EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

31 March 2011
EMA/HMPC/688212/2008
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Echinacea angustifolia* DC., radix

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

Draft

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Cut, dried underground parts of *Echinacea angustifolia* DC.
Herbal preparation(s)	a) Comminuted herbal substance b) Powdered herbal substance
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use. Herbal preparations in solid dosage forms for oral use.

Note: This Assessment Report is published to support the release for public consultation of the draft Community herbal monograph on *Echinacea angustifolia* DC., radix. It should be noted that this document is a working document, not yet fully edited, and which shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation' will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

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1. Introduction

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

- Herbal substance(s)

Echinaceae angustifoliae radix (European Pharmacopoeia)

Echinaceae angustifoliae radix consists of the whole or cut, dried underground parts of *Echinacea angustifolia* DC. It contains not less than 0.5% of echinacoside ($C_{35}H_{46}O_{20}$; M_r 786.5).

Constituents (Barnes *et al.* 2005, Barnes *et al.* 2007, Bauer *et al.* 1988b & 1989a, Bradley 2006, Cozzolino *et al.* 2006, Liersch *et al.* 1993, Mazza *et al.* 1999, Perry *et al.* 2001, Wolters Kluwer Health 2004):

- Alkamides (0.5%): mainly isobutylamides or 2-methylbutylamides 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-monoene units;

- Caffeic acid derivatives (1.0-1.4%): principally echinacoside (0.5-1.3%) with modest amounts of cynarin (0.12-0.14%), chlorogenic and cichoric acids;

- Polysaccharides and glycoproteins: two polysaccharides of molecular weight of 128 and 4.5 kDa, three glycoproteins of molecular weight of 17, 21 and 30 kDa, containing about 3% of protein, the dominant sugars were found to be arabinose (64-84%), galactose (2-5%) and glucosamine (6%). Fructans are also present;

- Volatile oil (0.1%): dodeca-2,4-diene-1-yl isovalerate, 8*Z*-pentadecene-2-one, pentadeca-1,8*Z*-diene, palmitinic acid, linolenic acid;

- Other constituents: saturated pyrrolizidine-type alkaloids tussilagine and isotussilagine (0.006%), phytomelanin, small amounts of polyacetylenic compounds (other than alkamides).

- Herbal preparation(s)

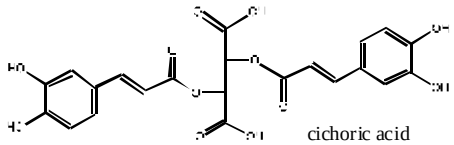
Herbal substance, powdered; comminuted herbal substance for decoctions and galenic preparations (PDR 2007, Blumenthal *et al.* 2000, ESCOP 2009);
dry extract (1.7-4.7:1), extraction solvent: ethanol 70% (V/V);
tincture (1:5), extraction solvent: ethanol 70% (V/V).

- 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.

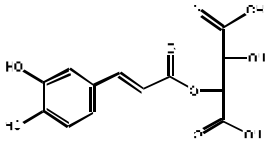
Not applicable.

Caffeic acid derivatives

with tartaric acid

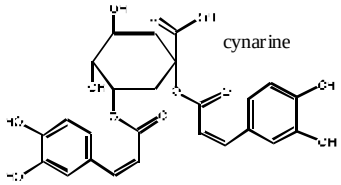


cichoric acid



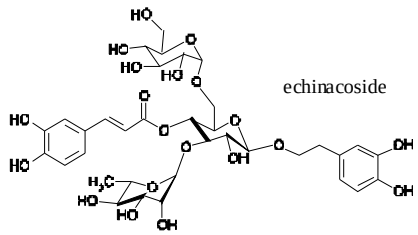
caftaric acid

with quinic acid



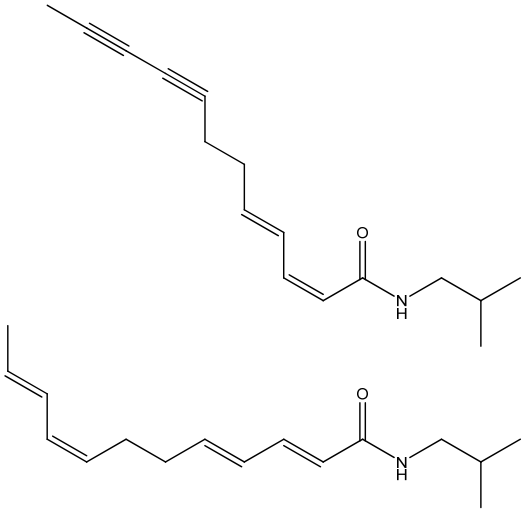
cynarine

with sugar



echinacoside

Alkamides



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

Herbal substance, powdered

Spain:

Preparations: herbal substance, powdered, (250 mg of herbal substance/capsule)

Preparation on the market: since 1990 (criteria of 30 years is not covered)

Pharmaceutical form: capsule, hard

Dry extracts

Sweden:

Preparations: dry extract (1.7-4.7:1) ethanol 70% (V/V); 100 mg of extract/capsule, equivalent to 170-470 mg of the herbal substance

Preparation on the market: since 2005 (criteria of 30 years is not covered)

Pharmaceutical form: capsule, hard

Liquid extracts

Latvia:

Preparations: tincture (1:5) ethanol 70% (V/V).

Preparation on the market: since 2002 (criteria of 30 years is not covered)

Pharmaceutical form: tincture

Hungary:

Preparations: 33.30 g liquid extract of *Echinacea angustifolia* radix (mother tincture according to HAB 2000); extraction solvent: ethanol 86 (m/m) % DER=2 : 1 in 100 g solution, ethanol 50% V/V)

Preparation on the market: since 1991 (criteria of 30 years is not covered)

Pharmaceutical form: oral solution

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
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:	No products.
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements.
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	One registered product (33,30 g liquid extract of *Echinacea* angustifolia radix extraction solvent: ethanol 86(m/m) % DER=2 : 1 in 100 g solution. Ethanol 50% V/V.), since 1991
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:	One registered preparation: tincture (1:5) ethanol 70% (V/V). since 2002 Pharm. form: tincture
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
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:	No answer.
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Comb. product (1)
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:	No products.

