toluene:ethyl acetate:cyclohexane:formic acid (24:6:3:0.9).

Sample Application:

5 µL of the test solution and 2 µL of the standards are applied each as a 10-mm band. Application position should be 8 mm from the lower edge of the plate.

Note: Application volume may need to be increased for older or degraded plant samples. It may also need to be adjusted when very concentrated hydroalcoholic extracts are applied in order to increase separation.

Development:

10 x 10 cm or 20 x 10 cm Twin Trough Chamber (CAMAG or equivalent), saturated for 10 minutes (filter paper inserted) with 5 or 10 mL developing solvent, respectively, per trough. Developing distance should be 60 mm from the lower edge of the plate. Dry plate in a stream of cold air for 5 minutes.

Detection:

a) UV 254 nm.

b) Anisaldehyde reagent: Immerse plate in reagent for 1 second, heat to 105 °C for 3 minutes. Examine the plate under white light.

c) Examine the derivatized plate under UV 366 nm.

Results:

Compare to the chromatograms provided (Figures 15-17).

2) Analysis of Phenolics

Sample Preparation

Place 1 g of freshly powdered herb and/or seed (or crushed tablets) in a flask, add 10 mL of methanol, and sonicate for 5 minutes. Filter the extract through a 0.2 µm syringe filter. This is the test solution. Hydroalcoholic extracts and juice preparations can be applied directly to the plate.

Standards Preparation

Individually dissolve 0.5 mg of caftaric acid* (AHP-Verified™), 0.5 mg of cichoric acid* (AHP-Verified™), 2 mg of echinacoside (AHP-Verified™), 2 mg of cynarin (AHP-

Verified™), 1 mg of chlorogenic acid (AHP-Verified™), and 1 mg of caffeic acid (AHP-Verified™) in 10 mL methanol each. These are the standard solutions.

* Required; other standards are optional.

Note: The identity and purity of AHP-Verified™ reference standards has been confirmed. AHP-Verified™ standards are available from ChromaDex, Santa Ana, CA.

Reagent Preparation

Natural Products reagent (NP reagent): 1 g of diphenylborinic acid aminoethylester is dissolved in 200 mL of ethyl acetate.

Chromatographic Conditions

Stationary Phase:

HPTLC plates 10 x 10 cm or 20 x 10 cm silica gel 60 F254 (Merck or equivalent).

Mobile Phase:

Ethyl acetate:ethylmethyl ketone:formic acid:water (15:9:3:3).

Sample Application:

5 µL (herb) or 20 µL (seeds) of the test solution and 2 µL of the standards are applied each as a 10-mm band. Application position should be 8 mm from the lower edge of the plate.

Note: Application volume may need to be adjusted when very concentrated hydroalcoholic extracts are applied in order to increase separation.

Development:

10 x 10 cm or 20 x 10 cm Twin Trough Chamber (CAMAG or equivalent), saturated for 10 minutes (filter paper) with 5 or 10 mL developing solvent, respectively, per trough. Developing distance should be 8 mm from the lower edge of the plate. Dry plate in a stream of cold air for 5 minutes.

Detection:

NP reagent: Heat the plate to 100 °C for 2 minutes, immerse the warm plate in the reagent for 1 second, then dry it in a stream of cold air. Examine the plate under UV 366 nm.

Note: Detection using UV 254 nm has not been included in the HPTLC assay for caffeic acid derivatives because it did not provide any additional information.

Results:

Compare to the chromatograms provided (Figures 18-20).

Note: In the analysis of liquid and dry extracts, the chromatographic fingerprint may differ substantially from the raw material depending on the manufacturing process, solvents used, and herb-to-extract ratio.

High Performance Liquid Chromatography (HPLC) for the Analysis of Total Alkamides in *Echinacea purpurea* Aerial Parts

The following method serves to quantify alkamides in *Echinacea* raw material and finished products. The method is based upon the gradient dual-wavelength detection method of Bauer and Remiger (1989) and has been subjected to the methods validation program of AHP using both raw material and a variety of finished products. Of the 4 naturally occurring alkamide reference standards used in this method, only the 2 dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide isomer standards are required for conformity with the quantitative portion of the AHP pharmacopoeial definition of *E. purpurea* aerial parts.

In the AHP *Echinacea purpurea* Root monograph (AHP 2004), an isocratic version of this method with only single-wavelength detection was adopted. This was very similar to the method adopted by the United States Pharmacopeia (USP 29-NF 24 2006) in their *Echinacea purpurea* Aerial Parts monograph, except that USP used reflux extraction rather than sonication. Sonication was found to give comparable results to the more time-intensive reflux procedure. The previous method of AHP and USP utilized a synthetic non-naturally occurring alkamide standard (2*E*,4*E*-hexadienoic acid isobutylamide). This was replaced with 4 newly available naturally occurring alkamide standards that account for the majority of the alkamides occurring in commercial *Echinacea* species. Separate calibration curves for the 4 standards were generated, allowing for a more accurate quantification of alkamides. Dual wavelength detection and a gradient mobile phase are used in order to optimize the method based on the new set of reference standards. These modifications to the original method increase the accuracy of the analytical results.

Reagents

Water, HPLC grade or Nanopure

Water, de-ionized (for sample preparation)

Ethanol, HPLC grade

Acetonitrile, HPLC grade

Sample Preparation

Aerial Parts: Accurately weigh 0.15 g of freshly powdered herb and/or seed and place it into a 40-mL amber vial. Add 3 mL of ethanol:water (70:30) and sonicate for 5 minutes. After allowing 5 minutes for the solid materials to settle down, collect the supernatant into a 10-mL volumetric flask. Repeat the sonication process 2 more times. Pool all of the supernatants and adjust to volume with ethanol:water (70:30).

Formulated *Echinacea* Extracts: Accurately weigh 0.15 g of the powdered sample, place it into a 40-mL amber flask, add 3 mL of de-ionized water, and shake for 1 minute by hand. Add 7 mL of ethanol:water (70:30) followed by sonication for 30 minutes. Filter the sample through a 0.45-µm polyvinylidene fluoride (PVDF) filter. The sample is then ready for HPLC analysis.

Liquid Extracts: Dilute the sample with ethanol:water (70:30) so the concentration of the individual analytes falls within the range of the corresponding calibration curve. Trials using different dilution levels may be necessary to determine the level that gives a response within the calibration range.

Standard Preparation

Prepare separate stock solutions of dodeca-2*E*,4*E*-dienoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide standards at a concentration of 1 mg/mL in ethanol:water (70:30). From each stock solution, prepare

(continued on page 28)

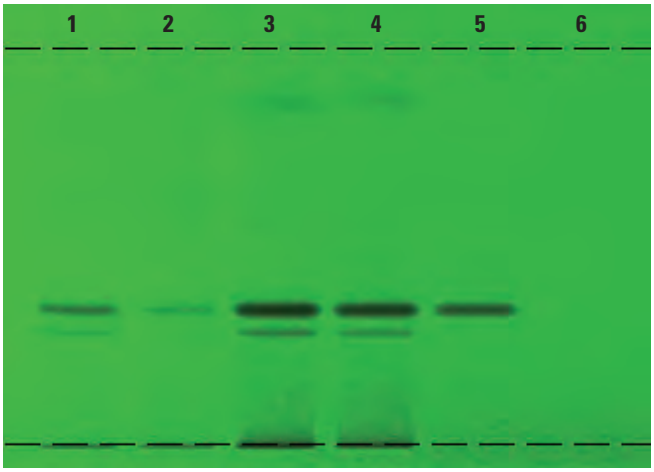

Figure 15a **UV 254 nm**

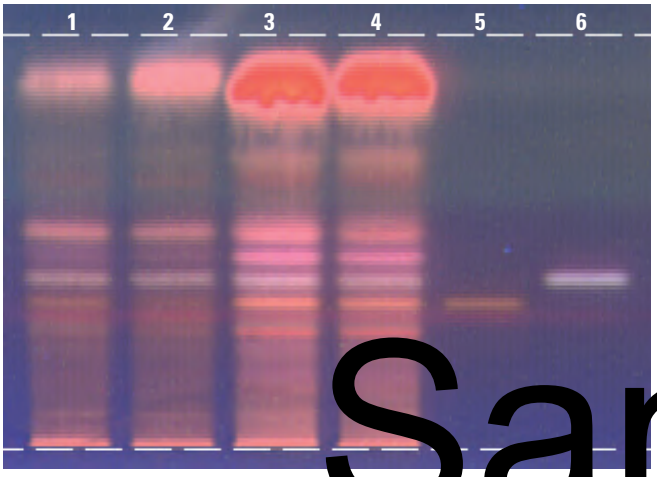

Figure 15b **Anisaldehyde reagent, [illegible] nm**

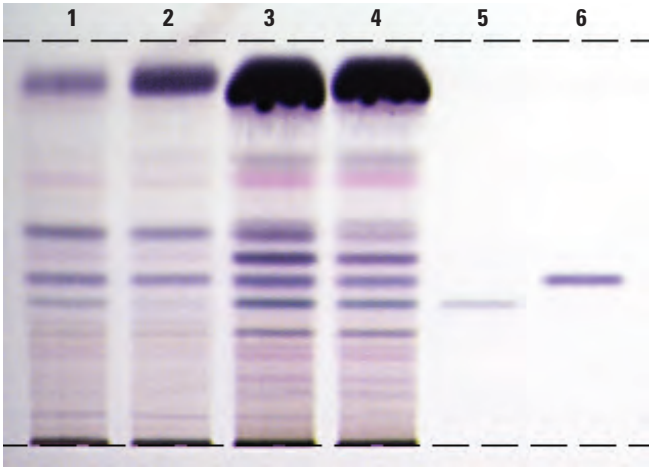

Figure 15c **Anisaldehyde reagent, white light**

Figure 15a-c HPTLC of alkamides in *Echinacea purpurea* aerial parts and fruit

Lane 1: *E. purpurea* aerial parts
Lane 2: *E. purpurea* aerial parts
Lane 3: *E. purpurea* fruit
Lane 4: *E. purpurea* fruit
Lane 5: dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic acid isobutylamide
Lane 6: β-sitosterol

Discussion of Chromatograms

15a) UV 254 nm: The isobutylamide standard (Lane 5) shows a fluorescence quenching band at R_f ~0.35. A band corresponding in color and position to the isobutylamide standard is found in *E. purpurea* aerial parts (Lanes 1 and 2) and fruit (Lanes 3 and 4), with higher concentrations in the fruit. There is an additional band below the isobutylamide standard in all samples and the fruit samples show a faint band near the solvent front. The standard β-sitosterol (Lane 6) is not visible using this method of detection.

15b) Anisaldehyde reagent, UV 366 nm: The isobutylamide standard (Lane 5) shows a faint orange-brown fluorescent band at R_f ~0.35 and the β-sitosterol standard (Lane 6) shows a pink to violet band at R_f ~0.40. Bands corresponding in color and position to the standards are found in *E. purpurea* aerial parts (Lanes 1 and 2) and fruit (Lanes 3 and 4), with higher concentrations in the fruit. All samples show an intense pink to red band near the solvent front. The *E. purpurea* aerial parts show 1 intense fluorescent band above β-sitosterol. Both fruit samples show 2 bands above β-sitosterol, 1 band below the isobutylamide standard, and another band at R_f ~0.70.

15c) Anisaldehyde reagent, white light: The isobutylamide standard (Lane 5) shows a gray-violet band at R_f ~0.35 and the β-sitosterol standard (Lane 6) shows a violet band at R_f ~0.40. Bands corresponding in color and position to the standards are found in *E. purpurea* aerial parts (Lanes 1 and 2) and fruit (Lanes 3 and 4). All samples show an intense band near the solvent front. The *E. purpurea* aerial parts show 1 intense fluorescent band above β-sitosterol. Both fruit samples show 2 bands above β-sitosterol, 1 band below the isobutylamide standard, and pink and violet zones at R_f ~0.70.

Note: The method is sensitive to changes in relative humidity. The chromatograms were developed at a relative humidity of 30% to 35%. If the humidity is higher (e.g. 45%), the R_f of the bands may change: dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic acid isobutylamide may change from R_f ~0.35 to R_f ~0.30, β-sitosterol will remain at R_f ~0.40, and the characteristic band above β-sitosterol in the samples may shift.

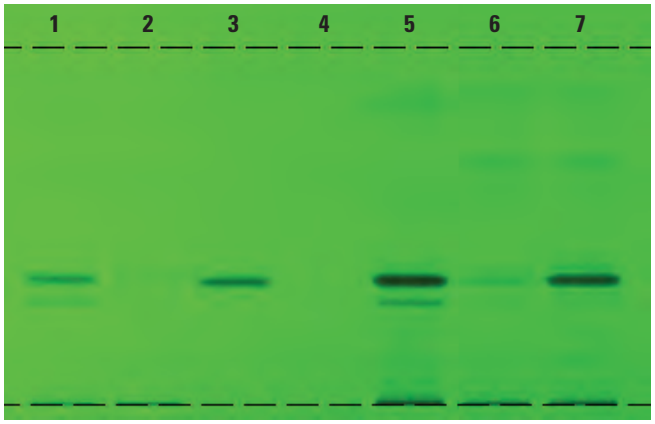

Figure 16a UV 254 nm

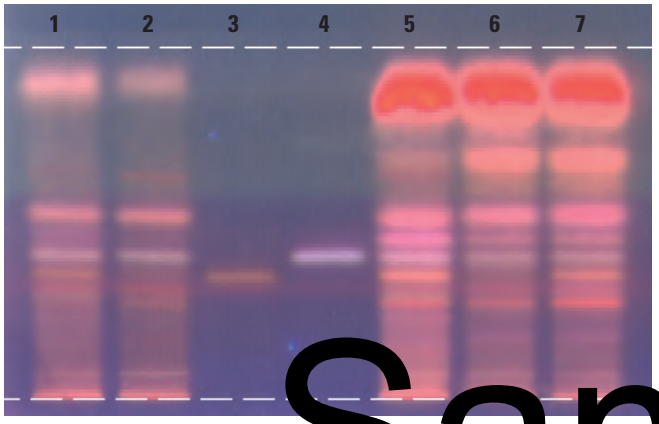

Figure 16b Anisaldehyde reagent, UV 366 nm

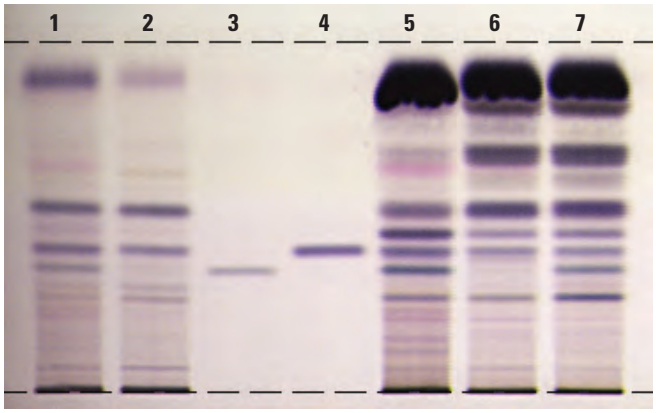

Figure 16c Anisaldehyde reagent, white light

Figure 16a-c HPTLC of alkamides in *Echinacea purpurea*, *E. angustifolia*, and *E. pallida* aerial parts and fruit

Lane 1: *E. purpurea* aerial parts
Lane 2: *E. angustifolia* aerial parts
Lane 3: dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide
Lane 4: β-sitosterol
Lane 5: *E. purpurea* fruit
Lane 6: *E. pallida* fruit
Lane 7: *E. angustifolia* fruit

Discussion of Chromatograms

16a) UV 254 nm: The isobutylamide standard (Lane 3) shows a fluorescence quenching band at R_f ~0.35. A band corresponding in color and position to the isobutylamide standard is found in the aerial parts (Lanes 1 and 2) and fruit samples (Lanes 5-7) of all the Echinacea species. There is an additional band below the isobutylamide standard in *E. purpurea* aerial parts (Lane 1) and fruit (Lane 5) and in *E. angustifolia* fruit (Lane 7). *E. pallida* (Lane 6) and *E. angustifolia* (Lane 7) fruit show a faint band at R_f ~0.70. All fruit samples show a band near the solvent front. The standard β-sitosterol (Lane 4) is not visible using this method of detection.

16b) Anisaldehyde reagent, UV 366 nm: The isobutylamide standard (Lane 3) shows a faint orange-brown fluorescent band at R_f ~0.35 and the β-sitosterol standard (Lane 4) shows a pink to violet band at R_f ~0.40. A band corresponding in color and position to the isobutylamide standard is found in *E. purpurea* aerial parts (Lane 1) and fruit (Lane 5) and in *E. angustifolia* fruit (Lane 7). The presence of isobutylamide in *E. angustifolia* aerial parts (Lane 2) and *E. pallida* fruit (Lane 6) is not definitive. A band corresponding to β-sitosterol is s[illegible] in all samples. [illegible] samples show an intense orange-to-red ban[illegible] near th[illegible] so[illegible]ent [illegible]nt. *E. purpurea* (Lane 1) and *E. angustifolia* (La[illegible] 2) ae[illegible] p[illegible]s h[illegible]ve similar fingerprints and show 1 intense fluo[illegible] band [illegible]bove [illegible]erol. Fruit samples from all 3 species sho[illegible] 2 bands above β-sitosterol and 1 band below the position of the isobutylamide standard. The fruits of *E. pallida* and *E. angustifolia* show an intense band and that of *E. purpurea* a faint band at R_f ~0.70.

16c) Anisaldehyde reagent, white light: The isobutylamide standard (Lane 3) shows a grayish-violet band at R_f ~0.35 and the β-sitosterol standard (Lane 4) shows a violet band at R_f ~0.40. A band corresponding in color and position to the isobutylamide standard is found in *E. purpurea* aerial parts (Lane 1) and fruit (Lane 5) and in *E. angustifolia* fruit (Lane 7). The presence of isobutylamide in *E. angustifolia* aerial parts (Lane 2) and *E. pallida* fruit (Lane 6) is not definitive. All samples show an intense gray to black band near the solvent front. *E. purpurea* and *E. angustifolia* aerial parts show 1 intense fluorescent band above β-sitosterol. Fruit samples from all 3 species show 2 bands above β-sitosterol, 1 band below the position of the isobutylamide standard, and pink and violet zones around R_f ~0.70. In *E. purpurea* fruit, the pink zone is dominant, while in *E. pallida* and *E. angustifolia* the violet zone is dominant.

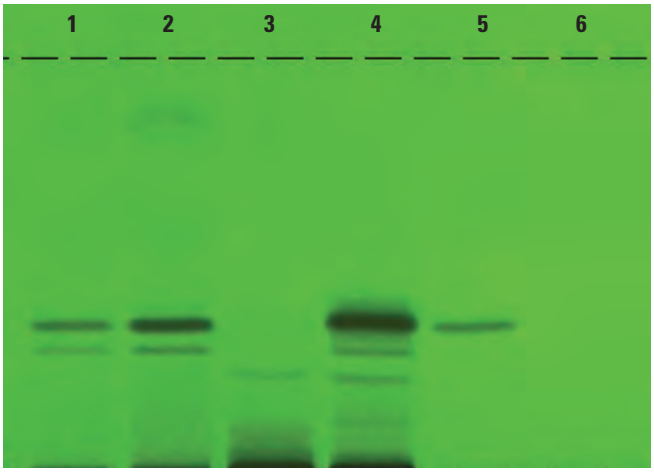

Figure 17a UV 254 nm

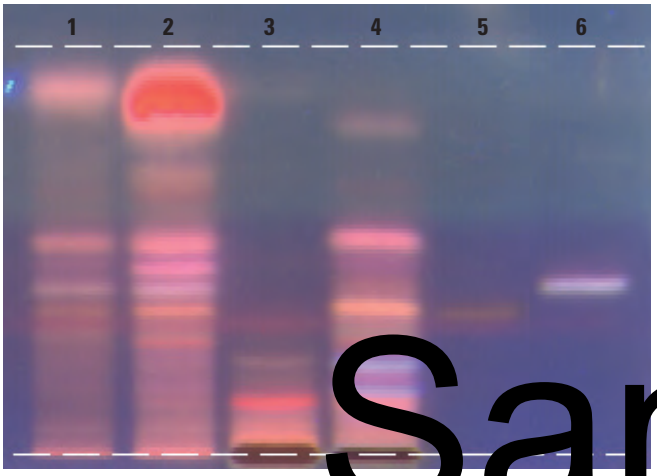

Figure 17b Anisaldehyde reagent, UV 366 nm

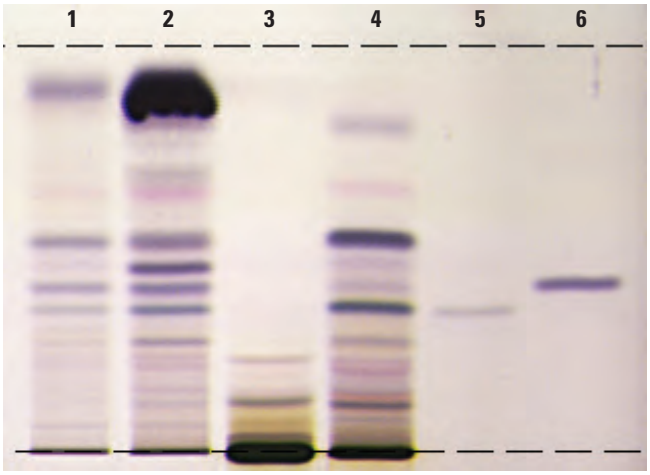

Figure 17c Anisaldehyde reagent, white light

Figure 17a-c HPTLC of alkamides in *Echinacea purpurea* aerial parts, fruit, and commercial products

Lane 1: *E. purpurea* aerial parts
Lane 2: *E. purpurea* fruit
Lane 3: *E. purpurea* expressed juice (stem, leaf, and flower juice in ethanol and water)
Lane 4: *E. purpurea* hydroalcoholic tincture (root, leaf, and flower juice, with mature fruit)
Lane 5: dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic acid isobutylamide
Lane 6: β-sitosterol

Discussion of Chromatograms

17a) UV 254 nm: The isobutylamide standard (Lane 5) shows a fluorescence quenching band at R_f ~0.35. A band corresponding in color and position to the isobutylamide standard, as well as another one below it, are found in *E. purpurea* aerial parts (Lane 1) and fruit (Lane 2) samples, as well as the tincture (Lane 4). The chromatogram of the expressed juice product (Lane 3) bears little similarity to the other samples and has very faint bands in the lower R_f region. The *E. purpurea* fruit sample shows a broad band near the solvent front. The standard β-sitosterol (Lane 6) is not visible using this method of detection.

