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Committee on Herbal Medicinal Products (HMPC)

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

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Echinacea purpurea</i> (L.) Moench, radix
Herbal preparation(s)	Dry extract (DER 5.5-7.5:1), extraction solvent ethanol 45% (V/V) Dry extract (DER 4:1); extraction solvent water
Pharmaceutical form(s)	Herbal preparation in solid dosage forms for oral and oromucosal use
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Introduction

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

- Herbal substance(s)

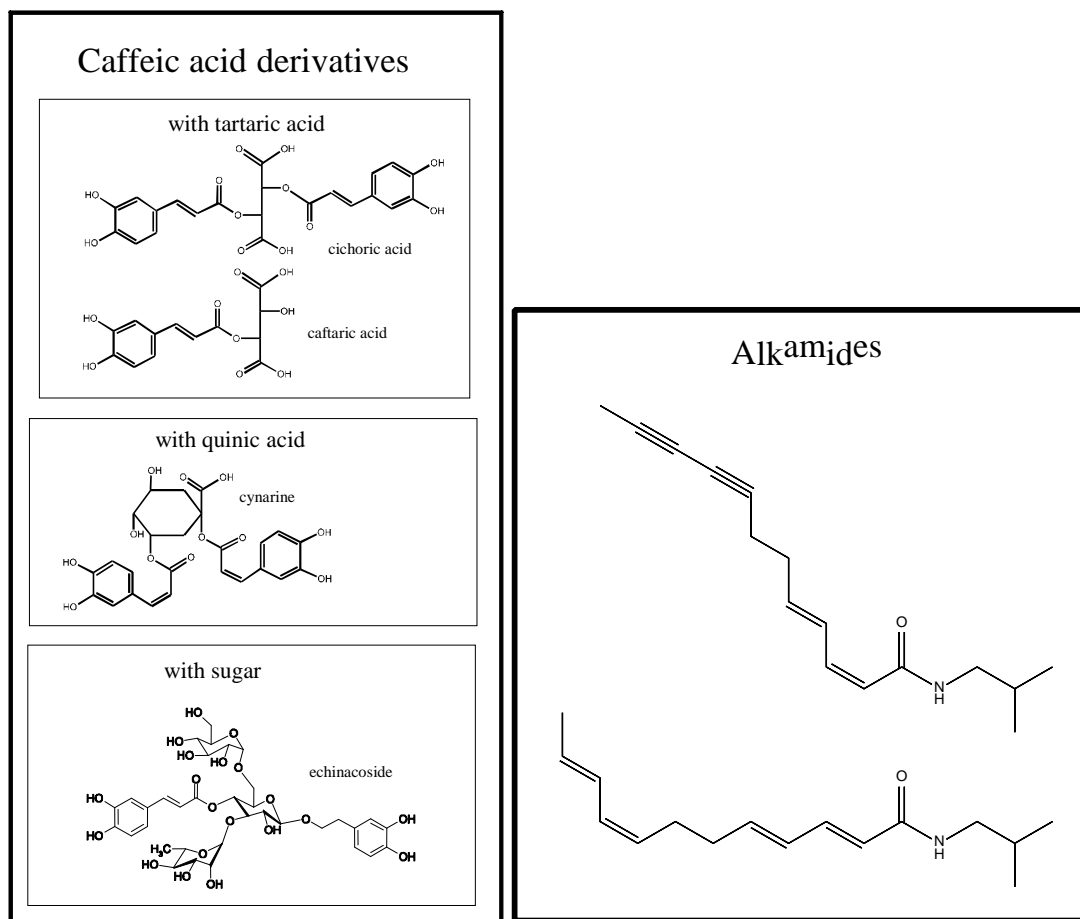
Echinaceae purpureae radix (European Pharmacopoeia monograph reference 01/2008: 1824)

Echinaceae purpureae radix consists of the whole or cut, dried underground parts of *Echinacea purpurea* (L.) Moench. It contains not less than 0.5% for the sum of caftaric acid (C₁₃H₁₂O₉; M_r 312.2) and cichoric acid (C₂₂H₁₈O₁₂; M_r 474.3) in dried drug.

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

- Alkamides (0.01-0.7%): mainly isobutylamides of straight-chain fatty-acids with olefinic and/or acetylenic bonds e.g. isomeric dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic isobutylamide. Undeca-2*Z*,4*E*-diene-8,10-dienoic acid isobutylamide is also prominent. Isobutylamides contain mainly 2,4-dienoic units.
- Caffeic acid derivatives (2.0-2.8%): principally cichoric acid (2,3-*O*-dicaffeoyltartaric acid, 1.7-2.4%) and caftaric acid (2-*O*-caffeoyltartaric acid, ca. 0.2-0.8%) also echinacoside, verbascoside, caffeylechinacoside, chlorogenic and isochlorogenic acids.
- Polysaccharides and glycoproteins: arabinogalactans, and an arabinogalactan-containing glycoprotein with a sugar component consisting of arabinose (64-84%), galactose (2-5%) and galactosamine (6%).
- Volatile oil (0.1%): caryophyllene, caryophyllene oxide, humulene, α -phellandrene, limonene, camphene, aldehydes and dimethyl sulphide.
- Other constituents: small amounts of polyacetylenic compounds polyynes (0.01 mg/100 g including trideca-1-en-3,5,7,9,11-pentaine, trideca-1,11-dien-3,5,7,9,-tetraene, trideca-8,10,12-triene-2,4,6-triene)
- Non toxic pyrrolizidine alkaloids: tussilagine, isotussilagine.

Baiciunaite *et al.* (2015) evaluated the protein content in dried roots of *Echinacea purpurea* after homogenization of roots with liquid nitrogen, extraction in 0.01 mol /L phosphate-buffered saline (PBS) and purification followed by fractionation of proteins using gel filtration chromatography. Total concentration of proteins was measured using the Bradford method, and evaluation of the molecular mass of proteins was accomplished by applying the SDS-PAGE gel electrophoresis. The Bradford assay revealed that the highest concentration of proteins in fractions collected after gel filtration chromatography was 4.66-6.07 mg/ml.



- Herbal preparation(s)

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

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

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

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

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

Not applicable

1.2. Search and assessment methodology

For the research, the databases of PubMed, ScienceDirect, Cochrane Database of Systematic Reviews and TOXLINE were used.

The research in PubMed contained the keywords "*Echinacea purpurea* radix" (42 results) and "*Echinacea purpurea* root" (53 results). All articles from 12 March 2010 to 05 April 2016 were included and then assessed due to their value for medicinal use of *Echinacea purpurea* radix. Articles with a reference to *Echinacea*'s health-related processes were included, whereas articles without any reference to physiological processes (e.g. the development of a new validation method) were excluded.

For the research in ScienceDirect, the keywords "*Echinacea purpurea* root" were used, including articles from 2010 to 08 April 2016. Therefore, 220 results were recommended and assessed as described above.

The same procedure was followed with EMBASE (3 results, 13 April 2016), BioMed Central (22 results, 13 April 2016) and Micromedex (0 result, 13 April 2016).

Reviews were searched in Cochrane Database of Systematic Reviews, typing in "*Echinacea purpurea* root". One result was obtained (13 April 2016).

For toxicological data, the advanced search in TOXLINE was carried out under the following conditions:

Search term: singular/plural, records with all of the words, all fields, "Add chemical synonyms and CAS numbers to search", "Include PubMed records", maximum records returned: 50 000, year of publication 2010 through 2016, all languages, all TOXLINE components except of PubMed. Three results were found (13 April 2016).

The articles were divided up first into clinical and non-clinical, *in vitro* and *in vivo* and then into more specific data, e. g. *Echinacea*'s immunomodulatory, anti-inflammatory and anti-infective effects (primary pharmacodynamics), its influence on other health-related processes (secondary pharmacodynamics, e.g antioxidant activity), pharmacokinetics, toxicological effects and interactions with other drugs.

Additionally, the research was about "*Echinacea purpurea* root" in WHO monographs (2 results, but before 2010), Health Canada monographs (1 result, "*Echinacea purpurea*") and UKPAR (all 13 April 2016).

Search engines used: Google

Scientific databases: PubMed, ScienceDirect, Embase

Medical databases: Medline, PubMed, ScienceDirect, BioMed Central, Micromedex

Toxicological databases: TOXLINE

Pharmacovigilance resources: UKPAR, WHO, Canadian monograph

Data from EU and non-EU regulatory authorities: UKPAR, WHO, Canadian monograph

Other resources: Library of the Faculty of Pharmacy of Ljubljana

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
IRELAND			
Dry extract from <i>Echinacea purpurea</i> radix (DER 6-7:1); Extraction solvent: Ethanol 30% V/V	A traditional herbal medicinal product used to relieve common cold and flu-like symptoms in adolescents and adults, Exclusively based on long-standing use	127 mg extract/film coated tablets. Adults, elderly and adolescents: 1 tablet, three times a day, if required. The use in children below 12 years of age is not recommended. Do not use the product for more than 10 days.	Traditional use registration; July 2014
Dry extract from <i>Echinacea purpurea</i> root (DER 6-8:1); extraction solvent: ethanol 75% V/V	A traditional herbal medicinal product used to relieve common cold and flu-like symptoms in adults and adolescents Over 12 years, exclusively based upon long-standing use.	140 mg extract/hard capsule Adults, older people and adolescents: Take one capsule 2 times daily. Duration of use: Do not use this product for more than 10 days.	Traditional use registration; January 2014
Dry extract) from <i>Echinacea purpurea</i> root (DER 6-8:1); extraction solvent: ethanol 75% V/V	A traditional herbal medicinal product used to relieve common cold and flu-like symptoms in adults and adolescents Over 12 years	140 mg extract/hard capsule Adults, older people and adolescents: Take one capsule 2 times daily. Duration of use: Do not use this product for more than 10 days.	Traditional use registration; January 2014

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
NETHERLANDS			
Dry extract of <i>ECHINACEA PURPUREA purpurea</i> radix (DER 6-8:1), extraction solvent: ethanol 75% V/V	Traditional medicinal products for supportive treatment of common cold.	Hard capsule Per capsule 140 mg dry extract ≥ 12 years: 1 capsule twice daily Consult a doctor if symptoms increase or remain after 10 days of use	Traditional use registration; 2011
SWEDEN			
Dry extract of <i>Echinacea purpurea</i> (purple coneflower) dried root, (DER 6.1-7:1), extraction solvent: ethanol 45% (V/V)	Traditional herbal medicinal product used for the relief of symptoms of common cold. The product is a traditional herbal medicinal product for use in the specified indication exclusively based upon long-standing use.	Chewable tablet, 1 tablet contains: 40 mg extract Adults and adolescents: 1 chewable tablet every second hour (max 9 chewable tablets per day). Should not be used for longer periods than 10 days.	2009, Traditional use registration (re-classification) Since 1978, on the market as a natural remedy under national legislation.
UNITED KINGDOM			
Dry extract from <i>Echinacea purpurea</i> root (DER 6-8:1); (extraction solvent: ethanol 75% V/V	A traditional herbal medicinal product used to relieve the symptoms of the common cold, and influenza type infections based on traditional use only.	200 mg extract/film coated tablet Adults, elderly, adolescents: 1 tablet twice a day Start at first signs of common cold Do not take for more than 10 days.	UK; 2012; Traditional use registration
Dry extract of <i>Echinacea purpurea</i> root (DER 6-8:1); extraction solvent: ethanol 75% V/V	A traditional herbal medicinal product used to relieve the symptoms of the common cold, and influenza type infections based on traditional use only.	50 mg extract/coated tablet Adults & elderly: 1 - 2 tablets, three times a day. Adolescents: 1 tablet night and morning. Start at first signs of common cold Do not take for more than 10 days.	UK; 2013; Traditional use registration

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
Dry extract from <i>Echinacea purpurea</i> root (DER 6-8:1); extraction solvent: ethanol 75% V/V		105 mg extract/film coated tablet Adults, elderly, adolescents: 1 tablet, twice a day. Start at first signs of common cold Do not take for more than 10 days.	UK; 2011; Traditional use registration
Dry extract from <i>Echinacea purpurea</i> root (DER 6-8:1); Extraction solvent: Ethanol 75% V/V		56 mg extract/film coated tablet Adults, elderly & adolescents: 1 - 2 tablets, twice a day. Start at first signs of common cold Do not take for more than 10 days.	UK; 2011; Traditional use registration
<i>Echinacea purpurea</i> root powder	A traditional herbal medicinal product used to relieve the symptoms of the common cold, and influenza type infections based on traditional use only.	250 mg dry root powder/hard capsule Adults, elderly & adolescents: 1-2 capsules, twice a day Start at first signs of common cold. Do not take for more than 10 days.	UK; 2013; Traditional use registration First approved in UK 1995 under national legislation
Dry extract of <i>Echinacea purpurea</i> root (DER 8.1-9.3:1); extraction solvent: Ethanol 45% V/V	A traditional herbal medicinal product used to relieve the symptoms of common cold and influenza type infections based on traditional use only.	30 mg extract/film coated tablet Adults, elderly & adolescents: 2 to 3 tablets, 3 times a day. Maximum: 9 tablets per day. Start at first signs of common cold. Do not take for more than 10 days.	UK; 2010; Traditional use registration First approved in UK 1993 under national legislation
Dry extract from <i>Echinacea purpurea</i> root (DER 6-7:1); extraction solvent: ethanol 30% V/V	A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections	71.5 mg/film coated tablet Adults, elderly and adolescents: 1-2 tablets, twice a day.	UK; 2010; Traditional use registration

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
	based on traditional use only.	Start at first signs of common cold. Do not take for more than 10 days.	
Dry extract from <i>Echinacea purpurea</i> root (DER 6-7:1); extraction solvent: ethanol 30% V/V	A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections based on traditional use only.	143 mg extract/film-coated tablet Adults, elderly and adolescents: 1 tablet, three times a day, if required. Start at first signs of common cold. Do not take for more than 10 days.	UK; 2010; Traditional use registration
Dry extract from <i>Echinacea purpurea</i> root (DER 6-7:1); extraction solvent: ethanol 30% V/V	A traditional herbal medicinal product used for the symptomatic relief of minor skin conditions, such as spots, pimples and blemishes based on traditional use only.	71.5 mg/film coated tablet Adults, elderly and adolescents: 1-2 tablets, three times a day Do not use the product for more than two weeks.	UK; 2010; Traditional use registration
Dry extract) from <i>Echinacea purpurea</i> root (DER 6-8:1); (extraction solvent: ethanol 75% V/V	A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections, based on traditional use only.	140 mg extract/hard capsule Adults, elderly & adolescents: 1 capsules twice a day. Start at first signs of common cold. Do not take for more than 10 days.	UK; 2011; Traditional use registration
Dry extract from <i>Echinacea purpurea</i> root (DER 6-8:1); extraction solvent: ethanol 75% V/V		105 mg extract/film coated tablet Adults, elderly & adolescents: 1 tablet twice a day. Start at first signs of common cold. Do not take for more than 10 days.	UK; 2011; Traditional use registration

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
Dry extract from <i>Echinacea purpurea</i> root (DER 6.6-7.6:1); extraction solvent: ethanol 30% V/V		70 mg extract/tablet Adults, elderly & adolescents: 1 tablet three times a day. Start at first signs of common cold. Do not take for more than 10 days.	UK; 2008; Traditional use registration
Dry extract from <i>Echinacea purpurea</i> root, (DER 6-8:); extraction solvent: ethanol 75% V/V	A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections based on traditional use only.	70 mg extract/film coated tablet Adults, elderly, adolescents: 1 tablet three times a day. Start at first signs of common cold. Do not take for more than 10 days.	UK; 2011; Traditional use registration
Dry extract of <i>Echinacea purpurea</i> root (DER 4:1); extraction solvent: water	A traditional herbal medicinal product used for the symptomatic relief of minor skin conditions such as spots, pimples, and blemishes, based in traditional use only.	50mg extract/coated tablet For oral use only. Adults and elderly: one or two tablets three times a day. Children over 12 years: One tablet night and morning. Not recommended for children under 12 years of age. If the symptoms worsen or persist for more than 2 weeks, a doctor or a qualified healthcare practitioner should be consulted. Duration of use: Do not take for longer than 2 weeks.	UK; 1970 – 2013; Traditional use registration

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Several combination products with *Echinacea purpurea* herba, *Baptisiae* rhizoma, *E. pallidae* radix; *Thujae occidentalis* herba and/or *S. officinalis* folium are authorised/registered in several MS. The indications are: "... prevention and supportive treatment of common cold ...".

Information on other products marketed in the EU/EEA (where relevant)

No data available.

