11 March 2010
EMA/HMPC/577786/2008
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Echinacea purpurea* (L.) Moench, radix

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Whole, cut, dried underground parts of *Echinacea purpurea* (L.) Moench
Herbal preparation(s)	Dry water-ethanol extracts
Pharmaceutical forms	Herbal preparation in solid dosage forms for oral and oromucosal use
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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Echinaceae purpureae radix (European Pharmacopoeia monograph reference 01/2008: 1824)

Echinaceae purpureae radix consists of the whole or cut, dried underground parts of *Echinacea purpurea* (L.) Moench. It contains not less than 0.5 % for the sum of caftaric acid ($C_{13}H_{12}O_9$; M_r 312.2) and cichoric acid ($C_{22}H_{18}O_{12}$; M_r 474.3) in dried drug.

Constituents (Barnes *et al.* 2005, Barnes *et al.* 2007, Bauer and Remiger 1989, Bradley 2006, ESCOP 2003 and 2009, Bauer and Liersch 1993, Mazza and Cottrell 1999, Wolters Kluwer Health 2004, PDR 2007):

- Alkamides (0.01-0.7%): mainly isobutylamides of straight-chain fatty-acids with olefinic and/or acetylenic bonds e.g. isomeric dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic isobutylamide. Undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide is also prominent. Isobutylamides contain mainly 2,4-dienoic units.

- Caffeic acid derivatives (2.0-2.8%): principally cichoric acid (2,3-*O*-dicaffeoyltartaric acid, 1.7-2.4%) and caftaric acid (2-*O*-caffeoyltartaric acid, ca. 0.2-0.8%) also echinacoside, verbascoside, caffeoylechinacoside, chlorogenic and isochlorogenic acids.

- Polysaccharides and glycoproteins: arabinogalactans, and an arabinogalactan-containing glycoprotein with a sugar component consisting of arabinose (64-84%), galactose (2-5%) and galactosamine (6%).

- Volatile oil (0.1%): caryophyllene, caryophyllene oxide, humulene, α-phellandrene, limonene, camphene, aldehydes and dimethyl sulphide.

- Other constituents: small amounts of polyacetylenic compounds polyynes (0.01 mg/%) including trideca-1-en-3,5,7,9,11-pentaine, trideca-1,11-dien-3,5,7,9,-tetraine, trideca-8,10,12-triene-2,4,6-triine)

- Effective pyrrolizidine alkaloids: tussilagine, isotussilagine (PDR 2007).

Caffeic acid derivatives

with tartaric acid

cichoric acid

caftaric acid

with quinic acid

cynarine

with sugar

echinacoside

Alkamides
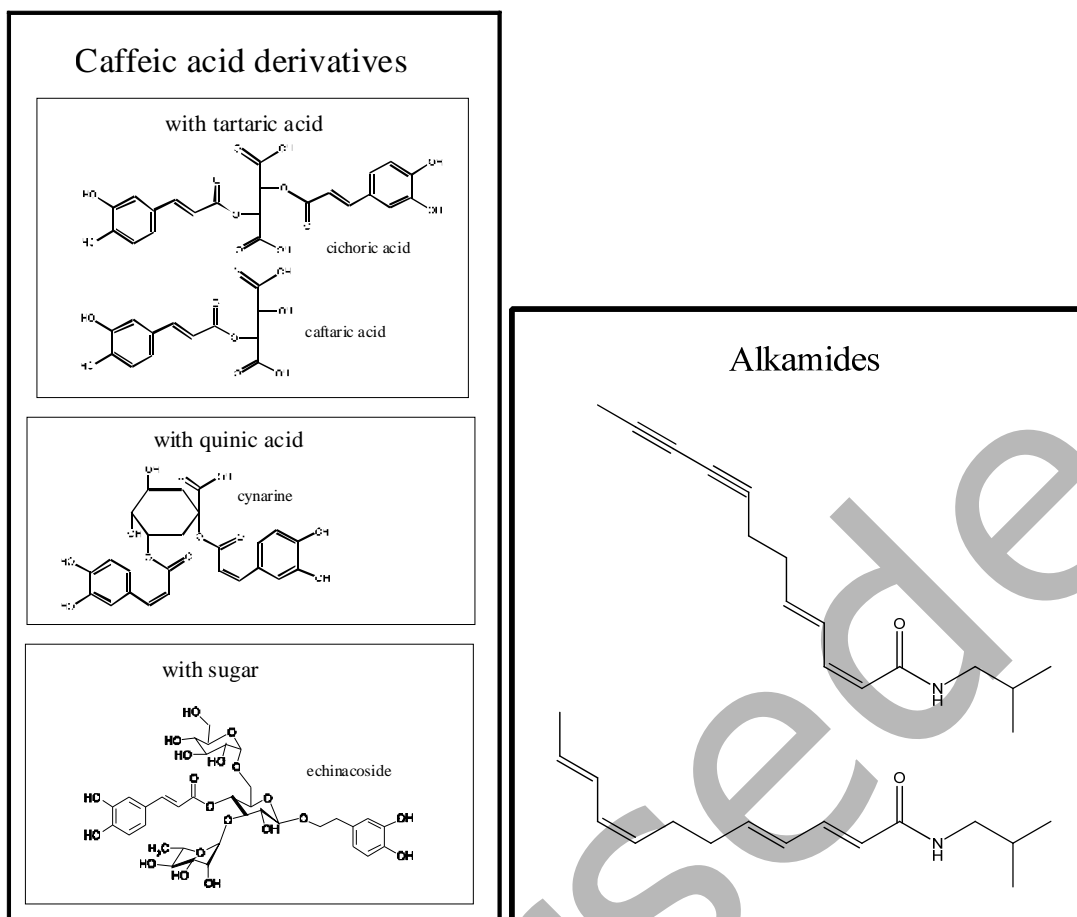
- Herbal preparation(s)

Comminuted herbal substance for decoctions and galenic preparations (PDR 2007, Blumenthal *et al.* 2000, ESCOP 2009)

Dry extract (6.5:1), extraction solvent: ethanol 45% (V/V).

Dry extract (5.5-7.5:1), extraction solvent: ethanol 45% (V/V)

Tincture (1:5), extraction solvent: ethanol 55% (V/V) (ESCOP 2009, Blumenthal *et al.* 2000, Bräunig *et al.* 1992, Barrett *et al.* 1999, Melchart *et al.* 1994).

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Information about products on the market in the Member States

Dry extracts

Spain

Preparations:

1)   Dry extract (5.5-7.5:1) ethanol 45% V/V. 30 mg of extract/tablet, equivalent to 200 mg of the herbal substance.

Pharmaceutical form:

1)   tablet

Sweden

Preparations:

1)   Ethanol extract (6.5:1), ethanol 45% V/V. 1 chewable tablet contains 40 mg extract corresponding to 260 mg radix.

Pharmaceutical form:

1)   chewable tablet

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Comb. product (1).
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Comb. product (1).
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements.
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Comb. prod. (3).
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Comb. products.
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Comb. prod. (4).
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements.
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.

Member State	Regulatory Status				Comments (not mandatory field)
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements.
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Comb. prod. (2).
Spain	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Sweden	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

Other: If known, it should be specified or otherwise add 'Not Known'

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Spain

The dry extract (5.5-7.5:1) ethanol 45% V/V has been on the market since 2002.

Sweden

The ethanol extract (6.5:1), ethanol 45% V/V) has been on the market since 1978.

2.2. Information on traditional/current indications and specified substances/preparations

Traditional indications for oral use of herbal teas and tincture.

Indication	References
Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold).	ESCOP 2003 and 2009, Barnes *et al.* 2005, Barnes *et al.* 2007, Bradley 2006, Bauer and Liersch 1993, Blumenthal *et al.* 2000, Bräunig *et al.* 1992, Barrett *et al.* 1999, Melchart *et al.* 1994)

Herbal substance

Not applicable.

Herbal preparations:

0.9 g comminuted or powdered dried roots as infusion several times daily between meals (Bauer and Liersch 1993, PDR 2007, Blumenthal *et al.* 2000, ESCOP 2009).

Infusion: Steep 0.9 g dried root in 150 ml boiled water for 10 min (Blumenthal *et al.* 2000).

Tincture: 60 drops (1:5 in ethanol 55%) three times daily, corresponding to three times 300 mg of dried root (Barnes *et al.* 2007, ESCOP 2003 and 2009, Blumenthal *et al.* 2000, Bräunig *et al.* 1992, Barrett *et al.* 1999, Melchart *et al.* 1994).

The duration of treatment should not exceed eight (8) weeks (Barnes *et al.* 2007, ESCOP 2003 and 2009).

There is no evidence of 30-year of medicinal use of the comminuted or powdered dried roots and of the tincture (1:5 in ethanol 55%) in the EU. These preparations are therefore not included in the monograph on Echinaceae purpureae radix.

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

Dry extracts

1) dry extract (5.5-7.5:1) ethanol 45% V/V. 30 mg of extract / tablet, equivalent to 200 mg of the herbal substance: 6-9 tablets a day.

2) dry extract (6.5:1) ethanol 45% V/V. Chewable tablet contains 40 mg extract corresponding to 260 mg radix: chewable tablet every second hour (maximum 9 tablets a day).

Indications:

1) Herbal medicinal product for the treatment of early symptoms of common cold.

Risks:

2) Special warning and precaution for use: Hypersensitivity reactions such as anaphylaxis and angioedema may occur in persons treated with *Echinacea* products. The risk appears higher in patients with atopic eczema. Treatment with *Echinacea* must be discontinued at first signs of hypersensitivity.
Undesirable effects: Hypersensitivity reactions such as skin reactions including urticaria may occur. A few cases of severe hypersensitivity reactions such as angioedema, dyspnea/bronchospasm and fall in blood pressure have been reported.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Immunomodulatory activity

In vitro **experiments**

Purified polysaccharides (EPS) prepared from the herb and root of *Echinacea purpurea* are shown to strongly activate macrophages. Macrophages activated with these substances develop pronounced extracellular cytotoxicity against tumour targets. The activation is brought about by EPS alone and is independent of any cooperative effect with lymphocytes. Also the production and secretion of oxygen

radicals and interleukin-1 (IL-1) by macrophages is increased after activation with EPS. Cells of the macrophages lineage seem to be the main target for the action of these polysaccharides. EPS has no effect on T lymphocytes. B lymphocytes show a comparatively modest proliferation after incubation with *E. purpurea* EPS. Thus, these compounds, which are at least in tissue culture completely nontoxic, may be suited to activate *in vivo* cells of the macrophage system to cytotoxicity. They may therefore be of relevance in tumour and infectious systems (Stimpel *et al.* 1984).

From the water or alcaline-water extracts of *Echinacea purpurea* (L.) Moench and *-angustifolia* DC., *Eupatorium cannabinum* L. and *-perfoliatum* L., *Chamomilla recutita* (L.) (Rauscher), *Calendula officinalis* L., *Baptisia tinctoria* (L.) R.B., *Achyrocline satureoides* DC., *Arnica montana* L., *Sabal serrulata* Roem et Schult. and *Eleutherococcus senticosus* Maxim. polysaccharide fractions with molecular weights in the range of 25 000 to 500 000 and higher have been isolated, which, according to the granulocytes- and carbon clearance tests, showed significant immunostimulating activities. They stimulated the activity of mouse macrophages; this activation included enhanced secretion of interleukin-1 (IL-1). The isolated compounds belong to the group of water-soluble, acidic heteroglycanes. The linkages in the different polysaccharides do not represent a uniform structure type (Wagner *et al.* 1984, Beuscher *et al.* 1990).

An ethanolic extract of purple coneflower root enhanced phagocytosis by 33% in the granulocyte smear test at a concentration of 10^{-4} mg/ml. Aqueous and lipophilic fractions from the ethanolic extract showed immunostimulatory activity (Bauer *et al.* 1989).