17b) Anisaldehyde reagent, UV 366 nm: The isobutylamide standard (Lane 5) shows a faint orange-brown fluorescent band at R_f ~0.35 and the β-sitosterol standard (Lane 6) shows a pink to violet band at R_f ~0.40. Bands corresponding in color and position to the standards are found in the *E. purpurea* aerial parts (Lane 1) and fruit (Lane 2) samples. A band corresponding to the isobutylamide standard is present in the tincture (Lane 4). The aerial part and fruit samples show an intense orange-to-red band near the solvent front. The aerial parts show 1 intense fluorescent zone above β-sitosterol, while the fruit shows 2 zones above β-sitosterol and 1 zone below the isobutylamide standard. The chromatogram of the expressed juice (Lane 3) bears little similarity to the other samples, with intense red and orange bands in the lower R_f region. The 2 light blue bands and 1 pink band in the lower R_f region of the tincture chromatogram (Lane 4) differ from what is seen in the raw material samples.

17c) Anisaldehyde reagent, white light: The isobutylamide standard (Lane 5) shows a grayish-violet band at R_f ~0.35 and the β-sitosterol standard (Lane 6) shows a violet band at R_f ~0.40. Bands corresponding in color and position to the standards are found in the *E. purpurea* aerial part (Lane 1), fruit (Lane 2), and tincture (Lane 4) samples. The aerial part and fruit samples show an intense gray to black band near the solvent front. The aerial parts show 1 intense zone above β-sitosterol and pink and violet zones at R_f ~0.70. The fruit shows 2 bands above β-sitosterol and 1 below the isobutylamide standard, with additional pink and a violet zones at R_f ~0.70 and fainter bands in the lower R_f region. The chromatogram of the expressed juice product (Lane 3) bears little similarity to the other samples and has 2 primary bands in the lower R_f region. The chromatogram of the tincture (Lane 4) is similar to that of the fruit in the middle R_f region in terms of band position and number, but not intensity.

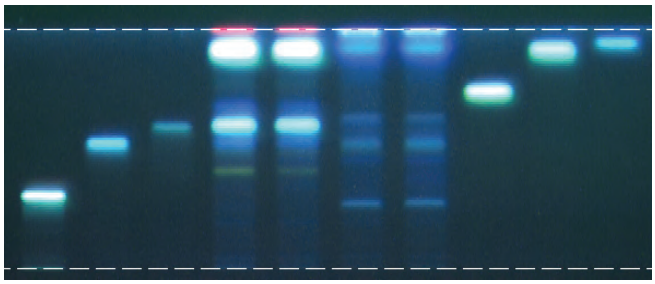

Figure 18 HPTLC of caffeic acid derivatives in *Echinacea purpurea* aerial parts and fruit. Natural Products reagent, UV 366 nm

Lane 1: Echinacoside (R_f ~0.25)
Lane 2: Chlorogenic acid (R_f ~0.45)
Lane 3: Caftaric acid (R_f ~0.50)
Lane 4: *E. purpurea* aerial parts
Lane 5: *E. purpurea* aerial parts
Lane 6: *E. purpurea* fruit
Lane 7: *E. purpurea* fruit
Lane 8: Cynarin (R_f ~0.65)
Lane 9: Cichoric acid (R_f ~0.84)
Lane 10: Caffeic acid (R_f ~0.87)

Discussion of Chromatogram

18) Natural Products reagent, UV 366 nm: All standards (Lanes 1-3 and 8-10) show faint to strong greenish-blue to white fluorescent bands. Bands corresponding in color and position to the cichoric acid (Lane 9), caftaric acid (Lane 3), and chlorogenic acid (Lane 2) standards only appear in *E. purpurea* aerial parts (Lanes 4 and 5). Several additional fluorescent bands are seen in the samples. In the fruit, a blue zone occurs slightly below the position of the echinacoside standard and should not be confused with that standard. In the aerial parts, faint yellow bands are seen at approximately R_f ~0.35 and a red fluorescent band is present at the solvent front. Echinacoside, cynarin, and caffeic acid appear to be lacking in all samples, which is generally consistent with the literature.*

*In some samples of *E. purpurea* aerial parts, echinacoside and caffeic acid may be present in trace amounts (Binns et al. 2002b; Letchamo et al. 1999, 2002).

Note: The R_f of the standards may vary with changes in relative humidity; however, the separation sequence will remain comparable.

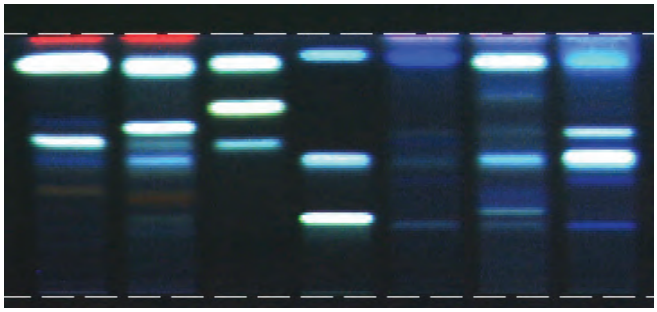

Figure 19 HPTLC of caffeic acid derivatives in *Echinacea purpurea*, *E. angustifolia*, and *E. pallida* aerial parts and fruit. Natural Products reagent, UV 366 nm

Lane 1: *E. purpurea* aerial parts
Lane 2: *E. angustifolia* aerial parts
Lane 3: Caftaric acid, cynarin, cichoric acid (with increasing R_f)
Lane 4: Echinacoside, chlorogenic acid, caffeic acid (with increasing R_f)
Lane 5: *E. purpurea* fruit
Lane 6: *E. pallida* fruit
Lane 7: *E. angustifolia* fruit

Discussion of Chromatogram

19) Natural Products reagent, UV 366 nm: All standards (Lanes 3 and 4) show faint to strong greenish-blue to white fluorescent bands. Bands corresponding in color and position to the cichoric acid (Lane 3, upper zone), caftaric acid (Lane 3, lower zone), and chlorogenic acid (Lane 4, middle zone) standards are found in *E. purpurea* and *E. angustifolia* aerial parts (Lanes 1 and 2). Cichoric acid is present in the fruit of *E. pallida* (Lane 6) and *E. angustifolia* (Lane 7) but not in that of *E. purpurea* (Lane 5). In all fruit samples a faint band corresponding to the chlorogenic acid standard is present, while caffeic acid is absent. A blue zone that occurs slightly below the position of the echinacoside standard in all fruit samples should not be confused with that standard. In the aerial parts samples, a red fluorescent band is present at the solvent front. Several additional fluorescent bands are seen in all samples. Echinacoside, cynarin, and caffeic acid appear to be lacking in all samples.

(continued from pg 23)

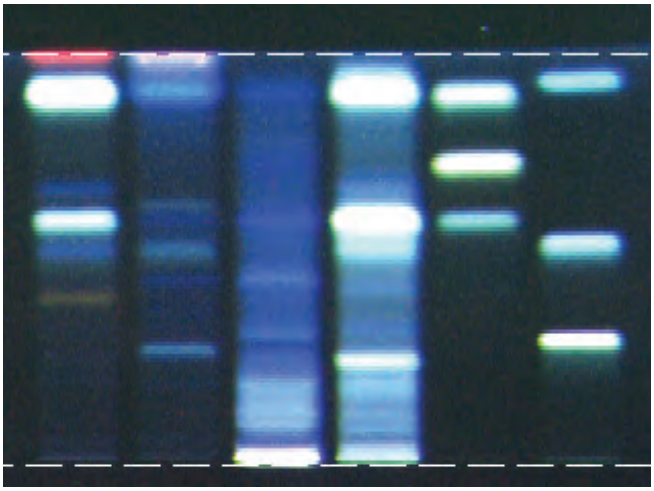

Figure 20 HPTLC of caffeic acid derivatives in *Echinacea purpurea* aerial parts, fruit, and commercial products. Natural Products reagent, UV 366 nm

Lane 1: *E. purpurea* aerial parts
Lane 2: *E. purpurea* fruit
Lane 3: *E. purpurea* expressed juice (stem, leaf, and flower juice in ethanol and water)
Lane 4: *E. purpurea* hydroalcoholic tincture (root, leaf, and flower juice, with mature fruit)
Lane 5: Caftaric acid, cynarin, cichoric acid (with increasing R_f)
Lane 6: Echinacoside, chlorogenic acid, caffeic acid (with increasing R_f)

Discussion of Chromatogram

20) Natural Products reagent, UV 366 nm: All standards (lanes 5 and 6) show faint to strong greenish-blue to white fluorescent bands. Bands corresponding in color and position to the cichoric acid (Lane 5, upper zone) and caftaric acid (Lane 5, lower zone) standards are found in *E. purpurea* aerial parts (Lane 1). In the fruit (Lane 2), there is a faint band corresponding to the chlorogenic acid standard (Lane 6, middle zone), caftaric acid is not present, and although there is a blue band at the level of caffeic acid, it is a slightly different color than the reference standard. In the fruit sample, a blue zone occurs slightly below the position of the echinacoside standard and should not be confused with that standard. In the aerial parts, a red fluorescent band is present at the solvent front. The tincture (Lane 4) shows bands corresponding to the cichoric acid, caftaric acid, and chlorogenic acid standards, as well as several additional blue fluorescent bands. The chromatogram of the expressed juice product (Lane 3) bears little similarity to the other samples. It is characterized by a white zone at the level of application, a broad blue zone in the lower R_f region, and 3 blue bands in the middle R_f region, including 1 band corresponding to the caftaric acid standard. Several additional fluorescent bands are seen in all samples.

a working standard mixture combining each of the standards at a concentration of 100 µg/mL. From the working standard, prepare calibration standards with concentrations of 1, 10, 20, and 50 µg/mL, and include the 100 µg/mL working standard in the calibration.

Note: Standards are available from ChromaDex, Santa Ana, CA. For purposes of species differentiation other alkamide standards are available.

Stability and Storage of Preparations

The sample and standard solutions are stored in amber vials and refrigerated.

Linearity Range

The linearity range of the method is 1 µg/mL to 100 µg/mL. The correlation coefficient (r^2) must be ≥ 0.995.

Chromatographic Conditions

Column:
Luna, 5 µm, C-18(2), 100A, 250 x 4.6 mm
(Phenomenex, Torrance, CA).
Mobile Phase: Gradient
A: De-ionized water.
B: Acetonitrile.

Time (min)	%A	%B
00.10	50	50
26.00	50	50
30.00	20	80
35.50	20	80
35.10	50	50
40.00	50	50

Flow Rate: 1.[illegible] mL/min
Detection: UV 210 & 259 nm dual wavelength monitoring
UV 210 nm is used for peaks 1 & 2: undeca-2*E*-ene-8,10-diynoic acid isobutylamide, dodeca-2*E*-ene-8,10-diynoic acid isobutylamide (for *E. angustifolia*).
UV 259 nm is used for peaks 3-5: dodeca-2*E*,4*E*-dienoic acid isobutylamide, and dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide (for *E. purpurea*).

Injection Volume:	5 µL.
Column Temperature:	30 °C.
Run Time:	40 min.

Elution Order:

Peak	Relative retention time (min)	Compound
1	~9.85	Undeca-2*E*-ene-8,10-diynoic acid isobutylamide
2	~13.9	Dodeca-2*E*-ene-8,10-diynoic acid isobutylamide
3	~23.1	Dodeca-2*E*, 4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide
4	~24	Dodeca-2*E*, 4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide
5	~34	Dodeca-2*E*, 4*E*-dienoic acid isobutylamide

Quantification

Inject each standard preparation one time and generate

a standard curve based on the peak area versus concentration in µg/mL for each compound. Quantify the alkamides in the samples using the linear equation based on least squares regression for each alkamide compound. This procedure quantifies the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide isomers as the sum of the 2 peaks, both clearly separated in the chromatogram (Figure 21), using the dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic isomer.

Calculation

Total alkamide content (% w/w) is calculated using the following equation:

$$\%w/w = \frac{Sum\_Concentration\ (\mu g/mL) \times Sample\_volume\ (mL)}{Sample\_mass\ (g) \times 1000000} \times 100$$

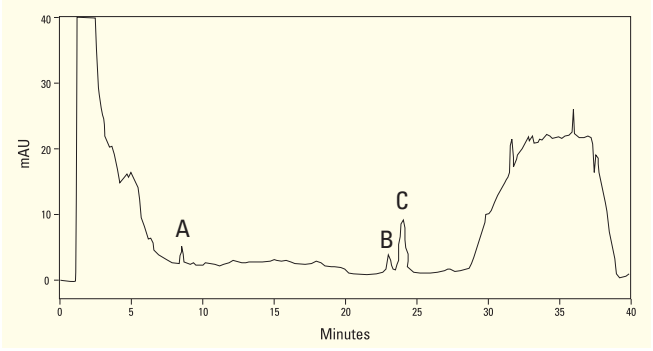

Figure 21a HPLC chromatogram of alkamides in the aerial parts of *Echinacea purpurea* using 2[illegible] nm detection (can be used for species differentiation between *E. purpurea* aerial parts and *E. angustifolia* roots).

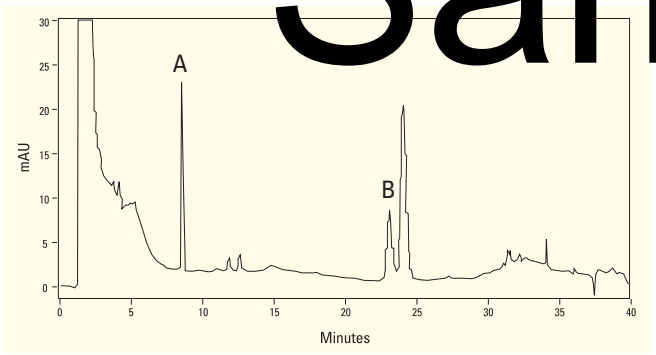

Figure 21b HPLC chromatogram of alkamides in the aerial parts of commercial species of *Echinacea purpurea* using 259 nm detection (primary chromatogram for identification of alkamides in *E. purpurea* aerial parts).

A. Undeca-3*E*, 4*Z*-diene-8,10-diynoic acid isobutylamide

B + C. Dodeca-2*E*,4*E*, 8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide.

High Performance Liquid Chromatography (HPLC) for the Analysis of Total Phenolics in *Echinacea purpurea* Aerial Parts

The following method for the HPLC analysis of total phenolics in *Echinacea* was validated for raw material using the single-lab validation procedure of AOAC International. The method may also be applied to finished products. Validation for various matrices is under way. The method is based upon the one adopted by AHP in its monograph on *Echinacea purpurea* Root (AHP 2004). That method was originally developed by the NSF International Methods Validation Program (NSF/MVP) of the Institute for Nutraceutical Advancement (INA). Of the 5 reference standards used in the method, only cichoric acid is required for conformity with the quantitative portion of the AHP pharmacopoeial definition of *E. purpurea* aerial parts.

Based on the results obtained in an extraction study, the original INA extraction procedure was modified to improve extraction efficiency. Due to the lack of readily available phenolics standards at the time, the original method used chlorogenic acid as the reference standard, and quantified other phenolics, including caftaric acid, cichoric acid, and echinacoside, with pre-determined correction factors. The limitation of using a single standard to quantify different compounds is that the correction factors may change with instrumentation, which makes it necessary to re-establish these correction factors when the method is used in a different laboratory. Since all these standards have become commercially available, modifications were made to employ 5 reference standard compounds to generate separate calibration curves instead of using chlorogenic acid alone as the calibration standard. The total phenolic content is represented as the sum of all the phenolic compounds present in the sample. These modifications make the method more reproducible, reliable and direct, especially when it is used in a multi-laboratory or multi-instrument context.

Reagents

Water, HPLC grade or Nanopure
Water, acidified (for sample preparation)
Methanol, HPLC grade
Acetonitrile, HPLC grade
Phosphoric acid (0.1% aqueous)

Sample Preparation

Grind the sample to 60-mesh size. Accurately weigh about 150 mg of ground raw material into a 50-mL centrifuge tube. Add exactly 25 mL of methanol:water (60:40) to the centrifuge tube. Agitate the sample on a rotator or wrist shaker for 60 minutes. Centrifuge the tube at 5000 rpm for 5 minutes to settle the solids. Filter a portion of the supernatant through a 0.45 µm polyvinylidene fluoride (PVDF) or polytetra-fluoroethylene (PTFE) syringe filter into an HPLC autosampler vial.

Standard Preparation

Calculate, based on the stated purity of the standard, the required amount of caftaric acid (AHP-Verified™), chlorogenic acid (AHP-Verified™), echinacoside (AHP-Verified™), cynarin (AHP-Verified™), and cichoric acid standard (AHP-Verified™) to obtain 1000 µg/mL when dissolved in 10 mL of methanol. Accurately weigh the required calculated amount of each of the phenolic standards into separate 10-mL amber volumetric flasks. Bring to half volume with methanol and mix. Bring to final volume using methanol and mix by inverting the flask. From the stock solutions, prepare a working standard mixture with caftaric acid, chlorogenic acid, cynarin, and cichoric acid standards

at a concentration of 100 µg/mL and with echinacoside at 200 µg/mL. From the working standard mixture, prepare calibration standards with concentrations of 2, 10, 20, 50, 100, 150, and 200 µg/mL for echinacoside; 1, 5, 10, 25, 50, 75, and 100 µg/mL for caftaric acid and cichoric acid; and 0.2, 1, 2, 5, 10, 15, and 20 µg/mL for chlorogenic acid and cynarin. Do not include the 0.2 µg/mL concentration for the generation of the chlorogenic acid and cynarin standard curves, as this value falls below the established linear range.

Note: The identity and purity of AHP-Verified™ reference standards has been confirmed. AHP-Verified™ standards are available from ChromaDex, Santa Ana, CA.

Stability and Storage of Preparations

The stock standard solutions are stored at -20 °C protected from light.

Linearity Range

The linearity range of the method is 2 µg/mL to 200 µg/mL for echinacoside and 1 µg/mL to 100 µg/mL for caftaric acid, chlorogenic acid, cynarin, and cichoric acid. The correlation coefficient is ≥ 0.995.

Chromatographic Conditions

Column*: Cosmosil 5C-18-AR-II, 4.6 x 150 mm, 5.0 µm.

Mobile Phase: Gradient

A: 0.1% Phosphoric acid in water.

B: Acetonitrile.

Time (min)	%A	%B
0.10	90	10
13.00	78	22
14.00	60	40
14.50	60	40

Post Time: 3.3 min.

Flow Rate: 1.5 mL/min.

Detection: 330 nm.

Injection Volume: 5 µL.

Column Temperature: 35 °C.

Run Time: 14.5 min.

Elution Order: Caftaric acid, chlorogenic acid, cynarin, echinacoside, cichoric acid.

* A column by Phenomenex Prodigy, 250 x 4.6mm, 5 µm, ODS(3) 100A was found to be an appropriate alternative.

Quantitation

Inject each standard preparation one time and generate a standard curve based on the peak area versus concentration in µg/mL for each compound. The samples are quantified using a linear equation based on least squares regression for each phenolic compound (Figure 22).

Calculation

The total phenolics content (%w/w) is calculated using the following equation:

$$\%w/w = \frac{Sum\_Concentration\ (\mu g/mL) \times Sample\_volume\ (mL)}{Sample\_mass\ (g) \times 1000000} \times 100$$

The following columns were evaluated and found to be ineffective in achieving analyte resolution under the specified gradient conditions. These columns may be suitable under different gradient/mobile phase conditions:

Phenomenex: Luna C-18(2), 250 x 4.6 mm, 5 µm. Cat #: 00G-4252-E0

Luna phenyl-hexyl, 250 x 4.6 mm, 5 µm. Cat #: 00G-4257-E0

Inertsil phenyl, 150 x 2.00 mm. Cat #: 00F-3137-B0

Gemini C-18 110A, 150 x 4.6 mm, 5 µm. Cat #: 00F-4435-E0

Restek: Ultra C-18, 250 x 4.6 mm, 5 µm. Cat#: 9174575-700

Ultra Aqueous C-18, 250 x 4.6 mm, 5 µm. Cat#: 9178575-700

Cosmosil: 5C-18-PAQ, 150 mm x 4.6 mm, 5 µm.

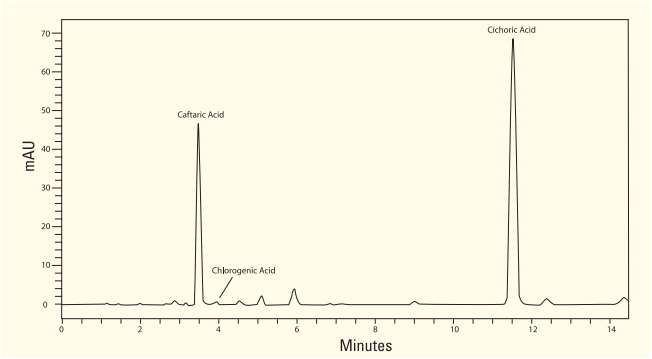

Figure 22 HPLC chromatogram of caffeic acid derivatives in the aerial parts of *Echinacea purpurea*

Quantitative Standards

Foreign Organic Matter:
Not to exceed 2% (USP 29-NF 24 2006).

Total Ash:
Not to exceed 10%, determined on 3 g of powdered plant material (USP 29-NF 24 2006).

Acid-insoluble Ash:
Not to exceed 2.5% (USP 29-NF 24 2006).

Loss of Moisture on Drying:
Not to exceed 12% (USP 29-NF 24 2006), determined on drying 1 g powdered plant material.

THERAPEUTICS

E. purpurea aerial parts, like all species of *Echinacea*, are best known for their effects on the immune system (Bauer 1999a; Bauer and Wagner 1991; Foster 1991; Hobbs 1990) and have been widely used in the treatment of the common cold and other forms of infection. The in vitro stimulation of various immune cells such as macrophages, other monocytes, and natural killer (NK) cells has been repeatedly demonstrated (Bauer 1998, 1999a; Burger et al. 1997; Möse 1983; Rininger et al. 2000). Some of the early pharmacological data on *E. purpurea* aerial parts also indicate that they may have an anti-inflammatory effect (Bauer 1999a; Büsing 1952; Wagner et al. 1989). However, if and how these immunomodulatory effects translate into improved human health is less well understood.

Some immune activities are beneficial, others harmful.

Most definitions of the immune system rest on the ability of the host to resist infection. Infection can be defined as the invasion and replication of infective particles (viruses, bacteria, fungi) within the host's biological boundaries. However, infection can occur with or without symptomatic illness, and significant immunological activity can occur with or without infection. Therefore, an expanded definition of beneficial immunomodulation could include the reduction of harmful host responses, such as inappropriate irritation or inflammation. This is important, as most infections for which *E. purpurea* aerial parts are commonly used are self-limiting and without sequelae, hence are harmful only as they affect the perceived health and function of the host. Theoretically, the goal of successful immunomodulation would be to decrease the severity and duration of symptoms while increasing the rate of elimination of infective pathogens. For an introduction to human immune function and the various mechanisms by which *Echinacea* may affect it, see the AHP monograph *Echinacea purpurea* Root.