2.1.2. Information on products on the market outside the EU/EEA

Not applicable

2.2. Information on documented medicinal use and historical data from literature

Herbal teas and tincture prepared of *Echinaceae purpureae* radix are mentioned in the literature.

Table 2: Overview of historical data

Herbal preparation	Documented use / Traditional use	Pharmaceutical form Posology Duration of use	Reference
Comminuted or powdered dried roots as infusion	Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold).	0.9 g several times daily between meals	Bauer and Liersch, 1993; PDR, 2007; Blumenthal <i>et al.</i> , 2000; ESCOP, 2009
Tincture (1:5 in ethanol 55% (V/V or m/m, not specified))	Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold).	60 drops three times daily, corresponding to three times 300 mg of dried root.	Bräunig <i>et al.</i> , 1992 referred by: Barnes <i>et al.</i> , 2007; ESCOP, 2003 and 2009; Blumenthal <i>et al.</i> , 2000; Barrett <i>et al.</i> , 1999; Melchart <i>et al.</i> , 1994 (it is not clear from the references if they report use in practice or use in the scope of the clinical trials)

There is no evidence of 30-year of medicinal use of the comminuted or powdered dried roots and of the tincture in the EU. These preparations are therefore not included in the monograph on *Echinaceae purpureae* radix.

2.3. Overall conclusions on medicinal use

Two herbal preparations with sufficient documentation for 30 years of traditional use are reported:

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
<i>Echinacea purpurea</i> (purple coneflower) dried root, dry extract (6.1-7:1), extraction solvent: ethanol 45 % V/V	Traditional herbal medicinal product used for the relief of symptoms of common cold.	Chewable tablet, 1 tablet contains: 40 mg extract corresponding to 243-279 mg radix Adults and adolescents: 1 chewable tablet every second hour (max 9 chewable tablets per day). Should not be used for longer periods than 10 days.	Since 1978, on the market as a natural remedy under national legislation in Sweden.
Dry extract of <i>Echinacea purpurea</i> root (DER 4:1) Extraction solvent: Water	A traditional herbal medicinal product used for the symptomatic relief of minor skin conditions such as spots, pimples, and blemishes, based in traditional use only.	50mg extract/ coated tablet For oral use only. Adults and elderly: one or two tablets three times a day. Children over 12 years: One tablet night and morning. Not recommended for children under 12 years of age. If the symptoms worsen or persist for more than 2 weeks, a doctor or a qualified healthcare practitioner should be consulted. Duration of use: Do not take for longer than 2 weeks.	UK; 1970 – 2013; THR

At the time of the preparation of the first monograph on *Echinacea purpurea* root in 2010, the product on the market with documented 30 years of medicinal use in Sweden had DER 6.5:1. Production of dry extract with a fixed DER (without any range) is not technically possible. Therefore it was decided in 2010 to extend the DER to 5.5–7.5:1, to cover other comparable preparations that were on the market at that time. The HMPC confirmed the MLWP proposal.

3. Non-Clinical Data

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

Many pharmacological investigations were performed with extract made from 95% aerial parts and 5% roots of *Echinacea purpurea*. They are presented in the assessment report of the *Echinacea purpurea* herba (EMA /HMPC/48704/2014 Corr).

3.1.1. Primary pharmacodynamics

Immunomodulatory activity

In vitro experiments

Ethanollic extracts

An ethanollic extract (strength of ethanol and DER not reported) of purple coneflower root enhanced phagocytosis by 33% in the granulocyte smear test at a concentration of 10^{-4} mg/ml. Aqueous and lipophilic fractions from the ethanollic extract showed immunostimulatory activity (Bauer *et al.*, 1989).

Using an older human adult model of influenza vaccination, peripheral blood mononuclear cells 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% ethanol tincture prepared from the roots of *E. purpurea*. Before being processed the 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. *E. purpurea* augmented IL-10 production, diminished IL-2 production, and had no effect on IFN- γ production. The results indicate that dried *Echinacea* roots stored for sixteen months maintain cytokine-modulating capacities (Senchina *et al.*, 2006).

An extract of *Echinacea purpurea* roots (50% alcoholic tincture, DER 1:9 w/v) was tested for immunomodulation in rhinovirus-infected and uninfected epithelial cells. Since immune modulation has been reported for similar extracts, cytokine antibody arrays were used to investigate the changes in the pro-inflammatory cytokines and chemokines released from a cultured line of human bronchial epithelial cells exposed to rhinovirus 14 and the chemically characterized *Echinacea* extract. Virus infection stimulated the release of at least 31 cytokine-related molecules, including several important chemokines known to attract inflammatory cells. Most of these effects were reversed by simultaneous exposure to the *Echinacea* extract. Furthermore, a number of these cytokines were stimulated by the same *Echinacea* preparation in uninfected cells (Sharma *et al.*, 2006).

The immunomodulatory properties of an *Echinacea* tincture from *E. purpurea* root (DER 1:9, ethanol strength: 50% V/V) after being stored at -20°C for 2 years was tested. The roots of other *Echinacea* species were tested separately for comparison. Two experimental techniques were employed using human peripheral blood mononuclear cells (PBMCs). In the first set of experiments, PBMCs were stimulated *in vitro* with tincture alone and assayed for proliferation and production of IL-10, IL-12, and

TNF- α . In the second set of experiments, subjects were immunized with influenza vaccine. PBMCs from vaccinated individuals were stimulated *in vitro* with the tincture and influenza virus; cytokine production (IL-2, IL-10, and IFN- γ) was compared prevaccination and postvaccination. In the first experiments, *E. purpurea* stimulated only IL-10. In the second experiment, the tincture did not diminish influenza-specific IL-2, and did not influence influenza-specific IL-10 or IFN- γ . (McCann *et al.*, 2007).

The effects of *Echinacea* and several of its phytochemical components on NF κ B 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 NF κ B expression levels. In the presence of endotoxin lipopolysaccharide (LPS), NF κ B expression was decreased. However, this decrease was significantly reversed by treatment with cichoric acid, an *Echinacea* root extract (prepared from both *E. angustifolia* and *E. purpurea*; 1:2 extraction solvent ethanol 60%) and the alkylamide fraction derived from this combination. For the phorbol myristate acetate stimulation of Jurkat cells, effects on NF κ B expression were mixed. Depending on the concentration, cichoric acid and a 2,4-diene alkylamide significantly induced NF κ B 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).

Todd *et al.* (2015) evaluated the effects of a 75% ethanolic root extract of *Echinacea purpurea*, prepared in accord with industry methods, on cytokine and chemokine production from RAW 264.7 macrophage-like cells. It was found that the extract displayed dual activities; the extract could itself stimulate production of the cytokine TNF- α , and also suppress production of TNF- α in response to stimulation with exogenous lipopolysaccharide LPS. Liquid: liquid partitioning followed by normal-phase flash chromatography resulted in separation of the stimulatory and inhibitory activities into different fractions, confirming the complex nature of this extract. The role of alkylamides in the suppressive activity of this *E. purpurea* extract was also studied. The fractionation method concentrated the alkylamides into a single fraction, which suppressed production of TNF- α , CCL3, and CCL5; however fractions that did not contain detectable alkylamides also displayed similar suppressive effects. Alkylamides, therefore, likely contribute to the suppressive activity of the extract but are not solely responsible for that activity. From the fractions without detectable alkylamides, xanthienopyran was purified, a compound not previously known to be a constituent of the *Echinacea* genus. Xanthienopyran suppressed production of TNF- α suggesting that it may contribute to the suppressive activity of the crude ethanolic extract. The authors ascertain that ethanolic extracts prepared from *E. purpurea* plants grown under sterile conditions and from sterilized seeds, do not contain LPS and do not stimulate macrophage production of TNF- α , supporting the hypothesis that the macrophage-stimulating activity in *E. purpurea* extracts can originate from endophytic bacteria. The findings indicate that ethanolic *E. purpurea* extracts contain multiple constituents that differentially regulate cytokine production by macrophages.

It was reported that with the increasing popularity of herbal medicines, many people make their own *Echinacea* extracts at home and storing them at refrigerator (4°C) temperatures. A 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 the roots of 5 different species (*E. angustifolia*, *E. pallida*, *E. purpurea*, *E. sanguinea*, *E. tennesseensis*) were prepared. Four *in vitro* immune assays (monocyte secretion of TNF- α , IL-10, and IL-12 and PBMC proliferation) using human blood was used to test extract efficacy at days 1 and 4 post-extraction. Two statistical analyses, traditional ANOVA and several statistical models

that account for endotoxin effects were used. Endotoxin was found to significantly impact immune outcomes only in 4-day old cold water infusions and not in all assays. Extracts showed the greatest stimulation in TNF- α assays. By extract type, 50% ethanol tinctures produced the most immune stimulation. By species, extracts from *Echinacea angustifolia* extracts were the most efficacious in the assays; extracts from *Echinacea sanguinea* showed the least activity overall (Senchina *et al.*, 2005).

Similarities and differences in immune response among *Echinacea* species, which are commonly used to treat upper respiratory infections were compared and investigated. The investigation involved two components: acquisition of immunomodulatory data reported for the first time according to the authors, and combined phenetic analysis of these data along with previous reports. Experimental data were obtained by stimulating human PBMC *in vitro* with extracts from *Echinacea* spp. and assaying production of three cytokines (IL-1 β , IL-2, and TNF- α). Phenetic analyses were employed to compare responses across the entire data set, including UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and neighbor-joining methods. In the immune experiments conducted for this investigation, *E. angustifolia*, *E. paradoxa*, *E. purpurea*, *E. simulata*, and *E. tennesseensis* extracts prepared from roots significantly augmented IL-1 β and TNF- α production, whereas no extracts significantly modulated IL-2. All phenetic methods produced similar dendrograms, revealing two species pairs (*E. angustifolia* + *E. simulata* and *E. pallida* + *E. sanguinea*) where both species cluster tightly and have similar immune-response profiles. These two species-pairs are maximally dissimilar from each other. The remaining species (*E. paradoxa*, *E. purpurea*, and *E. tennesseensis*) occupy intermediate positions in the dendrogram. The authors concluded that the results suggest that *Echinacea* spp. act heterogeneously on immune function (Senchina *et al.*, 2008).

Other extracts

Alcaline-water extracts of *Echinacea purpurea* (plant part not reported) polysaccharide fractions with molecular weights in the range of 25000 to 500 000 and higher have been isolated, which, according to the granulocytes- and carbon clearance tests, showed significant immunostimulating activities. They stimulated the activity of mouse macrophages; this activation included enhanced secretion of interleukin-1 (IL-1). The isolated compounds belong to the group of water-soluble, acidic heteroglycanes (Wagner *et al.*, 1984, Beuscher *et al.*, 1990).

Purple coneflower root powders and various extracts obtained from the market (details not reported) showed a macrophage activating capacity. Extracts standardised to 4% of phenolic compounds (such as chlorogenic and cichoric acid) or to alkylamides were inactive with respect to induction of macrophage cytokine production (Rininger *et al.*, 2000).

The research made by Benson *et al.* (2010) focused on defining the effects of *Echinacea purpurea* extracts in dendritic cells (DCs), which generate innate and adaptive immune responses. They hypothesized that *E. purpurea* extracts would enhance murine bone marrow-derived DC (BMDC) activation leading to increased immune responses. The fate and function of DCs that were obtained from C57Bl/6 mice was evaluated following 48h exposure to *E. purpurea* root and leaf extracts. The leaves were extracted with 75% ethanol, while the root extraction was aqueous. Flow cytometry revealed that the polysaccharide-rich root extract increased the expression of major histocompatibility complex (MHC) class II, CD86, and CD54 surface biomarkers whereas the alkylamide-rich leaf extract inhibited expression of these molecules. Production of IL-6 and TNF-alpha increased in a concentration-dependent manner with exposure to the root, but not leaf, extract. In contrast, the leaf but not root extract inhibited the enzymatic activity of cyclooxygenase-2. While both extracts decreased the uptake of ovalbumin by BMDCs, the leaf but not root extract inhibited the antigen-specific activation of naïve CD4(+) T cells from OT II/Thy1.1 mice. Collectively, these results suggest that *E. purpurea* can be immunostimulatory, immunosuppressive, and/or anti-inflammatory depending on the portion of the plant and extraction method.

Fast *et al.* (2015) describe the anti-inflammatory effect of a water extract of *Echinacea purpurea* roots (EPRW) that inhibited Pam3Csk4 stimulated production of TNF α by human monocytic THP-1 cells. The polyphenols and alkylamides typically found in *Echinacea* extracts were absent in EPRW suggesting that the anti-inflammatory component(s) was a polysaccharide. This anti-inflammatory activity was shown to be mediated by the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway as chemical inhibition of PI3K abolished the EPRW anti-inflammatory effect. Demonstration of phosphorylation of Akt and ribosomal S6 proteins, downstream targets of PI3K confirmed EPRW-mediated activation of this pathway. This result suggests that non-alkylamide/non-polyphenolic phytochemicals from *Echinacea* may contribute in part to some of the anti-inflammatory therapeutic effects such as reduced severity of symptoms that have been observed *in vivo* in the treatment of upper respiratory tract infections with *Echinacea*. But low bioavailability of polysaccharides in humans is not taken into account by the author.

The study carried out by Pugh *et al.* (2013) determined total bacterial load within *E. purpurea* samples (aerial and root plant parts) and ranged from 6.4×10^6 to 3.3×10^8 bacteria/g of dry plant material. To estimate total bacterial load, a PCR-based quantification method that circumvents the problems associated with nonviable/nonculturable cells (which precludes using plate counts) or the coamplification of mitochondrial or chloroplast DNA with the use of universal bacterial primers (which precludes the use of qPCR) was developed. Differences in total bacterial load within *Echinacea* samples were strongly correlated with the activity (NF- κ B activation in THP-1 cells) and content of bacterial lipopolysaccharides within extracts of this plant material. These results add to the growing body of evidence that bacteria within *Echinacea* are the main source of components responsible for enhancing innate immune function.

The study by Rizzello *et al.* (2013) aimed at investigating the capacity of selected lactic acid bacteria to enhance the antimicrobial, antioxidant and immune-modulatory features of *E. purpurea* with the prospect of its application as functional food, dietary supplement or pharmaceutical preparation. *E. purpurea* suspension (5%, w/v) in distilled water, containing 0.4% (w/v) yeast extract, was fermented with *Lactobacillus plantarum* POM1, 1MR20 or C2, previously selected from plant materials. Chemically acidified suspension, without bacterial inoculum, was used as the control to investigate functional features. *Echinacea* suspension fermented with *Lactobacillus plantarum* C2 exhibited a marked antimicrobial activity towards Gram-positive and -negative bacteria. Compared to control, the water-soluble extract from *Echinacea* suspension fermented with *Lactobacillus plantarum* 1MR20 showed twice time higher radical scavenging activity. Almost the same was found for the inhibition of oleic acid peroxidation. The methanol extract from *Echinacea* suspension had inherent antioxidant features but the activity of extract from the sample fermented with strain 1MR20 was the highest. The antioxidant activities were confirmed on Balb 3T3 mouse fibroblasts. *Lactobacillus plantarum* C2 and 1MR20 were used in association to ferment *Echinacea* suspension, and the water-soluble extract was subjected to ultra-filtration and purification through RP-FPLC. The antioxidant activity was distributed in a large number of fractions and proportional to the peptide concentration. The antimicrobial activity was detected only in one fraction, further subjected to nano-LC-ESI-MS/MS. A mixture of eight peptides was identified, corresponding to fragments of plantaricins PlnH or PlnG. Treatments with fermented *Echinacea* suspension exerted immune-modulatory effects on Caco-2 cells. The fermentation with *Lactobacillus plantarum* 1MR20 or with the association between strains C2 and 1MR20 had the highest effect on the expression of TNF- α gene.

Isolated substances

Purified polysaccharides (EPS) prepared from the herb and root of *Echinacea purpurea* are shown to strongly activate macrophages. Macrophages activated with these substances develop pronounced

extracellular cytotoxicity against tumour targets. The activation is brought about by EPS alone and is independent of any cooperative effect with lymphocytes. Also the production and secretion of oxygen radicals and interleukin-1 (IL-1) by macrophages is increased after activation with EPS. Cells of the macrophages lineage seem to be the main target for the action of these polysaccharides. EPS has no effect on T lymphocytes. B lymphocytes show a comparatively modest proliferation after incubation with *E. purpurea* EPS (Stimpel *et al.*, 1984).

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

Isolated alkamide dodeca-2Z,4E-diene-8,10-diynoic acid isobutylamide from *E. purpurea* and *E. pallida* roots exerted inhibition on lipopolysaccharide (LPS)-mediated activation of a murine macrophage line, RAW264.7 (Chen *et al.* 2005).