Extracts of *Echinacea purpurea* and *Panax ginseng* were evaluated for their capacity to stimulate cellular immune function by peripheral blood mononuclear cells (PBMC) from normal individuals and patients with either the chronic fatigue syndrome or the acquired immunodeficiency syndrome. PBMC isolated on a Ficoll-hypaque density gradient were tested in the presence or absence of varying concentrations of each extract for natural killer (NK) cell activity versus K562 cells and antibody-dependent cellular cytotoxicity (ADCC) against human herpes virus 6 infected H9 cells. Both *Echinacea* and *Ginseng*, at concentrations > or = 0.1 or 10 μg/kg, respectively, significantly enhanced NK-function of all groups. Similarly, the addition of either herb significantly increased ADCC of PBMC from all subject groups. Thus, extracts of *Echinacea purpurea* and *Panax ginseng* enhance cellular immune function of PBMC both from normal individuals and patients with depressed cellular immunity (See *et al.* 1997).

A high molecular weight fraction (M_r >10,000 D) containing polysaccharides and glycoproteins from purple coneflower root enhanced the proliferation of mouse spleen cells; stimulated the production of cytokines such as interferon (IFNα/β) in spleen cell cultures, and IL-1, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) in mouse macrophage cultures; increased immunoglobulin M production and the number of antibody-producing cells, and increased nitric oxide (NO) production of macrophages (Beuscher *et al.* 1995, Bodinet 1999). Incubation of this fraction with human monocytes also enhanced the production of IL-1, IL-6 and TNF-α (Bodinet 1999).

Purple coneflower root powders and various extracts showed a macrophage activating capacity. Extracts standardised to 4% of phenolic compounds (such as chlorogenic and cichoric acid) or to alkylamides were inactive with respect to induction of macrophage cytokine production (Rininger *et al*. 2000).

Isolated alkamides dodeca-2*Z*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide from *E. angustifolia* roots and dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide from *E. purpurea* roots and *E. pallida* roots exerted inhibition on lipopolysaccharide (LPS)-mediated activation of a murine macrophage line, RAW264.7. These data suggest that these alkamides may have anti-inflammatory activity (Chen *et al.* 2005).

With the increasing popularity of herbal medicines, many people make their own *Echinacea* extracts at home and storing them at refrigerator (4°C) temperatures. An hypothesis is that *Echinacea* extracts made using homemade methods change in immunomodulatory efficacy with storage at 4°C over a 4-day period. Three extract types (50% ethanol tincture, cold water infusion, hot water infusion) from 5 different species (*E. angustifolia*, *E. pallida*, *E. purpurea*, *E. sanguinea*, *E. tennesseensis*) were prepared. Four *in vitro* immune assays (monocyte secretion of TNF-α, IL-10, and IL-12 and PBMC proliferation) using human blood were used to test extract efficacy at days 1 and 4 post-extraction. Two statistical analyses, traditional ANOVA and several statistical models that account for endotoxin effects were used. Endotoxin was found to significantly impact immune outcomes only in 4-day old cold water infusions and not in all assays. Extracts showed the greatest stimulation in TNF-α assays. By extract type, 50% ethanol tinctures produced the most immune stimulation. By species, extracts from *E. angustifolia* extracts were the most efficacious in the assays; extracts from *E. sanguinea* showed the least activity overall (Senchina *et al.* 2005).

The effects of long-term (>1 year) dry storage on the capabilities of *Echinacea* spp. roots from mature individuals to modulate cytokine production are unknown. Using an older human adult model of influenza vaccination, peripheral blood mononuclear cells were collected from subjects 6 months post-vaccination and stimulated them *in vitro* with the two Type A influenza viruses contained in the trivalent 2004-2005 vaccine with a 50 % alcohol tincture prepared from the roots of one of seven *Echinacea* species: *E. angustifolia*, *E. pallida*, *E. paradoxa*, *E. purpurea*, *E. sanguinea*, *E. simulata*, and *E. tennesseensis*. Before being processed into extracts, all roots had been stored under dry conditions for sixteen months. Cells were cultured for 48 hours; following incubation, supernatants were collected and assayed for IL-2, IL-10, and IFN-γ production, cytokines important in the immune response to viral infection. Four species (*E. angustifolia*, *E. purpurea*, *E. simulata*, *E. tennesseensis*) augmented IL-10 production, diminished IL-2 production, and had no effect on IFN-γ production. *E. pallida* suppressed production of all cytokines; *E. paradoxa* and *E. sanguinea* behaved similarly, although to a lesser extent. The results from these *in vitro* bioactivity assays indicate that dried *Echinacea* roots stored for sixteen months maintain cytokine-modulating capacities. The authors concluded that the data support and extend previous research and indicate that tinctures from different *Echinacea* species have different patterns of immune modulation; further, they indicate that certain species may be efficacious in the immune response to viral infection (Senchina *et al.* 2006).

Extracts of *Echinacea purpurea* aerial parts (E1 – expressed juice) and roots (E2 – 50% alcoholic tincture, 1:9 w/v) were tested for immunomodulation in rhinovirus-infected and uninfected epithelial cells. Since immune modulation has been reported for similar extracts, cytokine antibody arrays were used to investigate the changes in the pro-inflammatory cytokines and chemokines released from a cultured line of human bronchial epithelial cells exposed to rhinovirus 14 and two different chemically characterized *Echinacea* extracts. Virus infection stimulated the release of at least 31 cytokine-related molecules, including several important chemokines known to attract inflammatory cells. Most of these effects were reversed by simultaneous exposure to either of the two *Echinacea* extracts, although the patterns of response were different for the two extracts. These results could explain the anti-inflammatory properties of *Echinacea* extracts. Furthermore, a number of these cytokines were stimulated by the same *Echinacea* preparations in uninfected cells. The authors concluded that these observations therefore provide support for the alleged beneficial uses of *Echinacea* extracts (Sharma *et al.* 2006).

The immunomodulatory properties of *Echinacea* tinctures from seven species after being stored at -20°C for 2 years were tested. Two experimental techniques were employed using human PBMCs. In the first set of experiments, PBMCs were stimulated *in vitro* with tinctures alone and assayed for proliferation and production of IL-10, IL-12, and TNF-α. In the second set of experiments, subjects were immunized with influenza vaccine. PBMCs from vaccinated individuals were stimulated *in vitro*

with *Echinacea* tinctures and influenza virus; cytokine production (IL-2, IL-10, and IFN-γ was compared prevaccination and postvaccination. In the first experiments, (1) tinctures from *E. angustifolia*, *E. pallida*, *E. paradoxa*, and *E. tennesseensis* stimulated proliferation and tended to increase IL-10, (2) *E. sanguinea* and *E. simulata* stimulated only proliferation, (3) *E. purpurea* stimulated only IL-10, and (4) none of the extracts influenced IL-12 or TNF-α. In the second experiments, (1) tinctures from *E. pallida*, *E. paradoxa*, *E. sanguinea*, and *E. simulata* diminished influenza-specific IL-2, and (2) none of the extracts influenced influenza-specific IL-10 or IFN-γ. For *in vitro* models using *Echinacea*, immune response may vary based on stimulus (*Echinacea* alone vs. *Echinacea* + recall stimulation with virus) (McCann *et al.* 2007).

The effects of *Echinacea* and several of its phytochemical components on NFκB expression by Jurkat cells (a human T-cell line) were investigated *in vitro*. In the absence of stimulation, *Echinacea* and its components exerted no significant effect on basal NFκB expression levels. In the presence of endotoxin (LPS), NFκB expression was decreased. However, this decrease was significantly reversed by treatment with cichoric acid, an *Echinacea* root extract (prepared from both *Echinacea angustifolia* and *Echinacea purpurea*; 1:2 extraction solvent ethanol 60%) and the alkylamide fraction derived from this combination. For the phorbol myristate acetate stimulation of Jurkat cells, effects on NFκB expression were mixed. Depending on the concentration, cichoric acid and a 2,4-diene alkylamide significantly induced NFκB levels, whereas a 2-ene alkylamide caused a significant inhibition. In contrast, both the *Echinacea* and the mixed alkylamide fraction exerted no effect. The alkylamide results indicate that the two basic forms of these compounds present in *Echinacea* may have opposing effects. These opposing effects demonstrate the importance of knowledge, not only of the phytochemical make-up of a herbal preparation, but also of the actions of each component and the consequences of differing relative amounts in the preparation being investigated (Matthias *et al.* 2008).

Standardized tincture Echinaforce® (made from aerial parts and roots of *E. purpurea*) was analysed and found that it induced de novo synthesis of TNF-α mRNA in primary human monocytes/macrophages, but not TNF-α protein. Moreover, LPS-stimulated TNF-α protein was potently inhibited in the early phase but prolonged in the late phase. A study of the main constituents of the extract showed that the alkylamides dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides, trienoic and dienoic acid derivatives are responsible for this effect. The upregulation of TNF-α mRNA was found to be mediated by CB2 receptors, increased cAMP, p38/MAPK and JNK signalling, as well as NF-jB and ATF-2/CREB-1 activation. The authors concluded that this study is the first to report a possible molecular mechanism of action of *Echinacea*, highlighting the role of alkylamides as potent immunomodulators and potential ligands for CB2 receptors (Gertsch *et al.* 2004).

Similarities and differences in immune response among *Echinacea* species, which are commonly used to treat upper respiratory infections were compared and investigated. The investigation involved two components: acquisition of immunomodulatory data reported for the first time according to the authors, and combined phenetic analysis of these data along with previous reports. Experimental data were obtained by stimulating human PBMC *in vitro* with extracts from *Echinacea* spp. and assaying production of three cytokines (IL-1β, IL-2, and TNF-α). Phenetic analyses were employed to compare responses across the entire data set, including UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and neighbor-joining methods. In the immune experiments conducted for this investigation, *E. angustifolia*, *E. paradoxa*, *E. purpurea*, *E. simulata*, and *E. tennesseensis* extracts significantly augmented IL-1β and TNF-α production, whereas no extracts significantly modulated IL-2. All phenetic methods produced similar dendrograms, revealing two species pairs (*E. angustifolia* + *E. simulata* and *E. pallida* + *E. sanguinea*) where both species cluster tightly and have similar immune-response profiles. These two species-pairs are maximally dissimilar from each other. The remaining species (*E. paradoxa*, *E. purpurea*, and *E. tennesseensis*) occupy intermediate positions in the dendrogram. The

authors concluded that the results suggest that *Echinacea* spp. act heterogeneously on immune function (Senchina *et al.* 2008).

In vivo experiments

A 3-fold increase in phagocytosis was demonstrated in the carbon clearance test in mice after oral administration of 10 ml/kg of a solution containing ca. 5 mg of an ethanolic extract of pale coneflower root (1:10, 90% ethanol V/V) in 30 ml of physiological saline, three times daily for 2 days. When chloroform and aqueous fractions of this extract were administered separately, the lipophilic fraction proved more active than the hydrophilic. However, the hydrophilic fraction showed considerably more activity than a hydrophilic fraction from *Echinacea pallida* root (Bauer *et al.* 1989, Bauer *et al.* 1988).

Production of the cytokines IL-1 and IL-6 in mice was enhanced by intravenous doses (50, 100 and 500 µg/animal) of a purified high molecular weight fraction containing glycoproteins and polysaccharides from purple coneflower root (Bodinet and Beuscher 1991, Beuscher *et al.* 1995). Oral administration of this fraction to mice significantly enhanced antibody production in Peyer'splaque cells (Bodinet 1999).

The combination preparation, comprising aqueous ethanolic extracts of *Echinacea purpurea* and *E. pallida* root, *Baptisia tinctoria* root and *Thuja occidentalis* herb, administered orally via the diet or drinking water to mice for 7 days enhanced the antibody response to sheep red blood cells (sRBC) (Bodinet and Freudenstein 1999).