Member State	Regulatory Status				Comments
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	One registered preparation- powdered herbal substance (250 mg of herbal substance / capsule) Since 1990 In hard capsules
Sweden	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	One registered preparation: dry extract (1.7-4.7:1) ethanol 70% (V/V). 100 mg of extract / capsule, equivalent to 170-470 mg of the herbal substance. Since 2005 In hard capsules
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

Not specified by the Rapporteur.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

See sections 1.2, 2.2 and 2.3.

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

Indications for oral use of herbal teas, tincture and liquid extracts.

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

Echinacea angustifolia roots were included in the 4th edition of USA National Formulary (National Formulary 1916).

The medical use of *Echinacea angustifolia* roots, mainly for treating upper respiratory tract infections and poisonous animal bites, was already reported at the end of 19th century (Felter 1898, Goss 1889, Kindscher 1989, Meyer 1887, Stevens 1898, Webster 1898),the first half of the 20th century (Ellingwood 1915, Fearn 1914, Felter & Lloyd 1905, Fyfe 1909, Gilmore 1919, Liebstein 1927, Lloyd 1904a, 1904b, 1917 & 1923, Kraemer & Sollenberger 1911, Niederkorn, 1910, Smith HH 1928, Vestal & Schultes 1939) and the 2nd half of the 20th century (Hocking 1965). A hundred years of history regarding harvesting of the plant, marketing and medical use is documented (Flannery 1999, Price & Kindscher 2007).

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

Herbal substance, powdered

2 capsules containing 250 mg of herbal substance; 2-3 times daily

Indications: for the relief of early symptoms of common cold

Risks: none given

Herbal substance, comminuted

1 g of comminuted root, boiled in 150 ml water for 10 minutes three times daily

Indications: Traditionally used for the relief of cold symptoms.

Dry extracts

dry extract (1.7-4.7:1) ethanol 70% (V/V): 2 capsules containing 100 mg of dry extract 2-3 times daily. Not to be used more than 14 days. Not recommended for children.

Indications: Traditionally used for the relief of cold symptoms.

Risks: Hypersensitivity reactions such as skin reactions including urticaria may occur in persons treated with *Echinacea* products. The risk appears higher in patients with atopic eczema. Severe hypersensitivity reactions such as angio oedema, dyspnoea/bronchospasm and fall in blood pressure have been reported. Treatment with *Echinacea* must be discontinued at first signs of hypersensitivity.

Liquid extracts

tincture (1:5) ethanol 70% (V/V): 2-2.5 ml 2 times daily
After recovering it is recommended to continue use until 8 weeks. In prophylaxis, use the same dose, the duration of use should not exceed 8 weeks. In children less than 12 years, should not be used without medical advice.

Indications: immunostimulant, antibacterial, antiviral, as an adjuvant in the treatment of common cold, influenza and fever; also for the stimulating the regeneration of tissue. As adjuvant in the treatment of furunculosis, septicaemia and similar infections; may be used in treatment of upper respiratory tract infections, laryngitis, tonsillitis, catarrhal conditions of the nose and sinus and viral infections; as well as in supportive treatment of weakened immune system

Risks:

Contra-indications: hyperaesthesia to plants of the *Asteraceae* (Compositae); progressive systemic diseases (tuberculosis, sarcoidosis), autoimmune diseases (collagenoses, multiple sclerosis), HIV infection, AIDS, hematological diseases (agranulocytosis, leukemia); serious hepatic diseases; epilepsy

Pregnancy and lactation: Contraindicated during pregnancy and lactation.

Adverse effects: Allergic reactions, short-lived feverish reactions, headache.

Oral administration

Herbal substance: 1 g by infusion three times daily (Barnes *et al.* 2007, PDR 2007) several times daily between meals (Liersch *et al.* 1993).

Herbal preparations:

Ticture: 2-5 ml (1:5 in 45% ethanol V/V) three times daily, or 1-2 ml (1:5 in 45% ethanol V/V) three times daily (Barnes *et al.* 2007, Blumenthal *et al.* 2000, ESCOP 2009).

Liquid extracts: a) 1 ml (1:11 in 30% ethanol V/V) three times daily (ESCOP 2009)
b) 0.5-1.0 ml (1:1 in 45% ethanol V/V) three times daily (ESCOP 2009)

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

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**

From water or alkaline-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 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).

An ethanolic extract of narrow-leaved coneflower root enhanced phagocytosis by 17% in the granulocyte smear test at a concentration of 10^{-3} mg/ml. Only lipophilic fractions from the ethanolic extract showed immunostimulatory activity (Bauer *et al.* 1989b).

A high molecular weight fraction (M_r >10,000 D) containing polysaccharides and glycoproteins from narrow-leaved coneflower root enhanced the proliferation of mouse spleen cells; stimulated the production of cytokines such as interferon (IFN$\alpha/\beta/\gamma$) in spleen cell cultures, and IL-1, 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 NO production of macrophages (Beuscher *et al.* 1995).