In vivo experiments

Ethanollic extracts

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

Isolated substances

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

Using male Sprague-Dawley rats (425–475 g), a study was conducted to examine the immunomodulatory effects of preparations of *Echinacea purpurea*. Cichoric acid, polysaccharide and alkylamide fractions were obtained from *Echinacea purpurea* plants by water-ethanol extraction of the roots or the aerial parts. The fractions were purified to ca. 95% purity and were used to make 4 extracts in 50% ethanol containing its components cichoric acid, polysaccharides and alkylamides in different concentrations. The rats were gavaged orally with these preparations two times daily for 4 days. Phagocytic activity of alveolar macrophage was increased with increasing concentrations of the *Echinacea* components. A trend of increase in TNF- α and NO release by the alveolar macrophages following an *in vitro* stimulation with LPS was also evident. An enhanced release of cytokines (such as TNF- α and $IFN-\gamma$) 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 , $IFN-\gamma$ and IL-2 by the splenocytes. These results suggest that the *Echinacea* preparations containing cichoric acid, polysaccharides and alkylamides are potentially effective in stimulating an *in vivo*, non-specific immune response in normal rats and that the alkylamides at a dose level of approximately 12

mg/kg body weight/day effectively stimulate alveolar macrophage function in healthy rats. The immunomodulatory effects of alkylamides appear to be more pronounced in lungs than in spleen (Goel *et al.*, 2002a, Goel *et al.*, 2002b).

Other preparations

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

The debate is still on-going with respect to the efficacy of ingesting *Echinacea purpurea* preparation intermittently, continuously, or only at the beginning of an affliction. It was sought, therefore, to find out if mice, receiving dietary *Echinacea* daily (commercial purple coneflower root extract, extraction solvent not reported), throughout life, from youth until late middle-age, demonstrated any longevity/survival differences, and/or any differences in their various populations of immune/hemopoietic cells. Sustained and/or high levels of these cells are crucial for longevity. Some mice were maintained on a regular chow diet to which was added *Echinacea purpurea* daily (2 mg/mouse), from puberty (7 week) until just beyond 13 months of age (late middle-age in mice). Control mice, identically housed and maintained, received identical chow without the herbal preparation. Mice consuming untreated diet had a 79% survival by 10 months of age, while those consuming *Echinacea* daily in the diet were still 100% alive by 10 months. At approximately 13 months of age, mice consuming untreated diet had a 46% survival rate while those consuming *Echinacea*, were 74% alive at this time. Moreover, the key immune cells, acting as the first line of defence against developing neoplasms in mice and humans, i.e. NK cells, were significantly elevated in absolute number both in their bone marrow production site, as well as in the major organ to which they traffic and function, i.e. the spleen. The cells of the myeloid/granulocyte lineages remained steadfastly at control levels in both the bone marrow and spleen in *Echinacea*-consuming mice. Thus, the authors concluded that it appears that regular intake of *Echinacea* may indeed be beneficial/prophylactic, if only for the reason that it maintains in an elevated state, NK cells, prime elements in immunosurveillance against spontaneous-developing tumours, a phenomenon which increases in frequency with progressive aging (Brousseau and Miller, 2005).

The research of Uluşık *et al.* (2012) determined the effect of ginseng and echinacea on the mRNA expression of IL-10, TNF- α , and TGF- β 1 in healthy rats. Six-week-old male Fischer 344 rats (n= 48) were used. The animals were divided into three equal group: control; ginseng; echinacea. While the control group was fed a standard rat diet (Purina) ad libitum for a period of 40 days, the ginseng and echinacea groups animals received the same diet containing 0.5 g/kg of *Panax ginseng* root powder and 0.75 g/kg of *E. purpurea* root powder, respectively. Blood samples were obtained from 8 rats in each group after 20 and 40 days of treatment, and the mRNA expression of IL-10, TNF- α , and TGF- β 1 was determined. After 20 days of treatment, the expression of IL-10 mRNA in the ginseng group was different from the control group (P< 0.05); however, after 40 days of treatment, there was no difference between the groups. There was no difference after 20 and 40 days of treatment between the groups with respect to the expression of TGF- β 1 mRNA. After 20 days of treatment, the expression of TNF- α mRNA in the echinacea group was higher (P< 0.05) than the control group. After 40 days of treatment, the expression of TNF- α mRNA was similar in all of the groups.

Barbour *et al.* (2015) evaluated an experimental *Salmonella enteritidis* (SE) bacterin and an indirect ELISA system to assess quantitatively the acquired immunity in Awassi ewes to the vaccine and/or *E. purpurea* dried roots. Four treatments of the ewes were included in the experimental design, with 6 ewes/treatment. The first treatment (T1) had the controls that were non-vaccinated and non-treated with *E. purpurea*. The T2 ewes were only treated with *E. purpurea*. The T3 and T4 ewes were

vaccinated at D1 (initiation of trial) and D10, while the T4 ewes were additionally administered the *E. purpurea* dried roots. Blood was collected from the jugular vein of all ewes at D1, D10, D21 and D45. The construction of the vaccine and the ELISA are detailed within the manuscript. The ELISA was able to detect quantitatively the significant acquired primary and secondary immunity to the vaccine in T3 and T4 ewes, compared to their low level of background immunities at initiation of the experiment ($p < 0.05$). In addition, the ELISA detected the absence of seroconversion at all blood sampling times ($p > 0.05$) in T1 control ewes, and in the T2 ewes that were given only the (EP) ($p > 0.05$). ELISA was able to uncover the significant seroconversion of secondary immune response in T4 ewes at D21 compared to that at D10 ($p < 0.05$), and the absence of significant seroconversion of secondary response in T3 ewes. The study reports the need to supplement the vaccination by the experimental SE bacterin with daily oral intake of 250 mg of *E. purpurea* dried roots, effective the first vaccination day and up to 21 days, for obtaining a statistically significant seroconversion.

Oral administration of 0.45 mg/day of commercial purple coneflower root extract (extraction solvent not reported) to 7-week-old mice for 2 weeks resulted in a doubling of the number of NK cells and monocytes in the bone marrow, and in the spleen (Sun *et al.*, 1999). Oral administration of the same amount of root extract to ageing mice (15-16 months old, with an average life span of 21 months) stimulated the production of new NK cells, leading to 30% increase in the absolute number of NK cells and a 20% increase in the total functional activity of NK cells in the spleen as measured by the lysis of lymphoma cells *in vitro* (Currier and Miller, 2000). Moreover, oral administration of the powdered root to mice injected with leukaemia cells increased their survival time compared to controls (Currier and Miller 2001). Powdered root also exhibited strong adjuvant effect on vaccination with inactivated leukaemia cells (Currier and Miller, 2002).

Combination preparations

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

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

El-Ashmawy *et al.* (2015) described the effect of *Echinacea purpurea* whole plant extract (extraction solvent 100% methanol) on the generation of immature dendritic cells (DCs) from monocytes, as well as its effect on DC differentiation. In addition, an *in vivo* experiment was conducted to investigate whether treatment of mice with extracts derived from *E. purpurea* has immunomodulatory effect on murine splenic DCs. Immature DCs were generated by incubating peripheral blood monocytes with cytokine cocktail (GM-CSF + IL-4) and matured by tumor necrosis factor- α (TNF- α). The cells were randomized to 5 groups to investigate *E. purpurea* effect in different stages. Phenotypic analysis of cell marker CD83-expressed on DCs was performed by flow cytometry. Mice were randomly divided into 3 groups; control, *E. purpurea* treated and *E. purpurea*-TNF- α treated group. The murine splenic DCs

were isolated and phenotyped for CD83 and CD11c by flow cytometry. Treatment of monocytes with *E. purpurea* prior to addition of the maturation factor TNF- α resulted in a significant decrease in the yield of DC expressing CD83. On the other hand, immature DCs generated in the culture in the presence of GM-CSF and IL-4, when treated simultaneously with *E. purpurea* and TNF- α , exhibited an insignificant change in the yield of CD83-expressing DCs compared with untreated control. The *in vivo* experiments showed that splenic DCs obtained from mice treated with *E. purpurea* with or without TNF- α did not exhibit significant changes in CD83 or CD11c compared with those obtained from control mice.

***In vitro* antimicrobial and antifungal activity**

Antibacterial activity against *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* has been demonstrated for a multi-herbal preparation containing *Echinacea purpurea* root extract, although it was stated that the observed antibacterial effects were most likely attributable to one of the ingredients, extract of onion (Westendorf, 1982).

Different commercially available extracts of different species and different parts of *Echinacea* (including roots of *E. purpurea*) and their fractions exhibited near UV-mediated phototoxic 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), *Trichophyton tonsurans*, *T. mentagrophytes*, *Microsporum gypseum* and *Pseudallescheria boydii*. Root extracts (extraction solvent 95 % ethanol, DER 1:10) of eight *Echinacea* taxa, including *E. purpurea* showed antifungal activity against most of the pathogenic fungi (Merali *et al.*, 2003).

***In vitro* antiviral activity**

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

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

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

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

***In vitro* anti-inflammatory activity**

Another study determined whether extracts and isolated alkylamides from *E. purpurea* would be useful for prevention of the inflammatory response that accompanies infections with H1N1 influenza A. Seventeen extracts and 4 alkylamides were tested for the ability to inhibit production of cytokines, chemokines, and PGE2 from RAW 264.7 macrophage-like cells infected with the H1N1 influenza A strain PR/8/34. The alkylamides undeca-2Z,4E-diene-8,10-diynic acid isobutylamide, dodeca-

2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide, dodeca-2E,4E-dienoic acid isobutylamide, and undeca-2E-ene-8,10-dienoic acid isobutylamide suppressed production of TNF- α and PGE₂ from infected cells. Dodeca-2E,4E-dienoic acid isobutylamide was especially effective at inhibiting production of these mediators and also strongly inhibited production of G-CSF, CCL2/MCP-1, CCL3/MIP-1 α and CCL5/RANTES. In contrast, the ethanol extracts (75%), which were prepared from dormant roots of *E. purpurea* grown in different locations throughout North Carolina, displayed a range of effects from suppression to stimulation of mediator production. Precipitation of the extracts with ethanol removed the stimulatory activity, however, even after precipitation; many of the extracts did not display any suppressive activity. Analysis of the extracts revealed slight variations in concentration of alkylamides, caftaric acid, and cichoric acid, but the activity of the extracts did not strongly correlate with concentrations of these compounds (Cech *et al.*, 2010).

***In vivo* anti-inflammatory activity**

Ethanol extracts

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

5-lipoxygenase-inhibiting activity of root extracts (extraction solvent 95 % ethanol, DER 1:10) of five wild and three commercially used species of the genus *Echinacea* were investigated to characterise anti-inflammatory activity of *Echinacea*. The inhibition of the 5-lipoxygenase (5-LOX) enzyme of the arachadonic acid pathway was determined by high-performance liquid chromatography (HPLC) detection of a direct metabolic product (LTB₄) of 5-LOX derived from stimulated rat basophilic cells. Root extracts of the three commercial species of *Echinacea* (*E. purpurea*, *E. pallida* var. *angustifolia*, *E. pallida* var. *pallida*) inhibited the 5-LOX enzyme (Merali *et al.*, 2003).

Inhibition of prostaglandin E₂ (PGE₂) production in LPS-stimulated RAW264.7 mouse macrophage cells was assessed with an enzyme immunoassay following treatments with *Echinacea* extracts (extraction solvent: ethanol (70, 95, or 100%), water, chloroform (100%), hexane (100%), or sequential extractions) or synthesized alkamides. Results indicated that ethanol extracts diluted in media to a concentration of 15 μ g/ml from the roots of *E. angustifolia*, *E. pallida*, *E. simulata*, and *E. sanguinea* significantly inhibited PGE₂ production. In further studies, PGE₂ production was significantly reduced by all synthesized alkamides assayed at 50 μ M, by Bauer alkamides 8, 12A analogue, and 14, Chen alkamide 2, and Chen alkamide 2 analogue at 25 μ M and by Bauer alkamide 14 at 10 μ M. Cytotoxicity did not play a role in the noted reduction of PGE₂ production in either the *Echinacea* extracts or synthesized alkamides. HPLC analysis identified individual alkamides present at concentrations below 2.8 μ M in the extracts from the six *Echinacea* species (15 μ g/ml crude extract). Because active extracts contained <2.8 μ M of specific alkamide and the results showed that synthetic alkamides must have a minimum concentration of 10 μ M to inhibit PGE₂, it is likely that alkamides may contribute toward the anti-inflammatory activity of *Echinacea* in a synergistic or additive manner (LaLone *et al.*, 2007).

Alcohol extracts from the roots (sequential extraction with 100% ethanol, 95% ethanol, chloroform and hexane, followed by solvent evaporation and dissolving in 95 % ethanol) of *E. angustifolia*, *E. pallida*, and *E. purpurea*, were investigated for immunomodulating properties. The three *Echinacea*

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

The effect of the same alcohol extracts as in previous paragraph on the production of inflammatory mediators (nitric oxide (NO), TNF- α and IL-1 β) in both LPS-stimulated RAW264.7 macrophages *in vitro* and murine peritoneal exudate cells (PECs) *in vivo* were investigated. As macrophages produce these inflammatory mediators in response to pathogenic infection, parallel cultures of macrophages were studied for phagocytosis and intracellular killing of *Salmonella enterica*. *E. pallida* and *E. purpurea* *in vitro* inhibited NO production and TNF- α release in a dose-dependent manner. RAW264.7 cells treated with *E. angustifolia* or *E. purpurea* showed decreased killing over 24 h, although *E. angustifolia* enhanced bacterial phagocytosis. Upon bacterial infection, RAW264.7 cells produce high levels of NO; however, an *Echinacea*-mediated decrease in NO production was observed. *Echinacea* alcohol extracts administered orally at 130 mg/kg per day for seven days had a weak effect on NO production and phagocytosis by LPS-stimulated PECs. The results indicated that all *Echinacea* species significantly decreased inflammatory mediators *in vitro*, however, only *E. angustifolia* and *E. 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 *et al.*, 2007b).

Isolated substances

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

A study made by Hou *et al.* (2010), demonstrates that the three most used medicinal *Echinacea* species, *E. purpurea*, *E. pallida*, and *E. angustifolia*, can be classified by the distribution and relative content of metabolites. Mixed alkamides and the major component, dodeca-2E,4E,8Z,10Z(E)-tetraenoic acid isobutylamides, were isolated from *E. purpurea* root extracts for further bioactivity elucidation. In macrophages, the alkamides significantly inhibited cyclooxygenase 2 (COX-2) activity and the lipopolysaccharide-induced expression of COX-2, inducible nitric oxide synthase and specific cytokines or chemokines [i.e., TNF- α , interleukin (IL)-1 α , IL-6, MCP-1, MIP-1 β] but elevated heme oxygenase-1 protein expression. Cichoric acid, however, exhibited little or no effect. The results of high-performance liquid chromatography/electron spray ionization/mass spectrometry metabolite profiling of alkamides and phenolic compounds in *E. purpurea* roots showed that specific compound contents changed under certain post-harvest or abiotic treatment.

The study of Hou *et al.* (2011) was about the anti-inflammatory and hepatoprotective effect of the major alkamides dodeca-2E,4E,8Z,10Z(E)-tetraenoic acid isobutylamides (Alk-8/9), isolated from *E. purpurea* roots (in the article it is not specified how they are isolated), against acute fulminant hepatitis induced by lipopolysaccharide/D-galactosamine (LPS/D-GalN) in mice. The results show that Alk-8/9 dose-dependently induced heme oxygenase (HO)-1 protein expression in LPS-stimulated murine macrophages that was likely regulated by the JNK-mediated pathway through increasing SAPK/JNK phosphorylation, c-jun protein expression, and phosphorylation, and transcription factor AP-1 binding consensus DNA activity. The HO-1 inhibitor or CO scavenger significantly reversed the inhibitory effect of Alk-8/9 on TNF- α expression, whereas N-acetyl-L-cysteine was observed to reduce Alk-8/9-induced HO-1 expression in LPS-treated macrophages. Furthermore, Alk-8/9 markedly induced c-jun and HO-1 protein expression and suppressed serum aminotransferase activities, TNF- α expression, and hepatocyte damage in liver tissues of LPS/d-GalN-treated mice.