In contrast with the extensive body of research supporting the immunostimulatory effect of Echinacea preparations, some recent work has reported a lack of effect. No evidence of NK cell activity or antibody formation was found in studies involving rats fed various preparations of *Echinacea*, including an alcoholic extract of *E. purpurea* root and an alcoholic extract of the roots of *E. angustifolia* and *E. pallida* in their diet (South and Exon 2001).

Oral administration of 0.45 mg/day of commercial purple coneflower root extract (Phyto Adrien Gagnon, La Prairie, QC, Canada) to 7-week-old mice for 2 weeks resulted in a doubling of the number of NK cells and monocytes in the bone marrow, and in the spleen (Sun *et al.* 1999). Oral administration of the same amount of root extract to ageing mice (15-16 months old, with an average life span of 21 months) stimulated the production of new NK cells, leading to 30% increase in the absolute number of NK cells and a 20% increase in the total functional activity of NK cells in the spleen as measured by the lysis of lymphoma cells *in vitro* (Currier and Miller 2000). Moreover, oral administration of the powdered root to mice injected with leukaemia cells increased their survival time compared to controls (Currier and Miller 2001). Powdered root also exhibited strong adjuvant effect on vaccination with inactivated leukaemia cells (Currier and Miller 2002).

Using male Sprague-Dawley rats (425–475 g), an *in vivo* study was conducted to examine the immunomodulatory effects of preparations of Echinacea containing its components cichoric acid, polysaccharides and alkylamides in different concentrations. The rats were gavaged orally with these preparations two times daily for 4 days. Phagocytic activity of alveolar macrophage was increased with increasing concentrations of the Echinacea components. A trend of increase in TNF-α and NO release by the alveolar macrophages following an in vitro stimulation with LPS was also evident. An enhanced release of cytokines (such as TNF-α and IFN-γ) in response to Echinacea components, was also apparent in rat's spleen macrophage, but at higher concentrations. Among the components, alkylamides at the dose level of 12 mg/kg body weight/day significantly increased the phagocytic activity as well as phagocytic index of the alveolar macrophages. None of the components at any concentration had any effect on the release of TNF-α, IFN-γ and IL-2 by the splenocytes. These results suggest that the *Echinacea* preparations containing optimal concentrations of cichoric acid,

polysaccharides and alkylamides are potentially effective in stimulating an *in vivo*, non-specific immune response in normal rats and that the alkylamides at a dose level of approximately 12 mg/kg body weight/day effectively stimulate alveolar macrophage function in healthy rats. The immunomodulatory effects of alkylamides appear to be more pronounced in lungs than in spleen (Goel *et al.* 2002a, Goel *et al.* 2002b).

A phytopharmaceutical containing an extract of Echinacea purpurea and Glycyrrhiza glabra root (Revitonil® tablets) was investigated for its suggested immunostimulating potential, using several *in vitro* tests and the *in vivo* carbon-clearance model in mice. In the *in vitro* phagocytosis test with human granulocytes, Revitonil® showed a 44-53% stimulating effect at a concentration of 100 µg/ml. Whereas in the chemoluminescence test at a concentration of 1.25 µg/ml, Revitonil® tablets exhibited a moderate enhancing effect only, a remarkable stimulating activity (30-50%) was observed in the T-lymphocyte CD69 bioassay at a concentration of 100 µg - 1 µg/ml. The highest immunological efficacy could be assigned to Revitonil® as revealed by the *in vivo* carbon clearance model in mice. With RCt/RCc-values of 2.0, Revitonil® exhibited a very high carbon elimination rate at oral administration. Because the Echinacea and Glycyrrhiza monoextracts alone showed lower RCt/RCc-values (1.3-1.7) than Revitonil®, a potentiating synergistic effect of the extract mixture in Revitonil® can be postulated (Wagner and Jurcic 2002).

Echinacea purpurea dry root powder (containing 1.5% total polyphenols, calculated as chlorogenic acid) increased the resistance of splenic lymphocytes to apoptosis; splenic lymphocytes were obtained from mice administered the *Echinacea* preparation orally at dosages of 30 or 100 mg/kg daily for 14 days (Di Carlo *et al.* 2003).

The debate is still on-going with respect to the efficacy of ingesting *Echinacea purpurea* preparation intermittently, continuously, or only at the beginning of an affliction. It was sought, therefore, to find out if mice, receiving dietary *Echinacea* daily (commercial purple coneflower root extract; Phyto Adrien Gagnon, La Prairie, QC, Canada), throughout life, from youth until late middle-age, demonstrated any longevity/survival differences, and/or any differences in their various populations of immune/hemopoietic cells. Sustained and/or high levels of these cells are crucial for longevity. Some mice were maintained on a regular chow diet to which was added *Echinacea purpurea* daily (2 mg/mouse), from puberty (7 week) until just beyond 13 months of age (late middle-age in mice). Control mice, identically housed and maintained, received identical chow without the herbal preparation. Mice consuming untreated diet had a 79% survival by 10 months of age, while those consuming *Echinacea* daily in the diet were still 100% alive by 10 months. At approximately 13 months of age, mice consuming untreated diet had a 46% survival rate while those consuming *Echinacea*, were 74% alive at this time. Moreover, the key immune cells, acting as the first line of defence against developing neoplasms in mice and humans, i.e., NK cells, were significantly elevated in absolute number both in their bone marrow production site, as well as in the major organ to which they traffic and function, i.e. the spleen. The cells of the myeloid/granulocyte lineages remained steadfastly at control levels in both the bone marrow and spleen in Echinacea-consuming mice. Thus, the authors concluded that it appears that regular intake of *Echinacea* may indeed be beneficial/prophylactic, if only for the reason that it maintains in an elevated state, NK cells, prime elements in immunosurveillance against spontaneous-developing tumours, a phenomenon which increases in frequency with progressive aging (Brousseau and Miller 2005).

In vitro antimicrobial activity

Antibacterial activity against *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* has been demonstrated for a multi-herbal preparation containing *Echinacea*

purpurea root extract, although it was stated that the observed antibacterial effects were most likely attributable to one of the ingredients, extract of onion (Westendorf 1982).

Extracts of purple coneflower root exhibited near UV-mediated phototoxic and antifungal activity, measured by inhibition of the growth of *Candida* spp. and *Saccharomyces cerevisiae*; the activity was attributed primarily to ketoalkenes and ketoalkynes (Binns *et al.* 2000).

Antifungal activity was tested against *Cryptococcus neoformans*, two *Candida albicans* isolates (D10 and CN1A), *Trychophyton tonsurans*, *T. mentagrophytes*, *Mycrosporum gypseum* and *Pseudallescheria boydii*. Root extracts of eight *Echinacea* taxa, including *E. purpurea* showed antifungal activity against most of the pathogenic fungi (Merali *et al.* 2003).

In vitro antiviral activity

Using mouse fibroblasts it was demonstrated that incubation with methanolic and aqueous extract of *Echinacea purpurea* root resulted in resistance to influenza A2, herpes, and vesicular stomatitis virus infection for 24 hours (Wacker and Hilbig 1978).

A high molecular weight fraction (Mr >10,000 D) containing polysaccharides and glycoproteins from purple coneflower root exhibited antiviral activity against *Herpes simplex* virus (HSV) and influenza virus (Beuscher *et al.* 1995).

A decoction and a 30% ethanolic extract of purple coneflower root inhibited the propagation of ECHO9 Hill virus in monkey kidney cell cultures (Skwarek *et al.* 1996).

Extracts of 8 taxa of the genus *Echinacea* were found to have antiviral activity against HSV Type I *in vitro* when exposed to visible and UV-A light. n-Hexane extracts of roots containing alkenes and amides were more active in general than ethyl acetate extracts containing caffeic acids. The most potent inhibitors of HSV were *E. pallida* var. *sanguinea* crude (70 % ethanol) inflorescence extract (MIC = 0.026 mg/ml), cichoric acid (MIC = 0.045 mg/ml) and *E. purpurea* n-hexane root extract (MIC = 0.12 mg/ml) (Binns *et al.* 2002).

In vivo anti-inflammatory activity

Polyunsaturated isobutylamides have been shown to exert anti-inflammatory activity in the 5-lipoxygenase assay (Wagner *et al.* 1989, Müller-Jakic *et al.* 1994). A fraction from purple coneflower root consisting of ten polyunsaturated isobutylamides had an inhibitory effect on 5-lipoxygenase of 92.5% at 60 µM (calculated for a mean relative molecular mass of 220) (Wagner *et al.* 1989).

Echinacea purpurea (L.) Moench (dry root powder) and *Hypericum perforatum* L. were evaluated for their anti-inflammatory activity against carrageenan-induced paw oedema in mice. Each drug was administered orally to mice at 30 and 100 mg/kg, twice daily. Only the higher dose significantly inhibited, time dependently, the formation of oedema, evaluated as area under the curve (*Echinacea* P < 0.01; *Hypericum* P < 0.05). Western blot analysis showed that *in vivo* treatment with these extracts could modulate lipopolysaccharide (LPS) and IFN-γ induced cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression in peritoneal macrophages. In particular, treatment with 100 mg/kg *Hypericum* inhibited both iNOS and COX-2 expression, whereas treatment with 100 mg/kg *Echinacea* down-regulated only COX-2 expression. The present study suggests that the anti-inflammatory effect of these extracts could be in part related to their modulation of COX-2 expression (Raso *et al.* 2002). Further exploration suggested that the observed effect may be due to down-regulation of COX-2 expression by the *Echinacea* preparation. *In vitro* inhibition of COX-1 and, to lesser extent, COX-2 has been described to alkamides isolated from *E. purpurea* roots (Clifford *et al.* 2002).

The anti-inflammatory and wound healing activities of echinacoside, compared with the ones of the total dry ethanolic root extract of *Echinacea purpurea* and *E. pallida*, were examined in rats, after topical application of gel containing 100 mg/ml of the extract. The tissues of the treated animals were evaluated after 24, 48 and 72 hours treatment and excised for histological observation at the end of the experiment. Results confirm the good anti-inflammatory and wound healing properties of *E. pallida* and of its constituent echinacoside, with respect to *E. purpurea* and control (*E. purpurea* was more effective over the first 24 hours but inferior at 48-72 hours). This activity probably resides in the antihyaluronidase activity of echinacoside (Speroni *et al.* 2002).

5-lipoxygenase-inhibiting activity of extracts of five wild and three commercially used species of the genus *Echinacea* were investigated to characterise anti-inflammatory activity of *Echinacea*. The inhibition of the 5-lipoxygenase (5-LOX) enzyme of the arachadonic acid pathway was determined by high-performance liquid chromatography (HPLC) detection of a direct metabolic product (LTB4) of 5-LOX derived from stimulated rat basophilic cells. Root extracts of the three commercial species of *Echinacea* (*E. purpurea*, *E. pallida* var. *angustifolia*, *E. pallida* var. *pallida*) inhibited the 5-LOX enzyme (Merali *et al.* 2003).

Inhibition of prostaglandin E(2) (PGE(2)) production in LPS-stimulated RAW264.7 mouse macrophage cells was assessed with an enzyme immunoassay following treatments with *Echinacea* extracts or synthesized alkamides. Results indicated that ethanol extracts diluted in media to a concentration of 15 µg/ml from *E. angustifolia*, *E. pallida*, *E. simulata*, and *E. sanguinea* significantly inhibited PGE2 production. In further studies, PGE2 production was significantly reduced by all synthesized alkamides assayed at 50 µM, by Bauer alkamides 8, 12A analogue, and 14, Chen alkamide 2, and Chen alkamide 2 analogue at 25 µM and by Bauer alkamide 14 at 10 µM. Cytotoxicity did not play a role in the noted reduction of PGE2 production in either the *Echinacea* extracts or synthesized alkamides. HPLC analysis identified individual alkamides present at concentrations below 2.8 µM in the extracts from the six *Echinacea* species (15 µg/ml crude extract). Because active extracts contained <2.8 µM of specific alkamide and the results showed that synthetic alkamides must have a minimum concentration of 10 µM to inhibit PGE2, it is likely that alkamides may contribute toward the anti-inflammatory activity of *Echinacea* in a synergistic or additive manner (LaLone *et al.* 2007).