Chemical investigation of the roots of *Echinacea angustifolia*, *Echinacea purpurea*, and *Echinacea pallida* yielded two new alkamides, identified by analysis of spectroscopic data and comparison with reported alkamides. The new compounds were dodeca-2*Z*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide from *Echinacea angustifolia* and dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide from *Echinacea purpurea* and *Echinacea pallida.* These two components, as well as previously identified alkamides, exerted inhibition on 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 are making their own *Echinacea* extracts at home and storing them at refrigerator (4°C) temperatures. The 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 (*Echinacea angustifolia*, *Echinacea pallida*, *Echinacea purpurea*, *Echinacea sanguinea*, *Echinacea tennesseensis*) were prepared. Four *in vitro* immune assays (monocyte secretion of TNF-α, IL-10, and IL-12; and peripheral blood mononuclear cell proliferation) using human blood were used to test extract efficacy at Day 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 *Echinacea angustifolia* extracts were the most efficacious in the assays; extracts from *Echinacea 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 (PBMC) were collected from subjects 6 months post-vaccination and stimulated *in vitro* with the two Type A influenza viruses contained in the trivalent 2004-2005 vaccine with a 50% alcohol tincture prepared from the roots of one of seven *Echinacea* species: *Echinacea angustifolia*, *Echinacea pallida*, *Echinacea paradoxa*, *Echinacea purpurea*, *Echinacea sanguinea*, *Echinacea simulata*, and *Echinacea 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 (*Echinacea angustifolia*, *Echinacea purpurea*, *Echinacea simulata*, *Echinacea tennesseensis*) augmented IL-10 production, diminished IL-2 production, and had no effect on IFN-γ production. *Echinacea pallida* suppressed production of all cytokines; *Echinacea paradoxa* and *Echinacea 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 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).

The immunomodulatory properties of *Echinacea* tinctures from seven species were investigated after being stored at -20°C for 2 years. Two experimental techniques were employed using human PBMC. 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 immunised 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 *Echinacea angustifolia*, *Echinacea pallida*, *Echinacea paradoxa*, and *Echinacea tennesseensis* stimulated proliferation and tended to increase IL-10, (2) *Echinacea sanguinea* and *Echinacea simulata* stimulated only proliferation, (3) *Echinacea purpurea* stimulated only IL-10, and (4) none of the extracts influenced IL-12 or TNF-α. In the second experiments, (1) tinctures from *Echinacea pallida*, *Echinacea paradoxa*, *Echinacea sanguinea*, and *Echinacea 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 ability of *Echinacea* and its components to alter the immune response was examined *in vitro* in a macrophage cell line under either basal or immunostimulated conditions. Potential immunostimulatory and inflammatory activity was determined using a nuclear transcription factor (NFκB) expression, TNF-α and nitric oxide (NO) production as biomarkers. In the absence of alternate stimulation, the only significant effects seen were a decrease in NFκB expression by a 2-ene alkylamide ((2*E*)-*N*-isobutylundeca-2-ene-8,10-diynamide (1)) and a decrease in TNF-α levels by cichoric acid and an Echinacea alkylamide fraction (EPL AA). When the cells were stimulated by lipopolysaccharide (LPS), inhibition of the increased NFκB expression levels was caused by cichoric acid, an *Echinacea* preparation , EPL AA and a 2,4-diene ((2*E*,4*E*,8*Z*,10*Z*)-N-isobutyldodeca-2,4,8,10-tetraenamide (2)). Increases in TNF-α levels were inhibited by cichoric acid, *Echinacea* preparation and EPL AA but enhanced by 1 in the presence of LPS, while only EPL AA was able to inhibit the stimulated increases in NO. When using phorbol myristate acetate to stimulate the cells, NFκB and NO levels were unaffected by *Echinacea* or its components while only cichoric acid and 2 inhibited TNF-α levels. Although cichoric acid was found to have an effect, it is probably not an important contributor to the *Echinacea* modulation of the immune response *in vivo*, as it is not bioavailable. *Echinacea* appears to attenuate the response of macrophages to an immune stimulus and its combination of phytochemicals exhibit different pharmacological properties to one or more of the isolated major individual components (Matthias *et al*. 2007a).

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 60% ethanol) 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).

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 here for the first time, 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 Unweighted Pair Group Method with Arithmetic Mean and neighbour-joining methods. In the immune experiments conducted for this investigation, *Echinacea angustifolia*, *Echinacea paradoxa*, *Echinacea purpurea*, *Echinacea simulata*, and *Echinacea 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 (*Echinacea angustifolia* + *Echinacea simulata* and *Echinacea pallida* + *Echinaceasanguinea*) where both species cluster tightly and have similar immune-response profiles. These two species-pairs are maximally dissimilar from each other. The remaining species (*Echinacea paradoxa*, *Echinacea purpurea*, and *Echinacea tennesseensis*) occupy intermediate positions in the dendrogram. The results suggest that *Echinacea* spp. act heterogeneously on immune function (Senchina *et al.* 2008).

***In vivo* experiments**

A 2-fold increase in phagocytosis was demonstrated in the carbon clearance test in mice after oral administration of 10 ml/kg of a solution containing circa 5 mg of an ethanolic extract of narrow-leaved 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, only the lipophilic fraction proved to be active (Bauer *et al.* 1988a, Bauer *et al.* 1989b).

The activity of phagocytosis was tested in the *in vitro* granulocyte test and the *in vivo* carbon-clearance-test in the mouse for an extract combination consisting of four plant extracts (*Echinacea angustifolia*, *Eupatorium perfoliatum*, *Baptisia tinctoria* and *Arnica montana*). In both immune models, a step by step stimulation of the activity of phagocytosis by the addition of the four plant extracts was shown with an increase in effectiveness of partially over 50% in comparison to the pure *Echinacea angustifolia* mono-extract. The extract combination showed also in both test models a higher efficiency than two other differently composed combination preparations and two *Echinacea* mono-preparations (Wagner *et al.* 1991).