Other preparations

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

Table 4: Overview of the main non-clinical data/conclusions

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Comparable/similar preparations to preparations of the monograph				
50% ethanol tincture of <i>E. purpurea</i> roots, drug solvent ratio 1:9 w/v	50 µg/ml	<i>In vitro</i>	Sharma <i>et al.</i> , 2006	Immunomodulatory activity though cytokine stimulation.
50% ethanol tincture of <i>E. purpurea</i> roots, drug solvent ratio 1:9	4 µl of extract per 50 µl of medium	<i>In vitro</i>	Senchina <i>et al.</i> , 2006	Immunomodulatory activity though cytokine stimulation.
50% ethanol tincture of <i>Echinacea E. purpurea</i> roots, drug solvent ratio 1:9	4 µl of extract per 50 µl of medium	<i>In vitro</i>	McCann <i>et al.</i> , 2007	Immunomodulatory activity though cytokine stimulation only partially confirmed.
Other preparations				
Purified polysaccharides prepared from the <i>Echinacea purpurea</i> herb and root (alkaline-water extract)	100 µg/ml	<i>In vitro</i>	Stimpel <i>et al.</i> , 1984	Polysaccharides activate <i>in vivo</i> cells of the macrophage system to cytotoxicity.
Ethanolic <i>E. purpurea</i> root extract	Various concentrations, 10 ⁻⁴ mg/ml showed effects	<i>In vitro</i>	Bauer <i>et al.</i> , 1989	Aqueous and lipophilic fractions showed immunostimulatory activity (enhancement of phagocytosis).
Polysaccharides and glycoproteins from <i>Echinacea E. purpurea</i> radix	High molecular weight fraction (Mr >10,000 D) of polysaccharides and glycoproteins	<i>In vitro</i>	Beuscher <i>et al.</i> , 1995, Bodinet <i>et al.</i> , 1999	Stimulation of cytokines, IL-1, IL-6, TNFα/β, IgM, number of antibody-producing cells, NO production of macrophages.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
<i>Echinacea purpurea</i> root powders and various extracts, <i>Echinacea purpurea</i> herb (whole plant)	Extract concentration 5-320 µg/ml; LPS 0.1 µg/ml; various media/solvents	<i>In vitro</i>	Rininger <i>et al.</i> , 2000	Macrophage activation, LPS showed most effect.
<i>Echinacea purpurea</i> extracts	Various concentrations	<i>In vitro</i>	Rininger <i>et al.</i> , 2000	No induction of macrophage cytokine production, but anti-inflammatory and antioxidant effect.
Root extract, prepared from both <i>Echinacea angustifolia</i> and <i>Echinacea purpurea</i> , 1:2 extraction with ethanol 60%, 300 mg/ml <i>Echinacea purpurea</i>	Alkylamide fraction and cichoric acid fraction tested in different concentrations	<i>In vitro</i>	Matthias <i>et al.</i> , 2008	Opposing effects of compounds on NFκB: Cichoric acid and a 2,4-diene alkylamide significantly induced levels, whereas a 2-ene alkylamide caused a significant inhibition. <i>Echinacea</i> and the mixed alkylamide fraction exerted no effect.
Aqueous extraction of <i>Echinacea purpurea</i> root	150 or 450 µg/ml	<i>In vitro</i>	Benson <i>et al.</i> , 2010	Increase of IL-6, TNF-alpha, expression of MHC class II, CD86, and CD54 surface biomarkers (in contrast to the leaf), decrease of the uptake of ovalbumin by BMDCs.
<i>Echinacea purpurea</i> samples (aerial and root plant parts), 95% ethanolic extraction, further extracted with 4% SDS	EC ₅₀ values see article	<i>In vitro</i>	Pugh <i>et al.</i> , 2013	Differences in total bacterial load within <i>Echinacea</i> samples correlate with the activity (NF-κB activation in THP-1 cells) and content of bacterial lipopolysaccharides within extracts.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
<i>Echinacea purpurea</i> powder suspension (5%, w/v) in distilled water, containing 0.4% (w/v) yeast extract	Various concentrations	<i>In vitro</i>	Rizzello <i>et al.</i> , 2013	Echinacea suspension exerted immunomodulatory effects on Caco-2 cells.
Water extract of <i>E. purpurea</i> root	Various concentrations, IC ₅₀ see article	<i>In vitro</i>	Fast <i>et al.</i> , 2015	Non-alkylamide/non-polyphenolic phytochemicals contribute to the anti-inflammatory effects.
75 % ethanol root extract of <i>E. purpurea</i>	Various concentrations, IC ₅₀ see article	<i>In vitro</i>	Todd <i>et al.</i> , 2015	Macrophage-stimulating activity in <i>E. purpurea</i> extracts can originate from endophytic bacteria.
Purified high molecular weight fraction containing glycoproteins and polysaccharides from <i>E. purpurea</i> root	Oral administration	<i>In vivo</i>	Bodinet, 1999	Enhancement of antibody production in Peyer's plaque cells.
75 % ethanolic extract of <i>E. purpurea</i> root	Tested in 6 rats/group via cracker, 50-250 mg/kg body weight	<i>In vivo</i>	South and Exon, 2001	No evidence of NK cell activity or antibody formation.
<i>E. purpurea</i> root extract (solvent not available/found)	Oral administration of 0.45 mg/day to 7-week-old mice	<i>In vivo</i>	Sun <i>et al.</i> , 1999	Doubling of the number of NK cells and monocytes in bone marrow and spleen.
<i>E. purpurea</i> root extract (solvent not available/found)	Oral administration of 0.45 mg/day to ageing mice	<i>In vivo</i>	Currier and Miller, 2000	Increase of NK cells.
Powdered <i>E. purpurea</i> root	Oral administration to mice injected with leukaemia cells	<i>In vivo</i>	Currier and Miller 2001	Increase of survival time compared to controls.
Powdered <i>E. purpurea</i> root	0.45 mg/day (dose per body weight) via chow	<i>In vivo</i>	Currier and Miller 2002	Strong adjuvant effect on vaccination with inactivated leukaemia cells.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Dry root powder of <i>E. purpurea</i> root (containing 1.5% total polyphenols, calculated as chlorogenic acid)	30 or 100 mg/kg in 7 mice/group	<i>In vivo</i>	Di Carlo <i>et al.</i> , 2003	Increase of resistance of splenic lymphocytes to apoptosis.
Commercial <i>E. purpurea</i> root extract (detailed description not further specified)	Tested in mice, 2 mg <i>E. purpurea</i> /6 g chow/day	<i>In vivo</i>	Brousseau and Miller, 2005	Regular intake lead to immunosurveillance against spontaneous-developing tumors.
<i>E. purpurea</i> root powder	0.75 g/kg in 6-week-old Fischer rats	<i>In vivo</i>	Uluşık <i>et al.</i> , 2012	Increase in expression of TNF- α mRNA.
<i>E. purpurea</i> (EP) dried roots	Assessment of the acquired immunity in 24 Awassi ewes to the vaccine and/or 250 mg dried roots tablet/daily, orally	<i>In vivo</i>	Barbour <i>et al.</i> , 2015	vaccination by the experimental <i>Salmonella enteritidis</i> bacterin with daily oral intake of 250 mg of <i>Echinacea purpurea</i> dried roots for obtaining a significant seroconversion.
Methanolic and aqueous extract of <i>Echinacea purpurea</i> root	Not available/found	<i>In vitro</i>	Wacker and Hilbig, 1978	Resistance to influenza A2, herpes, and vesicular stomatitis virus infection for 24 hours in mouse fibroblasts.
<i>E. purpurea</i> 75% ethanol extracts (detailed description not specified)	Various concentrations	<i>In vitro</i>	Cech <i>et al.</i> , 2010	Range of effects from suppression to stimulation of mediator production.
<i>E. purpurea</i> dry root extract (detailed description not specified)	Oral administration to mice at 30 and 100 mg/kg, twice daily	<i>In vivo</i>	Raso <i>et al.</i> , 2002	Anti-inflammatory effect, down-regulation of COX-2 expression.
<i>E. purpurea</i> root extract (detailed description not specified)	Topical application of gel containing 100 mg/ml in rats	<i>In vivo</i>	Speroni <i>et al.</i> , 2002	Less anti-inflammatory activity than <i>E. pallida</i> .

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
<i>E. purpurea</i> 95% ethanol root extract	Extract usually evaporated to 0.5 g/ml in stimulated rat basophilic cells	<i>In vivo</i>	Merali <i>et al.</i> , 2003	5-lipoxygenase-inhibiting activity.
Crude extract of <i>E. purpurea</i> , (not specified)	15 µg/ml (various solvents) in mouse macrophages	<i>In vivo</i>	LaLone <i>et al.</i> , 2007	Alkamides contribute to anti-inflammatory activity of <i>Echinacea</i> in a synergistic or additive manner.
<i>E. purpurea</i> alcohol extract, (not further specified)	130 mg/kg/daily, administered orally in mice	<i>In vivo</i>	Zhai <i>et al.</i> , 2007a	Modulation of innate and adaptive immune responses, less anti-inflammatory potential than <i>E. angustifolia</i> and <i>E. pallida</i> .
<i>E. purpurea</i> alcohol extract, (not specified)	Administered orally at 130 mg/kg per day both in macrophages <i>in vitro</i> and in murine peritoneal exudate <i>in vivo</i> (bacterial-infected)	<i>In vitro and in vivo</i>	Zhai <i>et al.</i> , 2007b	Anti-inflammatory (inhibition of NO production and TNFα <i>in vitro</i> , weaker effect <i>in vivo</i>).
<i>purpurea</i> root 100% methanol extract	Topic treatment on mouse skin (200 µl per site)	<i>In vivo</i>	Hou <i>et al.</i> , 2010	Anti-inflammatory (especially alkamides, not cichoric acid).
Single substances				
Isolated cichoric acid, polysaccharides and alkylamides	Various concentrations tested in 6 rats/group	<i>In vivo</i>	Goel <i>et al.</i> , 2002a, Goel <i>et al.</i> , 2002b	Cichoric acid, polysaccharides and alkylamides effective in stimulating <i>in vivo</i> , non-specific immune response; alkylamides at a dose level of approximately 12 mg/kg body weight/day stimulate alveolar macrophage function.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides, trienoic and dienoic acid derivatives from standardized tincture made from aerial parts and roots of <i>E. purpurea</i>	Various concentrations	<i>In vitro</i>	Gertsch <i>et al.</i> , 2004	Alkylamids = immunomodulators and potential ligands for CB2 receptors.
Undeca-2Z,4E-diene-8,10-diynic acid isobutylamide, dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide, dodeca-2E,4E-dienoic acid isobutylamide, and undeca-2E-ene-8,10-diynoic acid isobutylamide	Various concentrations	<i>In vitro</i>	Cech <i>et al.</i> , 2010	Suppression of production of TNF- α and PGE ₂ from influenza A infected cells.
Xanthienopyran from 75% ethanolic root extract of <i>E. purpurea</i>	Various concentrations	<i>In vitro</i>	Todd <i>et al.</i> , 2015	Suppression of production of TNF- α , IC ₅₀ = 3.4 μ g/ml
Alkamides from <i>E. purpurea</i> root dichloromethane extract	Concentration in final DMSO assay: 10 and 100 μ g/ml	<i>In vivo</i>	Clifford <i>et al.</i> , 2002	Inhibition of COX-1 and, lesser, of COX-2
Dodeca-2E,4E,8Z,10Z(E)-tetraenoic acid isobutylamides, isolated from <i>E. purpurea</i> root	Various concentrations tested in mice.	<i>In vivo</i>	Hou <i>et al.</i> , 2011	Anti-inflammatory

3.1.2. Secondary pharmacodynamics

***In vitro* antioxidant activity**

The protective effect of caffeoyl derivatives (echinacoside, chlorogenic acid, cichoric acid, cynarine, and caffeic acid, typical constituents of *Echinacea* species) on the free radical-induced degradation of Type III collagen has been investigated. The results indicate that this representative class of polyphenols of *Echinacea* species protects collagen from free radical damage through a scavenging effect on reactive oxygen species and/or C-, N-, S-centered secondary radicals, and provides an indication for the topical use of extracts from *Echinacea* species for the prevention/treatment of photodamage of the skin by UVA/UVB radiation, in which oxidative stress plays a crucial role (Maffei Facino *et al.*, 1995).

In the study of methanol extracts of freeze-dried roots of *Echinacea* species (*E. angustifolia*, *E. pallida*, and *E. purpurea*) on free radical scavenging capacities and antioxidant activities it was demonstrated that the mechanisms of antioxidant activity of extracts derived from *Echinacea* roots included free radical scavenging and transition metal chelating (Hu and Kitts, 2000).

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

The radical scavenging activity of *Echinacea* methanolic extracts was evaluated *in vitro* with a spectrophotometric method based on the reduction of an alcoholic DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical solution at 517 nm in the presence of a hydrogen donating antioxidant. As for pure compounds, echinacoside had the highest capacity to quench DPPH radicals ($EC_{50} = 6.6 \mu\text{M}$), while caftaric acid had the lowest ($EC_{50} = 20.5 \mu\text{M}$). The average EC_{50} values for *E. purpurea*, *E. pallida* and *E. angustifolia* were 134, 167 and 231 $\mu\text{g/ml}$, respectively. The radical scavenging activity of *Echinacea* root extracts reflected their phenolic composition (Pellati *et al.*, 2004).

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

The antioxidant activity of extracts of the stems, leaves, and roots of *Echinacea purpurea* was compared with the antioxidant activity of purified cichoric acid and alkamides, both constituents of *E. purpurea*. The antioxidant activity was determined using different methods: effect on oxygen consumption rate of a peroxidating lipid emulsion, and scavenging of radicals, i.e. DPPH, measured by two different techniques. The efficacy of the extracts in the reaction with DPPH correlated well with the amount of cichoric acid present in the various extracts. The alkamides alone showed no antioxidant

activity in any of the tests. Alkamides present in the extract increased, however, the antioxidative effect of cichoric acid in the peroxidating lipid emulsion. The activity was further compared with that of rosmarinic acid, a well-characterised antioxidant, and the extracts as well as cichoric acid were found to be efficient scavengers of radicals with an activity comparable to that of rosmarinic acid. Cichoric acid was found to have a stoichiometric factor of 4.0 in scavenging DPPH and to react in a second-order reaction with DPPH with a rate constant of 40 L/mol/s at 25°C in methanol (Thygesen *et al.*, 2007).

Other activities

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

Serial dilutions of 21 commercial ethanolic herbal extracts and tinctures, and 13 related pure plant compounds have been analyzed for their *in vitro* cytochrome P450 3A4 (CYP3A4) inhibitory capability via a fluorometric microtitre plate assay. Roughly 75% of the commercial products and 50% of the pure compounds showed significant inhibition of CYP3A4 metabolite formation. *Echinacea purpurea* root extract showed moderate inhibitory activity ($IC_{50} > 5\%$ and $< 10\%$ full strength) (Budzinski *et al.*, 2000).

In the study of the effect of *Echinacea purpurea* root extract (prepared with 50% aqueous ethanol, DER was not specified) on the weight of prostates in rats as well as on alterations of histological structure and separate blood cells with 3-month old male Wistar rats, it was observed a significantly important decrease of prostate weight of investigated rats, an increase in the number of lymphocytes as well as the alterations of histological structures after using *Echinacea* extract for 8 weeks (Skaudickas *et al.*, 2003).

The effect of *Echinacea purpurea* extract (prepared with 50% aqueous ethanol, DER was not given) on a rat testicle and epididymis was examined, the mass of these organs was determined, the proportion between the mass of the organ and the mass of a body was calculated, the changes in histological structures were evaluated in the study with the Wistar line 3-month old male rats. The histological structural changes were traced after 4 weeks of using the preparation; however they became more obvious after 8 weeks. Results of the study enabled to determine statistically significant reduction in the percentage of a testicle and the body mass, as well as changes in histological structures after 8 weeks of consuming extract of *E. purpurea* (Skaudickas *et al.*, 2004).

It was shown that the alkylamides dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (A1) and dodeca-2E,4E-dienoic acid isobutylamide (A2) bind to the CB2 receptor more strongly than the endogenous cannabinoids. Molecular modelling suggests that alkylamides bind in the solvent-accessible cavity in CB2, directed by H-bonding and π - π interactions. In a screen with 49 other pharmacologically relevant receptors, it could be shown that A1 and A2 specifically bind to CB2 and CB1. A1 and A2 elevated total intracellular Ca^{2+} in CB2-positive but not in CB2-negative promyelocytic HL60 cells, an effect that was inhibited by the CB2 antagonist SR144528. At 50 nM A1, A2, and the endogenous cannabinoid anandamide (CB2 $K_i > 200$ nM) up-regulated constitutive IL-6 expression in human whole blood in a seemingly CB2-dependent manner. A1, A2, anandamide, the CB2 antagonist SR144528 ($K_i < 10$ nM), and also the non-CB2-binding alkylamide undeca-2E-ene-8,10-dienoic acid isobutylamide all significantly inhibited LPS-induced TNF- α , IL-1 β , and IL-12p70 expression (5–500

nM) in a CB2-independent manner. Alkylamides and anandamide also showed weak differential effects on anti-CD3- versus anti-CD28-stimulated cytokine expression in human whole blood. Overall, alkylamides, anandamide, and SR144528 potently inhibited LPS-induced inflammation in human whole blood and exerted modulatory effects on cytokine expression, but these effects are not exclusively related to CB2 binding (Raduner *et al.*, 2006).