To date, there is limited evidence suggesting that oral doses of *E. purpurea* aerial parts can have a positive therapeutic effect in treating the common cold and other forms of infection. Below are presented data on the pharmacokinetics, pharmacodynamics, and clinical efficacy of *E. purpurea* aerial parts. Many of the preclinical and clinical studies presented test for the effects of Echinacin (Madaus AG), the alcohol-stabilized expressed juice of the flowering aerial parts of *E. purpurea* that has been on the market in Germany for 68 years (see preparations for full characterization). Also included are some trials using products containing a combination of *E. purpurea* aerial parts and root, or *E. purpurea* aerial parts and the root of other *Echinacea* species; the results of these studies cannot be extrapolated to effects of *E. purpurea* aerial parts alone. Table 6 provides a summary of the double-blind, randomized, placebo-controlled clinical trials (double-blind RCT) done to date on *E. purpurea* aerial parts.

Product characterization and dosage is given for each study. Study outcome should be interpreted in the light of overall study design, with careful attention given to product and dosage. Many studies use dosages far lower than those recommended by modern phytotherapists and constituent profile of an extract will depend upon the menstruum. It should be noted that bacterial endotoxins (lipopolysaccharides) have been found in both commercial and homemade *Echinacea* products (Morazzoni et al. 2005; Senchina et al. 2005). These compounds can be strong immunostimulants. Morazzoni and colleagues (2005) demonstrated confounding effects of endotoxins in both in vitro and in vivo tests of an *E. angustifolia* preparation on immune activity. In tests of homemade products, endotoxin content was correlated with an increased in vitro immune activity in a cold-water infusion stored for 4 days at 4° C (Senchina et al. 2005). Whenever researchers have analyzed their test products for endotoxins, it is noted in the text below.

Pharmacokinetics

To date, no individual compounds isolated from *E. purpurea* aerial parts have been reported to display the same level of apparent immunostimulatory effects as the total plant extract (Awang 1999; Bauer 1999b, 2000; Schug and Blume 2000). Data from preclinical immunoassays suggest that aerial parts-derived alkamides, caffeic acid derivatives, glycoproteins, and polysaccharides are all active and contributory to immunostimulatory effects (Alban et al. 2002; Al-Hassan et al. 2000; Bauer 1999a, 1999b; Bauer et al. 1988a; Blaschek et al. 1998; Sloley et al. 2001; Witthohn et al. 2000). Estimates of the relative contributions of various fractions to activity will require large comparative trials using varying doses of different types of extracts. Bauer (2000) challenged the utility of traditional in vitro dissolution and pharmacokinetic testing of individual compounds found in *Echinacea*, maintaining that since the total native extract is regarded as the active principle, it is dubious whether analysis of single constituents can yield useful information.

The available data suggest that alkamides cross the intestinal epithelium whereas caffeic acid derivatives do not. Matthias et al. (2005a, 2005c) analyzed plasma samples from 9 healthy volunteers following their ingestion of 4 tablets made from the dried ethanolic extract of *E. angustifolia* and *E. purpurea* roots. Each tablet contained the equivalent of 675 mg and 600 mg of the respective species and approximately 10.92 mg total alkamides and 32.5 mg caffeic acid derivatives. Nine of the subjects ingested the tablets immediately after eating a high-fat breakfast and 2 took them while fasting. Blood samples were drawn prior to ingestion and at 14 different times up to 12 hours post-treatment. Caffeic acid derivatives were not found in any of the plasma samples. Alkamides, on the other hand, were rapidly absorbed, detectable from 30 min to 12 h post-treatment, with a time to maximum concentration (T_{max}) of 2.3 h and a peak concentration (C_{max}) of 336 ng/mL total alkamides. When the data from 1 individual with unusually rapid absorption were removed, T_{max} increased to 2.5 h and C_{max} decreased to 215 ng/mL. The elimination rate constant (k_e) was 0.23 and the half-life ($T_{½}[e]$) 3.4 h. There was no effect of fasting on pharmacokinetic parameters. Using the same product, Agnew et al. (2005) reported that only the tetraene alkamides were found in the plasma of 11 healthy subjects 1 hour after ingestion of 1 tablet. The mean plasma concentration in 7 of the subjects was 11.5 ng equiv/mL.

In a similar study, Woelkart et al. (2005) reported rapid absorption of alkamides in 11 subjects given a single 2.5 mL oral dose of a 60% ethanolic extract of *E. angustifolia* root. T_{max} was reached within 20 to 30 minutes for the individual alkamides, with an average C_{max} of 10.88 ng/mL for the tetraene isomers. One of the isolated alkamides could not be detected (dodeca 2*E*,4*E*-dienoic acid isobutylamide). Given the rapid uptake of the alkamides, the authors suggested that the mouth and esophagus might be important absorption points when liquid extracts are given. Comparing the results of this study to those from Matthias et al. (2005a), who found a total alkamide T_{max} of 2.3 to 2.5 h for a tableted product, lends support to a hypothesis of more rapid absorption for liquid compared to solid dosage forms. Several years earlier, Dietz et al. (2001) published the first study to show

the absorption of the tetraene alkamides into human blood following oral dosing with *E. purpurea* aerial parts ethanolic mother tincture (equivalent to 4.3 mg tetraene alkamides).

The above data are in agreement with a number of in vitro studies on the pharmacokinetics of alkamides and caffeic acid derivatives. A previous report by Matthias et al. (2004) indicated that alkamides administered in the form of a 60% liquid ethanolic extract of *E. angustifolia* and *E. purpurea* root (200 and 300 mg/mL, respectively; *Echinacea* Premium, MediHerb) diluted 1:50 in a buffer solution, were able to cross Caco-2 monolayers, a model of the intestinal epithelium. Caffeic acid derivatives diffused across the monolayers only poorly at a rate indicating the likely absence of intestinal absorption. Similarly, Jager et al. (2002) reported the in vitro transport of the tetraenes across a layer of colon-derived epithelial cells. Studies by Gräfe and Veit (1999) and Nusslein et al. (2000) contribute to the evidence that cichoric acid is decomposed prior to absorption.

No pharmacokinetic work has been done on the glycoproteins, even though these compounds are also thought to contribute to the immunomodulatory activity of *E. purpurea* root. This is because analytical methods are lacking and because it is generally thought that polysaccharides and glycoproteins are broken down during digestion or in the large bowel by bacterial action and that only monosaccharides can be absorbed by the mucosa of the small intestine (Yamada et al. 2003). Given this chemistry, it is notable that the *Echinacea* polysaccharide fo[illegible]ion being developed for pharmaceutical use is des[illegible]ned as a injectable, being patterned after the earlier inj[illegible]le form[illegible] Ec[illegible]nac (Bauer 1999a).

The suggestion by Woelka[illegible]et al. (20[illegible]5) [illegible]at the mouth and esophagus might be importa[illegible]sorption p[illegible]ats for the alkamides in liquid products may point the way towards a different understanding of constituent absorption, namely that absorption of bioactive compounds by the intestines may not be necessary for immunological activity. In addition to oral absorption, activation could occur through receptor binding in mucosa-associated lymphoid tissue (MALT) on the intestinal wall, with immunological effects then mediated through the activation of immune cells which cross the intestinal wall and act systemically. Indeed, a recent study by Pugh et al. (2005) provides some evidence that melanin extracted from *E. purpurea* root and *E. angustifolia* leaf enhances ex vivo production of IgA and IL-6 from Peyer's patch cells. Melanin is poorly soluble in commonly used solvents, making its presence in many commercial *Echinacea* products unlikely.

A recent study by Matthias et al. (2005b) was the first to investigate the in vitro metabolism by human liver microsomes of alkamides isolated from a 60% ethanolic liquid extract of *E. angustifolia* and *E. purpurea* roots (200 and 300 mg/mL, respectively; *Echinacea* Premium, MediHerb). Their results suggest that individual alkamides are metabolized differently by the cytochrome P450 drug metabolizing liver enzymes and that there is an overall inhibitory effect on the P450s that may act to preserve alkamide bioavailability. The tetraenes appeared to be 10 times more sensitive to metabolism by P450 compared to 2*E*-*N*-isobutylundeca-2-ene-8,10-diynamide. When studied in mixture, the presence of the latter alkamide appeared to inhibit the metabolism of the tetraenes, apparently due to inactivation of the P450 enzymes. The authors suggested that the inhibitory activity might have been correlated with the presence of a terminal alkyne moiety on this alkamide, given that such a chemical a structure has been shown to be present in other compounds that inhibit the P450s. Both alkamides are present in *E. purpurea* aerial parts.

Pharmacodynamics and Clinical Efficacy

Immunomodulating Effects

The use of *Echinacea* as an immunomodulatory agent is based on Native American use of the plant, especially *E. angustifolia*, for things such as wounds, stings, snakebite, burns, rheumatism, and sore throat, indications associated with infection and/or inflammation. Building upon this Native American tradition, the Eclectic physicians that were active in the US during the late 1880s and early 1990s used *Echinacea* angustifolia for a wide variety of septic conditions (see Medical Indications Supported by Traditional or Modern Experience).

A good deal of preclinical work has been done to elucidate the immunomodulatory role of *Echinacea*. Summarizing work done on *E. angustifolia*, *E. pallida*, and *E. purpurea*, stimulation of macrophage phagocytosis and cytokine produ[illegible]ion, as well as anti-inflammatory and antioxidant effects h[illegible]ve been documented. However, there is [illegible]o g[illegible]d evi[illegible]enc[illegible] fro[illegible] immunoassays done in humans for[illegible] immunomo[illegible]ula[illegible]ory [illegible] *E. purpurea* aerial parts, alt[illegible]ough a nu[illegible]be[illegible] of [illegible]inical trials support a moderate degree [illegible] efficacy in preventing or treating upper respiratory infecti[illegible]s. Recent work by Gertsch et al. (2004) and Wölkart et al. (2004) is the first to report a molecular mechanism for the immunomodulatory effects of *Echinacea*. Their work suggests that the alkamides, and not the caffeic acid derivatives or polysaccharides, influence the proinflammatory cytokine TNF-α via an agonistic effect on cannabinoid receptors.

Below is presented the clinical and preclinical work done to date on the immunomodulatory effects of *E. purpurea* aerial parts. Another excellent, although somewhat dated, English language review of the hundreds of immune-related experiments using *Echinacea* is the chapter by Rudolph Bauer, the pre-eminent *Echinacea* phytochemist, in the 1999 volume Immunomodulatory Agents from Plants (Wagner 1999). Of comparable quality and comprehensiveness is an earlier review by Bauer and Liersch, published in German in Hager's Handbuch der Pharmazeutischen Praxis (1993).

Human Clinical Studies and Immunoassays

Activation of macrophage and polymorphonuclear (PMN) granulocytes are the most widely reported of the many claimed immunomodulatory activities of *E. purpurea* aerial parts (Bauer 1999a). Activation of human macrophages was reported in one early experiment in which 12 healthy young men were injected with 2 mL of Echinacin for 4

consecutive days, after which their blood was fractionated and immune cells were exposed to *Candida* albicans (Möse 1983). Using ex vivo cell uptake methods, he reported an increase in phagocytic efficiency" by macrophages of *Candida* albicans, but "no definite evidence of effect" on lymphocytes and natural killer (NK) cells. Physical examination and various urine and blood tests demonstrated no adverse reactions.

In a 1995 publication, Melchart et al. reported the results of a placebo-controlled study carried out in 1990 that tested the effect of a liquid 70% ethanolic extract on the phagocytic activity of PMN granulocytes. The extract contained 95% herb and 5% root of *E. purpurea* and was administered for 5 days at 30 drops 3 times daily (tid). Although described as randomized and blinded, the even numbers in treatment and placebo groups ($n = 12$ each) and the lack of description of allocation and concealment procedures limit interpretation somewhat. No significant differences in phagocytic indices or white cell counts were noted in the extract group. No adverse effects were noted despite monitoring using self-report and a small array of blood tests. Finding negative results, this team suggested using sick or immuno-compromised subjects rather than healthy volunteers for future studies. One criticism of this study is that the doses used were much lower than those prescribed by many practicing herbalists and naturopathic physicians.

In 1998, Berg et al. reported a double-blind RCT in which 42 triatheletes were randomized to either of the following 3 treatment regimens: magnesium (43 mg) and placebo drops (8 mL); placebo tablets (12) and placebo drops (8 mL); or *E. purpurea* juice from aerial parts (8 mL, Echinacin) and placebo tablets (12). The medications were taken daily in 3 divided doses during 28 days of training. Blood and urine samples were collected on days 0, 28, 29, and 30 (a competition was held on day 29). Outcome measures included changes in T and NK cell populations as well as serum and urine concentrations of interleukin (IL)-6 and soluble IL-2 receptor (sIL-2R). Although the authors claimed in the abstract that changes in urinary sIL-2R, IL-6, and cortisol were attributable to the active intervention, a close reading of the results shows that differences noted between the *Echinacea* and placebo groups were neither consistent nor significant. While 3 of 13 in the magnesium group and 4 of 13 in the placebo groups got colds, none of the athletes taking *Echinacea* developed respiratory infections. This trend toward benefit was not statistically significant, hence the observed 25% reduction in incidence of upper respiratory tract infection could be caused by *E. purpurea* aerial parts or by chance alone. No adverse events were reported in the *Echinacea* group, whereas 6 subjects each in the placebo and magnesium groups reported minor adverse events that were mainly symptoms of infections. No evidence of blinding was presented.

Schwarz et al. (2002) studied the non-specific immunostimulatory effects of an *E. purpurea* aerial part preparation in healthy young men using a double-blind, placebo-controlled, crossover design. Forty men were given 6 mL twice daily (bid) of the pressed juice from flowering *E. purpurea* aerial parts (Esberitox® mono, containing 64 mL of pressed juice, 22 mL ethanol, 14 mL sterilized water per 100 mL solution; Schaper and Brümmer) or a placebo. The placebo was developed by the same manufacturer and reportedly matched the verum in appearance, color, and flavor. Treatment duration was 14 days for each product, with a 4-day washout period. The endpoints were phagocytic activity of PMN leukocytes (PMNL) and monocytes, production of tumor necrosis factor (TNF)-α and IL-1β by blood monocytes. Safety parameters were also measured, including creatinin, glucose, protein, ferritin, and liver function. Blood was drawn on days 0, 7, and 14 of each treatment period. Intention-to-treat analyses were reportedly almost identical to per-protocol ($n = 35$) analyses, and hence are the only results presented here. Between days 1 and 14 of treatment with *Echinacea*, there was a very small but reportedly significant decrease in phagocytosis intensity ($P < 0.002$) and the density of monocytes ($P < 0.05$) and phagocytosis positive monocytes ($P < 0.025$). A comparison of verum vs. placebo found only a transient difference between groups in phagocytosis positive monocytes ($P = 0.03$) and phagocytosis intensity ($P = 0.05$) on day 7 that was no longer apparent at day 14. There was no difference between groups in PMNL phagocytic activity, although there was a significant decrease in the number of phagocytosis-positive PMNL in the *Echinacea* group after 14 days of treatment ($P = 0.046$). An analysis of the TNF-α data was not possible because concentrations were too close to the limit of detection. IL-1β levels were not affected by treatment. The meaning and clinical relevance of the transient decrease in monocytes in the *Echinacea* group is unclear. The decrease could possibly be explained by higher-than-normal monocyte baselines or by a trafficking of activated cells from the circulation into the periphery rather than from an immunosuppressive effect (Gooding 2005, personal communication to AHP, unreferenced). Interestingly, serum ferritin decreased significantly during treatment with *Echinacea* ($P = 0.0005$), while all other safety measures remained unchanged (ferritin is an iron-containing compound that is normally increased during inflammation).

Kim et al. (2002) compared the immunological activity of several *Echinacea* preparations. Using a double-blind RCT design, 48 women were divided into 6 groups and administered 1 of the following preparations daily on an empty stomach for 4 weeks: 1) *E. purpurea* "whole herb" extract standardized to 4% phenols (1500 mg) [EP]; 2) EP (780 mg) taken with ultra-refined *E. purpurea* "whole herb" plus *E. angustifolia* root (680 mg) [urEPA]; 3) EP (908 mg), ultra-refined *E. purpurea* "whole herb" (464 mg), and *E. angustifolia* root (36 mg) [EPA]; 4) EPA and larch arabinogalactan 90% (1500 mg) [EPALA]; 5) larch arabinogalactan 90% (1500 mg) [LA]; or 6) alfalfa and rice placebo. The *E. purpurea* "whole herb" preparation combined root and aerial parts (Kim 2003, personal communication to AHP, unreferenced). Outcome measures included complement properdin levels (CP), TNF-α, various hematological and immunological parameters (white blood cell, neutrophil, lymphocyte, and monocyte counts), as well as quality of

life and symptom (mood, sleep pattern, gastrointestinal function) assessments. EP alone had no significant effect on any of the outcome measures, whereas the combination preparations significantly increased CP (EPA, EPALA) and decreased TNF-α (urEPA, EPALA, as well as LA) ($P < 0.05$). Large differences between groups in baseline CP levels make interpretation of effects on CP difficult. The EP, LA, and placebo groups had among the highest baseline levels (approximately 95% to 110%), whereas the urEPA, EPA, and EPALA had lower levels (approximately 45% to 70%). It may be that the preparations had a stimulatory effect primarily when baseline CP levels were in the lower range. Interpretation of CP results are further complicated given that constituents of the complement system are generally thought to be constitutive rather than inducible. Only the EPA and EPALA groups showed significant improvements in quality of life and symptomology ($P < 0.05$). LA did not appear to add any activity to EPA based on any of the outcome measures. The authors noted that interpretation of the study is limited by variation in baseline CP levels, subject characteristics, insufficient sample size, and short duration. The use of crude alfalfa (*Medicago sativa*) as a placebo may also have introduced a confounding factor given the traditional and modern use of this botanical as a nutritive tonic (Barnes et al. 2002), its potential association with the exacerbation of autoimmune conditions (Herbert and Kasdan 1994; Light and Light 2003; Roberts and Hayashi 1983), and the fact that it contains melanin, a known immunomodulator (Pugh et al. 2005). Two subjects taking *Echinacea* experienced adverse events and withdrew from the study; 1 experienced anxiety and heart palpitations and the other bilateral arthritic symptoms similar to those experienced 20 years previously.

Animal and In Vitro Studies

Immunomodulatory Effects

The preclinical studies on the immunomodulating activity of *E. purpurea* aerial parts have focused on effects on macrophage and granulocyte phagocytosis and cytokine production, with some work also done on NK cell and immunoglobulin effects. Bauer (1999a) reviewed the many experiments done on the macrophage-activating properties of *E. purpurea* aerial parts using measures of yeast particle ingestion, carbon clearance, and cytokine levels. Orally administered ethanolic extract (1:10) of dried *E. purpurea* aerial parts (5 mg/kg given in 3 divided doses daily for 2 days) was correlated with a 40% in vitro increase in carbon clearance in mice, with the lipophilic fraction more stimulatory (110%) than the polar fraction (30%) (Bauer et al. 1989). The same study reported that a homeopathic mother tincture of the fresh aerial parts (1:10) given at the same dosage and duration produced a similar or slightly greater level of stimulation (110%). Several additional experiments have reported increased activity of macrophages from mouse liver and spleen following oral dosing of *E. purpurea* aerial parts extracts (Carr et al. 1998 [uncharacterized over-the-counter products used]; Rininger et al. 2000). Bukovsky et al. (1993) reported increased phagocytic, metabolic, and bactericidal activities of peritoneal macrophages as well as increased spleen weight among mice treated for 5 days with ethanolic extracts of the aerial parts of *E. purpurea*. Al-Hassan et al. (2000) reported no increase in phagocytic activity of human blood cells following in vitro exposure to the lipophilic and medium polar fractions of the alcohol-stabilized pressed juice of fresh flowering *E. purpurea*.

Goel et al. (2002b) found a dose-dependent in vivo stimulation of alveolar macrophage phagocytic activity in healthy rats given enriched 50% ethanolic extracts of *E. purpurea* roots or aerial parts bid for 4 days by oral gavage. Four extracts were made: 1 that delivered 40, 1000, and 4 μg/kg daily of cichoric acid, polysaccharides, and alkamides, respectively, and 3 others that delivered 3, 20, and 50 times those amounts. A vehicle control of 50% ethanol was used. The increase in phagocytic activity achieved statistical significance only with the 2 high-dose extracts ($P < 0.05$). A dose-dependent trend in increase in NO and TNF-α release by both alveolar and spleen macrophages was also apparent, with the exception that the high-dose extract had no effect on TNF-α. In a follow-up study using the same model, these same authors reported that the alkamides alone at a dosage of 12 μg/kg daily for 4 days outperformed cichoric acid and the polysaccharides with respect to alveolar macrophage stimulation and release of TNF-α and NO (Goel et al. 2002a). Stimulation of phagocytosis and cytokine secretion by splenocytes was not achieved using any of the individual compounds.

Increased activity of PMN granulocytes, in some cases dose-dependent, has been reported from a number of animal experiments using carbon clearance, bioluminescence, *Candida* ingestion, and modified Brandt granulocyte assays (Bauer 1998a; Bauer et al. 1999; Stotzem et al. 1992; Wildfeuer and Mayerhofer 1994; Wolf et al. 1998). Most of these experiments used Echinacin. A study measuring phagocytosis by granulocytes using chemoluminescence found that the effects of Echinacin depended on dosage and methodology (Gaisbauer et al. 1990). The authors concluded that standardization of procedures for measuring immunostimulatory effects on these immune cells are warranted.

Reports of increased phagocytic activity have been accompanied by several reports of enhanced cytokine production (Berger et al. 1997; Hwang et al. 2004; Rininger et al. 2000). Sharma et al. (2006) reported contrasting in vitro effects of *Echinacea* on cytokines depending upon whether treated cells had been exposed to rhinovirus or not. Human bronchial epithelial cells (BEAS-2B) that were exposed or left unexposed to rhinovirus 14 were either treated or left untreated with an *E. purpurea* aerial parts juice preparation or a 50% ethanolic *E. purpurea* root extract. Effects were determined using cytokine antibody array membranes. Cells exposed to rhinovirus reportedly produced at least 31 cytokine-related molecules associated with inflammatory responses. Treatment with either of the *Echinacea* products generally reversed rhinovirus-induced cytokine stimulation, while increasing cytokine production in cells not exposed to rhinovirus. The authors interpreted these results as consistent with an anti-inflammatory effect of *Echinacea* during

rhinovirus infection. They also suggested that *Echinacea* should not be used prophylactically given the cytokine stimulating effects in cells left unexposed to rhinovirus. These results are in contrast with other reports of in vitro cytokine release inhibition associated with *Echinacea* that are presented below (Chen et al. 2005; Dong et al. 2006; Gertsch et al. 2004; Matthias et al. forthcoming; Sasagawa et al. 2006).