A number of immunomodulatory effects have been attributed to the medicinal plants *Echinacea angustifolia* and Goldenseal (*Hydrastis canadensis*); however, little is known about whether treatment with these plants can enhance antigen-specific immunity. The antigen-specific *in vivo* immunomodulatory potential of continuous treatment with *Echinacea* and Goldenseal root extract was investigated over a period of 6 weeks using rats that were injected with the novel antigen keyhole limpet hemocyanin (KLH) and re-exposed to KLH after the initial exposure. Immunoglobulin production was monitored via ELISA continuously over a period of 6 weeks. The *Echinacea*-treated group showed a significant augmentation of their primary and secondary IgG response to the antigen, whereas the Goldenseal-treated group showed an increase in the primary IgM response during the first 2 weeks of treatment. These results suggest that medicinal plants like *Echinacea* or Goldenseal may enhance immune function by increasing antigen-specific immunoglobulin production (Rehman *et al.* 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 natural killer cell activity or antibody formation was found in studies involving rats fed various preparations of *Echinacea*, including an alcoholic extract of *Echinacea purpurea* root and an alcoholic extract of the roots of *Echinacea angustifolia* and *Echinacea pallida* in their diet (South *et al.* 2001).

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 nitric oxide

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 they 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 standardised hydroethanolic extract was obtained from *Echinacea angustifolia* roots containing echinacoside (>4%), the high molecular weight polysaccharide IDN 5405 (>5%) and an isobutylamide fraction (<0.1%). For *in vitro* tests, a bacterial LPS-free extract has been prepared in order to avoid non-specific responses of immunocompetent cells. The LPS-free extract enhanced the immune functions as highlighted by the proliferation rate and IFN-γ production in murine T-lymphocyte cell cultures stimulated by anti-CD3. The LPS-free extract did not have a direct role on macrophage response as measured in the nitric oxide production test using the J774 macrophage cells line. *In vivo*, the extract showed an immune stimulating activity by reducing the *Candida albicans* induced mortality both in normal and in cyclosporin A-treated mice (Morazzoni *et al.* 2005).

Antimicrobial activity

Extracts of narrow-leaved 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 *Echinacea angustifolia* showed antifungal activity against most of the pathogenic fungi (Merali *et al.* 2003).

Antiviral activity

A high molecular weight fraction (M_r >10,000 D) containing polysaccharides and glycoproteins from narrow-leaved coneflower root exhibited antiviral activity against herpes simplex virus and influenza virus (Beuscher *et al.* 1995).

A decoction and a 30% ethanolic extract of narrow-leaved coneflower root inhibited the propagation of $ECHO_9$ Hill virus in monkey kidney cell cultures (Skwarek *et al.* 1996).

Extracts of 8 taxa of the genus *Echinacea* (including *Echinacea angustifolia*) were found to have antiviral activity against *Herpes simplex* (HSV) virus 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 *Echinacea pallida var. sanguinea* crude (70% ethanol) inflorescence extract (MIC = 0.026 mg/ml), cichoric acid (MIC = 0.045 mg/ml) and *Echinacea purpurea* n-hexane root extract (MIC = 0.12 mg/ml) (Binns *et al.* 2002).

Anti-inflammatory activity

An aqueous extract from *Echinacea angustifolia* root dose-dependently inhibited oedema in the croton oil ear test in mice., both at the maximum (6 hours) and in the decreasing phase (18 hours), with a potency greater than that of benzydamine (Tragni *et al.* 1985); the topical anti-inflammatory activity was attributed to high molecular weight polysaccharides (Tragni *et al.* 1988).

Polyunsaturated isobutylamides have been shown to exert anti-inflammatory activity in the 5-lipoxygenase (5-LOX) assay (Wagner *et al.* 1989, Müller-Jakic *et al*. 1994). The *n*-hexane extract of *Echinacea angustifolia* inhibited cyclooxygenase (62.4% inhibition at 50 μg/ml) and 5-lipoxygenase (81.8% inhibition at 11.5 μg/ml) (Wagner *et al.* 1989).

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

The 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 synthesised alkamides. The results indicated that ethanol extracts diluted in media to a concentration of 15 μg/ml from *Echinacea angustifolia*, *Echinacea pallida*, *Echinacea simulata*, and *Echinacea sanguinea* significantly inhibited PGE(2) production. In further studies, PGE(2) production was significantly reduced by all synthesised 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 PGE(2) production in either the *Echinacea* extracts or synthesised alkamides. High-performance liquid chromatography 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 PGE(2), it is likely that alkamides may contribute toward the anti-inflammatory activity of *Echinacea* in a synergistic or additive manner (LaLone *et al.* 2007).

During past years inhibition of the cyclooxygenase-2 (COX-2) enzyme has been proven as an effective strategy to suppress pain and inflammation. Based on this and other mechanistic findings, interest has also renewed in the molecular pathways underlying the anti-inflammatory effects of herbal drugs. The impact of several polyunsaturated alkamides isolated from a CO_2 extract of the roots of *Echinacea angustifolia* DC. on both activity and expression of COX-2 was investigated. A 48-hour treatment of H4 human neuroglioma cells with the CO_2 extract led to a significant suppression of prostaglandin PGE(2) formation. Analysis of eight different alkamides revealed a contribution of undeca-2*Z*-ene-8,10-diynoic acid isobutylamide (A5), dodeca-2*E*-ene-8,10-diynoic acid isobutylamide (A7), and dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (A8) to this response. Using an established short-term COX-2 activity assay, all three alkamides were shown to interfere with COX-2 activity. In contrast, none of the COX-2-suppressing not any other tested alkamide was found to inhibit COX-2 mRNA and protein expression. Instead, increased COX-2 mRNA and protein levels were registered in the presence of the CO_2 extract and most of the analysed alkamides which caused, however, no stimulation of PG formation. Overall, results suggest that certain alkamides derived from *Echinacea angustifolia* roots may contribute to the pharmacological action of the herbal extract by inhibiting COX-2-dependent PGE2 formation at sites of inflammation (Hinz *et al.* 2007).