Potential *in vitro* cytotoxic and pro-apoptotic properties of hexanic root extract of the three medicinal *Echinacea* (Asteraceae) species (*E. pallida* (Nutt.) Nutt., *E. angustifolia* DC. var. *angustifolia*, *E. purpurea* (L.) Moench) were studied on the human pancreatic cancer MIA PaCa-2 and colon cancer COLO320 cell lines. The authors concluded that it was demonstrated, for the first time, that all the three species reduced cell viability in a concentration- and time-dependent manner (Chicca *et al.*, 2007).

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).

One likely mode of action is that alkamides from *Echinacea* bind to cannabinoid type 2 (CB2) receptors and induce a transient increase in intracellular Ca²⁺. The study of *E. purpurea* root extracts and constituents as potential regulators of intracellular Ca²⁺ levels made by Wu *et al.* (2010) shows that unidentified compounds from *Echinacea purpurea* induce cytosolic Ca²⁺ elevation in non-immune-related cells, which lack CB2 receptors and that the Ca²⁺ elevation is not influenced by alkamides. The data indicate that as yet unidentified constituents from *Echinacea* stimulate an IP3 receptor and phospholipase C mediation of cytosolic Ca²⁺ levels in non-immune mammalian cells. This pathway is distinct from that induced in immune associated cells via the CB2 receptor.

Multiple chromatographic separations of the CHCl₃-soluble extract of the roots of *Echinacea purpurea* led to the isolation of 19 compounds. Four natural products, three alkamides and nitidanin diisovalerianate, were identified, and five further compounds were detected for the first time in this species. Additionally, 10 known *Echinacea purpurea* metabolites were isolated. The bioactivity of the isolated compounds was studied in [³⁵S] GTPγS-binding experiments performed on rat brain membrane preparations. Both partial and inverse agonist compounds for cannabinoid (CB1) receptors were identified among the metabolites, characterized by weak to moderate interactions with the G-protein signaling mechanisms. The G-protein-modulating activities of the *Echinacea* compounds are rather far from the full agonist effects seen with the CB1 receptor agonist reference compound arachidonyl-2'-chloroethylamide (ACEA). However, upon coadministration with ACEA, a number of them proved capable of inhibiting the stimulation of the pure agonist, thereby demonstrating cannabinoid receptor antagonist properties (Hohmann *et al.*, 2011).

The study made by Shin *et al.* (2014) was conducted to investigate the effects of an ethanol extract of *Echinacea purpurea* root and herb material and its constituents on the insulin-induced adipocyte differentiation of 3T3-L1 preadipocytes. When adipocyte differentiation was induced with insulin plus 3-isobutyl-1-methylxanthine and dexamethasone, the accumulation of lipid droplets and the cellular triglyceride content were significantly increased by *Echinacea purpurea*. The expressions of PPARγ and C/EBPα in adipocytes treated with *Echinacea purpurea* were gradually increased as compared with control cells. Fat accumulation and triglyceride content of adipocytes treated with dodeca-2(E),4(E)-dienoic acid isobutylamide were significantly increased as compared with control cells. The expressions of PPARγ and C/EBPα in adipocytes treated with dodeca-2(E),4(E)-dienoic acid isobutylamide were significantly higher than in control cells. These results suggest *Echinacea purpurea* promotes the

adipogenesis that is partially induced by insulin and that dodeca-2(E),4(E)-dienoic acid isobutylamide appears to be responsible for enhanced adipocyte differentiation.

The purpose of the study carried out by Kotowska *et al.* (2014) was to identify the bioactive compounds responsible for the potential antidiabetic effect of the dichloromethane extract of *E. purpurea* roots using a bioassay-guided fractionation approach. Two novel isomeric dodeca-2E,4E,8Z,10E/Z-tetraenoic acid 2-methylbutylamides together with two known C12-alkamides and α -linolenic acid were isolated from the active fractions. The isomeric C12-alkamides were found to activate peroxisome proliferator-activated receptor γ , to increase basal and insulin-dependent glucose uptake in adipocytes in a dose-dependent manner, and to exhibit characteristics of a peroxisome proliferator-activated receptor γ partial agonist.

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

There are only a few publications where pharmacological tests were performed with preparations related to the *Echinacea purpurea* roots' preparations of the monograph. In most articles extracts prepared with 50% ethanol were tested, but the monograph describes the extract prepared with 45% ethanol. This does not present an issue regarding plausibility of the efficacy of the preparations concerned since a comparable composition of the extract is expected with so small difference in ethanol concentration as extraction solvent.

In many experiments concentrations are not available in the published reports. However, all researches refer to potential effects on different immunological parameters which indicate immunomodulatory effect.

The data obtained from other preparations differ, but show in most cases positive immunomodulatory effects.

Anti-inflammatory effects are described in several *in vitro* and *in vivo* experiments, but they were observed with preparations which are not comparable to the preparations of the monograph.

According to the literature both the immunomodulatory and the anti-inflammatory effects are attributed mainly to alkamides. The mechanism of action might be the inhibition of COX-1, COX-2 and 5-LO.

Less data are available for antibacterial, antiviral (mainly HSV and influenza virus) and antifungal effects. The data available allow only very limited conclusions on the contribution of the observed effects to the plausibility of the therapeutic effects of the preparations of the monograph.

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

Absorption, distribution, metabolism, elimination

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

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

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

The study made by Ardjomand-Woelkart *et al.* (2011) assessed the absolute and relative bioavailabilities of dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutylamides (tetraenes), the main bioactive constituents in *Echinacea*, administered as pure compounds or in the form of an *Echinacea purpurea* root extract preparation (extracted with 60 % ethanol) in rats. Tetraenes were administered orally by gavage or intravenously in a dose of 0.75 mg/kg. The extract was administered orally in a dose of 158.6 mg/kg which corresponds to the same amount of tetraenes. Pharmacokinetic parameters of tetraenes were calculated by non-compartmental analysis. Mean dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutylamide dose-normalized plasma area under the concentration-time curve (AUC - ∞/dose) was 3.24 ± 0.32 min · ng/ml/µg and 0.95 ± 0.16 min · ng/ml/µg after iv and oral administrations, respectively, and 1.53 ± 0.18 min · ng/ml/µg after oral administration of the *Echinacea* root extract. The absolute oral bioavailability of dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutylamides was 29.2 ± 2.3%, which was increased to 47.1 ± 7.2 % (1.6-fold) by administration of the *Echinacea* extract. Administration of an *Echinacea* extract increased blood exposure with no impact

on C_{max} , but prolonged the elimination half-life to 123.3 ± 15.7 min in comparison to 35.8 ± 6.5 min after administration of the pure dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutylamides.

Pharmacokinetic interactions

The six commonly used trade herbal products, St. John's wort, common valerian, common sage, *Ginkgo biloba*, *Echinacea purpurea* and horse chestnut, and ethanol, were investigated for their *in vitro* inhibitory potential of cytochrome P450 2D6 (CYP2D6)-mediated metabolism. Herbal components were extracted from commercially available products in a way that ensured the same composition of constituents in the extract as in the original trade products. c-DNA baculovirus expressed CYP2D6 was used with dextromethorphan as substrate. Quinidine was included as a positive control inhibitor. A validated HPLC methodology was used to quantify the formation of dextropropranolol (product of dextromethorphan O-demethylation). Ethanol showed a biphasic effect on CYP2D6 metabolism, increasing initially the CYP2D6 activity with 175% of control up to a concentration of 1.1%, where after ethanol linearly inhibited the CYP2D6 activity. All the investigated herbs inhibited CYP2D6 activity to some extent, but only St. John's wort, common sage and common valerian were considered possible candidates for *in vivo* clinically significant effects. They showed IC₅₀ values of $0.07 \pm 7 \times 10^{-3}$ mg/ml, 0.8 ± 0.05 mg/ml and 1.6 ± 0.2 mg/ml, respectively. St. John's wort inhibited CYP2D6-mediated metabolism in an uncompetitive manner, while common valerian and common sage in a non-competitive manner demonstrated interherb differences in inhibition patterns and differences when compared to the more homogenous competitive inhibitor quinidine. Common valerian was the only herb that showed a mechanistic inhibition of CYP2D6 activity and attention should be paid to a possible toxicity of this herb (Hellum and Nilsen, 2007).

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

Assessor's overall conclusions on pharmacokinetics

In pharmacokinetic studies only alkamides and caffeic acid conjugates were investigated. It was shown that alkamides (in contrast with caffeic acid conjugates) readily diffuse through the monolayers of Caco-2 cells. This supports the assumption that the alkamides can be easily transported from the intestine and hence may contribute to the *in vivo* effects of *Echinacea* preparations. Administration of an *Echinacea purpurea* root extract increased blood exposure with no impact on C_{max} , but prolonged the elimination half-life after administration of the pure dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutylamides in rats.

Study of metabolism suggests that alkamides of *Echinacea* are metabolised by cytochrome P450 and may affect the CYP450-mediated metabolism of other concurrently ingested pharmaceuticals. However, effects of *E. purpurea* root preparations on CYP450 were only shown *in vitro*.

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

3.3.1. Single dose toxicity

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

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

3.3.2. Repeat dose toxicity

No relevant data available.

3.3.3. Genotoxicity

No relevant data available.

3.3.4. Carcinogenicity

No relevant data available.

3.3.5. Reproductive and developmental toxicity

No relevant data available.

3.3.6. Local tolerance

No relevant data available.

3.3.7. Other special studies

No relevant data available.

3.3.8. Conclusions

In general, the toxicity of *E. purpurea* is considered to be low. However, the data on toxicity are limited and findings are sometimes difficult to interpret since there is a lack of details regarding the tested preparation or the part of the plant of *E. purpurea*. Tests on acute and chronic toxicity, reproductive toxicity, genotoxicity and carcinogenicity have not been performed on the dry ethanolic extract covered by the European Union herbal monograph.

3.4. Overall conclusions on non-clinical data

Non-clinical data on purple coneflower root activity supports the traditional use of *Echinacea purpurea* root dry extracts as traditional herbal medicinal product for the relief of symptoms of common cold.

The traditional use of *Echinacea purpurea* root dry extracts as traditional herbal medicinal product used for the relief of spots and pimples due to mild acne is not directly supported by relevant non-clinical data.

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety of purple coneflower root is scarce.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Based on long experience of use, oral administration of *Echinacea purpurea* root dry extracts can be regarded as safe at traditionally used doses.

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed, therefore a List Entry is not proposed.

4. Clinical Data

4.1. Clinical pharmacology

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

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

In a double-blind study, 24 healthy male volunteers took three times 30 drops of an ethanolic extract of purple coneflower root (detailed specifications of the extract are not given) or placebo daily for 5 days. By day 5 a significant increase in phagocytosis of 120% was observed in the verum group, compared to 20% in the placebo group. The effect was transient and phagocytotic activity returned to normal within 6 days (Jurcic *et al.*, 1989).

The effect of *Echinacea* tablets containing 675 mg of *E. purpurea* root extract and 600 mg of *Echinacea angustifolia* root extract, prepared by ethanol extraction (the concentration of ethanol is not declared in the article) on the expression of leucocyte heat shock protein 70 (hsp70), erythrocyte haemolysis, plasma antioxidant status, serum chemistry, haematological values and plasma alkylamide

concentrations. Eleven healthy individuals (26–61 years of age) were evaluated at baseline (day 1) and on day 15 after consuming two commercially blended *Echinacea* tablets daily for 14 days. *Echinacea* supplementation enhanced the fold increase in leucocyte hsp70 expression after a mild heat shock ($P = 0.029$). White cell counts (WCC) were also increased ($P = 0.043$). A preventative effect against free radical induced erythrocyte haemolysis ($P = 0.006$) indicative of an antioxidant effect was also observed. The authors suggest that *Echinacea* may invoke an immune response through altered expression of hsp70 and increased WCC (Agnew *et al.*, 2005).

Echinacea extract or placebo was administered to volunteers at the onset of their cold for a period of 7 days, with 8 doses (5 ml/dose) on day 1 and 3 doses on subsequent days. Extract was prepared by extraction of herb and root of *Echinacea purpurea* by water and ethanol (concentration not reported in the article), followed by purification of alkaloids, cichoric acid and polysaccharides to >95 % purity and their dissolution in 40 % ethanol to a final concentration of 0.25, 2.5 and 25.5 mg/ml respectively. Fasting blood samples were obtained before and during their colds. The decrease in total daily symptomatic score was more evident in the *Echinacea* group than in the placebo group. These effects of *Echinacea* were associated with a significant and sustained increase in the number of circulating total white blood cells, monocytes, neutrophils and NK cells. In the later part of the cold, the *Echinacea* treatment suppressed the cold-related increase in superoxide production by the neutrophils. The authors suggested that this extract, by enhancing the non-specific immune response and eliciting free radical scavenging properties, may have led to a faster resolution of the cold symptoms (Goel *et al.*, 2005).

Table 5: Overview of pharmacodynamic data

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Investigation of immunomodulatory activity, Melchart <i>et al.</i> , 1995	Placebo-controlled, randomized, double-blinded, duration 9-14 days	2: ethanolic extract corresponding to 333 mg of roots, for 5 days, orally, 3a: 380 mg of dried extract made with 50% ethanol (V/V), compared to <i>E. pallida</i> radix and placebo, acid-resistant capsules, one capsule 3x daily for 5 consecutive days, orally,	134 (18 female and 166 male) between 18 and 40 years, 1 drop out because of eosinophilia.	Healthy	Primary endpoint: relative phagocytic activity of polymorphonuclear neutrophil granulocytes (PNG) → significant increase, secondary endpoint: number of leukocytes in peripheral venous blood → no significant influence	2: Wilcoxon-Mann-Whitney-U-test; 3: Kruskal-Wallis test; 2: Maximal stimulation 54.0% (95 % CI 8.4-99.6%)	Immuno-modulating effect in study 2, no effect in study 3a
Influence on phagocytosis, Jurcic <i>et al.</i> , 1989	Placebo-controlled, double-blinded, duration 5 days	30 drops of an ethanolic <i>E. purpurea</i> root extract, orally	24 male	Healthy	Significant increase in phagocytosis of 120 % by day 5 (placebo: 20 %)	Not available/found	Small study, only males and healthy, randomization?
Effects on immune markers, Agnew <i>et al.</i> , 2005	Not available/found	Tablets containing 675 mg of <i>E. purpurea</i> root extract and 600 mg of <i>E. angustifolia</i> root extract, prepared by ethanol extraction Duration: 14 days	11 (5 male, 6 female), 26–61 years of age	Healthy	Leucocyte hsp70 expression increase (P=0.029); white cell counts increase (P=0.043); erythrocyte haemolysis increase (P=0.006) →antioxidant	Not available/found	Invoke an immune reponse, and protection of free radical;

Assessor's overall conclusions on pharmacodynamics

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

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

Serial plasma samples from 9 healthy volunteers who ingested 4 *Echinacea* tablets, each containing extract equivalent to 675 mg of *E. purpurea* root plus 600 mg of *E. angustifolia* root prepared from the dried ethanolic extracts (concentration of ethanol is not given in the article), 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. Alkylamides were rapidly absorbed and were measurable in plasma 20 min after tablet ingestion and remained detectable for up to 12 h. Concentration-time curves for 2,4-diene and 2-ene alkylamides were determined. The maximum concentrations for the sum of alkylamides 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 alkylamides in 2 additional fasted subjects who took the same dose of the *Echinacea* preparation (Matthias *et al.*, 2005b).

The relative oral bioavailability of alkylamides from two different *Echinacea* dosage forms (liquid and tablet) were compared in a small two-way crossover study in humans ($n = 3$). The liquid preparation investigated contained a mixture of *E. purpurea* root (300 mg/ml) and *E. angustifolia* root (200 mg/ml) extracted in 60% ethanol. The tablet preparation investigated was also a mixture of *E. purpurea* root (675 mg/tablet) and *E. angustifolia* root (600 mg/tablet), but was prepared from the dried 60% ethanolic extracts of these two *Echinacea* species. Alkylamides were found to be rapidly absorbed and measurable in plasma from both preparations. No significant differences in the tetraene alkylamide pharmacokinetic parameters for $T_{1/2}$, AUC_{0-t} and C_{max} in the two different preparations were found. T_{max} increased from 20 min for the liquid to 30 min for the tablet, which is not unexpected as the tablet required time for disintegration before absorption could occur. These results suggested that there was no significant difference in the bioavailability of alkylamides from the liquid and tablet *Echinacea* formulations. Furthermore, the results also indicated that the absorption site and any alkylamide loss due to digestive processes were similar in both preparations (Matthias *et al.*, 2007).