Alcohol extracts from three widely used *Echinacea* species, *E. angustifolia*, *E. pallida*, and *E. purpurea*, were investigated for immunomodulating properties. The three *Echinacea* species demonstrated a broad difference in concentrations of individual lipophilic amides and hydrophilic caffeic acid derivatives. Mice were gavaged once a day (for 7 days) with one of the *Echinacea* extracts (130 mg/kg) or vehicle and immunized with sRBC 4 days prior to collection of immune cells for multiple immunological assays. The three extracts induced similar, but differential, changes in the percentage of immune cell populations and their biological functions, including increased percentages of CD49+ and CD19+ lymphocytes in spleen and NK cell cytotoxicity. Antibody response to sRBC was significantly increased equally by extracts of all three *Echinacea* species. Concanavalin A-stimulated splenocytes from *E. angustifolia*- and *E. pallida*-treated mice demonstrated significantly higher T cell proliferation. In addition, the *Echinacea* treatment significantly altered the cytokine production by mitogen-stimulated splenic cells. The three herbal extracts significantly increased IFN-α production, but inhibited the release of TNF-γ and IL-1β. Only *E. angustifolia*- and *E. pallida*-treated mice demonstrated significantly higher production of IL-4 and increased IL-10 production. Taken together, these findings demonstrated that *Echinacea* is a wide-spectrum immunomodulator that modulates both innate and adaptive immune responses. In particular, *E. angustifolia* or *E. pallida* may have more anti-inflammatory potential (Zhai *et al.* 2007a).

It has been suggested that *Echinacea* has anti-inflammatory activity *in vivo*. Nitric oxide (NO), TNF-α and IL-1β are important mediators in the inflammatory response. The effect of alcohol extracts of *E. angustifolia* (EA), *E. pallida* (EPA) and *E. purpurea* (EP) on the production of these inflammatory

mediators in both LPS-stimulated RAW264.7 macrophages *in vitro* and murine peritoneal exudate cells (PECs) *in vivo* were investigated. As macrophages produce these inflammatory mediators in response to pathogenic infection, parallel cultures of macrophages were studied for phagocytosis and intracellular killing of Salmonella enterica. EPA and EP *in vitro* inhibited NO production and TNF-α release in a dose-dependent manner. RAW264.7 cells treated with EA or EP showed decreased killing over 24 h, although EA enhanced bacterial phagocytosis. Upon bacterial infection, RAW264.7 cells produce high levels of NO; however, an *Echinacea*-mediated decrease in NO production was observed. *Echinacea* alcohol extracts administered orally at 130 mg/kg per day for seven days had a weak effect on NO production and phagocytosis by LPS-stimulated PECs. The results indicated that all *Echinacea* species significantly decreased inflammatory mediators *in vitro*, however, only EA and EP reduced bacterial killing. Oral administration of *Echinacea* alcohol extracts did not adversely affect the development and anti-bacterial function of inflammatory PECs *in vivo*; however, NO production was decreased during bacterial infection of PECs (Zhai *et al.* 2007b).

In vitro antioxidant activity

The protective effect of caffeoyl derivatives (echinacoside, chlorogenic acid, cichoric acid, cynarine, and caffeic acid, typical constituents of *Echinacea* species) on the free radical-induced degradation of Type III collagen has been investigated. The macromolecule was exposed to a flux of oxygen radicals (superoxide anion and hydroxyl radical) generated by the xanthine/xanthine oxidase/Fe2+/EDTA system and its degradation assessed qualitatively by SDS-PAGE and quantitatively as the amount of soluble peptides (according to the 4-hydroxyproline method) released from native collagen after oxidative stress. The SDS-PAGE pattern of native collagen is markedly modified by free radical attack, with formation of a great number of peptide fragments with molecular masses below 97 kDa: in the presence of microM concentrations of echinacoside, there is a complete recovery of the native profile. Collagen degradation was, in fact, dose-dependently inhibited by all the compounds, with the following order of potency: echinacoside approximately cichoric acid > cynarine approximately caffeic acid > chlorogenic acid, with IC_{50} ranging from 15 to 90 microM. These results indicate that this representative class of polyphenols of *Echinacea* species protects collagen from free radical damage through a scavenging effect on reactive oxygen species and/or C-, N-, S-centered secondary radicals, and provides an indication for the topical use of extracts from *Echinacea* species for the prevention/treatment of photodamage of the skin by UVA/UVB radiation, in which oxidative stress plays a crucial role (Maffei Facino *et al.* 1995).

Methanol extracts of freeze-dried *Echinacea* (*E. angustifolia*, *E. pallida*, and *E. purpurea*) roots were examined for free radical scavenging capacities and antioxidant activities. Root extracts of *E. angustifolia*, *E. pallida*, and *E. purpurea* were capable of scavenging hydroxyl radical. Similar scavenging activities for each variety were found for both 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and ABTS radical. Meanwhile, antioxidant activities of all three varieties of Echinacea were found to delay the formation of conjugated diene hydroperoxide induced by the thermal decomposition of 2,2'-azobis(2-amidinopropane) dihydrochloride and extend the lag phase of peroxidation of soybean liposomes. *Echinacea* root extracts suppressed the oxidation of human low-density lipoprotein, as evaluated by reduced agarose electrophoretic mobility following oxidative modification by Cu2+. The mechanisms of antioxidant activity of extracts derived from *Echinacea* roots included free radical scavenging and transition metal chelating (Hu and Kitts 2000).

Alcoholic extracts of the roots and leaves of three *Echinacea* species (*E. purpurea*, *E. angustifolia* and *E. pallida*) were found to have antioxidant properties in a free radical scavenging assay and in a lipid peroxidation assay. Cichoric acid and verbascoside predominated in extracts of *E. purpurea* (Sloley *et al.* 2001).

The radical scavenging activity of *Echinacea* methanolic extracts was evaluated *in vitro* with a spectrophotometric method based on the reduction of an alcoholic DPPH radical solution at 517 nm in the presence of a hydrogen donating antioxidant. As for pure compounds, echinacoside had the highest capacity to quench DPPH radicals (EC_{50} = 6.6 µM), while caftaric acid had the lowest (EC_{50} = 20.5 µM). The average EC_{50} values for *E. purpurea*, *E. pallida* and *E. angustifolia* were 134, 167 and 231 µg/ml, respectively. The radical scavenging activity of *Echinacea* root extracts reflected their phenolic composition. The results indicate that *Echinacea* roots and derivatives are a good source of natural antioxidants and could be used to prevent free radical-induced deleterious effects (Pellati *et al.* 2004).

After extraction, fractionation, and isolation, the antioxidant activity of three extracts, one alkamide fraction, four polysaccharide-containing fractions, and three caffeic acid derivatives from *Echinacea purpurea* root was evaluated by measuring their inhibition of *in vitro* Cu(II)-catalyzed oxidation of human low-density lipoprotein. The antioxidant activities of the isolated caffeic acid derivatives were compared to those of echinacoside, caffeic acid, and rosmarinic acid for reference. The order of antioxidant activity of the tested substances was cichoric acid > echinacoside > derivative II > caffeic acid > rosmarinic acid > derivative I. Among the extracts the 80% aqueous ethanolic extract exhibited a 10 times longer lag phase prolongation (LPP) than the 50% ethanolic extract, which in turn exhibited a longer LPP than the water extract. Following ion-exchange chromatography of the water extract, the majority of its antioxidant activity was found in the latest eluted fraction (H2O-acidic 3). The antioxidant activity of the tested *Echinacea* extracts, fractions, and isolated compounds was dose-dependent. Synergistic antioxidant effects of *Echinacea* constituents were found when cichoric acid (major caffeic acid derivative in *E. purpurea*) or echinacoside (major caffeic acid derivative in *E. pallida* and *E. angustifolia*) were combined with a natural mixture of alkamides and/or a water extract containing the high molecular weight compounds. This contributes to the hypothesis that the physiologically beneficial effects of *Echinacea* are exerted by the multitude of constituents present in the preparations (Dalby-Brown *et al.* 2005).

The antioxidant activity of extracts of the stems, leaves, and roots of *Echinacea purpurea* was compared with the antioxidant activity of purified cichoric acid and alkamides, both constituents of *E. purpurea*. The extracts were quantified by HPLC. The antioxidant activity was determined using different methods: effect on oxygen consumption rate of a peroxidating lipid emulsion, and scavenging of radicals, i.e. DPPH, measured by two different techniques. The efficacy of the extracts in the reaction with DPPH correlated well with the amount of cichoric acid present in the various extracts. The alkamides alone showed no antioxidant activity in any of the tests. Alkamides present in the extract increased, however, the antioxidative effect of cichoric acid in the peroxidating lipid emulsion. The activity was further compared with that of rosmarinic acid, a well-characterised antioxidant, and the extracts as well as cichoric acid were found to be efficient scavengers of radicals with an activity comparable to that of rosmarinic acid. Cichoric acid was found to have a stoichiometric factor of 4.0 in scavenging DPPH and to react in a second-order reaction with DPPH with a rate constant of 40 L/mol/s at 25°C in methanol (Thygesen *et al.* 2007).

Other activities

Fibroblast-populated collagen lattice was used to study the influence of purple coneflower extracts on the collagen contracting ability of C3H10T1/2 mouse fibroblasts. An ethanolic extract (65% V/V) of purple coneflower root showed a dose-dependent inhibition of collagen gel contraction when added at the time of preparation of the gel. A corresponding amount of ethanol showed no influence. With increase of elapsed time between gel preparation and addition of extract, there was less inhibition of elongation of fibroblasts and of the processes leading to collagen linking. No effect was observed when the extract was added one hour after gel preparation (Zoutewelle and Van Wijk 1990).

Serial dilutions of 21 commercial ethanolic herbal extracts and tinctures, and 13 related pure plant compounds have been analyzed for their *in vitro* cytochrome P450 3A4 (CYP3A4) inhibitory capability via a fluorometric microtitre plate assay. Roughly 75% of the commercial products and 50% of the pure compounds showed significant inhibition of CYP3A4 metabolite formation. For each herbal product and pure compound exhibiting dose-dependency, the inhibition values were used to generate median inhibitory concentration (IC_{50}) curves using linear regression. Among the commercial extracts, *Hydrastis canadensis* (goldenseal), *Hypericum perforatum* (St. John's wort), and *Uncaria tomentosa* (cat's claw) had the lowest IC_{50} values at < 1% full strength, followed by *Echinacea angustifolia* roots, *Trifolium pratense* (wild cherry), *Matricaria chamomilla* (chamomile), and *Glycyrrhiza glabra* (licorice), which had IC_{50} values ranging from 1%-2% of full strength. *Echinacea purpurea* root extract showed moderate inhibitory activity (IC_{50} > 5% and < 10% full strength. Dillapiol, hypericin, and naringenin had the lowest IC_{50} values among the pure plant compounds at < 0.5 mM; dillapiol was the most potent inhibitor at 23.3 times the concentration of the positive CYP3A4 inhibitor ketoconazole (Budzinski *et al.* 2000).

The effect of *Echinacea purpurea* root extract (prepared with 50% aqueous ethanol, DER was not given) on the weight of prostates in rats as well as on alterations of histological structure and separate blood cells was studied. Experiments were carried out with 3-month old male Wistar rats, divided by 6 into 3 different groups. The first group was the control one. The rats of the second group were fed for 30 days with the usual food ration plus 50 mg/kg of *Echinacea* extract. The third group was fed for 60 days in the same way as the second one. After weighing the rats their prostates were removed and weighed. The weight of prostates in the first group of rats was 412.0±14.93 mg, in the second group 403.0±13.33 mg, and in the third group it was 388.0±14.66 mg. Having calculated the proportion between prostates of rats and their body weight it was estimated that in the first group it made 0.125±0.009%, in the second group 0.105±0.005%, and in the third group 0.091±0.007%. The percentage of lymphocytes in the first group was 72±1.41; in the second group 73±0.81; in the third group 79±1.86. The percentage of segmented neutrophils in the first group was 23±3.31; in the second group 23±2.25; in the third group 18±2.33. Having conducted analysis of the experimental results, a significantly important decrease of prostate weight of investigated rats, an increase in the number of lymphocytes as well as the alterations of histological structures after using *Echinacea* extract for 8 weeks were observed (Skaudickas *et al.* 2003).