Alcohol extracts from three widely used *Echinacea* species, *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea 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 immunised with sheep red blood cells 4 days prior to collection of immune cells for multiple immunological assays. The three herb 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 natural killer cell cytotoxicity. Antibody response to sheep red blood cells was significantly increased equally by extracts of all three *Echinacea* species. Concanavalin A-stimulated splenocytes from *Echinacea angustifolia*- and *Echinacea 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 tumour necrosis factor-gamma and IL-1β. Only *Echinacea angustifolia*- and *Echinacea 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, *Echinacea angustifolia* or *Echinacea 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 *Echinacea angustifolia*, *Echinacea pallida* and *Echinacea purpurea* on the production of these inflammatory mediators in both LPS-stimulated RAW 264.7 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*. *Echinacea pallida* and *Echinacea purpurea in vitro* inhibited NO production and TNF-α release in a dose-dependent manner. RAW 264.7 cells treated with *Echinacea angustifolia* or *Echinacea purpurea* showed decreased killing over 24 h, although *Echinacea angustifolia* enhanced bacterial phagocytosis. Upon bacterial infection, RAW 264.7 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 *Echinacea angustifolia* and *Echinacea purpurea* 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 2007b).

Antioxidant activity

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

Alcoholic extracts of the roots and leaves of three *Echinacea* species (*Echinacea purpurea*, *Echinacea angustifolia* and *Echinacea 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 *Echinacea 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 2,2-diphenyl-1-picrylhydrazyl (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 (EC50 = 6.6 μM), while caftaric acid had the lowest (EC50 = 20.5 μM). The average EC50 values for *Echinacea purpurea*, *Echinacea pallida* and *Echinacea 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).

Protective effect of echinacoside against free radicals was demonstrated in a study using oleic acid-induced acute lung injury in rats. The signals of free radicals in ECH-treated ALI rats were much lower than that of ECH-untreated at all time points. Echinacoside can clean out unstable free radicals, chelate the transition metal ions such as Fe, Cu and Zn to reduce the lipid peroxidation and stop the inflammatory cascade (Zhang *et al.* 2007).

Other activities

Serial dilutions of 21 commercial ethanolic herbal extracts and tinctures, and 13 related pure plant compounds have been analysed 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 strenght. 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).

Echinacea plant preparations are widely used in the prevention and treatment of common cold. However, so far no molecular mechanism of action has been proposed. The standardised tincture EchinaforceTM was analysed and it was 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-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (1/2), trienoic (3) and dienoic acid (4) 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 signaling, as well as NF-jB and ATF-2/CREB-1 activation. 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).

It was shown that the alkylamides dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (A1) and dodeca-2*E*,4*E*-dienoic acid isobutylamide (A2) bind to the CB2 receptor more strongly than the endogenous cannabinoids. The K_i values of A1 and A2 (CB_2~60 nM; CB_1>1500 nM) were determined by displacement of the synthetic high affinity cannabinoid ligand [^{3}H]CP-55,940. Molecular modelling suggests that alkylamides bind in the solvent-accessible cavity in CB_2, 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 CB_2 and CB_1. A1 and A2 elevated total intracellular Ca^{2+} in CB_2-positive but not in CB_2-negative promyelocytic HL60 cells, an effect that was inhibited by the CB_2 antagonist SR144528. At 50 nM A1, A2, and the endogenous cannabinoid anandamide (CB_2 K_i >200 nM) up-regulated constitutive IL IL-6 expression in human whole blood in a seemingly CB_2-dependent manner. A1, A2, anandamide, the CB_2 antagonist SR144528 (K_i <10 nM), and also the non-CB_2-binding alkylamide undeca-2*E*-ene-8,10-diynoic acid isobutylamide all significantly inhibited LPS-induced TNF-α, IL-1β, and IL-12p70 expression (5–500 nM) in a CB_2-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 CB_2 binding (Raduner *et al.* 2006, Woelkart *et al.* 2005a, Woelkart *et al.* 2007).

The 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 by Chicca was to investigate potential *in vitro* cytotoxic and pro-apoptotic properties of hexanic root extracts of the three medicinal *Echinacea* (*Asteraceae*) species (*Echinacea pallida* (Nutt.) Nutt., *Echinacea angustifolia* DC. *var. angustifolia*, *Echinacea purpurea* (L.) Moench.) on the human pancreatic cancer MIA PaCa-2 and colon cancer COLO320 cell lines. 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).

Recent studies showed that *Echinacea angustifolia* compounds and preparations interfere with doxorubicin chemotherapy. An evaluation of *Echinacea* interference with other chemotherapy agents is needed. Several *Echinacea* preparations, compounds and fractions of total extract (*n*-butanol, butyl

acetate, ethyl acetate, dichloromethane (acidic and neutral), hexane, chicoric acid, and cynarine) from the *Echinacea angustifolia* plant were tested on cervical cancer (HeLa) and breast cancer (MCF-7) cell lines for anti-hyaluronidase activity and the interference of 5-fluorouracil. Other anti-cancer drugs such as melphalan, vinblastine, and vincristine were tested on these cancer cell lines. Data indicated that the *Echinacea* preparations, compounds and extracts showed a significant ability to inhibit the activity of hyaluronidase and reduce the overall effect of chemotherapeutic agents in this study (Jensen *et al.* 2006, Jensen *et al.* 2007).

The *n*-hexane root extracts from *Echinacea pallida*, *Echinacea angustifolia* and *Echinacea purpurea* were evaluated for inhibition of the multidrug transporter P-glycoprotein (Pgp) activity, the product of the ABCB1 gene, involved in cancer multidrug resistance 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 *Echinacea 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 *Echinacea pallida* roots by a bioassay-guided fractionation, were found to be able to reduce Pgp activity. Pentadeca-(8*Z*,13*Z*)-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).

A constituent of the root oil of *Echinacea angustifolia* DC. and *Echinacea pallida* (Nutt.) Britt., inhibitory to Walker carcinosarcoma 256 and P-388 lymphocytic leukemia, was isolated and identified as (*Z*)-l,8-pentadecadiene. This compound occurs in these oils to the extent of approximately 44% and appears to be the first diene olefin reported to show *in vivo* antitumour activity. The corresponding *trans* isomer is less active (Voaden *et al.* 1972).