Cundell et al. (2003) reported that *E. purpurea* aerial parts temporarily increased circulating white cell counts in rats during the first 2 weeks of an 8-week treatment period ($P < 0.05$). The rats were fed 50 mg/kg daily of powdered herb (10.5 mg cichoric acid per 1.05 g powder; Nature's Resource) in peanut butter, or peanut butter alone. IL-2 levels increased during the final 5 weeks of treatment (more than 30-fold at maximum; $P < 0.05$), mononuclear cell:granulocyte ratio increased over the 8-week period ($P < 0.05$), while there was no effect on phagocytic activity.

Other indices of immune stimulation have also been reported. In one study, cultured cells infected with herpes virus 6 and exposed to *E. purpurea* juice from aerial parts (Echinacin) demonstrated an increased rate of presentation of viral antigen (Eichler and Krüger 1994). In another experiment, an extract of *E. purpurea* (0.1 µg/kg; plant part not specified) increased both antibody-dependent and innate NK-mediated activities against herpes virus infections in ex vivo cells from both normal and HIV-positive individuals. No negative effects on PBMC were reported at concentrations of up to [illegible] µg/mL after 4 hours (See et al. 1997). An increase in human peripheral blood NK cell cytotoxicity in response to an uncharacterized *E. purpurea* product was found in an in vitro study by Chan et al. (2003). Cytotoxicity increased by 10% over 4 hours of exposure to *Echinacea* at 0.1 µg/mL. Cowgill and Elek (1987) reported the increased production of lymphokines by lymphocytes harvested from 12 healthy adults taking a pressed juice product (Echinacin). A single dose (2 mL sc) stimulated immune response in 11 female patients with various skin ailments, whereas the same dose received daily for 1 week had a depressive effect in a skin test using recall antigens (Multitest Merieux). Increased white cell counts in peripheral blood have been noted following intramuscular (im) or iv injection of Echinacin, but not following oral dosing (Lorenz et al. 1972). In another set of experiments, *E. purpurea* root and herb decoctions and 20% ethanolic extracts were reported to induce type I interferon (IFN) in animal cells cultured with ECHO9 Hill virus (Skwarek et al. 1996).

Brokos et al. (1999) compared the in vitro immunostimulatory effects of *E. purpurea* aerial parts extract and fresh juice (Herbapol, Poland; further characterization unavailable), each diluted in 30% ethanol, with a 30% ethanol control. The test solutions were added to human blood in concentrations of 2.5% to 20% v/v x 10^{-2}. Both the juice and the extract significantly increased replication of lymphocytes, but the effect of the juice was twice that of the extract. Compared to controls, B lymphocytes decreased by 10% to 25%, while NK cells increased (30% to 90% with juice; 5% to 30% with the extract). Suppressor T lymphocyte frequencies decreased (20% to 25%) significantly compared to controls in response to the juice only; there was no effect on helper T lymphocytes. Free radical generation by resting granulocytes was enhanced at low concentrations and weakly reduced at high concentrations (up to 60% v/v x 10^{-2}).

Two studies were found that investigated the effect of *E. purpurea* on immunoglobulins in mice. Mice were immunized with sheep red blood cells (SRBC), after which they were treated for 4 days with 0.4 and 0.8 mL/kg daily of an oral 50% glycerin extract (250 mg herb/mL) (Freier et al. 2003). Compared to vehicle controls, both dosages were associated with an increase in IgM-specific antibody forming cell response. In a time course study, mice were treated at a daily dose of 0.6 mL/kg for 4 days before immunization and/or 4 days beginning 1 hour after SRBC challenge. An increase in IgM-specific antibody forming cell response was found in the mice treated only post-immunization, which the authors interpreted as an indication of acute rather than sub-chronic efficacy. These results contrast with those of Mishima et al. (2004) who administered the expressed juice from *E. purpurea* whole plant to mice at 360 mg/kg every other day for at least 3 weeks prior to exposure to radiation. Compared to vehicle (saline) controls, the *Echinacea* group showed an increase in populations of helper, suppressor, and killer T cells, and reduced levels of IgG and IgM (see Anti-inflammatory and Antioxidant Effects).

Recently, it was reported that melanin extracted from *Echinacea purpurea* root, *E. pallida* root, and *E. angustifolia* leaf enhanced in vitro monocyte activity, ex vivo IFN-γ production from spleen cells, and IgA and IL-6 production from Peyer's patch cells, with EC_{50} ranging from 1.0 to > 1000 µg/mL (Pugh et al. 2005). This is the first evidence supporting the hypothesis that *Echinacea* may act as a mucosal immune stimulant. Synthetic melanin was found to be inactive and there was up to a 100-fold difference in activity of melanin found in roots and leaves of the same plants and similar differences between species. Melanin is poorly soluble in solvents commonly used to make commercial *Echinacea* extract products, making its occurrence in such products unlikely but in need of further study.

Immunomodulatory Effects of Individual Constituents

A number of in vitro and laboratory animal studies have studied the immunomodulatory activity of various *E. purpurea* preparations and their isolated constituents, the results of which have unknown relevance to humans. Interpretation of these data must be made against the backdrop of what is known about the pharmacokinetics of *Echinacea* constituents. Based upon data reviewed above, it appears that the alkamides are readily absorbed by the intestinal mucosa, whereas the caffeic acid derivatives and polysaccharides are not. However, the hypothesis that *Echinacea* may act, at least in part, as a mucosal immune stimulant suggests the possibility that compounds not readily absorbed may still possess important bioactivity.

Very recent work reported that a 95% ethanolic extract of dried *E. purpurea* aerial parts inhibited in vitro IL-2 production by submaximally stimulated but not unstimulated human Jurkat T cells (Sasagawa et al. 2006).

Concentrations of 50 and 100 μg/mL were maximally effective ($P < 0.0001$) and cytotoxic, while 6.25 to 25 μg/mL were effective ($P < 0.003$) and not cytotoxic. Cells were stimulated with phytohemagglutinin and phorbol 12-myristate 13-acetate. The inhibitory activity correlated with alkamide but not caffeic acid concentration. Two reference standard alkamides found in *E. purpurea* aerial parts (dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*-dienoic acid isobutylamide) inhibited IL-2 production in a dose-dependent manner ($P < 0.08$) without cytotoxic effects. Earlier work by this same research group found that a dose-dependent decrease in IL-2 production in stimulated Jurkat E6-1 cells was noted in response to the 75% and 100% ethanolic extracts of *E. purpurea* flowering tops, but not the 50% or 25% ethanolic extracts (Sasagawa et al. 2003). The phenolic profile was fairly consistent between extracts, while the alkamide profile increased directly with ethanol concentration. The authors noted the increase in IL-2 was correlated with the increase in alkamide concentration in the high-ethanol extracts and emphasized the potential differences in bioactivity between preparations.

A study by Gertsch et al. (2004) suggested that although an *E. purpurea* aerial parts and root tincture induced the in vitro synthesis of the pro-inflammatory cytokine TNF-α mRNA by resting monocytes, it did not stimulate the production of the TNF-α protein itself. The product, Echinaforce (Bioforce, Switzerland), was applied at 10 to 25 μg/mL and was found to contain <0.5 EU/mL endotoxin. In monocytes artificially stimulated with LPS to produce TNF-α, the tincture inhibited TNF-α production. This effect continued for 20 hr, after which the tincture appeared to prolong TNF-α synthesis. An up-regulation of activated nuclear factor of activated T-cells and IL-8, as well as a down-regulation of IL-2 in the presence of Echinaforce, were also observed. The authors then isolated several alkamides from the tincture and found that these compounds alone upregulated TNF-α mRNA when applied at 0.5 and 5 ppm to resting cells, with an inhibitory effect on TNF-α protein production in LPS-stimulated cells. The alkamides tested were dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*, 8*Z*-trienoic acid isobutylamide, and dodeca-2*E*,4*E*-dienoic acid isobutylamide. Neither cichoric acid, chlorogenic acid, nor the polar fraction containing oligosaccharides had any effect on TNF-α.

The upregulation of TNF-α can be mediated by cannabinoid receptors. Gertsch et al. (2004) reported that a cannabinoid antagonist inhibited the alkamide-stimulated increase in TNF-α mRNA. The tetraene and diene alkamides had an agonistic effect on the cannabinoid receptors in a competitive binding assay, causing the authors to suggest that *Echinacea* exerts its effect on proinflammatory cytokines via these receptors, and particularly the CB2 receptor, which is thought to be important in immune modulation. The structure of one of the known cannabinoid substrates (anandamide) is very similar to some of the *Echinacea* alkamides. This is the first report providing evidence for a molecular mechanism behind the immunomodulatory effect of *Echinacea*.

Raduner et al. (2006) performed a study that supports the hypothesis that *Echinacea* alkamides act at least in part as cannabinoid receptor agonists. They reported that isolated dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*-dienoic acid isobutylamide bind more strongly to the CB2 receptor than does anandamide. These alkamides and anandamide increased constitutive IL-6 expression in human whole blood, consistent with a CB2 receptor-binding mechanism. However, these same 3 compounds, along with the non-CB2-binding alkamide undeca-2*E*-ene,8,10-diynoic acid isobutylamide, and also inhibited lipopolysaccharide-induced TNF-α, IL-1β, and IL-12p70 expression in what appeared to be a CB2-independent manner. The authors suggested that *Echinacea* exerts its immunomodulatory effects via multiple pathways. In contrast to the findings of Gertsch et al. (2004) and Raduner et al. (2006), 2 other studies cited above reported increased in vitro TNF-α production following exposure of cells to *E. purpurea* aerial parts (Goel et al. 2002b; Rininger et al. 2000).

In an unpublished study, alkamides isolated from a 60% ethanolic extract of *Echinacea* were tested for their in vitro effects on cytokine production by mouse macrophages (RAW 264.7) (Matthias et al. forthcoming). The liquid extract of *E. angustifolia* and *E. purpurea* roots (200 and 300 mg/mL, respectively) was the same used in the pharmacokinetic studies by Matthias et al. (2004, 2005b). Effects on nuclear factor kappa B (NF B) expression and the production of TNF-α and inducible NO in resting and LPS-stimulated cells were compared. Applied at concentrations of 12.2 2 ng/mL to LPS-stimulated cells, the extract, its alkamide fraction, and cichoric acid significantly decreased both NO and TNF-α. In LPS-stimulated cells, 2 ng/mL of the synthetic tetraene alkamide had a significant inhibitory effect on NF B expression, while undeca-2*E*-ene-8,10-diynoic acid isobutylamide had a significant stimulatory effect on TNF-α expression. NO levels were decreased in LPS-stimulated cells following co-incubation with the alkamide fraction of the extract only. The only significant effects on resting cells appeared to be a decrease in TNF-α production following incubation with cichoric acid and the alkamide fraction. The authors suggested that the effects of cichoric acid are irrelevant given the apparent poor absorption of this compound by humans (Matthias et al. 2004, 2005a, 2005c). They also cautioned that extrapolating results to recommended dosages in humans is not warranted due to the variability of in vitro dose response. In support of the results of Matthias et al. (forthcoming) and using the same model, Chen and colleagues (2005) reported that total alkamides (1.6 to 30 μg/mL) isolated from *E. angustifolia* root inhibited the production of NO in LPS-stimulated murine macrophages (RAW 264.7) compared to an LPS control. Cell toxicity (TD_{50}) was only apparent at the highest concentration.

Cichoric acid and other caffeic acid derivatives have demonstrated in vitro macrophage stimulating activity (Bauer 1999a). In contrast, Dong et al. (2006) reported that an uncharacterized *E. purpurea* extract (SaveOn Albertson's

Inc; plant part not specified) and cynarin isolated from the extract both bind to the CD28 T cell receptor and can inhibit CD28-dependent IL-2 expression. *Echinacea purpurea* is generally characterized by a lack of cynarin, bringing the identity of the raw material in the product into question.

A number of studies have investigated the immunomodulatory effects of the polysaccharide and glycoprotein fractions of *E. purpurea* aerial parts. Polymeric and glycoprotein fractions of Echinacin have been reported to have in vitro macrophage-activating, cytokine-generating, and antiviral activities (Witthohn et al. 2000). Arabinogalactan proteins from the non-alcohol stabilized, fresh pressed juice of the aerial parts have been reported to have in vitro complement activating effects (Alban et al. 2002) and to bind to human lymphocytes, monocytes, and granulocytes from a variety of donors (n = 8) (Thude et al. 2006). Investigators in other laboratories have also reported a wide variety of antigen-specific *E. purpurea* polysaccharides and proteoglycans (Egert and Beuscher 1992).

Polysaccharide-rich fractions from *E. purpurea* cell cultures were reported to increase carbon clearance in live mice and in vitro human PMN granulocytes (Bauer et al. 1999; Emmendörffer et al. 1999; Lohmann-Matthes and Wagner 1989; Schöllhorn et al. 1993; Wagner 1999; Wagner et al. 1984, 1988). Stimpel et al. (1984) found that polysaccharides isolated from cell cultures increased the cytotoxicity of macrophages, with [illegible] no effect on T and B lymphocytes. A number of [illegible]polysaccharide structures isolated from cell cultures, including a variety of arabinogalactans, have been reported active in macrophage activation and other immunological assays (Luettig et al. 1989; Wagner et al. 1988). The structure of the polysaccharides isolated from *E. purpurea* cell cultures differs from that of polysaccharides isolated from raw material, since cultured cells possess no secondary cell wall (Emmendörffer et al. 1999). Hence, the relevance of data from cell cultures to human use of *E. purpurea* aerial parts products may be marginal. Moreover, the polysaccharide hypothesis is problematic given that these compounds are thought to be poorly absorbed upon oral administration (see Pharmacokinetics).

Not too surprisingly, there is little consensus as to which extract type has the greatest desirable immunomodulatory properties. A variety of extracts, from alcoholic extracts expected to be rich in alkamides to expressed juice expected to be low in such compounds, have all given positive results in at least some pharmacological investigations. Gaisbauer et al. (1990) showed that not only dosage but also methodology affected outcome in studies using Echinacin, which serves as fair warning that products can only be justly compared when studied in the context of a comparative study. Three such studies were found: Bauer et al. (1989) reported that the lipophilic fraction of an orally administered ethanolic extract (1:10) of dried *E. purpurea* aerial parts (5 mg/kg daily for 2 days) increased macrophage activity to a greater degree than did the polar fraction (110% vs 30%) and that a homeopathic mother tincture of the fresh aerial parts (1:10) given at 0.5 mL/kg daily for 2 days produced an effect comparable to that of the lipophilic fraction (110%). Hwang et al. (2004) found that an aqueous preparation of powdered leaf with root increased cytokine secretion more than did a tincture (fresh root juice, mature seed, and fresh leaf and seed juice in 44% to 50% alcohol). According to Brokos et al. (1999), a fresh juice *E. purpurea* aerial parts preparation was twice as potent as an extract (both uncharacterized) in increasing lymphocyte replication, and was the only product that had a dose-dependent effect on increasing suppressor T cell frequency.

Summary

Evidence for an immunomodulatory effect of *E. purpurea* aerial parts following oral ingestion by humans is lacking. Of the 5 immunoassays done in humans (4 using an oral route, 1 iv), only 1 demonstrated an increase in macrophage phagocytic activity (Möse 1983). This study had numerous design flaws and used injectable aerial parts juice (Echinacin; 2 mL daily for 4 days), which is no longer available due to safety concerns. Two of the 3 negative studies used combination aerial parts-root products (Kim et al. 2002; Melchart et al. 1995), while the other used aerial parts juice (Echinacin; Berg et al. 1998). Oral dosages in the immunoassays ranged from 2.5 to 12 mL daily for liquid products, and 1500 mg daily for a dry product (Kim et al. 2002). Treatment duration ranged from 5 days to 4 weeks. Berg et al. (1998) employed a pressed juice dosage (8 mL daily) well within the range recommended by some modern phytotherapists (6 to 9 mL daily [Mills and Bone 2005]). More human immunoassays with larger patient populations and adequate dosages would be required in order to get a better indication of the immunomodulatory potential of *Echinacea*.

In contrast to the results from human immunoassays, there are considerable data from preclinical work suggesting that *E. purpurea* aerial parts can stimulate various aspects of the immune system, including phagocytic activity and cytokine secretion. Gertsch et al. (2004) reported that the effect of several *Echinacea* alkamides on the pro-inflammatory cytokine TNF-α appears to be mediated by an agonistic effect on cannabinoid receptors, offering the first suggestion of a molecular mechanism for the immunological effects of *Echinacea*. Although *Echinacea* increased the expression of TNF-α mRNA, it had no effect on TNF-α protein production except in cells artificially stimulated to increase their production of this cytokine.

Viral Resistance Effects

Studies on viral resistance have included 2 trials on induced single-strain rhinovirus infection (Sperber et al. 2004; Turner et al. 2000). These trials are included under Prevention and Treatment of Respiratory Infections because they are interpreted in terms of effects on naturally occurring colds.

Human Clinical Studies

The use of an *E. purpurea* root and aerial part product to prevent or decrease the frequency and severity of genital herpes recurrences was investigated by Vonau et al. (2001) using a prospective, double-blind, crossover RCT design. Fifty patients were divided equally and half received *E. purpurea*

extract (800 mg bid) or placebo for 6 months, after which groups switched interventions and continued treatment for another 6 months. The product used was Echinaforce® (Bioforce), a 65% alcoholic extract made from 95% aerial parts and 5% roots. A total of 19 dropouts occurred, only 4 of which were due to adverse events (mainly diarrhea) that may or may not have been associated with treatment. Only 2 patients in the control group experienced adverse events. Five patients switched interventions early (at 3 to 5 months). Changes in frequency of recurrence, duration, pain, and depression were assessed using the Visual Analogue Scale, and T lymphocyte and neutrophil counts were recorded. No statistically significant effect of treatment compared to placebo was found for any of the parameters measured. The authors noted that the high dropout rate affected the power of their study.

In Vitro Studies

As early as 1978, Wacker and Hilbig reported 50% to 80% resistance to Influenza, Herpes, and Vesicular stomatitis viruses in mouse L 929 cells incubated with the juice of *E. purpurea* aerial parts (Echinacin); after 48 hours the cells were virus-sensitive again. According to Bauer (1999a), the pressed juice used in this experiment was only active when mixed with DEAE dextran and dextran was inactive when applied alone. Similar results using the pressed juice had been reported 5 years earlier by Orinda et al. (1973). Eichler and Krüger (1994) reported that cultured cells infected with herpesvirus 6 and exposed to a[illegible]al pa[illegible] juice (Echinacin) demonstrated an increased r[illegible] of antigen [illegible]tati[illegible] However, no changes in replicatio[illegible]al load were note[illegible]

Cichoric acid and other caffeic ac[illegible] d[illegible]vative[illegible] ha[illegible] demonstrated antiviral activity (Che[illegible]nat[illegible] et al. 198[illegible] Cichoric acid has been shown to selectively inhibit HIV type 1 integrase (King and Robinson 1998; McDougall et al. 1998; Robinson 1998), an effect that may not be relevant to therapeutic efficacy given data indicating the poor absorption and bioavailability of this compound (see Pharmacokinetics).

Summary

Very limited work has been done on the effect of *E. purpurea* aerial parts on viral resistance. The one double-blind crossover RCT looking at effects on genital herpes recurrence reported no significant effect. In vitro tests have been too few and results not compelling enough to be taken as significant support for a possible clinical effect on viral infections.

Prevention and Treatment of Respiratory Infections

Human Clinical Studies

Induced Viral Colds

Turner et al. (2000) reported a double-blind RCT testing the efficacy of *Echinacea* in preventing or ameliorating the effects of experimental colds induced by a cultured rhinovirus type 23. Although the product used in this trial was undisclosed, an independent analysis of it commissioned by the authors found 0.16% cichoric acid and virtually no echinacosides or alkamides. A letter following the report inquired about the product used (Dennehy 2001) and the authors replied that it had been "labeled as a 4% phenolic extract of a mixture of *E. purpurea* and *E. angustifolia*" (Turner and Gangemi 2001). Adults were treated with 300 mg tid of the extract or placebo (uncharacterized) for 2 weeks, then challenged with rhinovirus and monitored for infection (ability to re-culture virus) and clinical colds (defined by symptoms). Treatment continued for 5 days post-exposure. Blinding was demonstrated. Rhinovirus infection developed in 22 of 50 *Echinacea*-treated participants (44%) and in 24 of 42 in the placebo group (57%). Of those infected, 11 (50%) of *Echinacea*-treated participants and 14 (59%) of placebo-treated participants developed clinical colds. These apparent absolute risk reductions of 13% and 9%, respectively, were not statistically significant, but may be clinically relevant. Similarly, there was no significant effect of *Echinacea* on total symptom score in infected subjects. No adverse events were noted in the verum group. The authors claimed that their experiment demonstrated the ineffectiveness of *Echinacea* for the prevention of experimental colds.

Sperber et al. (2004) conducted a double-blind RCT to determine whether the fresh pressed juice of *E. purpurea* aerial parts was effective as a preventive for experimentally induced rhinovirus type 39. Forty-eight healthy adults were randomized to 2.5 mL tid EchinaGuard® (= Echinacin) or a liquid placebo reportedly identical in appearance, taste, smell, and packaging (n = 24 in each group). Treatment was administered fo[illegible]7 days before and 7 days after intrana[illegible]sa[illegible]no[illegible]ation wit[illegible]rhi[illegible]virus. The occurrence of infection wa[illegible] assesse[illegible] u[illegible]ng [illegible]ures and blood tests. The oc[illegible]urre[illegible]ce of cli[illegible]ca[illegible]cold w[illegible] determined based upon the foll[illegible]wing symptoms: [illegible]rrhea, congestion, sneezing, cough, [illegible]re throat, headache, malaise, and chills. Infection occurred in 92% of subjects in the verum group and 96% of subjects taking placebo. Among infected individuals, clinical colds developed in 59% of subjects in the *Echinacea* group and 86% in the placebo group (P = 0.0883), giving an absolute risk reduction of 27%. The authors considered these results to indicate a trend towards the successful treatment of infections in the *Echinacea* group. They also noted a 23% reduction in total symptom score and a 29% to 31% reduction in the frequency of clinical colds in the *Echinacea* group. The authors acknowledged that the small sample size limited the possibility of detecting a statistically significant positive effect. In addition, blinding may have been inadequate given the difficulty in disguising the taste of *Echinacea* in liquid preparations. A total of 8 adverse events was reported by 4 subjects in the placebo group and 2 in the *Echinacea* group. The 2 adverse events reported in the *Echinacea* group, severe aphthous ulcers and insomnia, both resolved during treatment.