The effect of *Echinacea purpurea* extract (prepared with 50% aqueous ethanol, DER was not given) on a rat testicle and epididymis was examined, the mass of these organs was determined, the proportion between the mass of the organ and the mass of a body was calculated, the changes in histological structures were evaluated in this study. Experiments with the Wistar line 3-month old male rats were carried out. There were 3 experimental groups of rats. The first one was control group. The rats of the second group were fed on the usual food enriched with the *Echinacea* extract additive with the proportion of 50 mg/kg for 4 weeks. The rats in the third group were fed equally to the second one for 8 weeks. The rats were weighed, the testicles and epididymis were eliminated, and pathohistological examinations were carried out. Calculations of the relative quantity between the mass of the organs and the body weight were made and it was estimated that the testicles of the rats in the first group made up 0.496±0.399% of a body mass, in the second one 0.459±0.419%, and in the third one 0.429±0.410%. The epididymis in the control group made up 0.189±0.332% of a body mass; in the second one 0.1733±0.328%, and in the third one 0.1723±0.198%. The histological structural changes were traced after 4 weeks of using the preparation; however they became more obvious after 8 weeks. Results of the study enabled to determine statistically significant reduction in the percentage of a testicle and the body mass, as well as changes in histological structures after 8 weeks of consuming extract of *E. purpurea* (Skaudickas *et al.* 2004).

It was shown that the alkylamides dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (A1) and dodeca-2E,4E-dienoic acid isobutylamide (A2) bind to the CB2 receptor more strongly than the endogenous cannabinoids. The Ki values of A1 and A2 (CB2~60 nM; CB1>1500 nM) were determined by displacement of the synthetic high affinity cannabinoid ligand [3H]CP-55,940. Molecular modelling suggests that alkylamides bind in the solvent-accessible cavity in CB2, directed by H-bonding and π-π interactions. In a screen with 49 other pharmacologically relevant receptors, it could be shown that A1 and A2 specifically bind to CB2 and CB1. A1 and A2 elevated total intracellular Ca2+ inCB2-positive but not in CB2-negative promyelocytic HL60 cells, an effect that was inhibited by the CB2 antagonist SR144528. At 50 nM A1, A2, and the endogenous cannabinoid anandamide (CB2 Ki >200 nM) up-regulated constitutive IL-6 expression in human whole blood in a seemingly CB2-dependent manner. A1, A2, anandamide, the CB2 antagonist SR144528 (Ki <10 nM), and also the non-CB2-binding alkylamide undeca-2E-ene-8,10-diynoic acid isobutylamide all significantly inhibited LPS-induced TNF-α, IL-1β, and IL-12p70 expression (5–500 nM) in a CB2-independent manner. Alkylamides and anandamide also showed weak differential effects on anti-CD3- versus anti-CD28-stimulated cytokine expression in human whole blood. Overall, alkylamides, anandamide, and SR144528 potently inhibited LPS-induced inflammation in human whole blood and exerted modulatory effects on cytokine expression, but these effects are not exclusively related to CB2 binding (Raduner *et al.* 2006).

Intake of *Echinacea* preparations is common among patients with advanced malignancies enrolled onto phase I chemotherapy trials; however, no data are available regarding the possible direct effect of *Echinacea* species on human cancer cells. The purpose of the study was to investigate potential *in vitro* cytotoxic and pro-apoptotic properties of hexanic root extract of the three medicinal *Echinacea* (Asteraceae) species (*E. pallida* (Nutt.) Nutt., *E. angustifolia* DC. var. *angustifolia*, *E. purpurea* (L.) Moench) on the human pancreatic cancer MIA PaCa-2 and colon cancer COLO320 cell lines. The authors concluded that it was demonstrated, for the first time, that all the three species reduced cell viability in a concentration- and time-dependent manner. These results represent the starting point to establish viable scientific evidence on the possible role of *Echinacea* species in medical oncology (Chicca *et al.* 2007).

The curative efficacy of an *Echinacea* extract (Echinacin®; Madaus AG, Germany) in γ-irradiated mice is reported. *E. purpurea* was given to male mice that were divided into 5 groups (control, treated, irradiated, treated before irradiation & treated after irradiation) at a dose of 30 mg/kg body weight for 2 weeks before and after irradiation with 3 Gy of γ-rays. The results reflected the detrimental reduction effects of γ-rays on peripheral blood hemoglobin and the levels of red blood cells, differential white blood cells, and bone marrow cells. The thiobarbituric acid-reactive substances (TBARs) level, superoxide dismutase (SOD) and glutathione peroxidase (GSPx) activities and DNA fragmentation were also investigated. FT-Raman spectroscopy was used to explore the structural changes in liver tissues. Significant changes were observed in the microenvironment of the major constituents, including tyrosine and protein secondary structures. *E. purpurea* administration significantly ameliorated all estimated parameters. The radio-protection effectiveness was similar to the radio-recovery curativeness in comparison to the control group in most of the tested parameters. The radio-protection efficiency was greater than the radio-recovery in haemoglobin level during the first two weeks, in lymphoid cell count and TBARs level at the fourth week and in SOD activity during the first two weeks, as compared to the levels of these parameters in the control group (Abouelella et al. 2007).

Effect of Immunal forte® (containing dry ethanol-water extract of herb), Echinapur® (containing thick extract of herb) and Esberitox® (combination product containing extracts of *Thuja occidentalis*, *Baptisia tinctoria*, *Echinacea purpurea* and *Echinacea pallida*) on mice fetuses was studied to establish whether pharmaceuticals containing alcoholic extracts of *E. purpurea* given to pregnant mice influence angiogenic activity and tissue VEGF and bFGF production of their fetuses. It was shown that angiogenic activity of tissue homogenates was increased in Esberitox® group and diminished in case of Immunal

forte® as compared to standard diet group. In case of Echinapur® group no significant differences in angiogenic activity were found. VEGF and bFGF concentration were lower in all groups compared to the control. In the case of Echinapur® and Esberitox® number of fetuses in one litter were slightly lower as compared to control group, but the difference is on the border of statistical significance. In conclusion, there is some possibility that pharmaceuticals containing *E. purpurea* might influence fetal development in human also, because they may interfere with embryonal angiogenesis , and should not be recommended for pregnant women (Barcz *et al.* 2007).

The six commonly used trade herbal products, St. John's wort, common valerian, common sage, Ginkgo biloba, *Echinacea purpurea* and horse chestnut, and ethanol, were investigated for their *in vitro* inhibitory potential of cytochrome P450 2D6 (CYP2D6)-mediated metabolism. Herbal components were extracted from commercially available products in a way that ensured the same composition of constituents in the extract as in the original trade products. c-DNA baculovirus expressed CYP2D6 was used with dextromethorphan as substrate. Quinidine was included as a positive control inhibitor. A validated HPLC methodology was used to quantify the formation of dextrorphan (product of dextromethorphan O-demethylation). Ethanol showed a biphasic effect on CYP2D6 metabolism, increasing initially the CYP2D6 activity with 175% of control up to a concentration of 1.1%, where after ethanol linearly inhibited the CYP2D6 activity. All the investigated herbs inhibited CYP2D6 activity to some extent, but only St. John's wort, common sage and common valerian were considered possible candidates for *in vivo* clinically significant effects. They showed IC_{50} values of 0.07 +/- 7 x 10(-3) mg/ml, 0.8 +/- 0.05 mg/ml and 1.6 +/- 0.2 mg/ml, respectively. St. John's wort inhibited CYP2D6-mediated metabolism in an uncompetitive manner, while common valerian and common sage in a non-competitive manner demonstrated interherb differences in inhibition patterns and differences when compared to the more homogenous competitive inhibitor quinidine. Common valerian was the only herb that showed a mechanistic inhibition of CYP2D6 activity and attention should be paid to a possible toxicity of this herb (Hellum and Nilsen 2007).

The n-hexane root extracts from *Echinacea pallida*, *E. angustifolia* and *E. purpurea* were evaluated for inhibition of the multidrug transporter P-glycoprotein (Pgp) activity, the product of the ABCB1 gene, involved in cancer multidrug resistance (MDR) and in herb-drug or drug-drug interactions. The biological assay was performed using the human proximal tubule HK-2 cell line that constitutively expresses ABCB1. The n-hexane extracts of all three species reduced the efflux of the Pgp probe calcein-AM from HK-2 cells two-fold in a concentration-dependent manner, and *E. pallida* was found to be the most active species. For the first time, two polyacetylenes and three polyenes, isolated from the n-hexane extract of *E. pallida* roots by a bioassay-guided fractionation, were found to be able to reduce Pgp activity. Pentadeca-(8Z,13Z)-dien-11-yn-2-one was the most efficient compound, being able to decrease the calcein-AM efflux about three-fold with respect to the control at 30 µg/ml (Romiti *et al.* 2008).

The n-hexane extracts of the roots of three medicinally used *Echinacea* species exhibited cytotoxic activity on human cancer cell lines. Cytotoxic effects were assessed on human pancreatic MIA PaCa-2 and colonic COLO320 cancer cell lines. Cell viability was evaluated by the WST-1 assay and apoptotic cell death by the cytosolic internucleosomal DNA enrichment and the caspase 3/7 activity tests (Chicca *et al.* 2008).

Assessor's overall conclusions on pharmacology

For the extracts, fractions and isolated compounds of purple coneflower root, immunomodulatory (purified polysaccharides, glycoproteins, alkamides), antimicrobial, antiviral (fraction containing polysaccharides and glycoproteins), anti-inflammatory (polyunsaturated isobutylamides, echinacoside), antioxidant (caffeoyl derivatives), cytochrome enzyme inhibitory, antiandrogenic, cannabinoidomimetic

(alkylamides), radioprotective and antitumor effects (polyacetylenes, polyenes) were proven in several *in vitro* and *in vivo* tests. However, most of the pharmacological mechanisms and active compounds responsible for the effects still remain to be elucidated. Regarding combination products, it is difficult to say, which component is the active one, the pharmacological effects are probably achieved by synergistic effect.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Studies of transport of alkamides trough a cultured monolayer of colonic cells were performed on human adenocarcinoma colonic cell line Caco-2 (ATCC) as a model to assess the epithelial transport of dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides. 30 minutes after apical loading of 25 µg/ml, about 15% of these alkamides were detectable on the basolateral side. Close monitoring of the transport during 6 hours revealed a nearly complete transport to the basolateral side after 4 hours and no significant metabolism was observable. Transport experiments performed at 4oC showed only a slight decrease in transport, which is a strong hint that dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides cross biological membranes by passive diffusion. Nearly the same results were obtained after preincubation of the Caco-2 cells with LPS or phorbol 12-myristate-13-acetate to mimic an inflammatory status. These results support the assumption that the alkamides can be easily transported from the intestine and hence may contribute to the *in vivo* effects of Echinacea preparations (Jager *et al.* 2002).

Transport of 12 alkamides and 5 caffeic acid conjugates from a proprietary preparation of *Echinacea* (Echinacea Premium® Liquid; MediHerb, Austria) which contains 60% ethanol/water extract of *E. angustifolia* root (200 mg/ml) and *E. purpurea* root (300 mg/ml) was studied on Caco-2 monolayers. Almost all of the caffeic acid conjugates permeated poorly through the Caco-2 monolayers: their uptake was no better than that of control (mannitol). By contrast, both 2,4-diene and 2-ene alkamides readily diffused through the monolayers. Theses findings suggest that alkamides would be bioavailable following oral administration (Matthias *et al.* 2004).