Pharmacokinetic interactions

The study made by Moltó *et al.* (2011) investigated the potential of *E. purpurea* to interact with the boosted protease inhibitor darunavir-ritonavir. Fifteen HIV-infected patients receiving antiretroviral therapy including darunavir-ritonavir (600/100 mg twice daily) for at least 4 weeks were included. *E. purpurea* root extract capsules were added to the antiretroviral treatment (500 mg every 6 h) from days 1 to 14. Darunavir concentrations in plasma were determined by high-performance liquid chromatography immediately before and 1, 2, 4, 6, 8, 10, and 12 h after a morning dose of darunavir-ritonavir on days 0 (darunavir-ritonavir) and 14 (darunavir-ritonavir plus echinacea). Individual darunavir pharmacokinetic parameters were calculated by noncompartmental analysis and compared between days 0 and 14. The median age was 49 (range, 43 to 67) years, and the body mass index was 24.2 (range, 18.7 to 27.5) kg/m². Echinacea was well tolerated, and all participants completed the study. The geometric mean ratio for darunavir coadministered with echinacea relative to that for

darunavir alone was 0.84 (90% CI, 0.63-1.12) for the concentration at the end of the dosing interval, 0.90 (90% CI, 0.74-1.10) for the area under the concentration-time curve from 0 to 12 h, and 0.98 (90% CI, 0.82-1.16) for the maximum concentration. In summary, coadministration of *E. purpurea* with darunavir-ritonavir was safe and well tolerated. Individual patients did show a decrease in darunavir concentrations, although this did not affect the overall darunavir or ritonavir pharmacokinetics. Although no dose adjustment is required, monitoring darunavir concentrations on an individual basis may give reassurance in this setting.

The aim of an open-label, fixed-sequence study made by Moltó *et al.* (2012) was to investigate the potential *E. purpurea* to interact with etravirine, a nonnucleoside reverse transcriptase inhibitor of HIV. Fifteen HIV-infected patients receiving antiretroviral therapy with etravirine (400 mg once daily) for at least 4 weeks were included. *E. purpurea* root/extract-containing capsules were added to the antiretroviral treatment (500 mg every 8 h) for 14 days. Etravirine concentrations in plasma were determined by HPLC immediately before and 1, 2, 4, 6, 8, 10, 12, and 24 h after a morning dose of etravirine on day 0 and etravirine plus *E. purpurea* on day 14. Individual etravirine pharmacokinetic parameters were calculated by noncompartmental analysis and compared between days 0 and 14. The median age was 46 years (interquartile range, 41 to 50), and the median body weight was 76 kg (interquartile range, 68 to 92). *Echinacea* was well tolerated, and all participants completed the study. The geometric mean ratio for etravirine coadministered with *E. purpurea* relative to etravirine alone was 1.07 (90% CI, 0.81 to 1.42) for the maximum concentration, 1.04 (90% CI, 0.79 to 1.38) for the area under the concentration-time curve from 0 to 24 h, and 1.04 (90% CI, 0.74 to 1.44) for the concentration at the end of the dosing interval. The coadministration of *E. purpurea* with etravirine was safe and well tolerated in HIV-infected patients; the data suggest that no dose adjustment for etravirine is necessary.

Table 6: Overview of pharmacokinetic data

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Examination of plasma concentration, Matthias <i>et al.</i> , 2005b	Single dose study	4 tablets, each containing dried ethanolic extract equivalent to 675 mg of <i>E. purpurea</i> root plus 600 mg of <i>E. angustifolia</i> root; duration: 12 h	9 (male and female), 18-26 years; drop out not mentioned.	Healthy	C_{max} for the sum of alkamides in plasma were reached within 2.3 h post ingestion and averaged 336 ± 31 ng eq/ml plasma. No differences in 2 additional fasted subjects	Pharmacokinetic analysis	Availability of most alkamides after oral intake shown, but no caffeic acid conjugates.
Examination of bioavailability of alkylamides of two different dosage forms, Matthias <i>et al.</i> , 2007	Two-way crossover study	Liquid: mixture of <i>E. purpurea</i> root (300 mg/ml) and <i>E. angustifolia</i> root (200 mg/ml) extracted in 60% ethanol; Tablets: mixture of <i>E. purpurea</i> root (675 mg/tablet) and <i>E. angustifolia</i> root (600 mg/tablet), prepared from the dried 60% ethanolic extracts of <i>E. purpurea</i> and <i>E. angustifolia</i> , orally	3 male volunteers (39-62 years), no other medication, no drop out mentioned.	Healthy	No significant difference in bioavailability of alkylamides from the liquid and tablet Blood samples before start, at 10, 20, 30, 60, 90 and 120 min post-dose (liquid); before start, at 10, 30, 60, 90, 180, 210, 240 and 480 min post-dose	Pharmacokinetic analysis	Improvement of knowledge about bioavailability of alkylamides in two different dosage forms.

Assessor's overall conclusions on pharmacokinetics

Evidence were provided that alkamides are orally available from liquid extracts and tablets and that their pharmacokinetics is in agreement with the daily regimen already recommended for *Echinaceae purpureae radices extracts* 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.

In vivo, coadministration of *E. purpurea* with the protease inhibitor darunavir-ritonavir and the nonnucleoside reverse transcriptase inhibitor etravirine was safe and well-tolerated. Further pharmacokinetic testing is necessary to evaluate possible pharmacokinetic interactions of *E. purpurea* root preparations.

4.2. Clinical efficacy

Several reviews on studies of the effects of *Echinacea purpurea* herbal products (aerial parts, roots and combinations) in clinical trials have been published (Barnes *et al.*, 2005; Barnes *et al.*, 2007; Barrett, 2003; Bradley, 2006; ESCOP, 2003 and 2009; PDR, 2007; Blumenthal *et al.*, 2000; Bauer and Liersch, 1993; Karsch-Völk *et al.*, 2014, Linde *et al.*, 2006; Melchart *et al.*, 1994; Melchart *et al.*, 2004; Schoop *et al.*, 2006; Shah *et al.*, 2007). They all review the same studies, which are listed also in this AR and in the AR of *E. purpurea* herba.

4.2.1. Dose response studies

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

Table 7: Dose- response clinical studies on humans

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Effect on influenza, Bräunig <i>et al.</i> , 1992	Placebo-controlled, double-blinded, randomized	Tincture of <i>E. purpurea</i> root made with 55% ethanol (V/V) at daily dosages corresponding to 459 mg or 900 mg of dried root, or placebo, Orally; duration: 10 days; measurement points 3-4 and 8-10 days after intake	180 patients (18-60 years)	Patients with influenza.	Group taking the 900 mg dose showed a highly significant reduction in symptom score at both time points ($p < 0.0001$), but no difference in symptoms between the group taking the 450 mg dose and the placebo group	Not mentioned	900 mg <i>E. purpurea</i> root tincture with 55% ethanol (V/V) may have some effects on influenza

4.2.2. Clinical studies (case studies and clinical trials)

***Echinacea purpurea* radix as a single ingredient**

289 volunteers from four military establishments and one industrial plant participated in a double-blind, placebo-controlled study to investigate the efficacy of *Echinacea* extracts in the prevention of upper respiratory tract infections. Randomised groups were instructed to take twice daily for 12 weeks 50 drops (ca. 1 ml) of one of three trial preparations: ethanolic extract (1:11, ethanol 30%) of purple coneflower root (Group A, n = 99) or *E. angustifolia* root (Group B, n = 100), or an ethanolic placebo solution (Group C, n = 99). 244 participants fully conformed with the protocol: 85, 84 and 75 in Groups A, B and C, respectively. The average time until occurrence of first upper respiratory tract infections was 69, 66 and 65 days, and 29%, 32% and 37% of participants had at least one upper respiratory tract infection, in Groups A, B and C, respectively. Although these results show a trend of 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).

***Echinacea purpurea* radix in combination with other herbal drugs**

In a randomized, double-blind, placebo-controlled study the efficacy and safety of different doses and preparations of *Echinacea purpurea* in the treatment of common cold. 246 of 559 recruited healthy, adult volunteers caught a common cold and took 3 times daily 2 tablets of either 6.78 mg of *Echinacea purpurea* dry extract from 95% herba and 5% radix, (extraction solvent not reported in this article), *Echinacea purpurea* concentrate (same preparation at 7 times higher concentration), special *Echinacea purpurea* radix preparation (dry extract from root of *Echinacea purpurea*, 29.6 mg per tablet, extraction solvent not reported in this article) or placebo until they felt healthy again but not longer than 7 days. The primary endpoint was the relative reduction of the complaint index defined by 12 symptoms during common cold according to the doctor's record. *Echinacea purpurea* dry extract from 95% herba and 5% radix and its concentrated preparation were significantly more effective than the special *Echinacea* extract or placebo. All treatments were well tolerated. Among the *Echinacea* groups the frequency of adverse events was not significantly higher than in the placebo group. Therefore, *Echinacea* concentrate as well as *Echinacea purpurea* dry extract from 95% herba and 5% radix represent a low-risk and effective alternative to the standard symptomatic medicines in the acute treatment of common cold (Brinkeborn *et al.*, 1999).

A double-blind randomized placebo-controlled trial was performed on 263 patients to evaluate the use of commercially available fixed combination herbal remedy containing ethanolic-aqueous extracts of *Herba thujae occidentalis*, *Radix echinaceae* (*purpureae* + *pallidae* = 1 + 1) and *Radix baptisiae*, 2, 7.5 and 10 mg per tablet, respectively. The aim of the study was to verify clinical efficacy shown in recent studies under (i) good clinical practice (GCP) quality assurance and (ii) common situations at family doctors. Patients attending one of 15 study centres (practitioners) as a result of an acute common cold were randomised to the double-blind placebo-controlled study. Three tablets of study medication were applied t.i.d. for 7 to 9 days. Patients daily documented the intensity of 18 cold symptoms, as well as the cold overall, using a 10-point scale and estimated their general well-being using the Welzel-Kohnen colour scales. Additionally, the severity of illness was assessed by the physician on days 4 and 8 (CGI-1). The main and confirmatory outcome measure was expressed as a total efficacy value. This was gauged from the z-standardised AUC values of the primary endpoints (rhinitis score, bronchitis score, CGI-1 and general well-being). Adverse events, overall tolerability, vital signs and laboratory parameters were documented. For safety analysis, all patients were used. 259 patients were evaluable for primary efficacy analysis (ITT). Results were confirmed analysing only the 238 valid cases (VCs). The primary efficacy parameters showed the superiority of the herbal remedy over placebo ($p < 0.05$). Effect size was 20.6% of the standard deviation (90% CI: 0.04-41.1%; ITT) and 23.1% (1.7-44.5%;

VC). In relation to the general well-being, the effect size was 33.9% of the standard deviation (12.5-55.3%; VC). Patients who suffered from at least moderate symptom intensity at baseline showed response rates (at least 50% improvement of the global score, day 5) of 55.3% in the herbal remedy group and 27.3% in the placebo group ($p = 0.017$; NNT = 3.5). In the subgroup of patients who started therapy at an early phase of their cold, the efficacy of the herbal remedy was most prominent ($p = 0.014$ for the primary efficacy parameter). The therapeutic benefit of the herbal remedy had already occurred on day 2 and reached significance ($p < 0.05$) on day 4, and continued until the end of the treatment in the total score of symptoms, bronchitis score and rhinitis score, as well as in the patients' overall rating of the cold intensity. At that time, equal levels of improvement were reached three days earlier in the verum group than in the placebo group. In 26 patients receiving the herbal remedy and 23 patients receiving placebo, adverse events were reported. Adverse drug reactions were suspected in 2 patients in the verum group and in 4 patients in the placebo group. Serious adverse events did not occur. This study shows that the herbal remedy is effective and safe. The therapeutic benefit consists of a rapid onset of improvement of cold symptoms. If patients with colds are able to start the application of the herbal remedy as soon as practical after the occurrence of the initial symptoms, the benefit would be expected to increase (Henneicke-von Zepelin *et al.*, 1999).

The aim of another study was to determine the efficacy of an *Echinacea* compound herbal tea preparation comprising *E. purpurea* herb and extract of *E. purpurea* root equivalent to 1275 mg dried herb and root per tea bag, (further details about the product are not reported in the article) given at early onset of cold or flu symptoms in a random assignment double-blind placebo-controlled study. A total of 95 subjects with early symptoms of cold or flu (runny nose, scratchy throat, fever) were randomly assigned to receive *Echinacea* compound herbal tea 5 to 6 cups per day titrating to 1 over 5 days or placebo in a double-blind situation. Each participant completed a questionnaire 14 days after beginning the program. The efficacy, number of days the symptoms lasted and number of days for change were measured with a self scoring questionnaire. The study period was 90 days (1 January 1999 to 30 March 1999). There was a significant difference between the experimental group (*Echinacea* compound herbal tea preparation) and control group (placebo) for all 3 questions measured: $p < 0.001$. There were no negative effects reported by any of the subjects in either group. Treatment with *Echinacea* compound herbal tea at early onset of cold or flu symptoms was effective for relieving these symptoms in a shorter period of time than a placebo (Lindenmuth and Lindenmuth, 2000).

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

time failed to detect statistically significant differences between the *Echinacea* and placebo groups (Barrett *et al.*, 2002).

A formulation containing alkamides, cichoric acid, and polysaccharides at concentrations of 0.25, 2.5, and 25 mg/ml, respectively, was prepared from freshly harvested *Echinacea purpurea* herb and root. The objective of this study was to test the efficacy of this highly standardized formulation in reducing the severity and duration of symptoms of a naturally acquired common cold. In a randomized, double-blind, placebo-controlled trial, 282 subjects aged 18-65 years with a history of two or more colds in the previous year, but otherwise in good health, were recruited. The subjects were randomized to receive either *Echinacea* or placebo. They were instructed to start the *Echinacea* or placebo at the onset of the first symptoms related to a cold, consuming 10 doses the first day and 4 doses per day on subsequent days for 7 days. Severity of symptoms (10-point scale: 0, minimum; 9, maximum) and dosing were recorded daily. A nurse examined the subjects on the mornings of days 3 and 8 of their cold. A total of 128 subjects contracted a common cold (59 *Echinacea*, 69 placebo). The total daily symptom scores were found to be 23.1% lower in the *Echinacea* group than in placebo in those who followed all elements of the study protocol ($P < 0.01$). Throughout the treatment period, the response rate to treatments was greater in the *Echinacea* group. A few adverse event profiles were observed in both groups. Early intervention with a standardized formulation of *Echinacea* resulted in reduced symptom severity in subjects with naturally acquired upper respiratory tract infection. Further studies with larger patient populations appear to be warranted (Goel *et al.*, 2004).

Barrett *et al.* (2010) designed a randomized, controlled trial with the aim to assess the potential benefits of *Echinacea* as a treatment of common cold. There were 719 patients included, aged 12 to 80 years, with new-onset common cold. Patients were assigned to 1 of 4 parallel groups: no pills, placebo pills (blinded), *Echinacea* pills (blinded), or *Echinacea* pills (unblinded, open-label). *Echinacea* groups received the equivalent of 10.2 g of dried *Echinacea* root during the first 24 hours and 5.1 g during each of the next 4 days. Indistinguishable placebo tablets contained only inert ingredients. *Echinacea* tablets contained the equivalent of 675 mg of *E. purpurea* root and 600 mg of *E. angustifolia* root, each standardized to 2.1 mg of alkamides. The primary outcome was the area under the curve for global severity, with severity assessed twice daily by self-report using the Wisconsin Upper Respiratory Symptom Survey, short version. Secondary outcomes included interleukin-8 levels and neutrophil counts from nasal wash, assessed at intake and 2 days later. Of the 719 patients enrolled, 713 completed the protocol. Mean age was 33.7 years, 64% were female, and 88% were white. Mean global severity was 236 and 258 for the blinded and unblinded *Echinacea* groups, respectively; 264 for the blinded placebo group; and 286 for the no-pill group. A comparison of the 2 blinded groups showed a 28-point trend (95% CI, -69 to 13 points) toward benefit for *Echinacea* ($P = 0.089$). Mean illness duration in the blinded and unblinded *Echinacea* groups was 6.34 and 6.76 days, respectively, compared with 6.87 days in the blinded placebo group and 7.03 days in the no-pill group. A comparison of the blinded groups showed a nonsignificant 0.53-day (CI, -1.25 to 0.19 days) benefit ($P = 0.075$). Median change in interleukin-8 levels and neutrophil counts were also not statistically significant (30 ng/L and 1 cell/high-power field [hpf] in the no-pill group, 39 ng/L and 1 cell/hpf in the blinded placebo group, 58 ng/L and 2 cells/hpf in the blinded *Echinacea* group, and 70 ng/L and 1 cell/hpf in the open-label *Echinacea* group). Higher-than-expected variability limited power to detect small benefits. Illness duration and severity were not statistically significant with *Echinacea* compared with placebo. These results do not support the ability of this dose of the *Echinacea* formulation to substantively change the course of the common cold.