If the observed trends towards absolute risk reduction in the trials by Turner et al. (2000) and Sperber et al. (2004) are real, then they may be clinically significant even though they did not reach statistical significance. These effect sizes of 13% and 27%, respectively, are similar to the benefits expected with many standard treatments such as antibiotics

for middle ear or sinus infections (Glasziou et al. 2002; van Buchem et al. 1997). In order to determine whether the lack of statistically significant results in the induced rhinovirus trials was due to marginal efficacy or inadequate sample size, Schoop et al. (2006) performed a meta-analysis. Analyzing 3 studies that tested *Echinacea* for the prevention of induced rhinovirus colds (Turner et al. 2000, 2005 [the latter study used an *E. angustifolia* product]; Sperber et al. 2004), they found a 55% higher likelihood of experiencing a clinical cold among placebo subjects compared to *Echinacea* ($P < 0.04$). The authors concluded that standardized extracts of *Echinacea* were effective in preventing symptom development after exposure to rhinovirus, but that additional well-designed trials with adequate sample sizes would be necessary to confirm this finding. It should be noted that rhinoviruses account for only 10% to 40% of common colds in adults (Kirkpatrick 1996), and natural viral colds may not be single-strain, hence results based on induced single-strain viral infections may not be generalizable.

Prevention of Upper Respiratory Tract Infection (URTI)

In 1992, Schöneberger reported a double-blind RCT testing the efficacy of *E. purpurea* aerial parts juice (Echinacin) in the prevention of URTI among 108 patients. Inclusion criteria required a history of 3 or more infections in the previous year. Participants were randomized to either Echinacin or a similar-looking alcohol-based liquid placebo made by the same manufacturer (Madaus) at a dosage of 4 mL bid for 8 weeks. Participants were evaluated by a physician at baseline and again at 4 and 8 weeks by means of physical examination and blood analysis. All new infections developed during the study period were evaluated by physician and graded as mild, moderate, or severe and assessed for duration. The author reported a preventive effect, with 35.2% of the *Echinacea* group versus 25.9% of the placebo group remaining symptom-free and an increase in median time-to-onset among patients that did develop colds (40 vs. 25 days, respectively, in the *Echinacea* and placebo groups). In addition, symptom severity appeared to be lower in the *Echinacea* group. The author further noted that patients whose immune defenses were considered compromised at the onset of the study had a greater prophylactic benefit than other subjects. Interestingly, 7 years later Grimm and Müller (1999) published an English-language write-up of this trial, providing a distinctly less favorable interpretation. Without referencing the Schöneberger report, these authors described the trial in great detail, concluding that the *E. purpurea* juice did not significantly decrease the incidence, duration, or severity of URTI. However, they did note a non-significant trend toward benefit, with a relative risk reduction of 12% in the odds of catching a cold while taking Echinacin. There was no significant difference in the incidence of adverse events between the active and placebo groups. Adverse events were generally mild and transient, primarily affecting the nervous system or gastrointestinal tract.

The 1998 trial of Berg and colleagues reported above investigated the immunomodulatory effects of *E. purpurea* aerial parts juice (Echinacin) in athletes under training. The authors reported a statistically non-significant 25% reduction in the incidence of URTI in the *Echinacea* group, which can be interpreted as a preventive effect (see Immunomodulatory Effects). Weber et al. (2005) performed a secondary risk analysis of the children studied by Taylor et al. (2003) for the URTI treatment effect of an *E. purpurea* aerial parts juice product (see Treatment of Upper Respiratory Tract Infection). Among the 401 children reporting at least one URTI during the 4-month observation period, those randomized to *Echinacea* experienced a 28% reduction in the risk of developing a second URTI ($P = 0.01$). The authors cautioned that although this post hoc analysis suggests a preventive effect of the *E. purpurea* aerial parts product in children, such a result requires confirmation in a formal URTI prevention trial.

Treatment of Upper Respiratory Tract Infection

Echinacea is often used at the onset of URTIs in the hope of eliminating or reducing symptoms. Hoheisel et al. (1997) measured the effectiveness of *E. purpurea* aerial parts juice (EchinaGuard = Echinacin) against placebo among 120 participants with new onset common cold in a furniture factory work setting in Falköping, Sweden. Participants with a history of at least 3 colds in the prior 6 months were enrolled at the first sign of a cold and then randomized to either active treatment or placebo (a liquid identical in color and ethanol concentration to the active intervention). Twenty drops (approximately 1.33 mL) were given every 2 hours on the 1st day, and tid thereafter up to day 10. Although self-reported symptoms recorded daily in diaries did not differ between the groups, the authors claimed a 20% reduction in the number of participants who went on to develop a real cold in the active group compared to placebo ($P = 0.04$). The definition of "a real cold" was not provided, but appears to have been created and applied retrospectively, after unblinding. Of those who developed a "real cold", a median duration of 8 days was reported for the placebo group versus 4 days in the *Echinacea* group ($P < 0.0001$). No adverse events were reported and tolerability was high in both groups: 88.3%, Echinagard; 85%, placebo). Aside from the implausibility of self-reported symptom severities that reportedly did not differ between active and placebo groups alongside major reductions in duration and odds of developing an undefined "real cold", this trial suffers from the common problem of inadequate or undemonstrated blinding.

Schulten et al. (2001) described the results of a double-blind RCT that tested ethanol-stabilized *E. purpurea* aerial parts juice (Echinacin) in 80 employees of Madaus AG, the product manufacturer and sponsor of the trial. Participants were asked "to visit the company physician at the first sign of a cold", then randomized to *Echinacea* or placebo, and asked to take 5 mL bid for 10 days. Primary outcomes were severity and duration of self-reported symptoms, using the well-established Jackson criteria on daily symptom diary forms. Statistically significant reductions in both severity and duration were reported, with intention-to-treat analyses showing less benefit than per-protocol analyses (3 dropped out and 7 were excluded for insufficient compliance in dosing or because they did not develop cold symptoms). The

Table 6 Summary of results from double-blind, randomized, controlled clinical trials testing orally administered *Echinacea purpurea* aerial parts preparations

Reference	Indication	Group sizes*	Intervention	Dosage and duration	Outcome	Limitations†
Goel et al. 2004	Treat URTI	PP: E = 54, P = 57; ITT: E = 59, P = 69	Echinilin: EP aerial parts and root, yielding 0.25/2.5/25.5 mg/mL alkamides/cichoric acid/polysaccharides	4 mL in water 10 times on day 1, then qid for 6 days	17% (ITT) to 23% (PP) reduction in symptom severity ($P < 0.01$)	Uncertain randomization; statistical tests barely adequate; unvalidated measures; no test for baseline differences between groups
Sperber et al. 2004	Prevent induced viral URTI	E = 24, P = 24	EchinaGuard (= Echinacin)	2.5 mL tid for 7 days prior and 7 days after exposure to rhinovirus	No differences between groups; trend in symptom score reduction (23%) and frequency of clinical colds (29% to 31%) in E group	Induced rhinovirus exposure; small sample size; low dosage
Yale and Liu 2004	Treat URTI	E = 63, P = 65	EchinaFresh capsules: freeze-dried juice of fresh EP aerial parts containing 2.4% soluble fructofuranosides	100 mg tid upon onset of symptoms until resolution, up to 14 days	No differences between groups	Treatment not always begun upon onset of symptoms; low power; low dosage
Aldous et al. 2003	Treat URTI to prevent recurrent otitis media	90 children: ~22 in each of 4 treatment groups	EP root and seed 1:1, 50% ethanolic extract	0.5 mL tid for 3 days, then 0.25 mL tid for 7 days	No differences between groups	Outcome measure not directly associated with intervention; low dosage
Taylor et al. 2003; Weber et al. 2005	Treat URTI	407 children: 337 URT[illegible] w[illegible] E and 370 U[illegible] treated with P	Echinacin, non-alcohol stabilized	3.75 mL bid in 2- to 5-yr-olds; 5 mL bid in 6- to 11-yr-olds; up to 10 days	No differences bet[illegible]en groups in ma[illegible] outcome me[illegible]ures; [illegible]% risk rec[illegible]tion[illegible] develop-ing subs[illegible]uent cold in E group (P = 0.01)	Parent assessment of outcome measures; low dosage; baseline differences between groups; low power
Barrett et al. 2002	Treat URTI	E = 73, P = 75	E = EP herb and root (62 mg each), EA root (123 mg), thyme (49 mg), peppermint leaf (31 mg), citric acid (3 mg) per capsule; P = alfalfa (333 mg)	4 capsules 6 t[illegible]es on day 1, then 4 capsules tid until symptoms resolved, up to 10 days	No differences between groups	Unvalidated measures; potentially active placebo
Kim et al. 2002	Immunoassay	E = 8, P = 8	EP aerial parts and root extract (4% phenolics) (other treatments discussed in text)	1500 mg daily for 4 weeks	No differences between groups in TNF-α, complement properdin, or other parameters	Small sample size; Baseline differences between groups
Schwarz et al. 2002	Immunoassay	n = 40, crossover	Esberitox mono: 64 g pressed juice of EP aerial parts per 100 g 22% ethanol	6 mL bid for 14 days	No influence on monocyte populations, phagocytosis, or cytokine production; decrease in serum ferritin (P = 0.0005)	Small sample size
Schulten et al. 2001	Treat URTI	E = 41, P = 39	Echinacin	5 mL bid for 10 days	Severity and duration reduced in most self-reported outcomes	Subjects were employees of manufacturer; unvalidated measures
Vonau et al. 2001	Prevent recurrent genital herpes	n = 31, crossover	Echinaforce: 65% ethanolic extract of fresh EP aerial parts (95%) and roots (5%)	800 mg bid for 6 months	No differences between groups	Small sample size; high drop-out rate

Reference	Indication	Group sizes*	Intervention	Dosage and duration	Outcome	Limitations†
Lindenmuth and Lindenmuth 2000	Treat URTI	E = 48, P = 47	E = Echinacea Plus tea: EP and EA aerial parts, EP root extract (6:1) (total 1275 mg E /bag), lemon grass, spearmint; P = Eater's Digest tea: peppermint leaf, fennel seed, ginger root, rose hip, papaya leaf, alfalfa leaf, cinnamon bark	5-6 cups daily, titrating to 1 cup by day 5	Effectiveness index higher in E group ($P < 0.01$)	Alternate allocation; poor outcome measures (retrospective global assessment); potentially active placebo
Turner et al. 2000	Prevent induced viral URTI	E = 50, P = 42	EP and EA extract containing 4% phenolics, plant parts unknown	300 mg tid for 14 days prior and 5 days after exposure to rhinovirus	No differences between groups; 13% absolute risk reduction in E group (trend, $P > 0.05$)	Induced rhinovirus exposure; unvalidated measures; low dosage
Brinkeborn et al. 1999	Treat URTI	E1 = 41, E2 = 49, E3 = 44, P = 46	Echinaforce (40 mg/tablet); Echinaforce 7:1 (284 mg/ tablet); EP root extract (148 mg/tablet)	2 tablets tid for up to 7 days	Reduction in complaint index in Echinaforce groups ($P < 0.03$)	Unvalidated measures
Berg et al. 1998	Immunoassay	E = 14, Mg = 13, P = 13	Echinacin + P tablets; Mg + P drops; P drops + P tablets	40 drops (8 mL) tid of liquid products (= 43 mg Mg); 4 tablets tid: for 28 days	No differences between groups; 25% reduction in incidence of URTI in E group (trend, $P > 0.05$)	Small sample size; low dosage
Hoheisel et al. 1997	Treat URTI	E = 60, P = 60	EchinaGuard (= Echinacin)	20 drops (~1.33 mL) every 2 h on day 1, then tid to day 7	20% reduction in development of a "real cold" in E group ($P = 0.04$), decreased duration in E group ($P < 0.0001$)	Unvalidated measures; selective reporting of outcomes; outcomes may have been defined retrospectively
Melchart et al. 1995 (1990 trial)	Immunoassay	24 (group sizes not given)	Ethanolic extract of EP aerial parts (95%) and root (5%)	30 drops tid (~2.5 mL daily) for 5 days	No differences in phagocytic activity or white cell counts between groups	Uncertain randomization; unvalidated measures; small sample size; low dosage
Schöneberger 1992 (= Grimm and Müller 1999)	Prevent URTI	E = 54, P = 54	Echinacin	4 mL bid for 8 weeks	Relative risk reduction of 12% in E group (trend, $P > 0.05$)	Unvalidated measures; selective reporting of outcomes; low dosage

Key: URTI = upper respiratory tract infection; EP = *E. purpurea;* EA = *E. angustifolia;* E = *Echinacea* group (when more than one type of ***Echinacea*** preparation was used, E1, E2, E3 refer to the products listed in the order given in the Intervention column); P = placebo group; ITT = intention-to-treat patient group; PP = per-protocol patient group; bid = 2 times daily; tid = 3 times daily; qid = 4 times daily.

* = Per-protocol patient group sizes have been given unless otherwise indicated.

† = Barrett et al. (2002), Taylor et al. 2003, Turner et al. (2000), and Yale and Liu (2004) were the only trials that adequately demonstrated intact concealment (blinding); blinding of liquid products was particularly suspect.

median duration was reported as 6 days in the *Echinacea* group and 9 days in the placebo group. However, graphics showing the time-severity curve and the survival curve for days of illness suggest a somewhat smaller, yet probably clinically significant, effect size. The main drawback of this study was the lack of evidence of blinding. Liquid *Echinacea* preparations are notoriously difficult to match with placebo. With employees of the manufacturer as the study participants, this represents a very significant limitation. Neither the incidence of adverse events nor tolerability (reported as good and very good by "most" patients) differed between groups. All adverse events were mild, with the majority affecting the gastrointestinal tract.

According to a multi-center double-blind RCT of children, the non-alcohol stabilized fresh pressed juice from *E. purpurea* flowering aerial parts (Madaus AG) was found to be ineffective in the treatment of URTI (Taylor et al. 2003). Healthy children (n = 407 per-protocol; n = 524 intent-to-treat) aged 2 to 11 years were followed over a 4-month period or up to 3 URTI. At the onset of symptoms, the children received either *Echinacea* or a placebo until symptoms resolved or for up to 10 days (blinding demonstrated). The placebo was an inactive syrup and the active intervention was matched to it by combining it in the same syrup. Children 2- to 5-years-old received 3.75 mL bid, while 6- to 11-year-olds received 5 mL bid. According to the authors, these dosages were equivalent to 50% and 67% of the adult dosage recommended by the manufacturers. The primary outcome measures were duration and the severity of symptoms and adverse events as recorded by parents. Secondary outcome measures included maximal daily symptom severity score, duration of peak severity, duration of fever, and global assessment of symptom severity by parents. Of the 707 URTI that occurred, 337 were treated with *Echinacea* and 370 with placebo. No significant between-group differences were apparent in any of the outcome measures in the per-protocol population. However, a follow-up analysis of the data by Weber et al. (2005) reported that among the 401 children who developed an URTI, there was a 28% reduction in the risk of developing a subsequent URTI among the children randomized to the *Echinacea* product (P = 0.01). The overall rate of adverse events did not differ between groups. However, 2 of the verum subjects experienced a sudden onset of stridor (harsh sound upon breathing due to obstruction of the air passages) after taking their medication that required outpatient treatment with steroids at an emergency room. These children were removed from the study. Analyzed alone, rashes occurred more frequently in the *Echinacea* group (7.1%) compared to the placebo group (2.7%; P = 0.008).

This study by Taylor et al. (2003) is one of the best-designed clinical trials on *Echinacea* to date, with demonstrated randomization. Its greatest shortcoming was the parent assessment of outcome measures. In addition, the difficulty in pinpointing onset of symptoms in children may have made it challenging to begin treatment early enough to have the greatest chance of efficacy. This last point suggests caution in extrapolating results to the adult population. In follow-up commentary, the fact that subjects in the placebo group took significantly more vitamins and minerals compared to the *Echinacea* group was highlighted (Kim et al. 2004b). If such supplements can influence the course of an URTI, this difference between groups may have affected outcome. According to the authors, the sample size only gave them enough statistical power to detect a 20% decrease in duration.

Yale and Liu (2004) used a double-blind RCT design to study the effect of *E. purpurea* aerial parts on the treatment of the common cold in adults. Subjects were randomized to 1 capsule tid of either *E. purpurea* (n = 63) or an identical-appearing lactose placebo (n = 65). The verum was EchinaFresh®, made of the freeze-dried juice of *E. purpurea* aerial parts (100 mg/capsule, standardized to 2.4% soluble β-1,2-D-fructofuranosides; Enzymatic Therapy). Treatment was begun within 24 hrs of symptom onset and continued until symptoms resolved or up to 14 days. The occurrence of an URTI was confirmed by a physician. The primary outcome measures were patient-assessed duration and symptom severity (sneezing, nasal discharge, nasal congestion, headache, sore or scratchy throat, hoarseness, muscle aches, and cough). No statistically significant effect of *Echinacea* vs. placebo was found for any of the outcome measures. The adverse events reported were minor and most could be considered to be symptoms of the common cold (mouth irritation, bad taste, headache, dizziness, dry mouth). Abdominal pain and nausea were also reported. Although a between-group comparison of adverse event incidence was not reported, a bar graph indicated that the overall incidence of adverse events may have been higher in the *Echinacea* group compared to placebo. This appears to be true for the individual symptoms of nausea, abdominal pain, dizziness, and dry mouth. Overall, this was a very well designed study, with evidence for blinding. One of the limitations of the study was the small sample size that provides little power to detect a positive effect. Another limitation was that subjects were required to begin treatment between 6 and 24 hrs after the onset of symptoms (mean = 15 hrs). The window of efficacy for *Echinacea* is unknown, but reports from herbalists indicate that it is most effective when begun immediately upon the onset of symptoms (see Medical Indications Supported by Traditional or Modern Experience). In addition, the dosage was low compared to recommendations made by herbalists (e.g. Mills and Bone 2005).

At least 4 clinical studies on the use of *E. purpurea* aerial parts in the treatment of URTI used combination products made of various parts of 1 or more *Echinacea* species, and 3 included several other plant species for flavor. The results of these studies cannot be extrapolated to *E. purpurea* aerial parts alone. Brinkeborn et al. (1999) reported a double-blind RCT trial comparing 3 formulations of *E. purpurea* with a similar appearing placebo. A total of 246 adults with new onset URTI were randomized to placebo or 1 of 3 active preparations: 1) Echinaforce® (95% herb, 5% root; 40 mg dried herb equivalents/tablet; Bioforce, Switzerland); 2) a 7:1 Echinaforce® concentrate (284 mg dried herb/tablet);

and 3) a "special" *E. purpurea* root extract (148 mg dried herb/tablet). The per-protocol group sizes were 41, 49, and 44, respectively. Participants took 2 tablets tid while they had a cold, but not longer than 7 days. Using a 4-point severity scale, 12 symptoms were assessed daily by participants and on days 1 and 8 by a physician. According to per-protocol and intent-to-treat analyses, symptomatic benefits were significant in the Echinaforce groups, while trends toward benefit in the "special" root extract group did not reach statistical significance. Randomized allocation and concealment methods were adequately described, but some question of concealment was left open with the authors' statement that the treatments "could almost not be distinguished from one another by their smell or taste" and by the fact that no test of blinding was reported. Reported benefits were greater for physician-assessed than for patient-assessed symptoms. Although statistical significance was reached, clinical significance remains in doubt, as day-by-day symptom scores and duration benefits were not reported. There was no difference between groups in the incidence of adverse events.

Lindenmuth and Lindenmuth (2000) reported a double-blind RCT in which 95 employees of a nursing and rehabilitation center were treated with an *Echinacea* tea beginning at the onset of cold or flu symptoms (runny nose, scratchy throat, fever). Each presenting participant was alternately given either a packet of 21 *Echinacea* Plus® tea bags (flavored with lemon grass and spearmint) or a packet of "placebo" tea bags (Eat[illegible] Digest®, flavored with peppermint, ginger, and cinnamon and also containing alfalfa leaf) and instructed to drink 5 or 6 cups of tea on the 1st day of symptoms, titrating to 1 cup daily by day 5. Both teas are manufactured by Tra[illegible]ditional Medicinals (California); *Echinacea* Plus tea contains leaves, flowers, and stems of *E. angustifolia* and *E. purpurea*, with a water-soluble dry extract of *E. purpurea* root (6:1), to give a total of 1275 mg of dried herb per tea bag. Outcomes were measured by a questionnaire that was given to subjects 14 days after they started the program. The questionnaire asked the participants to use 5-point Likert scales to retrospectively judge: 1) overall effectiveness; 2) length of illness; and 3) number of days it took to notice a difference. T-test comparison of means yielded significant ($P < 0.01$) differences favoring the *Echinacea* group. No mention was made of whether the participants thought they took *Echinacea* or placebo, hence evidence of blinding was either not assessed or not reported. With alternate allocation and distinctly different tasting preparations likely influencing concealment, the unbiased nature of these results is suspect. The use of alfalfa in the placebo is also problematic, given the known immunomodulating properties of this botanical (see references above). The retrospective assessment of outcomes further limits interpretation. No adverse events were reported by any of the subjects.

Barrett et al. (2002) reported the results of a double-blind RCT using a combination product (Shaklee Tecnica) containing *E. purpurea* (herb and root, 62 mg each), *E. angustifolia* (root, 123 mg), thyme (*Thymus vulgaris*, 49 mg), peppermint (*Mentha* x *piperita*, 31 mg), and citric acid (3 mg). Participants were college students presenting within 36 hours of the 1st symptom of a cold. Subjects in the treatment group ($n = 69$) received a dose of 6 g (4 capsules x 6) on the 1st day and 3 g (4 capsules tid) on each subsequent day, as long as they had symptoms or for a maximum of 10 days. The placebo group ($n = 73$) received an encapsulated placebo consisting of alfalfa (333 mg) that was indistinguishable from the verum to investigators and participants (blinding demonstrated). Participants were monitored by daily self-report of 15 symptoms, both on paper and on a web-based data collection system, for up to 10 days of illness. No significant differences were noted between the *Echinacea* and placebo groups, either in symptom severity or in duration. The authors hypothesized that the lack of a detectable effect could have been due to the product (low dose crude *Echinacea*), participants (young adults with intact immune systems), or measurement methods (unvalidated Likert-scale questionnaires). Another consideration may be the use of alfalfa as the placebo for the reasons previously noted. No serious adverse events were noted and their incidence did not differ between groups.