The metabolism by human liver microsomes of the alkylamide components from an *Echinacea* preparation as well as that of pure synthetic alkylamides was investigated. No significant degradation of alkylamides was evident in cytosolic fractions. Time- and NADPH-dependent degradation of alkylamides was observed in microsomal fractions suggesting they are metabolised by cytochrome P450 (CYP450) enzymes in human liver. There was a difference in the susceptibility of 2-ene and 2,4-diene pure synthetic alkylamides to microsomal degradation with (2E)-N-isobutylundeca-2-ene-8,10-diynamide (1) metabolised to only a tenth the extent of (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide (3) under identical incubation conditions. Markedly less degradation of 3 was evident in the mixture of alkylamides present in an ethanolic *Echinacea* extract, suggesting that metabolism by liver P450s was dependent both on their chemistry and the combination present in the incubation. Co-incubation of 1 with 3 at equimolar concentrations resulted in a significant decrease in the metabolism of 3 by liver microsomes. This inhibition by 1, which has a terminal alkyne moiety, was found to be time- and concentration-dependent, and due to a mechanism-based inactivation of the P450s. Alkylamide metabolites were detected and found to be the predicted epoxidation, hydroxylation and dealkylation products. These findings suggest that *Echinacea* may affect the P450-mediated metabolism of other concurrently ingested pharmaceuticals (Matthias *et al.* 2005a).

Assessor's overall conclusions on pharmacokinetics

In pharmacokinetic studies only alkamides and caffeic acid conjugates were investigated. It was shown that alkamides (in contrast with caffeic acid conjugates) readily diffuse through the monolayers of

Caco-2 cells. This supports the assumption that the alkamides can be easily transported from the intestine and hence may contribute to the *in vivo* effects of *Echinacea* preparations. Study of metabolism suggests that alkamides of *Echinacea* are metabolised by cytochrome P450 and may affect the CYP450-mediated metabolism of other concurrently ingested pharmaceuticals.

These data must be confronted with clinical data – see section 'Drug interactions' under 5.5.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

In general, animal studies with different preparation and fractions of *Echinacea* species have indicated low toxicity (Barrett 2003).

Acute toxicity of *Echinacea purpurea* root extract was > 3000 mg/kg after p.o. application to NMRI mice. Specifications for the extract are not available (Bauer and Liersch 1993).

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

Six major herbal references published from 1996 to 2000 were selected to evaluate the adequacy of their toxicological information in light of published adverse events. To identify herbs most relevant to toxicology, herbal-related calls to regional California Poison Control System, San Francisco division (CPCS-SF) in 1998 were reviewed and 12 herbs were identified (defined as botanical dietary supplements) most frequently involved in these CPCS-SF referrals. Medline was searched (1966 to 2000) to identify published reports of adverse effects potentially related to these same 12 herbs. Each herbal reference text was scored on the basis of information inclusiveness for the target 12 herbs, with a maximal overall score of 3. The herbs, identified on the basis of CPCS-SF call frequency were: St John's wort, ma huang, echinacea, guarana, ginkgo, ginseng, valerian, tea tree oil, goldenseal, arnica, yohimbe and kava kava. The overall herbal reference scores ranged from 2.2 to 0.4 (median 1.1). The Natural Medicines Comprehensive Database received the highest overall score and was the most complete and useful reference source. All of the references, however, lacked sufficient information on management of herbal medicine overdose, and several had incorrect overdose management guidelines that could negatively impact patient care. Current herbal reference texts do not contain sufficient information for the assessment and management of adverse health effects of botanical therapies (Haller *et al.* 2001).

Reports to poison control centers (PCCs) were characterised involving two widely used herbal dietary supplements (HDSs), *Echinacea*, and St. John's wort (SJW). METHODS: Data were purchased from the American Association of Poison Control Center's (AAPCC) toxic exposure surveillance system (TESS(R)) on reports made to PCCs in 2001 involving Echinacea or SJW. Analyses were limited to those cases in which *Echinacea* or SJW were the only associated products, and in which these HDSs were deemed primary to observed adverse effects. Descriptive statistics were generated for selected demographic and exposure-related variables. During 2001, PCCs were contacted regarding 406 exposures involving *Echinacea* and 356 exposures involving SJW. Most of the reported exposures for both HDSs occurred among children 5 years and younger, and the majority of exposures were coded as unintentional. For both HDSs, exposures among patients >/=20 years old were more likely to be associated with adverse effects. Intentional exposures accounted for 21% of SJW cases and 3% of *Echinacea* cases, with 13% of SJW exposures reported as 'suspected suicidal'. TESS represents a potentially important means of

assessing and characterizing HDS-related adverse effects. Detailed studies validating the clinical events and outcomes of a sample of exposures reported to TESS(R) might offer substantial insights into adverse events that could be systematically studied with other, established pharmacoepidemiological study designs (Gryzlak *et al.* 2007).

Assessor's overall conclusions on toxicology

In general, the toxicity of *Echinacea purpurea* is low. However, the data on purple coneflower root toxicity are limited and findings are sometimes difficult to interpret since there is a lack of detail regarding the tested preparation or the part of the plant of *E. purpurea*. Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed on the dry ethanolic extract included in the Community herbal monograph.

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Five placebo-controlled randomized studies investigating the immunomodulatory activity of preparations containing extracts of *Echinacea* in healthy volunteers were studied. A total of 134 (18 female and 116 male) healthy volunteers between 18 and 40 years of age were studied. Two studies (2 and 3a) tested oral alcoholic extracts of roots of *E. purpurea*, (2: ethanolic extract, corresponding to 333 mg of roots, 3a: 380 mg of dried extract made with 50% ethanol (V/V)) one study an extract of *E. pallida* roots (study 3b), and one study an extract of *E. purpurea* herb (study 4). Test and placebo preparations were applied for four (study 5) or five (studies 1-4) consecutive days. The primary outcome measure for immunomodulatory activity was the relative phagocytic activity of polymorphonuclear neutrophil granulocytes (PNG), measured in studies 1 and 2 with a microscopic method and in studies 3, 4, and 5 with two different cytometric methods. The secondary outcome measure was the number of leukocytes in peripheral venous blood. Safety was assessed by a screening program of blood and other objective parameters as well as by documentation of all subjective side effects. In studies 1 and 2 the phagocytic activity of PNG was significantly enhanced compared with placebo [maximal stimulation 22.7% (95% confidence interval [CI] 17.5-27.9%) and 54.0% (8.4-99.6%), respectively], while in the other studies no significant effects were observed. Analysis of intragroup differences revealed significant changes in phagocytic activity during the observation periods in five test and three control groups. Leukocyte number was not influenced significantly in any study. Side effects due to the test preparations could not be detected. The study showed immunomodulatory activity of the *E. purpurea* radix extract tested in study 2. The negative results of the other 3 studies are difficult to interpret due to the different methods for measuring phagocytosis, the relevant changes in phagocytic activity within most placebo and treatment groups during the observation period, and the small sample sizes. Further studies should be performed on patients rather than healthy volunteers and use standardized or chemically defined monopreparations of *Echinacea* (Melchart *et al.* 1995).

In a double-blind study, 24 healthy male volunteers took three times 30 drops of an ethanolic extract of purple coneflower root (detailed specifications of the extract are not given) or placebo daily for 5 days. By day 5 a significant increase in phagocytosis of 120% was observed in the verum group,

compared to 20% in the placebo group. The effect was transient and phagocytotic activity returned to normal within 6 days (Jurcic *et al.* 1989).

The effect of Echinacea Premium® tablets containing 675 mg of *E. purpurea* root extract and 600 mg of *E. angustifolia* root extract, prepared by ethanol extraction (MediHerb, Warwick, Australia) on the expression of leucocyte heat shock protein 70 (hsp70), erythrocyte haemolysis, plasma antioxidant status, serum chemistry, haematological values and plasma alkylamide concentrations. Eleven healthy individuals (26–61 years of age) were evaluated at baseline (day 1) and on day 15 after consuming two commercially blended *Echinacea* tablets daily for 14 days. *Echinacea* supplementation enhanced the fold increase in leucocyte hsp70 expression after a mild heat shock (P = 0.029). White cell counts (WCC) were also increased (P = 0.043). A preventative effect against free radical induced erythrocyte haemolysis (P = 0.006) indicative of an antioxidant effect was also observed. The pilot study suggests that *Echinacea* may invoke an immune response through altered expression of hsp70 and increased WCC (Agnew *et al.* 2005).

Echinilin® (extract of herb and root of *Echinacea purpurea*) or placebo were administered to volunteers at the onset of their cold for a period of 7 days, with 8 doses (5 ml/dose) on day 1 and 3 doses on subsequent days. Fasting blood samples were obtained before and during their colds. The decrease in total daily symptomatic score was more evident in the *Echinacea* group than in the placebo group. These effects of *Echinacea* were associated with a significant and sustained increase in the number of circulating total white blood cells, monocytes, neutrophils and NK cells. In the later part of the cold, the *Echinacea* treatment suppressed the cold-related increase in superoxide production by the neutrophils. These results suggested that Echinilin®, by enhancing the non-specific immune response and eliciting free radical scavenging properties, may have led to a faster resolution of the cold symptoms (Goel *et al.* 2005).

Assessor's overall conclusions on pharmacodynamics

Increased phagocytosis was observed in two studies on purple coneflower root extract as a single ingredient, unfortunately detailed composition of extracts is not available. In other two studies combination herbal products were investigated and it is difficult to interpret which plant or part of *E. purpurea* plant was responsible for the effect (leucocyte hsp70 expression, increased WCC, preventative effect against free radical induced erythrocyte haemolysis, antioxidant effect, suppressed cold-related increase in superoxide production by the neutrophils).

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Serial plasma samples from 9 healthy volunteers who ingested *Echinacea* tablets manufactured from both ethanolic liquid extracts of *E. angustifolia* and *E. purpurea* immediately after a standard high fat breakfast were examined. Caffeic acid conjugates could not be identified in any plasma sample at any time after tablet ingestion. Alkamides were rapidly absorbed and were measurable in plasma 20 min after tablet ingestion and remained detectable for up to 12 h. Concentration-time curves for 2,4-diene and 2-ene alkamides were determined. The maximal concentrations for the sum of alkamides in human plasma were reached within 2.3 hours post ingestion and averaged 336±31 ng eq/ml plasma. No obvious differences were observed in the pharmacokinetics of individual or total alkamides in 2 additional fasted subjects who took the same dose of the *Echinacea* preparation. (Matthias *et al.* 2005b).

The relative oral bioavailability of alkylamides from two different *Echinacea* dosage forms (liquid and tablet) were compared in a small two-way crossover study in humans (n = 3). The liquid preparation investigated contained a mixture of *E. purpurea* root (300 mg/ml) and *E. angustifolia* root (200 mg/ml)

extracted in 60% ethanol. The tablet preparation investigated was also a mixture of *E. purpurea* root (675 mg/tablet) and *E. angustifolia* root (600 mg/tablet), but was prepared from the dried 60% ethanolic extracts of these two *Echinacea* species. Alkylamides were found to be rapidly absorbed and measurable in plasma from both preparations. No significant differences in the tetraene alkylamide pharmacokinetic parameters for $T_{1/2}$, AUCt-lin and C_{max} in the two different preparations were found. T_{max} increased from 20 min for the liquid to 30 min for the tablet, which is not unexpected as the tablet required time for disintegration before absorption could occur. These results suggested that there was no significant difference in the bioavailability of alkylamides from the liquid and tablet *Echinacea* formulations. Furthermore, the results also indicated that the absorption site and any alkylamide loss due to digestive processes were similar in both preparations (Matthias *et al.* 2007).