Jawad *et al.* (2012) investigated the safety and efficacy of *E. purpurea* extract in the prevention of common cold episodes in a large population over a 4-month period creating a randomized, double-blind and placebo-controlled study. Therefore, 755 healthy subjects were allocated to receive either placebo or an alcohol extract from freshly harvested *E. purpurea*. Extract was prepared by alcoholic

(57.3% m/m) extraction from freshly harvested *E. purpurea* with a combination of 95% herba (DER = 1:12) and 5% roots (DER = 1:11). The sample was microbiologically tested and proven to be free of endotoxins. The batch used in this study (027643) was standardized to contain 5 mg/100 g of dodecatetraenoic acid isobutylamide, based on high performance liquid chromatography measurements. Participants were required to record adverse events and to rate cold-related issues in a diary throughout the investigation period. Nasal secretions were sampled at acute colds and screened for viruses. A total of 293 adverse events occurred with *Echinacea* and 306 with placebo treatment. Nine and 10% of participants experienced adverse events, which were at least possibly related to the study drug (adverse drug reactions). Thus, the safety of *Echinacea* was noninferior to placebo. *Echinacea* reduced the total number of cold episodes, cumulated episode days within the group, and pain-killer medicated episodes. *Echinacea* inhibited virally confirmed colds and especially prevented enveloped virus infections ($P < 0.05$). *Echinacea* showed maximal effects on recurrent infections, and preventive effects increased with therapy compliance and adherence to the protocol. Compliant prophylactic intake of *E. purpurea* over a 4-month period appeared to provide a positive risk to benefit ratio.

Tiralongo *et al.* (2012) examined whether a standardised *Echinacea* formulation (tablets containing 112.5 mg *E. purpurea* 6:1 root extract and 150 mg *E. angustifolia* 4:1 root extract, extraction solvent is not reported in the article) is effective in the prevention of respiratory and other symptoms associated with long-haul flights. Therefore, 175 adults participated in a randomised, double-blind placebo-controlled trial travelling back from Australia to America, Europe, or Africa for a period of 1-5 weeks on commercial flights via economy class. Participants took *Echinacea* (root extract, standardised to 4.4 mg alkylamides) or placebo tablets. Participants were surveyed before, immediately after travel, and at 4 weeks after travel regarding upper respiratory symptoms and travel-related quality of life. Respiratory symptoms for both groups increased significantly during travel ($P < 0.0005$). However, the *Echinacea* group had borderline significantly lower respiratory symptom scores compared to placebo ($P = 0.05$) during travel. Supplementation with standardised *Echinacea* tablets, if taken before and during travel, may have preventive effects against the development of respiratory symptoms during travel involving long-haul flights.

The study by Barth *et al.* (2015) was performed with an oral solution (KJ) containing a fixed combination of aqueous ethanolic extracts of *Justicia adhatoda* L. leaf, *E. purpurea* root, and *Eleutherococcus senticosus* (Rupr. & Maxim.) Harms root. The KJ solution contained 9 mg/ml genuine extract (in 55% ethanol) from 23–63 mg dried root of *Echinacea purpurea* radix, 14 mg/ml genuine extract (in water) from 49–70 mg dried leaves of *Justicia adhatoda* folium and 2mg/ml genuine extract (in 70 % ethanol) from 34–60 mg dried root of *Eleutherococcus senticosus* radix. The solution base comprised sorbitol, aroma, ginger extract, peppermint oil, dark syrup, benzoate and water. The preparation was standardized on contents of vasicine (0.2mg/ml), chicoric acid (0.8mg/ml), and eleutherosides B and E (0.03 mg/ml). It is approved in Scandinavia as an herbal medicinal product for respiratory tract infection treatment. The clinical trial aimed to compare the antitussive effect of KJ with placebo (PL) and bromhexine (BH) among patients of 18-65 years old with non-complicated upper respiratory infections (URI). The study performed a parallel-group, randomized, double-blinded, placebo-controlled trial in 177 patients with acute URI over a 5 day period. It investigated the antitussive effects of a KJ (30 ml/day; 762 mg genuine extracts with standardized contents of 0.2 mg/ml vasicine, 0.8 mg/ml chicoric acid, and 0.03 mg/ml eleutherosides B and E), bromhexine hydrochloride (24 mg/30 ml/day) and PL on cough and blood markers. The primary outcome was cough relief, which was assessed as the change of cough frequency from baseline (cough index). Secondary outcomes were safety with regards to reported adverse events (AEs) and hematological data. Both KJ and BH relieved cough more effectively than placebo. On the third and fourth days of treatment, faster improvement in the group receiving KJ compared to in the groups receiving BH

(100%) or PL (100%) was observed, indicating a slightly shorter recovery time in the KJ group. KJ showed a good tolerability and safety profile. KJ exerted significant antitussive effects in URI.

Rauš *et al.* (2015) performed a randomized, double-blind, double-dummy, multicenter, controlled clinical trial in which they compared *Echinacea* with the neuraminidase inhibitor oseltamivir. Following informed consent, 473 patients with early influenza symptoms (≤ 48 hours) were recruited in primary care in the Czech Republic and randomized to either 5 days of oseltamivir followed by 5 days of placebo, or 10 days of an *E. purpurea*-based hotdrink formulation. The hotdrink contained the ethanolic extract (65% V/V) of freshly harvested *E. purpurea*. The tinctures from herba (drug extraction ratio, DER 1:12) and from the roots (DER 1:11) are combined at a ratio of 95% to 5%. 240 mg of above active ingredient was concentrated to extractum spissum, which was supplemented with 276.5 mg of *Sambucus fructus succus recentis* (elderberry) and excipients were added sufficient to give 1 ml of hotdrink. The proportion of recovered patients (influenza symptoms rated as absent or mild in the evening) was analyzed for noninferiority between treatment groups using a generalized Wilcoxon test with significance level $\alpha = 0.05$ (2-sided) and using a CI approach in the per-protocol sample. Recovery from illness was comparable in both treatment groups at 1.5% versus 4.1% after 1 day, 50.2% versus 48.8% after 5 days, and 90.1% versus 84.8% after 10 days of treatment with *E. purpurea*-based hotdrink formulation and oseltamivir, respectively. Noninferiority was demonstrated for each day and overall (95% CI, 0.487–0.5265 by generalized Wilcoxon test). Very similar results were obtained in the group with virologically confirmed influenza virus infections and in a retrospective analysis during the peak influenza period. The incidence of complications was lower with *E. purpurea*-based hotdrink formulation than with oseltamivir (2.46% vs 6.45%; $P = 0.076$) and fewer adverse events (particularly nausea and vomiting) were observed with *E. purpurea*-based hotdrink formulation. In this study it was demonstrated that *E. purpurea*-based hotdrink formulation was as effective as oseltamivir in the early treatment of clinically diagnosed and virologically confirmed influenza virus infections with a reduced risk of complications and adverse events.

Table 8: Clinical studies on humans with *Echinaceae purpureae* radix and its combinations

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Prevention of upper respiratory tract infections, Melchart <i>et al.</i> 1998	Placebo-controlled, double-blinded, randomized	Twice daily for 12 weeks 50 drops (ca. 1 ml) of one of three trial preparations: group A: ethanolic extract (1: 11, ethanol 30%) of <i>E. purpurea</i> radix (n = 99), group B: ethanolic extract (1: 11, ethanol 30%) of <i>E. angustifolia</i> root (n = 100), group C: ethanolic placebo solution (n = 99); duration: 12 weeks	289, 45 drop out	Not mentioned	Primary endpoint: time until first occurrence of first upper respiratory tract infection → relative reduction in risk of infection of 20 % for <i>E. purpurea</i> root, but not significant; secondary endpoint: number of participants with at least one infection	Not mentioned	Effects on prevention of upper respiratory tract infections could not be shown.
Treatment of common cold, Barrett <i>et al.</i> 2010	Placebo-controlled, randomized; placebo and <i>Echinacea</i> pills blinded, <i>Echinacea</i> pills unblinded (open-label)	4 groups: 1) no pills, 2) placebo pills (blinded), 3) <i>Echinacea</i> pills (blinded), 4) <i>Echinacea</i> pills (unblinded, open-label) <i>Echinacea</i> tablets = equivalent of 675 mg of <i>E. purpurea</i> root and 600 mg of <i>E. angustifolia</i> root, each	719 patients (12-80 years), 64 % female, 88 % white; 6 drop out	Patients with new-onset common cold (Jackson and colleagues' criteria)	Primary outcome = area under the curve for global severity, with severity assessed twice daily by self-report using the Wisconsin Upper Respiratory Symptom Survey, short version; secondary outcomes included interleukin-8 levels and neutrophil counts from nasal wash,	Mann-Whitney-U-Test, Box-Cox transformation	<i>Echinacea</i> cannot substantively change course of common cold.

		standardized to 2.1 mg of alkamides; <i>Echinacea</i> groups received the equivalent of 10.2 g of dried <i>Echinacea</i> root during the first 24 hours and 5.1 g during each of the next 4 days.			assessed at intake and 2 days later		
Safety and efficacy in treatment of common cold, Jawad <i>et al.</i> 2012	Placebo-controlled, double-blinded, randomized, parallel trial	Ethanollic extract from freshly harvested <i>E. purpurea</i> (95% herba and 5% root) or placebo; Regimen: 3 x 0.9 ml/day (2400 mg extract/day), 5 x 0.9 ml/day (4000 mg extract/day) during acute stages of cold; duration: 4 months; each application dose was diluted in water and retained in the mouth for 10 s	755 adults, 82 drop out (Echinaforce: 244 female, 111 male; placebo: 227 female, 135 male)	Healthy, but experience of at least two colds/year.	Cold-related issues' diary	ITT yes, chi-square test	Reduction of total number of cold episodes, "positive risk to benefit ratio", the largest study up to date.
Prevention of respiratory and other symptoms associated with long-haul flights;	Placebo-controlled, double-blinded, randomized	Tablets containing 112.5 mg <i>E. purpurea</i> 6:1 root extract and 150 mg <i>E. angustifolia</i> 4:1 root extract or placebo, orally; duration: 4 weeks; Regimen: treatment 14 days	175 adults randomized, 5 drop out before allocated to intervention. → 170	Long-haul flight from Australia to America, Europe or Africa for a period of 1-5 weeks on	Primary endpoint: Respiratory symptom score (Quality of Life) → significantly lower in the <i>Echinacea</i> group; secondary endpoint:	ITT yes, Non parametric Kolmogoro v-Smirnov test, 2 x 2 chi-	Supplementation with standardised <i>Echinacea</i> tablets, if taken before and during travel, may have

Tiralongo <i>et al.</i> 2012		prior to travel, during travel and 14 days after travel	allocated (113 female, 67 male), on average 43 years old	commercial flights via economy class.	occurrence and duration of jet lag, headache, sleep pattern, herpes simplex sores	squared test, <i>t</i> -test	preventive effects against the development of respiratory symptoms during travel. However, secondary endpoints did not reveal a significance.
Evaluation of use of available combinations for treatment of the common cold or flu; Lindenmuth and Lindenmuth 2000	Placebo-controlled, double-blinded, randomized	<i>Echinacea</i> compound herbal tea (<i>E. purpurea</i> herb and extract of <i>E. purpurea</i> root equivalent to 1275 mg dried herb and root per tea bag), placebo: cinnamon, ginger, peppermint; Regimen: 5 to 6 cups/day titrating to 1 over 5 days; duration of treatment: 90 days	95 patients (81 female, 14 male), 24-62 years, drop out not available/found	Hospital patients with early symptoms of cold and flu.	Self-scoring questionnaire with regard to cold/flu symptoms → significant difference between <i>Echinacea</i> group and placebo group	Not available / found	<i>Echinacea</i> compound herbal tea at early onset of cold or flu may shorten symptom period; limitations: mainly females and hospital patients.

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

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

Echinacea purpurea root extract was not statistically significant superior than placebo in preventing of upper respiratory tract infection in one double-blind, placebo-controlled study (Melchart *et al.*, 1998). In only one double-blind, placebo-controlled study on patients with influenza, a tincture of purple coneflower root (1:5, ethanol 55%) showed significant reduction in symptom score at both time points (after 3-4 and 8-10 days) and the effect was dose-dependent (Bräunig *et al.*, 1992).

In many double-blind, placebo-controlled trials *Echinacea purpurea* root in combination with other herbal drugs (*Echinacea purpurea* herb, *E. angustifolia* root or other plant species) was reported to be efficient in reducing the severity of symptoms of common cold or flu. One placebo-controlled, double-blinded and randomized trial found that *Echinacea purpurea* root in combination with other herbal drugs could reduce the total number of cold episodes. Another PCR trial attributed benefits in prevention of upper respiratory infections (URI) to a combination including *Echinacea purpurea* root and one in treatment of an URI. There were no significant differences between placebo and verum group in two double-blind, placebo-controlled trials in reducing the severity of common cold or flu. The results are difficult to interpret, it is difficult to say which component is the active one, and the pharmacological effects are probably achieved by synergistic effect.

The clinical evidence of efficacy is not sufficient for *Echinacea purpurea* root extract to be considered as well-established medicinal product but it supports plausibility of efficacy of the relief of symptoms of common cold based on the long standing traditional use.

There are no clinical evidence on the efficacy of *Echinacea purpurea* root dry extracts for the relief of spots and pimples due to mild acne.

5. Clinical Safety/Pharmacovigilance

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

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

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

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

Table 9: Clinical safety data from clinical trials

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
Brinkeborn <i>et al.</i> , 1999	Placebo-controlled, double-blinded, randomized	3 times daily 2 tablets of either (<i>Echinacea purpurea</i> -preparation from 95% herba and 5% radix), <i>Echinacea purpurea</i> concentrate (same preparation at 7 times higher concentration), special <i>Echinacea purpurea</i> radix preparation (dry extract from root, 29.6 mg per tablet) or placebo until they felt healthy again but not longer than 7 days.	246 volunteers	Patients with common cold	"Frequency of adverse events was not significantly higher than in the placebo group".	Safe treatment of common cold.
Barrett <i>et al.</i> , 2010	Placebo-controlled, randomized; placebo and <i>Echinacea</i> pills blinded, <i>Echinacea</i> pills unblinded (open-label)	4 groups: 1) no pills, 2) placebo pills (blinded), 3) <i>Echinacea</i> pills (blinded), 4) <i>Echinacea</i> pills (unblinded, open-label) <i>Echinacea</i> tablets = equivalent of 675 mg of <i>E. purpurea</i> root and 600 mg of <i>E. angustifolia</i> root, each standardized to 2.1 mg of alkamides; <i>Echinacea</i> groups received the equivalent of 10.2 g of dried <i>Echinacea</i> root during the first 24 hours and 5.1 g during each of the next 4 days	719 patients (12-80 years), 64% female, 88% white; 6 drop out	Patients with new-onset common cold (Jackson and colleagues' criteria)	Frequency of potential adverse effects was similar (statistically indistinguishable) in the 4 groups. The only possible exception was headache (62% in the no-pill group, fewer than 50% in the 3 pill groups. Responses to openended questions about possible adverse effects during monitoring showed no patterns of adverse effects attributable to <i>Echinacea</i> .	Headache might be a possible adverse event, but no responses to openended questions about adverse events.