A recent double-blind RCT investigated the use of an *E. purpurea* root and aerial part tincture in the early treatment of URTIs (Goel et al. 2004). At the onset of the 1st symptoms of a naturally-acquired cold, adult subjects ($n = 54$) were asked to take 4-mL doses of EchinilinMC with a glass of water 10 times over the first 24 hours, followed by 4 doses daily for the next 6 days. According to product labeling, Echinilin contains 0.25 mg/mL alkamides, 2.5 mg/mL cichoric acid, and 25.5 mg/mL polysaccharides. The placebo was reportedly made to look, taste, and smell like the active preparation. According to the manufacturer, spearmint oil was added to both the *Echinacea* and placebo preparations to create a similar taste and aroma between them. Symptom severity was assessed daily by patients using a 10-point scale and by physicians on days 3 and 8 using a 4- or 10-point scale. Symptoms monitored included: sore throat, runny nose, sneeze, stuffy nose, watery eyes, chills, malaise, fever, headache, sore muscles, hoarseness, shortness of breath, and cough. Total daily symptom score was significantly lower in the *Echinacea* group in both the intent-to-treat (17.6% reduction, $P < 0.05$) and per-protocol (23%, $P < 0.01$) analyses. Especially in the per-protocol analysis, many individual symptom scores also averaged lower in the *Echinacea* compared to the placebo group, although differences were not always significant. There was no duration benefit in the intent-to-treat group and a significant one ($P < 0.05$) only for sore throat and nasal congestion in the per-protocol group. Adverse events were mild (heartburn, constipation, itching, burning, numbness of the tongue) and equally distributed between groups.

The study by Goel et al. (2004) would have benefited by a comparison of symptom severity between groups on day 1 in order to ensure that the benefits found were not confounded with greater baseline symptom severity in the *Echinacea* group. In addition, more thorough evidence of blinding and a multivariate analysis of effects would have improved study quality. Nonetheless, this is a well-designed study supporting a modest benefit for those randomized to

Echinacea at a dosing regimen that is relatively consistent with that used by herbal practitioners.

Treatment of Bronchitis and Pertussis

Clinically, bronchitis is distinguished from URTI by the lack of nasal and throat symptoms and by the prominence of cough, sputum production, and fever (Oeffinger et al. 1997). Many experts consider URTI and bronchitis to be different ends of the same spectrum, since both are usually viral in nature and relatively unresponsive to antibiotics (Bent et al. 2000; Fahey et al. 2004). In 1988, Baetgen reported a retrospective analysis of 1280 children with bronchitis who had received either *E. purpurea* aerial parts juice (Echinacin) im (n = 468), antibiotics alone (n = 482), or a combination of the 2 (n = 330). Information on dosage was not available. The duration of illness in the *Echinacea*-only group was reportedly shorter than in the *Echinacea*-plus-antibiotics group, which was shorter than in the antibiotics-only group. As neither randomization nor blinding was attempted, and as data extraction was performed retrospectively, any inferences on effectiveness would be highly suspect. One case of redness at the injection site and 3 cases of allergic skin reaction were reported among the 798 children injected with *Echinacea* (Huntley et al. 2005).

In 1984, Baetgen reported a retrospective analysis of 170 children with clinically defined pertussis (whooping cough) who received either *E. purpurea* aerial parts juice (Echinacin; n = 77), antibiotics alone (n = 30), or a combination of the 2 (n = 63) (Baetgen 1984; Melchart et al. 1994). The dosage regime is not entirely clear from the report, but it appears that 2 mL Echinacin was administered daily over a period of 10 days in 19 different treatment plans. The most frequent treatment model was 2 mL IM daily for 3 consecutive days (n = 89). The author claimed a duration benefit for the *Echinacea* groups. Unfortunately, the open-label, non-randomized, retrospective methodology limits the strength of this claim. The author reported that the Echinacin injections were well tolerated, with 1 incident of erythema or localized pain at the injection site in the Echinacin only group.

Treatment of Children with Recurrent Ear Infections

Aldous et al. (2003) investigated the use of *E. purpurea* root and seed extract in the early treatment of pediatric URTIs in an attempt to prevent recurrent inner ear infections (otitis media) (see also Mark et al. 2001). Ninety children 1- to 5-years-old with a history of at least 3 ear infections in 6 months, or 4 in 1 year, were enrolled in this double-blind RCT. Children were divided into 4 groups and given either of the following treatments upon onset of every URTI contracted over a 3-month period: 1) *Echinacea* and cranial osteopathic manipulative treatment (COMT); 2) *Echinacea* and no COMT; 3) placebo and COMT; or 4) placebo and no COMT. *E. purpurea* was administered as a 50% alcoholic 1:1 g/mL root (fresh) and seed (dry) extract (Eclectic Institute) at a dosage of 0.5 mL tid for 3 days, followed by 0.25 mL tid for the subsequent 7 days. Children received 5 doctor's visits during which a COMT was received or not. The primary outcome measure was the occurrence of physician-reported otitis media. Only 93% of the 90 children enrolled had complete medical records at the end of 3 months and compliance with regard to COMT was only 64%. No effect of treatment on the incidence of otitis media was found. Children taking *Echinacea* were just as likely as those taking placebo to develop inner ear infections (relative risk of 65% vs. 55%), with similar results for COMT. The possibility of proper blinding in this study is suspect despite the fact that the placebo was reported to be identical to the active treatment, since liquid *Echinacea* preparations have such a distinctive taste. However, the parents could not tell the difference between the products their children were given (Aldous 2004, personal communication to AHP, unreferenced). Moreover, the findings of this study cannot be extrapolated to typical aerial parts preparations, since the product contained seed and root only. Adverse events were not addressed in the report available.

Evidence-based Reviews

Several evidence-based reviews have been done on the use of *Echinacea* for the prevention or treatment of URTI. Linde et al. (2006) recently updated the Cochrane Review on *Echinacea* for preventing and treating the common cold. Sixteen randomized controlled trials considered to be of reasonable to good methodology were included in the final analysis; all but 1 of the trials were double-blind. Grouping all trials, 22 comparisons of an *Echinacea* product and a control group were made, 3 of which addressed prevention and 19 of which addressed treatment. Two of the trials used products made from *E. angustifolia*; 4, *E. purpurea* root; 6, the fresh juice of *E. purpurea* flowering aerial parts; 4, *E. purpurea* root and aerial parts combined; 1, *E. pallida* root; and 3, several *Echinacea* species. The variation in products and study design prevented any statistical analysis of pooled data. The authors concluded that some *E. purpurea* aerial parts preparations (hydroalcoholic extracts and expressed juice) might be useful for the early treatment of colds in adults, but that results were too inconsistent to generalize. They found no convincing evidence for a treatment effect of other preparations and no clear preventive effect of *Echinacea* or benefit in children. The authors noted a publication bias towards positive trials. Adverse events associated with treatment were minor, infrequent, and generally similar to those found in the placebo groups.

The Natural Standards evidence-based review of *Echinacea* (Ulbricht and Basch 2005) considered 10 trials on the treatment and 6 on the prevention of upper respiratory infection. One of the trials used a product made from *E. angustifolia* root; 3, *E. purpurea* root; 5, the fresh juice of *E. purpurea* flowering aerial parts; 1, *E. purpurea* root and aerial parts combined; 1, *E. pallida* root; 3, several *Echinacea* species; and 4, *Echinacea* and other species (2 trials compared 2 products). The 1 trial using an *E. pallida* root preparation (Bräunig 1993; Dorn et al. 1997) was mistakenly reported twice. The authors considered there to be good scientific evidence for the efficacy of *Echinacea* in the treatment of URTI and unclear scientific evidence for a preventive effect. They acknowledged that most of the trials considered were methodologically flawed and noted a lack of benefit in children.

Caruso and Gwaltney (2005) recently reviewed 9 placebo-controlled trials on the use of *Echinacea* for the prevention or treatment of the common cold. Two of the trials used products made from *E. purpurea* root; 1, *E. angustifolia*; 2, *E. purpurea* root and tops; 3, the fresh juice of *E. purpurea* flowering aerial parts; and 2, several *Echinacea* species. They evaluated the studies for methodological rigor in terms of case definition, quantifiable hypothesis, sample-size calculation, randomization, double-blinding, proof of blinding, compliance testing, drop-out rate calculation, use of intent-to-treat analysis, and measurement of probability. The authors concluded that the therapeutic efficacy of *Echinacea* has not been established.

Giles et al. (2000) identified 5 double-blind RCT published between 1997-2000 on the use of *Echinacea* to treat the common cold, 2 of which compared multiple *Echinacea* products. Two of the trials used products made from *E. purpurea* root; 1, *E. angustifolia*; 1, *E. pallida* root; 1, *E. purpurea* root and tops; and 2, the fresh juice of *E. purpurea* flowering aerial parts. They reported that 3 of the trials concluded that *Echinacea* was efficacious in reducing frequency, duration, and severity of the common cold, whereas 2 concluded that it was ineffective. All of the trials suffered from design flaws. Based upon these studies and a review of previous evidence-based reviews (Barrett et al. 1999a; Melchart et al. 1994), the authors concluded that the therapeutic efficacy of *Echinacea* for treating the common cold was inconclusive, although it appeared to be safe.

Among the 13 double-blind RCT reviewed by Barrett et al. (1999b) were 9 treatment trials and 4 prevention trials. Two of the 13 trials included in the review used products made from the fresh juice of *E. purpurea* flowering aerial parts; 1, *E. purpurea* root and tops; 1, *E. purpurea* root; 1, *E. pallida*; and 6 used combination products containing other plant species in addition to *Echinacea* (Esberitox® or Resistan®). The authors concluded that *Echinacea* might confer some benefit in the early treatment of URTI, but found very little evidence for a preventive effect. They considered the methodological quality of trials modest.

Summary

It seems reasonable to conclude from the evidence outlined above that *E. purpurea* aerial parts preparations may have a modest therapeutic benefit in the early treatment of the common cold and a possible preventive effect as well. Of the 9 double-blind RCT investigating a treatment effect, 5 had positive outcomes (Brinkeborn et al. 1999; Goel et al. 2004; Hoheisel et al. 1997; Lindenmuth and Lindenmuth 2000; Schulten et al. 2001). One of these trials had significant design limitations (Hoheisel et al. 1997) and none of them provided adequate proof of blinding. The alcohol-stabilized fresh juice of *E. purpurea* aerial parts was used in 2 of the 5 studies, while the other 3 used aerial part/root combinations or a combination with *E. angustifolia*. Three of the studies began treatment with high doses on the 1st day, followed by a lower maintenance dosage on subsequent days. Lindenmuth and Lindenmuth (2000) used a combination tea containing 1275 mg dried herb per bag that was taken 6 times on day 1 titrating to once daily by day 5. The other 2 variable-dose studies used a liquid preparation at approximately 14 to 40 mL on day 1 in divided doses, followed by 4 to 16 mL daily on subsequent days (Goel et al. 2004; Hoheisel et al. 1997). Looking at maintenance dosage across 4 of the positive studies (Lindenmuth and Lindenmuth [2000] excluded due to continual decrease in dosage), liquid preparations were administered at 10 to 40 mL daily for 6 to 10 days and tablets at 80 to 568 mg daily for up to 7 days. All of the studies on the treatment of URTI claimed to begin treatment at the 1st sign of symptoms, although the time period was not always specified and in 1 of the trials with a negative outcome, treatment was allowed to begin as late as 36 hours after symptom onset (Barrett et al. 2002).

Three additional double-blind RCT addressing the preventive effects of *Echinacea* showed positive trends towards risk reduction of 9% to 27%, which may be clinically significant (Schöneberger 1992; Sperber et al. 2004; Turner et al. 2000), although this benefit was in response to induced rhinovirus in 2 of the studies. A meta-analysis of the induced rhinovirus trials found a significant preventive effect. Two other double-blind RCT observed preventive trends as well, although prevention was not a primary outcome measure of these studies. Weber et al. (2005) found a significant decrease in risk of future infection in *Echinacea*-treated children, while Berg et al. (1998) found a trend showing a 25% reduction in incidence of URTI. Of the 5 trials addressing URTI prevention, 4 used the alcohol-stabilized fresh pressed juice of *E. purpurea* aerial parts at 7.5 to 10 mL daily for 10 days to 8 weeks, while 1 used an uncharacterized combination of *E. purpurea* and *E. angustifolia* at 900 mg daily for 19 days.

Summarizing the above data, 10 of the 12 URTI studies included in Table 3 had positive outcomes or trends. Of these 10, 7 used the alcohol-stabilized fresh pressed juice of *E. purpurea* aerial parts. Five of 7 studies detected a 10% to 40% reduction in symptom severity in the *Echinacea* group (Brinkeborn et al. 1999; Goel et al. 2004; Schöneberger 1992; Schulten et al. 2001; Sperber et al. 2004), and 4 of 6 studies observed a duration benefit (Hoheisel et al. 1997; Lindenmuth and Lindenmuth 2000; Schöneberger 1992; Schulten et al. 2001). A preventive effect was found in one study in children (Weber et al. 2005) and trends toward prevention were observed in 4 studies (Berg et al. 1998; Schöneberger 1992; Sperber et al. 2004; Turner et al. 2000). Positive effects on symptom severity and duration were not found in children (Aldous et al. 2003; Taylor et al. 2003) or healthy young adults (Barrett et al. 2002). These results parallel the lack of benefit to children of conventional cold medicines that is reported in the literature (Clemens et al. 1997; Smith and Feldman 1993; Taylor et al. 1993). Benefits for children with pertussis and bronchitis were reported by Baetgen (1984, 1988), but these results are of limited value given the methodological flaws in study design.

Anti-inflammatory and Antioxidant Effects

Human Clinical Studies

In a small, open, uncontrolled trial, Randolph et al. (2003) investigated the effects of a combination product on the in vivo expression of human peripheral leukocyte genes associated with an inflammatory response (IL-1,-1β,-8,-10, TNF, and intracellular adhesion molecule [ICAM]). The product contained *E. purpurea* root and aerial parts, *E. angustifolia* root (506 mg total *Echinacea* per 3 tablets) and 100 mg citrus multiflavonoid complex per 3 tablets (Triple Guard *Echinacea*; Nutrilite). Six healthy adults were given 3 tablets tid for 2 days, with 1 additional dose on day 3. No change was found in serum chemistry or hematologic values. Some subjects showed a slight decrease in the expression of genes associated with acute inflammation through day 5, returning to baseline by day 12. The decrease was statistically significant only for TNF-α expression on day 5. IFN-α2 expression increased to significance on day 12, which the authors interpreted as consistent with an antiviral response. No conclusion regarding *Echinacea* alone can be made due to the bioflavonoid complex in the product. In addition, the small patient population compromises adequate statistical analysis and the generalizing of results. In contrast to these results, this same study found an in vitro dose-dependent up-regulation of IL-1,-1β,-8,-10, TNF-α, and ICAM gene expression in THP-1 cells following their incubation in 10, 50 and 250 μg/mL of an *E. purpurea* aerial parts extract (endotoxin-free).

In 1978, Viehmann reported a longitudinal observational study in which 4598 patients under the care of 58 doctors were treated with an Echinacin ointment for a variety of skin lesions, including [illegible], herpes simplex, and varicose ulcers. The authors reported a success rate of 85% and stated that side effects occurred in 2.3% of cases. Despite these encouraging results, properly designed trials demonstrating clinically useful wound-healing properties are lacking.

Animal and In Vitro Studies

A number of animal and in vitro studies lend mechanistic credibility to claims regarding an anti-inflammatory effect of *E. purpurea* aerial parts. Inhibition of hyaluronidase was among the earliest pharmacological properties attributed to *Echinacea* (Bauer 1999a; Büsing 1952; Koch and Haase 1952). Hyaluronidase hydrolyzes hyaluronic acid and chondroitin, allowing penetration of the ground substance by fluids containing pro-inflammatory cytokines. A tincture of *E. purpurea* flowers was reported to have an anti-inflammatory effect on rat paw edema induced by formalin, hyaluronidase, serotonin, and trypsin (Voitenko et al. 1996). Reference standard grade cichoric and caftaric acids have been shown to have in vitro antihyaluronidase activity (IC_{50} = 0.42 and 0.61 mM, respectively) (Facino et al. 1993).

It has also been proposed that *E. purpurea* aerial parts ameliorate inflammation by interfering with arachidonic acid metabolism via the inhibition of cyclooxygenase and 5-lipoxygenase. In one set of experiments, arachidonic acid metabolism and prostaglandin E2 production were reduced by several uncharacterized *E. purpurea* products standardized to 3% to 4% phenolic acids; this effect was not found with powdered aerial parts (Rininger et al. 2000).

Mishima et al. (2004) reported radiation-protective and antioxidant effects of *E. purpurea* aerial parts in mice, as well as activation of cell-mediated immune responses. Powdered dried juice of *E. purpurea* whole plant was mixed into saline solution and either it or a saline placebo was administered into the abdominal cavity at 360 mg/kg every other day for at least 3 weeks prior to experimentation and continued during experimental treatment. *Echinacea* reportedly suppressed radiation-induced leukopenia and hastened recovery of blood cell counts. It also increased peripheral blood antioxidant activity. Enhancement of free radical-scavenging activity by *Echinacea*, including suppression of oxidation of human low-density lipoprotein, has been reported by some laboratories (Rininger et al. 2000; Sloley et al. 2001). Other research suggests that the phenolic compounds from *Echinacea* are associated with antioxidant activity (Dalby-Brown et al. 2005; Facino et al. 1995; Pellati et al. 2005).

Wound healing effects in animals have been reported. An Echinacin-based ointment applied to surgically induced wounds in guinea pigs resulted in more rapid healing compared to controls (Kinkel et al. 1984). Enhanced fibroblast activity has been reported in fibrin grafts exposed to Echinacin (Tünnerhoff and Schwabe 1956). In contrast, an experiment using mouse fibroblasts reported that both herb and root extracts from *E. purpurea* inhibited fibroblast-mediated collagen contraction in vitro (Zoutewelle and van Wijk 1984). The results of this latter study are suggestive of an anti-inflammatory effect which could aid wound healing. Clinical data regarding the effects of *E. purpurea* aerial parts on wound healing are needed. Historically, the first recorded use of *Echinacea* was for treating saddle sores in horses using an external preparation.

Summary

No reliable data from humans on the anti-inflammatory or antioxidant effects of *E. purpurea* aerial parts alone were found, although limited in vitro and laboratory animal studies support such effects. Anti-inflammatory effects may be mediated by the inhibition of hyaluronidase and/or arachidonic acid metabolism. More work is required to better understand the influence of *E. purpurea* aerial parts on wound healing in humans.

Antifungal Effects

Human Clinical Studies

Coeugniet and Kühnast (1986) reported a trial testing the expressed juice of *E. purpurea* aerial parts (Echinacin) for its ability to affect recurrent vaginal yeast infections. Women with laboratory-confirmed *Candida* infections were treated with topical econazole, then allocated to either oral (30 drops tid; n = 60) or injected (0.5 to 2 mL twice weekly; im, n = 60; iv, n = 20; subcutaneous [sc], n = 20) Echinacin, with 43 reserved as controls. Treatment continued for 10 weeks. Recurrence of yeast infection and response to the Merieux Multitest® (antigenic skin test) were measured dur-

ing the treatment period and recurrence was followed over an additional 6-month monitoring period. Echinacin-treated groups demonstrated increased skin reactivity and decreased recurrence of vaginal candidiasis over the 6-month monitoring period. While 60% of controls got new infections, only 5% (im), 15% (sc and iv), and 17% (po) of women in the treatment groups were diagnosed with recurrent vaginal infections ($P < 0.05$). No adverse events were experienced in the group receiving oral treatment. Among subjects receiving parenteral Echinacin, im administration was associated with the highest incidence of both local and systemic adverse events. Randomization and blinding methods were not described, hence it can only be assumed that allocation was non-random and blinding was not attempted.

Animal and In Vitro Studies

Binns et al. (2000) suggested that the roots of *E. purpurea* possess greater antifungal activity than do the aerial parts. These researchers tested *n*-hexane extracts from *E. purpurea* herb and root for antifungal activities in a series of in vitro experiments testing near-ultraviolet (UV)-mediated and light-independent activity against Saccharomyces cerevisiae and several *Candida* strains, including different strains of *Candida* albicans, the most common fungal cause of human skin disease. In these experiments, only *E. purpurea* *n*-hexane root extract caused significant inhibition of C. shehata. Near UV-mediated antifungal activity was significantly greater than light-independent activity ($P = 0.05$). Following isolation and testing of various of the alkamides from *E. purpurea* tops and roots, significant photo[illegible] against cervisiae was attributed to tride[illegible]ne-3,7,9,11-pentadecatetraynyne, one of the ketoalkynes from the roo[illegible]

At least 2 laboratories have reported in vitro increases in the phagocytosis of *Candida* following exposure of human granulocytes to the pressed juice of *E. purpurea* aerial parts (Stotzem et al. 1992; Wildfeuer and Mayerhofer 1994). See et al. (1997) found that in vitro phagocytosis of *Candida* by human macrophages and NK cells was enhanced following exposure to *E. purpurea* extracts (plant part not specified). Polysaccharides extracted from *E. purpurea* cell cultures have been shown to stimulate mouse macrophage activity against *Candida* (Lohmann-Matthes and Wagner 1989) and decrease the infection and death rates of immunosuppressed mice infected with *Candida* (Steinmüller et al. 1993). As noted above, polysaccharides extracted from *E. purpurea* cell cultures differ from those isolated from the aerial parts of the plant.

Summary

Few studies have investigated the antifungal effects of *E. purpurea* aerial parts. One clinical study with unknown randomization and blinding suggested that oral and parenteral application of the aerial parts might decrease recurrence of vaginal candidiasis. Results from in vitro studies have been limited and contrasting.

Anticancer Effects

Specific and nonspecific immune targeting of cancer cells forms the basis of successful mammalian response to transformation of normal cells into cancerous ones. Although immunostimulation makes good theoretical sense, few if any immune-stimulating therapies have proven effective in combating human cancer. A few modest attempts at using *E. purpurea* aerial parts against cancer have been attempted.

Human Clinical Studies

Lersch et al. (1990, 1992, 1994) treated patients having advanced cancer of the liver ($n = 5$), colorectum ($n = 15$), and gastrointestinal tract ($n = 56$) with the expressed juice of *E. purpurea* aerial parts (Echinacin) and thymostimulin. The interventions were given at 60 and 30 mg/m^2 im, respectively, on days 3 to 10 following cyclophosphamide chemotherapy, then twice a week. No controls were used. Although increases in the activity of NK cells, lymphokine activated T cells, and PMNs were reported, no dramatic health benefits were noted. Mean survival time after the first dose was 2.5 months (liver), 4 months (colorectal), and 5.7 months (GI).