Assessor's overall conclusions on pharmacokinetics

Evidence were provided that alkamides are orally available from liquid extracts and tablets and that their pharmacokinetics is in agreement with the daily regimen already recommended for *Echinacea* and there was no significant difference in the bioavailability of alkylamides from the liquid and tablet *Echinacea* formulations. In contrast, caffeic acid conjugates could not be identified in any plasma sample; therefore their oral bioavailability is questionable.

4.2. Clinical Efficacy

Several papers have been published reviewing studies of the effects of *Echinacea purpurea* herbal products (aerial parts, roots and combinations) in clinical trials (Barnes *et al.* 2005, Barnes *et al.* 2007, Barrett 2003, Bradley 2006, ESCOP 2003 and 2009, PDR 2007, Blumenthal *et al.* 2000, Bauer and Liersch 1993, Linde *et al.* 2006, Melchart *et al.* 1994, Melchart *et al.* 2004, Schoop *et al.* 2006, Shah *et al.* 2007).

4.2.1. Dose response studies

In a double-blind, placebo-controlled study, 180 patients (18-60 years old) with influenza, randomized into three groups of 60, were given a tincture of purple coneflower root made with 55% ethanol (V/V) at daily dosages corresponding to 459 mg or 900 mg of dried root, or placebo. After 3-4 days and 8-10 days, there was no statistical difference in symptoms between the group taking the 450 mg dose and the placebo group. In contrast, the group taking the 900 mg dose showed a highly significant reduction in symptom score at both time points ($p < 0.0001$). An effect from the higher dose was seen after 3 to 4 days, but the full effect was not seen for 8-10 days (Bräunig *et al.* 1992). This trial is frequently referred to as a plausible evidence of the efficacy of preparations of *E. purpurea* root, for the treatment of symptoms associated with common cold (Blumenthal *et al.* 2000, Melchart *et al.* 1994, Barrett *et al.* 1999).

4.2.2. Clinical studies (case studies and clinical trials)

Echinacea purpurea **as a single ingredient**

289 volunteers from four military establishments and one industrial plant participated in a double-blind, placebo-controlled study to investigate the efficacy of *Echinacea* extracts in the prevention of upper respiratory tract infections. Randomised groups were instructed to take twice daily for 12 weeks 50 drops (ca. 1 ml) of one of three trial preparations: ethanolic extract (1: 11, ethanol 30%) of purple coneflower root (Group A, n = 99) or *E. angustifolia* root (Group B, n = 100), or an ethanolic placebo solution (Group C, n = 99). 244 participants fully conformed with the protocol: 85, 84 and 75 in Groups A, B and C, respectively. The average time until occurrence of first upper respiratory tract

infections was 69, 66 and 65 days, and 29%, 32% and 37% of participants had at least one upper respiratory tract infection, in Groups A, B and C, respectively. Although perhaps suggesting a relative reduction in risk of infection of 20% for purple coneflower root compared to placebo, the results were not statistically significant (Melchart *et al.* 1998).

In a randomized, double-blind, placebo-controlled study the efficacy and safety of different doses and preparations of *Echinacea purpurea* in the treatment of common cold. 246 of 559 recruited healthy, adult volunteers caught a common cold and took 3 times daily 2 tablets of either Echinaforce® (*Echinacea purpurea*-preparation from 95% herba and 5% radix), *Echinacea purpurea* concentrate (same preparation at 7 times higher concentration), special *Echinacea purpurea* radix preparation (dry extract from root, 29.6 mg per tablet) or placebo until they felt healthy again but not longer than 7 days. The primary endpoint was the relative reduction of the complaint index defined by 12 symptoms during common cold according to the doctor's record. Echinaforce® and its concentrated preparation were significantly more effective than the special *Echinacea* extract or placebo. All treatments were well tolerated. Among the *Echinacea* groups the frequency of adverse events was not significantly higher than in the placebo group. Therefore, *Echinacea* concentrate as well as Echinaforce® represent a low-risk and effective alternative to the standard symptomatic medicines in the acute treatment of common cold (Brinkeborn *et al.* 1999).

Echinacea purpurea in combination with other herbal drugs

A double-blind randomized placebo-controlled trial was performed on 263 patients to evaluate the use of commercially available fixed combination herbal remedy containing ethanolic-aqueous extracts of *Herba thujae occidentalis*, *Radix echinaceae* (*purpureae* + *pallidae* = 1 + 1) and *Radix baptisiae*, 2, 7.5 and 10 mg per tablet, respectively. The aim of the study was to verify clinical efficacy shown in recent studies under (i) good clinical practice (GCP) quality assurance and (ii) common situations at family doctors. Patients attending one of 15 study centres (practitioners) as a result of an acute common cold were randomised to the double-blind placebo-controlled study. Three tablets of study medication were applied t.i.d. for 7 to 9 days. Patients daily documented the intensity of 18 cold symptoms, as well as the cold overall, using a 10-point scale and estimated their general well-being using the Welzel-Kohnen colour scales. Additionally, the severity of illness was assessed by the physician on days 4 and 8 (CGI-1). The main and confirmatory outcome measure was expressed as a total efficacy value. This was gauged from the z-standardised AUC values of the primary endpoints (rhinitis score, bronchitis score, CGI-1 and general well-being). Adverse events, overall tolerability, vital signs and laboratory parameters were documented. For safety analysis, all patients were used. 259 patients were evaluable for primary efficacy analysis (ITT). Results were confirmed analysing only the 238 valid cases (VCs). The primary efficacy parameters showed the superiority of the herbal remedy over placebo ($p < 0.05$). Effect size was 20.6% of the standard deviation (90% CI: 0.04-41.1%; ITT) and 23.1% (1.7-44.5%; VC). In relation to the general well-being, the effect size was 33.9% of the standard deviation (12.5-55.3%; VC). Patients who suffered from at least moderate symptom intensity at baseline showed response rates (at least 50% improvement of the global score, day 5) of 55.3% in the herbal remedy group and 27.3% in the placebo group ($p = 0.017$; NNT = 3.5). In the subgroup of patients who started therapy at an early phase of their cold, the efficacy of the herbal remedy was most prominent ($p = 0.014$ for the primary efficacy parameter). The therapeutic benefit of the herbal remedy had already occurred on day 2 and reached significance ($p < 0.05$) on day 4, and continued until the end of the treatment in the total score of symptoms, bronchitis score and rhinitis score, as well as in the patients' overall rating of the cold intensity. At that time, equal levels of improvement were reached three days earlier in the verum group than in the placebo group. In 26 patients receiving the herbal remedy and 23 patients receiving placebo, adverse events were reported. Adverse drug reactions were suspected in 2 patients in the verum group and in 4 patients in the placebo group. Serious adverse

events did not occur. This study shows that the herbal remedy is effective and safe. The therapeutic benefit consists of a rapid onset of improvement of cold symptoms. If patients with colds are able to start the application of the herbal remedy as soon as practical after the occurrence of the initial symptoms, the benefit would be expected to increase (Henneicke-von Zepelin *et al.* 1999).

The aim of this study was to determine the efficacy of an *Echinacea* compound herbal tea preparation Echinacea Plus® (comprising *E. purpurea* herb and extract of *E. purpurea* root equivalent to 1275 mg dried herb and root per tea bag) given at early onset of cold or flu symptoms in a random assignment double-blind placebo-controlled study. A total of 95 subjects with early symptoms of cold or flu (runny nose, scratchy throat, fever) were randomly assigned to receive Echinacea Plus® tea 5 to 6 cups per day titrating to 1 over 5 days or placebo in a double-blind situation. Each participant completed a questionnaire 14 days after beginning the program. The efficacy, number of days the symptoms lasted and number of days for change were measured with a self scoring questionnaire. The study period was 90 days (1 January 1999 to 30 March 1999). There was a significant difference between the experimental group (Echinacea Plus®) and control group (placebo) for all 3 questions measured: p < 0.001. There were no negative effects reported by any of the subjects in either group. Treatment with Echinacea Plus® tea at early onset of cold or flu symptoms was effective for relieving these symptoms in a shorter period of time than a placebo (Lindenmuth and Lindenmuth 2000).

The efficacy of dried, encapsulated, whole-plant *Echinacea* as early treatment for the common cold in a randomised, double-blind, placebo-controlled community-based trial at the University of Wisconsin, on 148 students with common colds of recent onset was assessed. Each active capsule contained a dried mixture of *E. angustifolia* root (50% [123 mg]), *E. purpurea* root (25% [62 mg]), and *E. purpurea* herb (25% [62 mg]). *Echinacea* capsules also contained thyme (49 mg) and peppermint (31 mg) to disguise taste and flavour, as well as citric acid (3 mg) as a preservative. The placebo capsules contained 333 mg of alfalfa. The patients took 4 capsules 6 times during the first 24 hours of the study, and 4 capsules 3 times each day thereafter until symptoms resolved, for a maximum of 10 days. Severity and duration of self-reported symptoms of upper respiratory tract infection were recorded. No statistically significant differences were detected between the *Echinacea* and placebo groups for any of the measured outcomes. Trajectories of severity over time were nearly identical in the 2 groups. Mean cold duration was 6.01 days in both groups as a whole, 5.75 days in the placebo group, and 6.27 days in the *Echinacea* group (between-group difference, -0.52 day [95% CI, -1.09 to 0.22 days]). After controlling for severity and duration of symptoms before study entry, sex, date of enrolment, and use of nonprotocol medications, researchers found no statistically significant treatment effect (adjusted hazard ratio, 1.24 [CI, 0.86 to 1.78]). Multivariable regression models assessing severity scores over time failed to detect statistically significant differences between the Echinacea and placebo groups (Barrett *et al.* 2002).

A formulation containing alkamides, cichoric acid, and polysaccharides at concentrations of 0.25, 2.5, and 25 mg/ml, respectively, was prepared from freshly harvested *Echinacea purpurea* herb and root (commercially available as Echinilin®, Natural Factors Nutritional Products, Inc., Vancouver, BC, Canada). The objective of this study was to test the efficacy of this highly standardized formulation in reducing the severity and duration of symptoms of a naturally acquired common cold. In a randomized, double-blind, placebo-controlled trial, 282 subjects aged 18-65 years with a history of two or more colds in the previous year, but otherwise in good health, were recruited. The subjects were randomized to receive either *Echinacea* or placebo. They were instructed to start the Echinacea or placebo at the onset of the first symptom related to a cold, consuming 10 doses the first day and 4 doses per day on subsequent days for 7 days. Severity of symptoms (10-point scale: 0, minimum; 9, maximum) and dosing were recorded daily. A nurse examined the subjects on the mornings of days 3 and 8 of their cold. A total of 128 subjects contracted a common cold (59 *Echinacea*, 69 placebo). The total daily symptom scores were found to be 23.1% lower in the *Echinacea* group than in placebo in

those who followed all elements of the study protocol (P < 0.01). Throughout the treatment period, the response rate to treatments was greater in the *Echinacea* group. A few adverse event profiles were observed in both groups. Early intervention with a standardized formulation of *Echinacea* resulted in reduced symptom severity in subjects with naturally acquired upper respiratory tract infection. Further studies with larger patient populations appear to be warranted (Goel *et al.* 2004).

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No data available.

4.3. Overall conclusions on clinical pharmacology and efficacy

Purple coneflower root extract did not prove to be successful in preventing of upper respiratory tract infection in one double-blind, placebo-controlled study, it also did not prove to be successful in the treatment of common cold in other study. In only one double-blind, placebo-controlled study on patients with influenza, a tincture of purple coneflower root (1:5, ethanol 55%) showed significant reduction in symptom score at both time points (after 3-4 and 8-10 days) and the effect was dose-dependent.

In 3 double-blind, placebo-controlled trials *Echinacea purpurea* root in combination with other herbal drugs was reported to be successful in reducing the severity of symptoms of common cold or flu. There was no significant difference between placebo and verum group in one double-blind, placebo-controlled trial. The results are difficult to interpret, it is difficult to say which component is the active one, and the pharmacological effects are probably achieved by synergistic effect.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

See below.