The biological effect of *Echinacea augustifolia* extract on cell viability and cell differentiation was demonstrated in mammary epithelial cell lines. These effects have been observed in two different cell lines derived from mouse (HC11) and bovine (BME-UV). *Echinacea* extract enhanced cell liability from 100 to 1000 ng/ml in association with growth factors, epidermal growth factor (EGF) or insulin, but also without EGF ($p<0.05$) up to 37% vs. control. This effect may be modulated by MAPK and Akt activation that *Echinacea* extract treatment increased and/or by a reduction of caspase 3 activity, showed a dose-response decrease after *Echinacea* treatment. Finally *Echinacea* extract was able to increase ($p<0.05$) at 100 ng/ml beta-casein expression in association with PRL (5 μg/ml). These data demonstrate that *Echinacea angustifolia* extract can stimulate mammary epithelial cell physiology and may be considered a candidate to support mammary gland activity during a mammogenetic and lactogenetic state (Starvaggi Cucuzza *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).

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-2*E*,4*E*,8*Z*,10*E*/*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 4°C showed only a slight decrease in transport, which is a strong hint that dodeca-2*E*,4*E*,8*Z*,10*E*/*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 *Echinacea angustifolia* root (200 mg/ml) and *Echinacea 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 (CYP 450) 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 (2*E*)-*N*-isobutylundeca-2-ene-8,10-diynamide (1) metabolised to only a tenth the extent of (2*E*,4*E*,8*Z*,10*Z*)-*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).

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 (Barret *et al.* 2003).

Pyrrolizidine alkaloids tussilagine and isotussilagine have a 1,2-saturated necine scaffold and therefore exhibit no hepatotoxic activity (Liersch *et al.* 1993).

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 were reviewed to regional California Poison Control System, San Francisco division (CPCS-SF) in 1998 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. The authors concluded that 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).

3.4. Overall conclusions on non-clinical data

Pharmacology:

For the extracts, fractions and isolated compounds of narrow-leaved 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, cannabinoidomimetic (alkylamides), cell physiology stimulative (extract) and antitumour effects (*Z*)-1,8-pentadecadiene) 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.

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 P450-mediated metabolism of other concurrently ingested pharmaceuticals.

Toxicology:

In general, the toxicity of *Echinacea angustifolia* is low. The data on narrow-leaved coneflower root toxicity are limited and findings are sometimes difficult to interpret since there is a lack of detail regarding the preparation of *Echinacea angustifolia*. Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed.

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 randomised 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 tested intravenous homeopathic complex preparations containing *Echinacea angustifolia* D1 (study 1) and D4 (study 5). Two studies (2 and 3a) tested oral alcoholic extracts of roots of *Echinacea purpurea*,

one study an extract of *Echinacea pallida* roots (study 3b), and one study an extract of *Echinacea 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 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 studies provide evidence for immunomodulatory activity of the homeopathic combination tested in study 1 and the *Echinacea purpureae* radix extract tested in study 2. The negative results of the other three 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. The authors concluded that future studies should be performed on patients rather than healthy volunteers and use standardised or chemically defined monopreparations of *Echinacea* (Melchart *et al.* 1995).

The effect of *Echinacea* Premium tablets containing 675 mg of *Echinacea purpurea* root extract and 600 mg of *Echinacea 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 was tested. 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).

Assessor's overall conclusions on pharmacodynamics

Increased phagocytosis was observed in one study on narrow-leaved coneflower root extract as a single ingredient, unfortunately a detailed composition of extracts is not available. In another study a combination herbal product was investigated and it is difficult to interpret which plant was responsible for the effect (leucocyte hsp70 expression, increased white cell counts, preventative effect against free radical induced erythrocyte haemolysis, antioxidant effect).

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 ethanolic liquid extracts of *Echinacea angustifolia* and *Echinacea 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 minutes 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. This single dose study provides (Matthias *et al.* 2005b).

Many studies have been done over the years to assess the effectiveness of *Echinacea* as an immunomodulator. The potential bioavailability of alkyl- amides and caffeic acid conjugates was assessed using Caco-2 monolayers and compared to their actual bioavailability in a Phase I clinical trial. The caffeic acid conjugates permeated poorly through the Caco-2 monolayers. Alkylamides were found to diffuse rapidly through Caco-2 monolayers. Differences in diffusion rates for each alkylamide correlated to structural variations, with saturation and *N*-terminal methylation contributing to decreases in diffusion rates. Alkylamide diffusion is not affected by the presence of other constituents and the results for a synthetic alkylamide were in line with those for alkylamides found in an ethanolic *Echinacea* preparation. The plasma from healthy volunteers was examined for 12 hours after ingestion of *Echinacea* tablets manufactured from an ethanolic liquid extract. Caffeic acid conjugates could not be identified in any plasma sample at any time after tablet ingestion. Alkylamides were detected in plasma 20 minutes after tablet ingestion and for each alkylamide, pharmacokinetic profiles were devised. The data are consistent with the dosing regimen of one tablet three times daily and supports their usage as the primary markers for quality *Echinacea* preparations (Matthias *et al.* 2005c).

Six alkamides have been isolated from the roots of *Echinacea angustifolia* as major lipophilic constituents and have been investigated regarding their pharmacokinetics. A sensitive and specific method has been developed for the identification and quantification of these alkamides in human plasma using liquid chromatography electrospray ionization ion-trap mass spectrometry. The method was applied to analyse plasma samples obtained from a randomised, open, single-dose, crossover study after oral administration of a 60% ethanolic extract from the roots of *Echinacea angustifolia* to 11 healthy subjects. The maximum concentration of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides, the main alkamides in the roots of *Echinacea angustifolia*, appeared already after 30 minutes and was 10.88 ng/ml for the 2.5-ml dose (Woelkart *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 *Echinacea purpurea* root (300 mg/ml) and *Echinacea angustifolia* root (200 mg/ml) extracted in 60% ethanol. The tablet preparation investigated was also a mixture of *Echinacea purpurea* root (675 mg/tablet) and *Echinacea 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 $T_{1/2}$, $AUC_{t\text{-}lin}$ and C_{max} in the two different preparations were found. T_{max} increased from 20 minutes for the liquid to 30 minutes 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.* 2007b).