A pilot study investigated the use of polysaccharides isolated from *E. purpurea* herb cell cultures to improve immune function and counteract chemotherapy-induced leukopenia (reduction in leukocytes) in 13 patients with advanced gastric cancer (Melchart et al. 2002). Beginning on day 3 prior to commencement of chemotherapy (etoposide, leucovorin, and 5-fluorouracil), each patient received 2 mg iv daily of the polysaccharides for 10 days. By days 14 to 16 post-chemotherapy, leukocyte levels were slightly but significantly higher in the polysaccharide group compared to control gastric cancer patients receiving the same chemotherapy regimen but no polysaccharides ($P < 0.015$). However, once baseline variation between individuals was accounted for, the effect was no longer significant. No clinically relevant effects on the phagocytic activity of granulocytes or on lymphocyte populations were found. A total of 68 adverse events were recorded, most likely due to chemotherapy and cancer progression, but their association with the polysaccharide treatment could not be ruled out and the authors noted that further study was required. Currently, there are no large-scale randomized trials testing *E. purpurea* aerial parts for cancer prevention or treatment, although some herbalists do employ it as part of cancer treatment programs.

Animal Studies

Hayashi et al. (2001) investigated the effect of oral doses of *E. purpurea* leaf powder on spontaneous leukemia caused by endogenous recombinant murine leukemia virus (MuLV) in 4-week-old female mice. Experimental mice received 7.5 mg of the powder weekly for 8 weeks, while controls received buffered saline. Mean survival age was prolonged, the enlargement of thymic lymphoma was suppressed, and the proliferation of MuLV in the thymus was inhibited in verum vs. control mice. The production of IFN-γ was reportedly increased, while effects on TNF-α and IL-12 were small.

Studying induced tumors in mice inoculated with P815 malignant tumor cells, Simpson et al. (2001) found that tumor growth and maximum size were reduced in mice

given an aqueous extract of *E. purpurea* (plant part not specified) compared to controls (statistics not presented). *Echinacea* was applied at 25 μg/g ip beginning 1 day before inoculation. These effects were correlated with an increase in NK cell activity. Despite differences in tumor growth rate and size, T cell rejection of the tumors occurred at equivalent times in both groups.

Summary

To date, very little study has been made of the effects of *E. purpurea* aerial parts on the prevention or treatment of cancer. Although some laboratory animal studies indicate that *E. purpurea* leaf may be beneficial in fighting cancer, the limited work done in humans has shown no clinical benefit of the pressed juice from aerial parts. Similarly, there is no evidence that the polysaccharides isolated from *E. purpurea* cell cultures can help ameliorate chemotherapy-induced leukopenia or improve immune function in cancer patients undergoing conventional treatment.

Conclusion

Although *E. purpurea* aerial parts have been relatively well studied, there are still important gaps in the knowledge base. The most widely reported pharmacological activity, immunomodulation, is only partially understood. In vitro and experimental animal studies have demonstrated the ability of *E. purpurea* aerial parts preparations to enhance the activities of various cells of the non-specific immune system, with no firm evidence to suppo[illegible]s use in the stimulation of acquired immunity. Yet the me[illegible]hanism of imm[illegible]odul[illegible]tion is little understood. In vitro w[illegible]as suggested [illegible]at [illegible] agonistic effect of *Echinacea* alkamides [illegible] th[illegible]anni[illegible]oid receptors may be at least pa[illegible] respo[illegible]sib[illegible] for [illegible]ort[illegible] effects on TNF-α, but this requires confirmation in humans. Active constituents, bioavailability, and pharmacokinetics are not known in sufficient detail. Recent pharmacokinetic work indicates that the alkamides are better absorbed than the caffeic acid derivatives. However, other compounds, possibly the polysaccharides, must also contribute to the therapeutic efficacy of *E. purpurea* aerial parts given that the juice of fresh flowering aerial parts, which contains only trace amounts of alkamides, has been shown to have a therapeutic benefit in some clinical trials.

The most widespread modern use of *E. purpurea* aerial parts for the treatment of acute URTI is tentatively supported by a number of the available clinical studies. Reduction or a trend towards reduction in symptom severity with early treatment has been reported in 5 of 12 double-blind RCT of varying quality. Benefits appear to be modest, with a 10% to 40% reduction of symptoms as the most widely reported outcome. A benefit as a cold preventive is apparent as well, with an estimated effect size of 9% to 27% that may well be clinically significant. Efficacy for the treatment of acute URTI in children has not been upheld, although 1 trial identified a preventive effect in this patient population. Most of the trials used low dosages by the standards of modern herbalists. Additional well-designed trials of adequate size and using dosages congruent with those suggested by herbalists for the prevention and treatment of URTI (Mills and Bone 2000, 2005; see Medical Indications Supported by Traditional or Modern Experience) are necessary in order to better understand the usefulness of *E. purpurea* aerial parts for these indications.

If *Echinacea*'s reported minor-to-moderate benefits as a cold preventive and treatment are real, the implications at a population level are quite significant, as the common cold is humanity's most universal illness. In addition to the discomfort and loss of productivity due to colds and flu-like syndromes, deaths in high-risk persons are not uncommon (Abdullah 2000; Evans and Kaslow 1997; Gwaltney 1985; Monto 1995; Temte 2000). Choices in therapy should be tempered with knowledge regarding various alternatives. In the case of the common cold, few if any conventional medical interventions have been shown to be safe and effective (Turner 2001), making the evidence on *Echinacea* all the more salient.

Medical Indications Supported by Clinical Trials

Based on the positive findings from 7 of 13 double-blind RCT clinical trials, most of which had design limitations, there is evidence to suggest a benefit of *E. purpurea* aerial parts in reducing the incidence or severity of URTI. *E. purpurea* aerial parts showed some benefit in the prevention of URTI in the 3 double-blind RCT addressing this indication, and although this effect did not reach statistical significance, it may [illegible]ell be clinically significant. Additional [illegible]ll-de[illegible]igned trials [illegible]e need[illegible] to substantiate the use of this bo[illegible]nic[illegible] for th[illegible] tre[illegible]me[illegible]t and [illegible]revention of URTI. Given th[illegible] ow incide[illegible]e o[illegible]ad[illegible]se events associated with oral use of *[illegible]chi[illegible]cea*[illegible]s u[illegible] m[illegible] have[illegible] very high benefit-to-risk ratio.

Medical Indications Supported by Traditional and Modern Experience

The 3 primary commercial species of *Echinacea* are used virtually identically among modern herbal practitioners. A more complete presentation of the use of *Echinacea* by practitioners is available in the AHP *Echinacea purpurea* Root monograph (AHP 2004). Summarizing from that monograph, herbalists use all species of *Echinacea* predominantly for acute infections (viral and bacterial), immunomodulation, and bites. Infections for which *Echinacea* is used include URTI (treatment and prevention), skin infections (including topical use for bites and herpes), followed by infections of the eyes, ears, nose, throat, urinary tract, and lymphatic system (Romm and Treasure 2002). Modern commercial use has focused almost exclusively on prevention and treatment of URTI. Some herbalists regard URTI as a secondary rather than primary indication for *Echinacea* and consider its prominent use for these indications to be based on commercial influences rather than medical specificity. These herbalists rely on the oral and topical use of *Echinacea* primarily for septic infections due to wounds or bites, or systemic infections associated with more aggressive stages of inflammation (Tierra 2003, Treasure 2003, personal communications to AHP, unreferenced). Such

indications are consistent with the use of *E. angustifolia* root by the Eclectics, who considered it to be a specific for blood poisoning, meningitis, venomous bites, and various states of septicemia, among numerous other indications (Felter and Lloyd 1898; Lloyd 1923). The Eclectics mention the use of *Echinacea* against URTI, but this was not a frequently cited indication. They did not use *E. purpurea* and in fact, the Eclectic physician Finley Ellingwood reported that *E. purpurea* root was inferior to *E. angustifolia* (Ellingwood 1919). Similarly, there is little historical record of Native American use of *E. purpurea*, leaving little guidance from the traditional literature for appropriate indications for this species.

The use of *E. purpurea* aerial parts as an adjuvant therapy and prophylaxis against recurrent infections of the upper respiratory tract (common colds) and urogenital tract is supported by the European Scientific Cooperative on Phytotherapy (ESCOP 2003). Similarly, the World Health Organization considers the oral administration of the aerial parts to be useful as supportive therapy for colds and infections of the respiratory and urinary tracts and of benefit when applied externally for the promotion of wound healing and inflammation (WHO 2004).

Modern herbalists have reported a preference for *E. angustifolia* over *E. purpurea*. Some prefer root to leaf, while others use a mixture of root and aerial parts. However, all are consistent in their opinion that material must be as freshly harvested as possible noting the rapid loss of potency that occurs over time. Herbalists have reported a preference for hydroalcoholic extracts of varying strengths (e.g. 1:2-5 g/mL; 55% to 70% alcohol) for acute infections and during periods of increased risk of infection, while both solid and liquid dosage forms are employed for general prophylaxis (Gruenwald and Treasure 2002). An optimal dosage range has yet to be clearly defined. To treat acute infections, clinicians may use up to 15 g daily in divided doses in various dosage forms, including powder, decoction, and liquid extracts of varying compositions and strengths (see Dosage). In a recent clinical study reporting positive outcomes, patients were given 40 mL (herb to extract ratio not specified) of an *E. purpurea* root and aerial part tincture upon onset of a cold followed by 16 mL daily for the next 6 days (Goel et al. 2004). This dosage is relatively consistent with modern use by professional herbalists who typically increase dose or frequency if risk of infection increases or at the 1st sign of cold symptoms. As a preventive agent and for treatment of chronic conditions, Mills and Bone (2000, 2005) recommend a daily dose of 2.5 to 6 g of dry *Echinacea* aerial parts; 3 to 5 mL of a 1:1 fresh plant extract (root and aerial parts; equivalent of 3 to 5 g); or 6 to 9 mL of the expressed juice of *E. purpurea* aerial parts. For acute conditions they recommend up to 15 g daily of *E. angustifolia* root.

Some herbalists are of the opinion that *Echinacea* is best used acutely for short periods of time and the German Commission E suggests that duration of use be no more than 8 weeks, although no rationale for this precaution is given. Based on the immunostimulatory nature of *Echinacea*, a few herbal practitioners expressed concern that its long-term use may mask the symptoms of general immune weakness and exacerbate any underlying immune deficiency (Bergner 2001; Hedley 2003, personal communication to AHP, unreferenced). Other herbalists have reported on cases where *Echinacea* was considered to be ineffective due to the inherent deficiency of the patients, and have suggested that in such cases, the effects of *Echinacea* can be supported by the concomitant use of immune modulators such as Astragalus (Astragalus mongholicus), Atractylodes (Atractylodes spp.), or eleuthero (Eleutherococcus senticosus) (Hedley 2003, Tierra 2003, Upton 2003, personal communications to AHP, unreferenced). Yet other herbalists do not feel a temporal limit is warranted and, as noted above, consider *Echinacea* useful for long-term immune support (Bone 2004).

Actions

Clinical: There is modest formal clinical evidence to suggest that *Echinacea purpurea* leaf juice elicits a cold prophylaxis effect and mitigates cold symptoms and duration.

Traditional: There are no well-determined actions and effects for *Echinacea purpurea* aerial parts. In modern traditional use, *Echinacea purpurea* aerial parts are used similarly to *Echinacea* root by some though root is preferred by most.

Pharmacology: Increased phagocytic activity and cytokine secretion (TNF-α, IL-1α,-1β,-6,-2,-10, NO) of macrophages; increased activity of PMN granulocytes; enhanced number and lytic activity of NK cells; antifungal; antioxidant; anti-inflammatory.

Substantiation for Structure and Function Claims

Echinacea possesses various immune modulating effects including increased activity of macrophages and NK cells, as well as antioxidant and anti-inflammatory effects.

Dosages

The dosage range for the alcohol-stabilized fresh juice of *E. purpurea* aerial parts is based upon the following double-blind RCT trials showing positive trends toward efficacy for Echinacin: Berg et al. 1998; Schöneberger 1992; Schulten et al. 2001; Sperber et al. 2004; Taylor et al. 2003. This dosage range is consistent with the daily dosage of 8 to 9 mL recommended by the German Commission E (Blumenthal et al. 2000) and with that recommended by modern herbalists (6 to 9 mL daily [Mills and Bone 2005]). Dosage recommendations for dried raw material and fluid extract represent prophylactic or chronic dosages typically prescribed by modern practitioners (Mills and Bone 2000, 2005). Higher dosages are generally recommended for acute conditions (Mills and Bone 2005). For a more detailed discussion of dosages used by herbal clinicians, see Medical Indications Supported by Traditional or Modern Experience.

Generalizing dosage by product type is difficult given that constituent concentration within a product type can be expected to vary due to differences in the quality of raw material used during manufacture and possible constituent degradation during processing. In the case of hydroalcoholic preparations, potency and hence dosage will depend upon alcohol concentration. In addition to daily dosage, efficacy

will depend upon treatment duration, and dosage and duration will vary with indication.

Fresh juice (21.6% ethanol):
7.5 to 10 mL daily in divided doses.

Dried raw material:
2.5 to 6 g daily (Mills and Bone 2000).

Fluid Extract (1:1):
3 to 5.5 mL daily (whole plant; Mills and Bone 2000).

Safety Profile

Adverse Reactions

Based upon findings from clinical trials, numerous critical reviews, and the widespread use of *E. purpurea* aerial parts by consumers and herbal health care practitioners, this botanical appears to be relatively safe as a supplement or herbal medicine (Barrett 2003; Linde et al. 2006; McGuffin et al. 1997; Mills and Bone 2005; Ulbricht and Basch 2005). Adverse events associated with *Echinacea* (products variously characterized or uncharacterized) are generally mild in nature, often affecting the gastrointestinal tract (nausea, diarrhea) and skin. Rare reports of serious but reversible allergic reactions have been made. No serious adverse events have been associated specifically with the oral use of *E. purpurea* aerial parts.

Table 7 gives the incidence of adverse events reported in the 19 human clinical studies reported in this monograph (trials on cancer were not considered due to concurrent use of chemotherapy). Of these studies, only one reported significantly more adverse events in the *Echinacea* group compared to placebo except Taylor et al. (2003). While these authors found no difference between groups in total adverse events, they did find a significant increased incidence of rashes in the *Echinacea* group compared to placebo (P = 0.008). Five of the studies did not present an analysis of between-group differences in adverse event rate. Four of the studies provided no information on adverse events. No serious events were reported and the most common adverse effect reported was gastrointestinal upset. Three of the four studies using injectable preparations (Echinacin) reported adverse effects associated with the active treatment (Baetgen 1984, 1988 [neither study had a control]; Coeugniet and Kühnast 1986).

Parnham (1996a, 1996b, 1999) has reviewed the literature on the benefit and risks of the fresh juice of *E. purpurea* aerial parts, much of it in German, untranslated, and available only with manufacturer permission. He states that between 1989 and 1995, 13 adverse events that may have been associated with orally administered Echinacin were reported to the German federal health authority (Barrett 2003). Of these, only 4 cases involving an allergic skin reaction were considered to possibly have been associated with the herbal medication. During that same period, several million people in Germany took an *Echinacea* product. Parnham reported that an unpublished open-label study by Madaus found a self-reported adverse event rate of 5% in 1231 children and young adults aged 2 to 20 who were treated with Echinacin lozenges. Of the 62 reported events, unpleasant taste was the most frequent, with 21 reports (1.7%). Nausea, vomiting, sore throat, abdominal pain, diarrhea, and difficulty swallowing were each reported by 6 or less (< 0.5%) persons.

Parnham also reported an early trial in which Echinacin was administered iv to 500 children with tuberculosis at increasing doses from 0.5 to 5 mL over 2 days and repeated up to 15 times over several weeks. In some of the children, an improvement in condition was accompanied by acute symptoms of shivering, headache, vomiting, and fever within 2 to 4 hours of injection. The symptoms disappeared over the course of 1 to 2 hours (Heesen 1964, cited in Parnham 1996a). Parnham noted that this suite of severe adverse reactions may have been associated with immunostimulation. These kinds of strong reactions are likely to have been associated with the parenteral route. In contrast, women with post-partum infections who were injected iv with 0.1 to 1.2 mL of Echinacin reported no ill effects (case series by Röseler 1952 [n = 226] and Moell 1951 [n = 120], cited by Parnham 1999). The original German Commission E monograph on *E. purpurea* aerial parts noted that parenteral application could result in the following side effects, depending upon dosage: short-term fever, nausea and vomiting, and in individual cases immediate allergic reactions (Blumenthal et al. 2000). The parenteral application of *E. purpurea* aerial parts is no longer marketed in Germany.

A recent systematic review of the safety of *Echinacea* products (excluding combination and homeopathic preparations) considered published case reports, reports from spontaneous reporting programs and 23 product manufacturers in addition to clinical trials (Huntley et al. 2005). Spontaneous report programs from Australia, Germany, the UK, USA (FDA), and WHO were examined. The authors concluded that adverse event reports related to the short-term use of *Echinacea* were uncommon. Gastrointestinal upset and rashes were the most frequently reported adverse events, with rare instances of severe allergic reactions. A similar review considering spontaneous reports of adverse events submitted to WHO (259 reports of 537 adverse reactions as of the end of 2004) reported that the large majority of reactions involved the gastrointestinal tract, angioedema, dyspnea, and various skin rashes (Barnes et al. 2005). The authors cautioned that spontaneous reports are based on a suspected but unproven relationship between the *Echinacea* product and the adverse reaction.

The potential allergenicity of *Echinacea* has received a fair amount of attention (Bielory 2002), although there are very few data on *E. purpurea* aerial parts alone. As noted above, 4 cases of allergic skin reactions possibly associated with the oral administration of Echinacin were reported to the German health authority between 1989 and 1995. Rashes were also a reported side effect of oral Echinacin in the recent trial on the treatment of URTI in children by Taylor et al. (2003). Other potential reports of allergenicity have not been definitively associated with *E. purpurea* aerial parts and are presented in the AHP monograph *Echinacea*

Table 7 **Adverse events reported in clinical trials using *Echinacea purpurea* aerial parts preparations *(continued)***

Reference	Study design	Intervention*	Adverse events (AE) in Echinacea group	Adverse events (AE) in placebo group	Statistical difference between groups
Goel et al. 2004	DBRCT	Echinilin po (EP aerial parts and root): 4 mL in water 10 times on day 1, then qid for 6 days	8 of 59 subjects reported nausea, heartburn, or constipation; 8 of 59 subjects reported itching, burning, and numbness of tongue	6 of 69 subjects reported nausea, heartburn, or constipation; 8 of 69 reported itching, burning, and numbness of tongue	No
Sperber et al. 2004	DBRCT	EchinaGuard (= Echinacin) po: 2.5 mL tid for 14 days	2 of 24 subjects reported sleeplessness and severe oral aphthous ulcers	4 of 24 subjects reported AE	No
Yale and Liu 2004	DBRCT	Echinafresh capsules po (EP aerial parts juice): 100 mg tid for up to 14 days	7 AE among 63 subjects: nausea, abdominal pain, mouth irritation, bad taste, headache, dizziness, dry mouth	5 AE among 65 subjects	Not analyzed
Taylor et al. 2003	DBRCT	Echinacin po (no alcohol): 3.75 mL bid in 2- to 5-yr-olds; 5 mL bid in 6- to 11-yr-olds; for up to 10 days	152 AE among 337 URTI treated: itchiness, rash, hyperactivity, diarrhea, vomiting, stomachache, headache, drowsiness	146 AE among 370 URTI treated; AE of same types as experienced by verum subjects	Overall no; yes for rashes ($P = 0.008$)
Barrett et al. 2002	DBRCT	EA root extract, EP root and herb, thyme, peppermint, citric acid: 4 capsules 6 times on day 1, then 4 capsules for up to 10 days	13 AE reported by 8 of 13 subjects: sleeplessness, heartburn, nausea, stomachache, upset stomach, bad taste in mouth	9 AE reported by 7 of 75 subjects: stomachache, nausea, belching, thirst, diarrhea	No
Kim et al. 2002	DBRCT	EP whole herb extract po): 1500 mg for 4 weeks	1 of 8 subjects reported recurrence of arthritis	None	No
Schulten et al. 2001	DBRCT	Echinacin po: 5 mL bid for 10 days	8 AE reported by 6 of 41 subjects: GI tract, respiratory tract	9 AE reported by 6 of 39 subjects	No
Vonau et al. 2001	DBRCT, crossover	Echinaforce po (EP aerial parts and root): 800 mg bid for 6 months	4 of 50 subjects reported nausea, diarrhea	2 of 50 subjects reported nausea	Not analyzed
Lindenmuth and Lindenmuth 2000	DBRCT	Echinacea Plus tea: 5-6 cups daily, titrating to 1 cup by day 5	None	_	
Turner et al. 2000	DBRCT	Extract po (EP and EA, plant part unknown): 300 mg tid for19 days	None	_	
Brinkeborn et al. 1999	DBRCT	Echinaforce po (EP aerial parts and root) or Echinaforce 7:1 po: 2 tablets tid for up to 7 days	Echinaforce = 7 of 55 subjects; Echinaforce 7:1 = 8 of 64 subjects. AE reported: GI tract, whole body, nervous system, skin, urinary tract	6 of 64 subjects reported AE affecting GI tract, nervous system, skin	No

Table 7 Adverse events reported in clinical trials using *Echinacea purpurea* aerial parts preparations

Reference	Study design	Intervention*	Adverse events (AE) in Echinacea group	Adverse events (AE) in placebo group	Statistical difference between groups
Berg et al. 1998	DBRCT	Echinacin po: 40 drops tid for 28 days	None	_	
Hoheisel et al. 1997	DBRCT	EchinaGuard (= Echinacin) po: 20 drops (~1.33 mL) every 2 hr on day 1, then tid up to day 10	None	_	
Melchart et al. 1995 (1990 trial)	DBRCT	Extract po: 30 drops tid for 5 days	None	_	
Schöneberger 1992	DBRCT	Echinacin po: 4 mL bid for 8 weeks	11 of 54 subjects reported nausea, heartburn, constipation, fatigue, vertigo, headache, irritability, urinary tract, eczema, hair loss	7 of 54 subjects reported nausea, heartburn, constipation, urinary tract, sweating, paraesthesia	No
Coeugniet and Kühnast 1986	Open, Controlled	Echinacin po (30 drops tid or ~2.4 mL daily), sc, im, or iv (0.5 to 2 mL twice weekly) for 10 weeks	0 of 60 subjects po; 4 of 20 sc; 9 of 60 im; 2 of 20 iv: local reactions, fever (38.5 ° C), low energy	None	No
Baetgen 1988	Retrospective	Echinacin im: dosage not available	None	_	
Baetgen 1984	Retrospective	Echinacin im 1–2 mL daily for 10 days	1 of 7 subjects reported erythema, localized pain at injection site	3 of 63 subjects reported erythema or pain at injection site	Not analyzed
Möse 1983	DBRCT	Echinacin iv: 2 mL daily for 4 days	None	_	

purpurea Root (AHP 2004). Allergenicity to the pollen proteins from plants in the sunflower family (*Asteraceae*) is common (e.g. ragweed). It is possible to hypothesize that *E. purpurea* aerial parts products have more potential to elicit allergic reactions in atopic individuals than do root products. That said, allergenic pollen proteins have relatively poor solubility in aqueous ethanolic extracts, suggesting there may be less of a propensity for allergenicity with alcoholic extracts compared to solid dosage forms (Bone 2004). This latter assertion has not been confirmed.