5.2. Patient exposure

A systematic review, based on clinical studies, case reports and surveillance programmes of national medicines regulatory authorities and WHO, concluded that *Echinacea* products have a good safety profile when taken in the short term, while data on long-term use is not available. If adverse events occur they tend to be transient and reversible, the most common being gastrointestinal or skin related (Huntley *et al.* 2005).

No adverse events were reported in a clinical study in which 180 patients randomised in 3 groups of 60 patients with influenza received oral treatment daily for 10 days with a tincture of purple coneflower root made with ethanol 55% (V/V), equivalent to 450 mg or 900 mg of dried root; the preparation was well tolerated (Bräunig *et al.* 1992).

In a clinical study, 10 out 99 subjects who took 2 times 50 drops (2 times 1 ml) of a hydroethanolic extract of purple coneflower root (detailed specifications of the extract are not given) daily for 12 weeks reported adverse effects, compared to 11 out of 90 subjects in the placebo group; none of the adverse effects were serious or required therapeutic action (Melchart *et al.* 1998).

5.3. Adverse events and serious adverse events and deaths

In rare cases hypersensitivity reactions e.g. skin reactions may occur (ESCOP 2003 and 2009, Bauer and Liersch 1993, Blumenthal *et al.* 2000, Mullins 1998). Individuals with allergic tendencies, particularly those with known allergy to other members of the Asteraceae family should be advised to avoid *Echinacea* (Barnes *et al.* 2007, Bauer and Liersch 1993).

In an analysis of the Australian Adverse Drug Reactions Advisory Committttee's database of events of IgE-mediated hypersensitivity reactions, 51 reports were found to be related to *Echinacea* use. 26 reactions including urticaria, angio-oedema, asthma and anaphylaxis were confirmed to be IgE-mediated reactions to *Echinacea*. More than half of the affected patients had a history of asthma, allergic rhinitis or atopic dermatitis. Four persons required hospitalization due to their reactions and no deaths occurred. In 94% of patients, the symptoms appeared within 24 hours of *Echinacea* ingestion. 80% of the patients included were female and the medium age was 32 years (PDR 2007, Mullins & Heddle 2002).

Serious adverse events and deaths

None reported.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

Intrinsic (including elderly and children)/extrinsic factors

As with all immunostimulants, use is not recommended in progressive systemic diseases such as tuberculosis, diseases of the white blood cells system, collagenoses, multiple sclerosis, AIDS, HIV infections, and other immune diseases (Barnes *et al.* 2005, Barnes *et al.* 2007, ESCOP 2003 and 2009, Bauer and Liersch 1993, Blumenthal *et al.* 2000).

In accordance with the 'Guideline on the Summary of Product Characteristics' dated September 2009, the statement 'Not recommended in cases of progressive systemic diseases such as tuberculosis, diseases of the white blood cells system, collagenoses, multiple sclerosis, AIDS, HIV infections and other immune diseases' appears in the section 'Warnings and precautions for use' of the monograph on *Echinaceae purpureae radix* (not as a contraindication).

Atopic patients and those with asthma should be cautious since rare allergic reactions have been reported (Barnes *et al.* 2005, Barnes *et al.* 2007, Huntley *et al.* 2005).

Use in children

There are no sufficient data on safety of purple coneflower root preparations in children; therefore the use of *Echinacea purpurea* root and preparations thereof is not recommended. This appears as a warning in the monograph and not as a contraindication in accordance with the 'Guideline on the Summary of Product Characteristics' dated September 2009.

Drug interactions

The effect of *Echinacea* (*Echinacea purpurea* root) on CYP activity *in vivo* was assessed by use of the CYP probe drugs caffeine (CYP1A2), tolbutamide (CYP2C9), dextromethorphan (CYP2D6), and

midazolam (hepatic and intestinal CYP3A). Twelve healthy subjects (6 men) completed this 2-period, open-label, fixed-schedule study. Caffeine, tolbutamide, dextromethorphan, and oral and intravenous midazolam were administered before and after a short course of *Echinacea* (400 mg 4 times a day for 8 days) to determine *in vivo* CYP activities. *Echinacea* administration significantly increased the systemic clearance of midazolam by 34%, from 32 +/- 7 L/h to 43 +/- 16 L/h (P =0.003; 90% CI, 116%-150%), and significantly reduced the midazolam area under the concentration-time curve by 23%, from 127 +/- 36 microg. h/L to 102 +/- 43 microg. h/L (P =0.024; 90% CI, 63%-88%). In contrast, the oral clearance of midazolam was not significantly altered (P =0.655; 90% CI, 75%-124%), 137 +/- 19 L/h compared with 146 +/- 71 L/h. The oral availability of midazolam after Echinacea dosing was significantly increased (P =0.028; 90% CI, 108%-153%), from 0.23 +/- 0.06 to 0.33 +/- 0.13. Hepatic availability (0.72 +/- 0.08 versus 0.61 +/- 0.16; P =0.006; 90% CI, 73%-90%) and intestinal availability (0.33 +/- 0.11 versus 0.61 +/- 0.38; P =0.015; 90% CI, 125%-203%) were significantly altered in opposite directions. Echinacea dosing significantly reduced the oral clearance of caffeine, from 6.6 +/- 3.8 L/h to 4.9 +/- 2.3 L/h (P =0.049; 90% CI, 58%-96%). The oral clearance of tolbutamide was reduced by 11%, from 0.81 +/- 0.18 L/h to 0.72 +/- 0.19 L/h, but this change was not considered to be clinically relevant because the 90% CIs were within the 80% to 125% range. The oral clearance of dextromethorphan in 11 CYP2D6 extensive metabolizers was not affected by *Echinacea* dosing (1289 +/- 414 L/h compared with 1281 +/- 483 L/h; P =0.732; 90% CI, 89%-108%). *Echinacea* (*E. purpurea* root) reduced the oral clearance of substrates of CYP1A2 but not the oral clearance of substrates of CYP2C9 and CYP2D6. *Echinacea* selectively modulates the catalytic activity of CYP3A at hepatic and intestinal sites. The type of drug interaction observed between *Echinacea* and other CYP3A substrates will be dependent on the relative extraction of drugs at hepatic and intestinal sites. Caution should be used when *Echinacea* is co-administered with drugs dependent on CYP3A or CYP1A2 for their elimination (Gorski *et al.* 2004).

An increasing number of cancer patients are using complementary and alternative medicines (CAM) in combination with their conventional chemotherapeutic treatment. Considering the narrow therapeutic window of oncolytic drugs, this CAM use increases the risk of clinically relevant herb-anticancer drug interactions. Recently identified nuclear receptors, such as the pregnane X receptor, the constitutive androstane receptor, and the vitamin D-binding receptor, play an important role in the induction of metabolizing enzymes and drug transporters. This knowledge has already been an aid in the identification of some CAM probably capable of causing interactions with anticancer drugs: kava-kava, vitamin E, quercetin, ginseng, garlic, beta-carotene, and echinacea. Evidently, more research is necessary to prevent therapeutic failure and toxicity in cancer patients and to establish guidelines for CAM use (Meijerman *et al.* 2006).

The review by Freeman and Spelman assessed the occurrence of drug interactions with one of the top selling botanical remedies, *Echinacea* including *E. angustifolia*, *E. pallida*, and *E. purpurea*. Only eight papers containing primary data relating to drug interactions were identified. Herbal remedies made from *E. purpurea* appear to have a low potential to generate cytochrome P450 (CYP450) drug–herb interactions including CYP 450 1A2 (CYP1A2) and CYP 450 3A4 (CYP3A4). Currently there are no verifiable reports of drug–herb interactions with any *Echinacea* product. However, further pharmacokinetic testing is necessary before conclusive statements can be made about *Echinacea* drug–herb interactions. The authors concluded that given the findings, the estimated risk of taking *Echinacea* products (1 in 100 000), the number of *Echinacea* doses consumed yearly (> 10 million), the number of adverse events (< 100) and that the majority of use is short term, *E. purpurea* products (roots and/or aerial parts) do not appear to be a risk to consumers (Freeman and Spelman 2008).

Use in pregnancy and lactation

A review on safety of *Echinacea* during pregnancy and lactation was published recently (Perri *et al.* 2006). They searched 7 electronic databases and compiled data according to the grade of evidence found. They found good scientific evidence from a prospective cohort study that oral consumption of *Echinacea* during the first trimester does not increase the risk for major malformations. Low-level evidence based on expert opinion shows that oral consumption of *Echinacea* in recommended doses is safe for use during pregnancy and lactation. They concluded that *Echinacea* is non-teratogenic when used during pregnancy. Using *Echinacea* during lactation is not recommended until further high quality human studies can determine its safety.

Pregnancy outcome in women that used *Echinacea* during pregnancy was studied to evaluate the safety of *Echinacea*. There is no specification which species of *Echinacea* was evaluated. Since at least half of all pregnancies are unplanned, many women inadvertently use *Echinacea* in their first trimester. The study group consisted of 206 women who were prospectively followed up after contacting the Motherisk Program regarding the gestational use of *Echinacea*, 112 women used the herb in the first trimester. This cohort was disease-matched to women exposed to non-teratogenic agents by maternal age, alcohol, and cigarette use. Rates of major and minor malformations between the groups were compared. There were a total of 195 live births, including 3 sets of twins, 13 spontaneous abortions, and 1 therapeutic abortion in the *Echinacea* group. Six major malformations were reported, including 1 chromosomal abnormality, and 4 of these malformations occurred with *Echinacea* exposure in the first trimester. In the control group, there were 206 women with 198 live births, 7 spontaneous abortions, and 1 therapeutic abortion. Seven major malformations were reported. There were no statistical differences between the study and control groups for any of the endpoints analysed. The authors concluded that gestational use of *Echinacea* during organogenesis is not associated with an increased risk for major malformations (Gallo *et al.* 2000).

Overdose

No case of overdose has been reported.

Drug abuse

No case of drug abuse has been reported.

Withdrawal and rebound

No data available.

Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

5.6. Overall conclusions on clinical safety

Hypersensitivity reactions e.g. skin reactions were observed in rare cases; therefore individuals with allergic tendencies particularly those with known allergy to other members of the Asteraceae family should avoid *Echinacea purpurea* preparations. Atopic patients and those with asthma should be cautious since rare allergic reactions have been reported.

There are no sufficient data on safety of purple coneflower root preparations in children; therefore the use of *Echinacea purpurea* root and preparations thereof is not recommended.

Echinacea purpurea should not be used in progressive systemic diseases such as tuberculosis, diseases of the white blood cells system, collagenoses, multiple sclerosis, AIDS, HIV infections, and other immune diseases.

Due to unreliable studies, administration during pregnancy and lactation is not generally recommended in accordance with general medical practice. *Echinacea purpurea* preparations should not be used during pregnancy or lactation without medical advice.

Herbal remedies made from *Echinacea purpurea* appear to have a low potential to generate cytochrome P450 (CYP450) drug–herb interactions including CYP 450 1A2 (CYP1A2) and CYP 450 3A4 (CYP3A4). Currently there are no verifiable reports of drug–herb interactions with any *Echinacea* product. However, further pharmacokinetic testing is necessary before conclusive statements can be made about *Echinacea* drug–herb interactions.

6. Overall conclusions

The pharmacological effects of *Echinacea purpurea* root preparations on immune system of adults were proved but the pharmacological mechanisms and active compounds still remain mainly unclear. So far the only compounds for which the oral availability has been established are alkamides. Clinical efficacy in children is not certain. The data about the toxicity of purple coneflower root preparations are very limited although it has been used for decades.

Well-established use of *Echinacea purpurea* root for the preventive or supportive treatment of common cold is not possible, due to insufficient clinical data.

Traditional use of *Echinacea purpurea* root in this indication is possible, although toxicological data are limited; however a certain level of safety could be expected due to the long-standing use of *Echinacea purpurea* root preparations with no serious side effects reported.

Annex

List of references