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 one dose three times 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 effects of *Echinacea* herbal product in clinical trials (Barnes *et al.* 2005, Barnes *et al.* 2007, Barret 2003, Basch *et al.* 2005, Bradley 2006, Islam *et al.* 2005, Liersch *et al.* 1993, Linde *et al.* 2006, Melchart *et al.* 1994 & 2004, Melchart & Linde 1999, Schoop *et al.* 2006, Shah *et al.* 2007).

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

***Echinacea angustifolia* 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, 30% ethanol) of purple coneflower root (Group A, n = 99) or *Echinacea 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).

The effect of chemically defined extracts from *Echinacea angustifolia* roots on rhinovirus infection was evaluated. Three preparations of *Echinacea*, with distinct phytochemical profiles, were produced by extraction from *Echinacea angustifolia* roots with supercritical carbon dioxide, 60% ethanol, or 20% ethanol. A total of 437 volunteers were randomly assigned to receive either prophylaxis (beginning seven days before the virus challenge) or treatment (beginning at the time of the challenge) either with one of these preparations or with placebo. The treatments were given three times each day as a 1.5 ml tincture containing the equivalent of 300 mg of *Echinacea* root. The results for 399 volunteers who were challenged with rhinovirus type 39 and observed in a sequestered setting for five days were included in the data analysis. There were no statistically significant effects of the three *Echinacea* extracts on rates of infection or severity of symptoms. Similarly, there were no significant effects of treatment on the volume of nasal secretions, on polymorph nuclear leukocyte or IL-8 concentrations in nasal-lavage specimens, or on the quantitative-virus titer. The results of this study indicate that extracts of *Echinacea angustifolia* root, either alone or in combination, do not have clinically significant effects on infection with a rhinovirus or on the clinical illness that results from it (Turner *et al.* 2005).

There were two responses to this study:

The trial by Turner *et al.* (July 28 issue) could have benefited from the inclusion of additional treatment groups with higher daily doses of *Echinacea*. The dose equivalent of 900 mg of dried root derives from the German Commission E monograph for *Echinacea pallida* root, a different species from *Echinacea*

angustifolia. *Echinacea angustifolia* root is not officially approved and thus has no recommended dosage in Germany, owing to a lack of sufficient supporting data. The 1999 World Health Organization monograph on *Echinacea angustifolia* root cites a 3 g daily dose, which is 330% of the dose used in this trial. The 3 g daily dose is also recommended by the Canadian Natural Health Products Directorate. Outcomes of rigorously designed clinical trials usually pertain only to the specific variables of such trials. A higher dosage, approximating the levels used by many consumers and recommended by many alternative health care providers, might have made this trial more relevant to the real-world use and potential benefits of *Echinacea* (Blumenthal 2005).

Turner *et al.* recently reported the results of a trial that evaluated the efficacy of *Echinacea angustifolia* in the prevention and treatment of experimental rhinovirus type 39 infection. Although the trial was well designed and adequately powered, there are several caveats that need to be highlighted. First, there was no evidence that the herb used was *Echinacea angustifolia*, given that product identification and validation tests, such as chromatography, were not performed. Second, there was some confusion as to whether the product was an extract or a tincture and, therefore, some uncertainty about the strength of the formulation. Hence, it is difficult to ascertain whether the administered dose of *Echinacea* was adequate. Finally, the authors inappropriately conclude that *Echinacea* is ineffective in the treatment of rhinovirus infection, with only 1 of more than 100 subtypes of rhinovirus having been tested. Thus, any claim that *Echinacea* is ineffective in the prophylaxis and treatment of the common cold cannot be validated without further tests to determine the efficacy of the extract on other viral agents (Leach 2005).

Echinacea angustifolia in combination with other herbal drugs

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 University of Wisconsin, on 148 students with common colds of recent onset was assessed. Each active capsule contained a dried mixture of *Echinacea angustifolia* root (50% [123 mg]), *Echinacea purpurea* root (25% [62 mg]), and *Echinacea 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 four capsules six times during the first 24 hours of the study, and four capsules three 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 two 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 (Barret *et al.* 2002).

The immunomodulating effects of two *Echinacea* species, *Echinacea purpurea* and *Echinacea angustifolia* and larch arabinogalactan extracted from *Larix occidentalis* were examined in a randomised, double-blind, placebo-controlled, prospective four-week clinical trial at a naturopathic medical school research centre. Forty-eight healthy female volunteers (22-51 years) were randomly assigned to one of six groups: extract of *Echinacea purpurea* ; ultra-refined *Echinacea purpurea*/*Echinacea angustifolia* ; *Echinacea purpurea*/*Echinacea angustifolia* (EPA); *Echinacea purpurea*/*Echinacea angustifolia* plus larch arabinogalactan (EPALA); larch arabinogalactan ; or

placebo. Immunological tests with enumerative measurements, stool cultures for *Lactobacillus acidophilus* and yeast, and health-related quality of life using the Medical Outcomes Study derived SF-36 self-administered questionnaire were assessed at baseline and at four weeks. Complement properdin increased by 21% in the EPA group ($p<0.05$) and by 18% in the EPALA group ($p<0.05$), compared to the placebo group ($p>0.05$). SF-36 showed improvements in overall physical health, vitality, and emotional health in the same two groups (EPA and EPALA). Volunteers in the EPA and EPALA groups had increased production of complement properdin after four weeks of intervention. The increased complement properdin may be an indication of one aspect of immune system stimulation in patients treated with either *Echinacea purpurea*/*Echinacea angustifolia* or *Echinacea purpurea*/*Echinacea angustifolia* plus larch arabinogalactan.