There is a single case report of an individual with diverticulitis developing granulomatous hepatitis while using an unidentified species of *Echinacea* (Moore et al. 2004). Causality could not be definitively determined. The authors reported that granuloma formation results from the accumulation of macrophages in response to a foreign agent. As one of the reported effects of *Echinacea* is enhanced macrophage activity, the authors felt that a connection between *Echinacea* and the occurrence was possible.

Three studies have noted transient leukopenia (low white cell count) following the intravenous use of *E. purpurea* aerial parts (Echinacin) (Coeugniet and Elek 1987; Moell 1951; Röseler 1952; the last 2 studies cited in Parnham 1996a). A case report by Kemp and Franco (2002) that suggests a possible association between an uncharacterized *Echinacea* product (450 mg tid) and leukopenia is presented in the AHP monograph *Echinacea purpurea* Root (AHP 2004). The woman reported on was also taking Ginkgo biloba, bupropion, vitamins, and calcium.

Echinacea's potential immunostimulatory activity has caused some concern regarding its use in people with autoimmune disorders. However, definitive data supporting a connection between *Echinacea* and autoimmune symptom exacerbation are lacking. In a published case report, Lee

and Werth (2004) describe an individual with chronic uveitis who had been in remission for a year and who became symptomatic again following the onset of an URTI for which he took an *Echinacea* product. A definitive causal relationship between *Echinacea* and the flare-up could not be made. Flare-ups of autoimmune symptoms are more likely in patients experiencing concurrent acute infections regardless of whether additional medications are prescribed (Macdonald 2005). According to a report from one herbal practitioner, an exacerbation of symptoms following the use of *E. purpurea* or *E. angustifolia* (products uncharacterized) was observed in 11 patients with autoimmune disorders such as systemic lupus, ulcerative colitis (autoimmune etiology uncertain), glomerulonephritis, and multiple sclerosis (Bergner 2001). In "some" of these cases, symptom exacerbation was reported upon re-challenge with *Echinacea*. In contrast, another practitioner reported use of low doses of *Echinacea* over 10-day periods for opportunistic infections in several patients with rheumatoid arthritis, with no observable exacerbation of the condition (Hedley 2003, personal communication to AHP, unreferenced).

A survey of medical herbalists (n = 25) was conducted specifically to ask about the use of *Echinacea* in autoimmune conditions (Macdonald 2005). Forty-eight percent of the respondents (n = 12) reported having used *Echinacea* specifically for autoimmune conditions. Of these, 92% reported beneficial effects while 8.5% said they had observed a worsening of sym[illegible]. There are 2 case reports of an association betwee[illegible]an *Ech[illegible]acea* product and an acute asthma attack (Mull[illegible]and Hedd[illegible]2002). Alt[illegible]ugh asthma is not an autoimmune disea[illegible]it is a d[illegible]ulation of the immune system. In th[illegible]irst case[illegible]he attack b[illegible]an within 10 minutes of ingestion of a[illegible]*inacea*-containing tea. In the other, a documented new onset asthma attack was associated with *Echinacea* tablets. Three separate re-challenges resulted in difficulty breathing and coughing within 2 hours of tablet ingestion. One herbal practitioner reported an occasional temporary worsening of asthma symptoms lasting approximately a week in patients given *Echinacea* spp., with "considerable benefit" thereafter (Bone 2005, personal communication to AHP, unreferenced).

Interactions

There is evidence for a potential inhibitory effect of *Echinacea* on the cytochrome P450 (CYP) family of drug metabolizing enzymes based upon in vivo and in vitro work done with extracts of *E. purpurea* root or whole plant and/or *E. angustifolia* root. These results could have implications for those taking CYP substrates such as immunosuppressants, anticoagulants, benzodiazepines, and calcium channel blockers. The interpretation of in vitro studies on CYP effects is difficult, given that in vitro CYP inhibition studies (e.g. of milk thistle [*Silybum marianum*] and St John's wort [*Hypercium perforatum*] and their isolates) have produced numerous false positives based upon results from comparative human studies (Brinker 2005). When assessing the data below, it is also important to bear in mind that any potential effects on the CYP enzymes will be expected to vary with product given the differences in constituent profile between the various preparations of *Echinacea*.

No studies testing *E. purpurea* aerial parts alone were available. Gurley et al. (2004) performed an in vivo test in healthy humans (n = 12) of long-term supplementation with *E. purpurea* whole plant extract (800 mg bid for 28 days) on cytochrome P450 activity. An inhibitory effect approaching significance was found on CYP1A2 ($P < 0.07$). The authors suggested that the effects of *Echinacea* on P450 may be more complex than their own study was able to assess, involving different effects on different organs. This viewpoint was discussed in light of the findings of Gorski et al. (2004), who reported both inhibitory and stimulatory in vivo CYP effects of *E. purpurea* root powder depending on the substrate and the relative extraction of the drug at hepatic vs. intestinal sites (see AHP monograph *Echinacea purpurea* Root [AHP 2004]). Yale and Glurich (2005) reported that *E. purpurea* (product uncharacterized) demonstrated mild inhibitory or inducing in vitro effects on CYP 3A4 depending upon which model substrate was used. There was little effect observed on CYP 2D6 and moderate inhibition of CYP 2C9.

The recent study by Matthias et al. (2005b) presented above (see Pharmacokinetics) tested the in vitro metabolism by human liver microsomes of alkamides isolated from a 60% ethanolic extract of *E. angustifolia* and *E. purpurea* roots. Their work suggested that individual alkamides also known to be present in *E. purpurea* aerial parts are metabolized differently by the CYP enzymes and that there is an overall inhibitory effect on the CYPs that may act to preserve alkamide bioavailability. Several other studies have suggested that some of the constituents found in *E. purpurea* aerial parts (i.e. cichoric acid, dodecatrienoic acid isobutylamides) have variously inhibitory or inducing effects on liver drug metabolizing enzymes (Allen et al. 2001; Ciolino et al. 1999; Ho et al. 2001; Nguyen et al. 2003; Raucy 2003).

Some have expressed a theoretical concern that *Echinacea* could decrease the effectiveness of immunosuppressant agents due to its immunostimulatory activity (Ang-Lee et al. 2001; Izzo and Ernst 2001). Data supporting or refuting this hypothesis have not been published to date.

One study in healthy mice reported that *E. purpurea* root extract (0.45 mg daily) and melatonin (0.0142 mg daily) administered together, but not alone, had a detrimental effect on levels of mature granulocytes in both bone marrow and spleen (Currier et al. 2001). Melatonin is a neurohormone commonly taken as a sleep aid. It is also a potent stimulator of NK cell populations (Currier and Miller 2001). The authors suggested that because the observed effect was correlated with an increase in myeloid progenitor cells in both organs, that melatonin and *Echinacea* together had a deleterious effect on the maturation of granulocyte progenitors. It is unknown whether the observed effect has any clinical relevance.

Reproductive and Developmental Effects

A definitive determination of the safety of *Echinacea* in pregnancy has not been made. However, *Echinacea* has been reported to be the most common botanical used by women throughout pregnancy (Tsui et al. 2001). Despite this widespread use, no case reports of adverse effects of *Echinacea* associated with pregnancy have been found. A prospective controlled cohort study was conducted and published by the Motherisk Program in an attempt to provide women with an evidence-based assessment of the safety or risk of *Echinacea* use in pregnancy (Gallo et al. 2000; see AHP monograph *Echinacea purpurea* Root [AHP 2004]). Although no evidence of increased gestational risk was noted, the study lacked the statistical power and methodological rigor to determine pregnancy-associated risks with any degree of confidence. In addition, the dosages consumed were generally much lower than those often employed by herbalists.

Much of the data available on the potential reproductive and developmental effects of *E. purpurea* aerial parts comes from in vitro or experimental animal studies and hence cannot be extrapolated to oral use of *Echinacea* by humans. Limited preclinical data indicate that *E. purpurea* aerial parts in the form of the commercial product Echinacin are unlikely to cause negative reproductive or developmental effects in laboratory animals. Oral doses up to 2700 mg/kg of Echinacin did not cause embryotoxicity in rats or rabbits or affect postnatal development in rats (Mengs et al. 2000). Studies looking for gene mutations, malignant transformation, or chromosome aberrations in bacteria, mouse lymphoma cells, cultured hamster cells, or human lymphocytes have found no evidence of mutagenicity of Echinacin (Mengs et al. 1991, 2000). A polysaccharide isolated from *E. purpurea* aerial parts cell cultures showed no evidence of mutagenicity in a genotoxicity human lymphocyte assay (Schimmer et al. 1989).

Concern has been expressed that agents such as *Echinacea* that have demonstrated in vitro NK-stimulating activity may be contraindicated, particularly in the 1st half of pregnancy (Chow et al. 2005). This is because NK cell activity is suppressed during normal pregnancy to prevent immunological attack of the fetus. In a study of pregnant mice, an *E. purpurea* root extract (Santé Naturelle [AG] Ltée) was administered as part of their diet (0.45 mg/mouse daily) from onset of pregnancy to gestational days 10 to 14 (Chow et al. 2005). Splenic lymphocytes and nucleated erythroid cells, which are normally significantly elevated in the maternal spleens of mice, were reduced to levels comparable to non-pregnant mice in mice that ingested the preparation. Hematopoiesis from the bone marrow was not affected. A reduced number of viable fetuses during the 1st half of the gestational period were found in the *Echinacea* group. In the 2nd half of gestation, the viable fetuses were clinically normal and of control weight. This led the authors to conclude that use of *Echinacea* in the 1st half of pregnancy warrants caution.

Ondrizek et al. (1999b) reported that high concentrations of *E. purpurea* (0.6 mg/mL, plant part undisclosed) applied directly to sperm impaired sperm motility and suggested this may have a negative effect on male fertility. Motility was inhibited at 24 and 48 hours after incubation. They reported similar in vitro results using hamster sperm and oocytes (Ondrizek et al. 1999a).

Carcinogenicity

No data available.

Toxicology

The low incidence of adverse events in the human clinical studies reported offers some empirical evidence of the lack of toxicity of typical acute and chronic oral dosages of *E. purpurea* aerial parts in humans. Modern herbalists have reported use of up to 30 mL of a 1:5 tincture in a single dose with no adverse effect (Schoenbart 2005, personal communication to AHP, unreferenced).

There are very limited data from laboratory animals on the acute and chronic toxicity of the unstabilized fresh juice of *E. purpurea* aerial parts. These experiments have failed to demonstrate significant risks. According to Mengs et al. (1991, 2000), rats (mice) given a single oral dose greater than 15,000 mg/kg (30,000 mg/kg) or an iv dose greater than 5000 mg/kg (10,000 mg/kg) of the juice experienced no ill effects other than sedation and dyspnea at the high iv dose. The authors concluded that the lethal dose could not be found. Oral treatment of rats for 4 weeks at doses of up to 8 g/kg daily of the same product failed to cause adverse effects on body weight gain, organ weights, or histopathology (Mengs et al. 1991). The data reported are reassuring, suggesting that there is a wide therapeutic window of safety between the typical doses consumed daily by humans in clinical trials (200 to 900 mg in 70 to 90 kg adults, equivalent to 2.5 to 40 mg/kg) and the doses used on the experimental animals. In a different series of experiments, Lenk (1989) reported that varying doses of concentrated polysaccharide fractions from *E. purpurea* aerial parts cell cultures injected into 18 mice led to an estimated LD_{50} of 2500 mg/kg. Coeugniet and Elek (1987) reported that Echinacin had no adverse effect on the viability of human leukocytes and lymphocytes except at the highest clinically irrelevant concentration (1/100).

Contraindications

No definitive contraindications are known for either internal or external application of *E. purpurea* aerial parts (see Precautions). Of professional herbalists surveyed in the US, 59% did not recognize any contraindications for *Echinacea* in general, while 31% did (Romm and Treasure 2002). Among the contraindications given were: immune malfunction (10); organ transplant recipients (2); ragweed allergy (2); and in the 1st trimester of pregnancy (1). Of 46 respondents, 22% contraindicated *Echinacea* in autoimmune conditions, 26% did not know if this was necessary, and 52% did not agree with such a contraindication. Seventy percent of 52 respondents had used *Echinacea* in patients with an autoimmune condition, in most cases (63%) not for the autoimmune disease itself, but for a concurrent condition such as an infection. In a recent survey that interviewed 25 herbalists in the UK, US, and Australia regarding their

use of *Echinacea* in patients with autoimmune disorders, 4 described their clinical experience as supporting a contraindication of *Echinacea* in patients with autoimmune disorders, while 21 did not (Macdonald 2005). The German Commission E contraindicates the use of *E. purpurea* aerial parts in cases of progressive systemic diseases such as tuberculosis, leucosis, collagenosis, and multiple sclerosis (Blumenthal et al. 2000). No rationale was given for this contraindication (Mills and Bone 2000).

Precautions

There are no clear precautions regarding the use of *Echinacea*. However, because the possibility of immune-related adverse consequences due to *Echinacea* has not been ruled out in individuals with atopy, autoimmune, or inflammatory diseases, these patient populations should use *Echinacea* with caution and discontinue use if these conditions appear to be exacerbated with *Echinacea* use. More investigation in this area is clearly needed.

Atopic patients especially should avoid use of *Echinacea* products made from flowering aerial parts or use them with care and discontinue use if an exacerbation of symptoms appears. Sharp (1997) cautioned that *Echinacea* may be contraindicated in asthmatics because of its effect on TNF-α, a cytokine that increases the inflammatory process in asthmatics. There have been 2 published case reports of an acute asthma attack associated with *Echinacea* (Mullins and Heddle 2002) and a report of [illegible]ional acute worsening of asthma symptoms lasting approximately 1 week following the oral use of *Echinacea* (B[illegible]2005, personal communication to AHP, unreferenced).

Some practitioners are of the opinion that *Echinacea* is best used acutely for short periods of time (Percival 2000; Tesch 2001; Thomas 2001) and the German Commission E suggests a duration limit of 8 weeks, although no rationale for this precaution is given. Based upon the immunostimulatory nature of *Echinacea*, there has been a concern among herbalists that its chronic use may mask the symptoms of an immune system weakened by poor diet or lifestyle factors (Bergner 2001; Hedley 2003, personal communication to AHP, unreferenced) or may exhaust the immune system through over-stimulation. Yet other herbalists do not feel a temporal limit is warranted and, as noted above, consider *Echinacea* useful for long-term immune support (Bone 2004). Of 45 respondents, the AHG survey reported that 60% recommended that the use of *Echinacea* be limited in duration, while 40% did not (Romm and Treasure 2002). The most frequent way of applying limits was to: 1) dose acutely for 3 to 10 days; 2) use for moderate durations, such as 2 to 12 weeks; or 3) to "pulse" dose, for example by taking a product for 10 days, then discontinuing for 3 days, and repeating this pattern.

There is no firm evidence to conclude the debate on duration limits. Long-term use of *Echinacea* in healthy mice has not shown evidence of a deleterious effect on immune cells. One study reported that immune reactivity in mice was greater after 10 weeks of continuous oral doses of Echinacin than after 2 weeks (Coeugniet and Kühnast 1986). In a study by Miller and Brousseau (2004), low dose chronic administration of *Echinacea* in mice resulted in the preservation of NK cell activity. Natural killer cell activity normally decreases with age and contributes to increased mortality. Use of *Echinacea* increased the life span of most of the mice (Miller and Brousseau 2004). Considering the widespread inclusion of *Echinacea* in multivitamins intended for daily use, juices, and a myriad of herbal supplements, further investigation of effects of long-term use is warranted.

Lactation

No data available. Based upon the experience of modern practitioners, no adverse effects are to be expected. Considering that *Echinacea* consists of compounds generally considered to be non-toxic, little or no toxicity is expected to occur in nursing infants (Hale 2000).

Influence on Driving

No data available. Based upon widespread use, no adverse effects are to be expected.

Overdose

No overdoses have been reported in the literature. The Eclectic physician Ellingwood (1919) reported that "extreme" yet undefined doses of *E. angustifolia* root resulted in: slow pulse, dry mouth, thirst, headache, tendency to fainting upon standing up, flushed face, joint and muscle pain, vomiting, and watery diarrhea. He further noted that to his knowledge no fatal case of poisoning with *Echinacea* had been reported. It should be noted that *E. angustifolia* root was one of the most widely prescribed botanicals of the Eclectics for a period of almost 50 years.

Treatment of Overdose

No data available.

Classification of the American Herbal Products Association

The Botanical Safety Handbook of the American Herbal Products Association (AHPA) assigns *E. purpurea* aerial parts the following classification (McGuffin et al. 1997): Class 1: Herbs that can be safely consumed when used appropriately.

Conclusion

E. purpurea aerial parts appear to have a strong safety record, with no serious and irreversible adverse events reported in any of the clinical trials published to date or in any case reports submitted by medical herbalists and consumers. A comprehensive review of adverse effects associated with all *Echinacea* products was conducted that included systematic literature reviews and reviews of pharmacovigilance data from the World Health Organization, other national drug safety organizations, and several manufacturers (Huntley et al. 2005). According to this review, adverse effects associated with *Echinacea* use are not commonly reported. The most common adverse effects reported were gastrointestinal upset and rash, with rare reports of severe allergic reactions.

Nonetheless, because of the potential immunostimulatory effects of *E. purpurea* aerial parts, they should be avoided or taken with caution by individuals with atopy, autoimmune, and/or inflammatory diseases. Data regarding its safety during pregnancy and lactation are insufficient to make a conclusive determination of safety for these subject populations.

Perhaps the most convincing argument for the safety of *Echinacea* is based upon the ratio of reported serious adverse events (less than 100 ever reported in the medical literature) to the estimated number of courses of treatment annually (conservatively, more than 10 million), which yields a risk estimate of less than 1 in 100,000 (Barrett 2003). Herbal medicines are estimated to be taken by between 15% and 40% of American adults in a given year (Astin 1998; Eisenberg et al. 1998; Landmark Healthcare [LH] 1998). According to Brevoort (1998), *Echinacea* accounted for approximately 10% of the US herbal market in 1998. Using these numbers, it can be estimated that 1% to 4% of the general population uses *Echinacea* in a given year. With a US population of more than 200 million adults and self-treating adolescents, it can be estimated that 2 to 8 million Americans use an *Echinacea* product at least once in a given year. With no deaths and few significant adverse events reported, and if the minor-to-moderate benefits as a cold preventive and treatment are real, the overall benefit:risk ratio appears favorable, especially when compared with the thousands of deaths each year attributed to over-the-counter non-steroidal anti-inflammatory drugs (aspirin, ibuprofen, naproxen) and decongestants (phenylpropanolamine, pseudoephedrine) (Bates et al. 199[illegible]; Brower and Coldiron 199[illegible]; Cetaruk and Aaron 1994).

International Status

United States
Marketed as a dietary supplement (USC 1994) with allowable non-specific structure/function claim statements such as "supports the immune system", or as a homeopathic medicine with specific therapeutic claim statements. Included in the United States Pharmacopeia (USP 29-NF 24 2006), with limits provided for cichoric acid and dodecatetraenoic acid isobutylamides.

Australia
E. purpurea herb is a substance that may be used as an active ingredient in 'Listed' medicines (TGA 2004a). There are numerous listed medicines that contain various preparations of *E. purpurea* herb included in the Australian Register of Therapeutic Goods (TGA 2004b). Indications: Product-specific depending on level of evidence submitted with product license application. An approved "traditional use" indication is: "*Echinacea* helps support the immune system especially during the winter colds and flu season. This herb has been used traditionally for hundreds of years and now scientific evidence suggests that it may assist in supporting immune function" (TGA 2001).

Canada
E. purpurea herb in various dosage forms is regulated as a Natural Health Product (NHP) requiring pre-marketing authorization and assignment of a Natural Product Number. *Echinacea* NHPs include traditional herbal medicines and homeopathic medicines (HC 2003). Indications: Oral: Traditionally used to fight off colds, flus and infections, especially of the respiratory tract. Topical: Helps to promote wound healing and in the relief of inflammatory skin conditions (HC-NHPD 2004).

Council of Europe
Regulated as a Traditional Herbal Medicine Product in the 25 EU-Member States (European Parliament and Council of European Union [EPCEU 2004]). Quality control monograph for *E. purpurea* dried, whole or cut flowering aerial parts has been proposed for inclusion in the *European Pharmacopoeia* (Pharmeuropa 2004), with a limit provided for total phenols calculated as the sum of caftaric acid and cichoric acid.

European Scientific Cooperative for Phytotherapy
A therapeutic monograph for *E. purpurea* fresh or dried flowering aerial parts has been published by ESCOP. Internal Indications: Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common colds) and also of the urogenital tract. External Indications: Adjuvant for the treatment of superficial wounds (ESCOP 2003).

Switzerland
[illegible] containing *E. purpurea* herb require pre-marketing authorization and assignment of an IKS product license number from Swissmedic; retail availability without prescription is limited to authorized establishments such as pharmacies, drugstores, and clinic dispensaries (Swissmedic 2004). Indications: Product-specific depending on level of evidence submitted with product license application. For example, a licensed product containing a tincture of the fresh herb and root (EchinaMed®; IKS No. 54825) is indicated for stimulating the body's immune system when a person is susceptible to cold and flu conditions as well as feverish cold (Morant and Ruppanner 2005).

World Health Organization (WHO)
A therapeutic and standards monograph for fresh or dried aerial parts harvested at full bloom has been published by WHO. Internal Indications: Supportive therapy for colds and infections of the respiratory and urinary tracts. External Indications: Promotion of wound healing and treatment of inflammatory skin conditions (WHO 2004).

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Echinacea purpurea

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