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

The effectiveness and safety of a preparation containing *Echinacea*, propolis, and vitamin C in the prevention of respiratory tract infections in children during a 12-week winter period was evaluated in a randomised, double-blind, placebo-controlled study. Four hundred thirty children, aged 1 to 5 years, were randomised to an herbal extract preparation (n = 215) or a placebo elixir (n = 215). A herbal preparation containing 50 mg/ml of *Echinacea*, 50 mg/ml of propolis, and 10 mg/ml of vitamin C, or placebo (5.0 ml and 7.5 ml twice daily for ages 1 to 3 years and 4 to 5 years, respectively were administrated) for 12 weeks. Significant mean +/- SD reductions of illnesses were seen in the verum group in the number of illness episodes, 138 vs 308 (55% reduction); number of episodes per child, 0.9 +/- 1.1 vs 1.8 +/- 1.3 (50% reduction, P<.001); and number of days with fever per child, 2.1 +/- 2.9 vs 5.4 +/- 4.4) (62% reduction, P<.001). The total number of illness days and duration of individual episodes were also significantly lower in the verum group. Adverse drug reactions were rare, mild, and transient. A preventive effect of a preparation containing *Echinacea*, propolis, and vitamin C on the incidence of respiratory tract infections was observed (Cohen *et al.* 2004).

4.3. Overall conclusions on clinical pharmacology and efficacy

Narrow-leaved 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 rhinovirus infection in another randomised, double-blind, placebo-controlled study.

In a randomised, double-blind, placebo-controlled trial, *Echinacea angustifolia* root in combination with *Echinacea purpurea* root and herb did not prove to be successful in early treatment of the common cold. On the other hand it showed improvements in overall physical health, vitality, and emotional health when combined with *Echinacea purpurea* and larch arabinogalactan in another randomised, double-blind, placebo-controlled, prospective clinical trial.

Echinacea angustifolia in combination with propolis and vitamin C was reported to be successful in reducing severity and duration of illness in children in a randomised, double-blind, placebo-controlled study; however it is difficult to say which component is responsible for the effect or if there was a synergistic action.

5. Clinical Safety/Pharmacovigilance

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

See sections 4.1, 4.2 and 4.3.

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).

In a clinical study, 18 out 100 subjects who took two times 50 drops daily (2 times 1 ml) of a hydroethanolic extract of narrow-leaved coneflower root 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 (Liersch *et al.* 1993). 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, Liersch *et al.* 1993).

Reports to poison control centers (PCCs) were characterised involving two widely used herbal dietary supplements (HDSs), *Echinacea*, and St. John's wort. METHODS: Data were purchased from the American Association of Poison Control Center's toxic exposure surveillance system (TESS(R)) on reports made to PCCs in 2001 involving *Echinacea* or St. John's wort. Analyses were limited to those cases in which *Echinacea* or St. John's wort 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 St. John's wort. 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 St. John's wort cases and 3% of *Echinacea* cases, with 13% of St. John's wort exposures reported as 'suspected suicidal'. TESS represents a potentially important means of assessing and characterising 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 (AEs) that could be systematically studied with other, established pharmacoepidemiological study designs (Gryzlak *et al.* 2007).

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 not recommended in progressive systemic diseases such as: tuberculosis, leucoses, collagenoses, multiple sclerosis, AIDS, HIV infections, and other autoimmune diseases (Barnes *et al.* 2005, Barnes *et al.* 2007, Liersch *et al.* 1993).

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).

Drug interactions

A publication by Meijerman *et al.* (2006) summarises as follows: 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 antitumor 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).

A publication by Freeman *et al.* (2008) summarises as follows: Review assessed the occurrence of drug interactions with one of the top selling botanical remedies, *Echinacea* including *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea*. Only eight papers containing primary data relating to drug interactions were identified. Herbal remedies made from *Echinacea angustifolia* appear to have a low potential to generate cytochrome P450 (CYP 450) 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. 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, *Echinacea purpurea* products (roots and/or aerial parts) do not appear to be a risk to consumers (Freeman *et al.* 2008).

Use in pregnancy and lactation

A review on safety of *Echinacea* during pregnancy and lactation was published (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. Caution with using *Echinacea* during lactation is 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*. 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 *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 end points 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 angustifolia* 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 narrow-leaved coneflower preparations in children; therefore the use of herbal drug and preparations is not recommended.

Echinacea angustifolia should not be used in progressive systemic diseases such as: tuberculosis, leucoses, collagenoses, multiple sclerosis, AIDS, HIV infections, and other autoimmune diseases.

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

Herbal remedies made from *Echinacea angustifolia* appear to have a low potential to generate cytochrome P450 (CYP 450) 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.

6. Overall conclusions

The pharmacological effects of *Echinacea angustifolia* root preparations on immune system of adults were not proved so far; the only evidence for immunomodulatory action is based on *in vitro* and *in vivo* animal experiments. 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; *Echinacea angustifolia* was only tested in combination with propolis and vitamin C. The data about the toxicity of narrow-leaved coneflower root preparations are limited although it has been used for decades.

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

Traditional use of *Echinacea angustifolia* root in this indication (for supportive treatment of common cold) is possible regarding bibliographic evidence. Although toxicological data are limited a certain level of safety could be expected due to the long-time use of *Echinacea angustifolia* root preparations with no serious side effects reported.

The pharmaceutical forms which are proposed in the monograph (comminuted and powdered herbal substance) do not fulfil the criterion of 30 years for traditional use within the Community. However; they are documented in medicinal use for over 15 years in the Community and there is bibliographical evidence on their traditional use (over 30 years) in the USA. Therefore the following herbal preparations are suggested for acceptance for traditional use: a) comminuted herbal substance for preparation of herbal tea and b) powdered herbal substance in solid dosage form for oral use.

Annex

List of references