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
Jawad <i>et al.</i> , 2012	Placebo-controlled, double-blinded, randomized, parallel trial	Ethanol extract from freshly harvested <i>E. purpurea</i> (95% herba and 5% root) or placebo; Regimen: 3 x 0.9 ml/day (2400 mg extract/day), 5 x 0.9 ml/day (4000 mg extract/day) during acute stages of cold; Duration: 4 months; Each application dose was diluted in water and retained in the mouth for 10 s	755 adults, 82 drop out (<i>Echinacea</i> : 244 female, 111 male; placebo: 227 female, 135 male)	Healthy, but experience of at least two colds/year	293 adverse events (but no serious) occurred with <i>Echinacea</i> and 306 with placebo treatment. Nine and 10% of participants experienced adverse events.	No serious adverse events, <i>Echinacea</i> appears to be safe.
Rauš <i>et al.</i> , (2015)	Randomized, double-blind, double-dummy, multicenter, controlled	Group 1: <i>Echinacea</i> hotdrink commercial preparation= a hydro-ethanolic extract (65% V/V) of freshly harvested <i>Echinacea purpurea</i> . The tinctures from herba (drug extraction ratio, DER 1:12) and from the roots (DER 1:11) are combined at a ratio of 95% to 5%. 240 mg of above active ingredient was concentrated to extractum spissum, which was supplemented with 276.5 mg of <i>Sambucus fructus succus recentis</i> (elderberry) and excipients were added sufficient to give 1 ml of <i>Echinacea</i> hotdrink.	473 patients, 12-70 years, Czech Republic, drop out: 50 patients	Patients; Inclusion criteria: early influenza symptoms (≤ 48 hours): at least one respiratory symptom, constitutional symptom and fever, symptoms not present for more than 48 hours;	26/229 patients in the <i>Echinacea</i> hotdrink commercial preparation group reported adverse events, 4 were assessed to be related to <i>Echinacea</i> . Fewer adverse events (particularly nausea and vomiting) were observed with <i>Echinacea</i> hotdrink commercial preparation compared to oseltamivir.	<i>Echinacea</i> appears to be a safer treatment than oseltamivir. Adverse events were rare; no serious adverse events were discovered.

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
		<p>Group 2:</p> <p>5 days oseltamivir followed by 5 days of placebo;</p> <p>Duration of treatment: 10 days;</p> <p>Regimen:</p> <p>group 1: days 1-3: 5 x 5 ml <i>Echinacea</i> hotdrink commercial preparation verum syrup, dissolved in 150 ml hot water;</p> <p>days 4-10: 3 x 5 ml;</p> <p>group 2: oseltamivir/placebo days 1-10: 2 x 1 capsule, orally</p>		negative pregnancy test, body weight of > 40 kg, good general health, signed informed consent.		
Henneicke-von Zepelin et al., 1999	Placebo-controlled, double-blinded, randomized	<p>Ethanollic-aqueous extracts of <i>Herba thujae occidentalis</i>, <i>Radix echinaceae</i> (<i>purpureae</i> + <i>pallidae</i> = 1 + 1) and <i>Radix baptisiae</i>, 2, 7.5 and 10 mg per tablet, compared to placebo</p> <p>Regimen:</p> <p>3 tablets t.i.d. for 7 to 9 days, orally</p>	<p>263 patients (male and female), 18-70 years, drop out: 21,</p> <p>238 valid cases for secondary efficacy analysis</p>	Patients who were attending their family doctor for acute common cold.	2 questionable, 1 possible adverse event (insomnia), no serious events.	<i>Echinacea</i> can be stated as safe.
Lindenmuth and Lindenmuth,	Placebo-controlled, double-blinded, randomized	Herbal tea <i>Echinacea</i> preparation (comprising <i>E. purpurea</i> herb and extract of <i>E. purpurea</i> root equivalent to 1275 mg dried herb and root per tea bag),	95 patients (81 female, 14 male), 24-62 years, drop out not	Hospital patients with early symptoms of cold and flu.	No adverse reactions reported.	Safe treatment

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
2000		Placebo: cinnamon, ginger, peppermint; Regimen: 5 to 6 cups/day titrating to 1 over 5 days; Duration of treatment: 90 days	available/found			
Goel <i>et al.</i> , 2004	Placebo-controlled, double-blinded, randomized	Formulation containing alkamides, cichoric acid, and polysaccharides at concentrations of 0.25, 2.5, and 25 mg/ml, prepared from freshly harvested <i>E. purpurea</i> herb and root (Echinilin®), Placebo; Regimen: 10 doses the first day, 4 doses per day on subsequent days for 7 days	282 subjects (18-65 years) randomized, 128 contracted a cold and were subjected to ITT, drop out: 17/128	Subjects with a history of two or more colds in the previous year, but otherwise in good health.	"A few adverse event profiles were observed in both groups" (in the article, it is not further specified).	Lack of information about adverse events in the article.
Barth <i>et al.</i> , 2015	Placebo-controlled, double-blinded, randomized, parallel-group	Kan Jang® oral solution (KJ) = fixed combination aqueous ethanolic extracts of <i>Justicia adhatoda</i> leaf, <i>E. purpurea</i> root, and <i>Eleutherococcus senticosus</i> root, 30 ml/day, orally; 762 mg genuine extracts with standardized contents of 0.2 mg/ml vasicine, 0.8 mg/ml chicoric acid, and 0.03 mg/ml eleutherosides B and E), compared with placebo and bromhexine (24 mg/30 ml/day); duration: 5 days	177 patients (101 female, 76 male), 18-65 years, drop out not available / found	Patients with non-complicated upper respiratory infections (URI; i.e., common cold).	2 minor adverse events in the KJ group (3.9 %), minor adverse events: pruritus, diarrhea, abdominal pain, skin rash; no serious adverse events.	Safe treatment

Table 10: Pharmacovigilance reports from Member States (causality has not been evaluated)

Finland	<p>Literature: Case report in the Finnish Medical Journal (3/2002): A man with chronic alcoholism used for years high-concentration ethanol extract of <i>Echinacea</i> during his drinking binges as he had no money to buy liquor but stealing these herbal products containing 60 to 70% of ethanol from natural product stores was easy (as opposed to stealing liquor from state liquor stores). He had twice been hospitalized with a blood concentration of ethanol of 4,8 promilles. Similar use was stated to be common in adolescents at that time.</p> <p>(The applicant has been requested to equip the products with a childproof closure but this variation is pending).</p>
Ireland	<p>There have been 13 case reports associated with <i>Echinacea</i> containing products with 26 adverse reactions as follows:</p> <p>3 x abdominal pain, 2 x headache, 2 x urticaria, 1 x amenorrhoea, arthralgia, attention deficit/hyperactivity disorder, bone disorder, chest pain, dizziness, fatigue, feeling drunk, goitre, hypoglycaemia, oedema, parkinsonism, pharyngitis, pregnancy on oral contraceptive, purpura, renal impairment, white blood cell disorder, abdominal discomfort, pruritus</p>

5.2. Patient exposure

Data obtained from 2935 patients, tested for safety during clinical trials described above, showed the following results: Only few minor adverse events were reported, e. g. pruritus, diarrhea or headache, and no serious adverse events occurred. Some articles do not specify adverse events reported. According to these data, preparations of *Echinacea purpurea* radix in combination with other botanical preparations can be assessed as safe.

Aside from data about the presence of many products containing *Echinacea purpurea* radix on the market and data from studies, there are no concrete data concerning patient exposure.

5.3. Adverse events, serious adverse events and deaths

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

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

Six major herbal references published from 1996 to 2000 were selected to evaluate the adequacy of their toxicological information in light of published adverse events. To identify herbs most relevant to toxicology, herbal-related calls to regional California Poison Control System, San Francisco division (CPCS-SF) in 1998 were reviewed and 12 herbs were identified (defined as botanical dietary supplements) most frequently involved in these CPCS-SF referrals. Medline was searched (1966 to 2000) to identify published reports of adverse effects potentially related to these same 12 herbs. Each herbal reference text was scored on the basis of information inclusiveness for the target 12 herbs, with a maximal overall score of 3. The herbs, identified on the basis of CPCS-SF call frequency were: St John's wort, ma huang (*Ephedra* spp.), echinacea, guarana, ginkgo, ginseng, valerian, tea tree oil, goldenseal (*Hydrastis canadensis*), arnica, yohimbe (*Pausinystalia johimbe*) and kava kava (*Piper methysticum*). 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.*, 2002).

Reports to poison control centers (PCCs) were characterised involving two widely used herbal dietary supplements (HDSs), *Echinacea*, and St. John's wort (SJW). METHODS: Data were purchased from the American Association of Poison Control Center's (AAPCC) toxic exposure surveillance system (TESS(R)) on reports made to PCCs in 2001 involving *Echinacea* or SJW. Analyses were limited to those cases in which *Echinacea* or SJW were the only associated products, and in which these HDSs were deemed primary to observed adverse effects. Descriptive statistics were generated for selected demographic

and exposure-related variables. During 2001, PCCs were contacted regarding 406 exposures involving *Echinacea* and 356 exposures involving SJW. Most of the reported exposures for both HDSs occurred among children 5 years and younger, and the majority of exposures were coded as unintentional. For both HDSs, exposures among patients ≥ 20 years old were more likely to be associated with adverse effects. Intentional exposures accounted for 21% of SJW cases and 3% of *Echinacea* cases, with 13% of SJW exposures reported as 'suspected suicidal'. TESS represents a potentially important means of assessing and characterizing HDS-related adverse effects. It was concluded that detailed studies validating the clinical events and outcomes of a sample of exposures reported to TESS(R) might offer substantial insights into adverse events that could be systematically studied with other, established pharmacoepidemiological study designs (Gryzlak *et al.*, 2007).

Goel *et al.* (2004) mention that there were "a few adverse event profiles" observed in both groups of their trials, but they are not further specified in their article.

In the clinical trial by Jawad *et al.* (2012), 755 healthy subjects received either an ethanolic extract from freshly harvested *E. purpurea* or placebo and were requested to report adverse events. *Echinacea* preparation was prepared by ethanolic (57.3% m/m) extraction from freshly harvested *E. purpurea* with a combination of 95% herba (DER = 1:12) and 5% roots (DER = 1:11). The sample was microbiologically tested and proven to be free of endotoxins. The batch used in this study (027643) was standardized to contain 5 mg/100 g of dodecatetraenoic acid isobutylamide, based on highperformance liquid chromatography measurements. A total of 293 adverse events occurred with *Echinacea* and 306 with placebo treatment. Nine and 10% of participants experienced adverse events, which were at least possibly related to the study drug. In the article it is not announced which kind of adverse events appeared.

The clinical trial by Rauš *et al.* (2015) mentions particularly nausea and vomiting as adverse events. The *Echinacea* preparation was associated with a reduced risk of complications and adverse events compared to oseltamivir.

Barth *et al.* (2015) tested an oral solution combination containing *E. purpurea* radix. Two out of 66 patients in the *Echinacea* group reported minor adverse events (pruritus, diarrhea, abdominal pain, skin rash), but proportion of patients reporting adverse events did not significantly differ between groups. Although it is not specified which minor adverse events were observed in the *Echinacea* group, the oral solution was assessed as well-tolerated.

Serious adverse events and deaths: None reported.

5.4. Laboratory findings

Barth *et al.* (2015) examined hematologic parameters between verum (*Echinacea purpurea* radix in combination) and placebo group, but did not find any statistically significant differences.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

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

Godwin *et al.* (2013) determined how common it is for parents to give natural health products (NHPs) to their children, A total of 53.4% of the 378 eligible parents who were contacted completed the

survey. This represented 333 children. Mean (SD) age of the children was 5.1 (3.3) years. Overall, 28.7% of parents reported using nonvitamin NHPs for their children: teas (primarily chamomile and green teas), *Echinacea*, fish or omega-3 oils, and a large category of "other" products. These NHPs were most commonly used to improve general health, improve immunity, and prevent colds and infections. Approximately half of the parents (51.7%) believed their children had benefited from taking NHPs, and 4.4% believed their children had experienced adverse side effects.

5.5.2. Contraindications

In case of hypersensitivity to the active substance or to other plants of the *Asteraceae* (*Compositae*) family the use is contraindicated. No other concerns requiring a contraindication were identified.

5.5.3. Special warnings and precautions for use

Based on the assumption that *Echinacea purpurea* radix has immunomodulatory effects, some authors declared, that its use is contraindicated in progressive systemic diseases such as tuberculosis, diseases of the white blood cells system, collagenoses, multiple sclerosis, AIDS, HIV infections, and other immune diseases (Barnes *et al.*, 2005; Barnes *et al.*, 2007; ESCOP 2003 and 2009; Bauer and Liersch, 1993; Blumenthal *et al.*, 2000). None of them, however, had made a thorough assessment on this issue.

At present there is a lack of reliable clinical evidence to support the immunomodulatory effects of *Echinacea*, but in the view of the seriousness of the conditions listed above it is appropriate to avoid use in these disorders until further information is available (Barnes *et al.*, 2005; Barnes *et al.*, 2007).

In accordance with the 'Guideline on the Summary of Product Characteristics' dated September 2009, the statement that *Echinacea purpurea radix* is not recommended in progressive systemic disorders, autoimmune diseases, immunodeficiencies, immunosuppression and diseases of the white blood cell system appears in the section 'Warnings and precautions for use' of the monograph on *Echinaceae purpureae radix* (not as a contraindication).

There is a possible risk of allergic reactions in sensitive individuals. Those patients should consult their doctor before using *Echinacea*.

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). None of these references presented any details on these patients with allergic reactions. There is a possible risk of anaphylactic reactions in atopic patients. Atopic patients should consult their doctor before using *Echinacea*.

5.5.4. Drug interactions and other forms of interaction

The effect of *Echinacea* (*Echinacea purpurea* root) on CYP activity *in vivo* was assessed by use of the CYP probe drugs caffeine (CYP1A2), tolbutamide (CYP2C9), dextromethorphan (CYP2D6), and midazolam (hepatic and intestinal CYP3A). Twelve healthy subjects (6 men) completed this 2-period, open-label, fixed-schedule study. Caffeine, tolbutamide, dextromethorphan, and oral and intravenous midazolam were administered before and after a short course of *Echinacea* (400 mg 4 times a day for 8 days) to determine *in vivo* CYP activities. *Echinacea* administration significantly increased the systemic clearance of midazolam by 34%, from 32 +/- 7 L/h to 43 +/- 16 L/h (P =0.003; 90% CI, 116%-150%), and significantly reduced the midazolam area under the concentration-time curve by 23%, from 127 +/- 36 microg. h/L to 102 +/- 43 microg. h/L (P =0.024; 90% CI, 63%-88%). In contrast, the oral clearance of midazolam was not significantly altered (P =0.655; 90% CI, 75%-124%), 137 +/- 19 L/h compared with 146 +/- 71 L/h. The oral availability of midazolam after

Echinacea dosing was significantly increased ($P = 0.028$; 90% CI, 108%-153%), from 0.23 ± 0.06 to 0.33 ± 0.13 . Hepatic availability (0.72 ± 0.08 versus 0.61 ± 0.16 ; $P = 0.006$; 90% CI, 73%-90%) and intestinal availability (0.33 ± 0.11 versus 0.61 ± 0.38 ; $P = 0.015$; 90% CI, 125%-203%) were significantly altered in opposite directions. *Echinacea* dosing significantly reduced the oral clearance of caffeine, from 6.6 ± 3.8 L/h to 4.9 ± 2.3 L/h ($P = 0.049$; 90% CI, 58%-96%). The oral clearance of tolbutamide was reduced by 11%, from 0.81 ± 0.18 L/h to 0.72 ± 0.19 L/h, but this change was not considered to be clinically relevant because the 90% CIs were within the 80% to 125% range. The oral clearance of dextromethorphan in 11 CYP2D6 extensive metabolizers was not affected by *Echinacea* dosing (1289 ± 414 L/h compared with 1281 ± 483 L/h; $P = 0.732$; 90% CI, 89%-108%). *Echinacea* (*E. purpurea* root) reduced the oral clearance of substrates of CYP1A2 but not the oral clearance of substrates of CYP2C9 and CYP2D6. *Echinacea* selectively modulates the catalytic activity of CYP3A at hepatic and intestinal sites. The type of drug interaction observed between *Echinacea* and other CYP3A substrates will be dependent on the relative extraction of drugs at hepatic and intestinal sites. The authors recommended that caution should be used when *Echinacea* is co-administered with drugs dependent on CYP3A or CYP1A2 for their elimination (Gorski *et al.*, 2004).

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

A report on possible drug-herbal interaction between *Echinacea* (details of drug administration not stated) and etoposide was published in 2012 (Bossauer & Odle, 2012). A 61-year-old man newly diagnosed with nonsmall cell lung cancer began concurrent chemoradiation with cisplatin and etoposide. He was admitted to the hospital on day 8 of his first cycle and found to be thrombocytopenic. His platelet count eventually reached a nadir of 16×10^3 /l, requiring platelet transfusion support. Upon admission, it was discovered that he was taking vitamin B12, vitamin E, vitamin D, vitamin C, *Echinacea* and 'vitamin B17' (laetrile-apricots kernel), which were discontinued. He received his next cycle of chemotherapy without taking herbal products and vitamins and with the addition of pegfilgrastim. His platelet count decreased to a nadir of 44×10^3 /l, but he did not require platelet transfusions. Since the patient stopped taking *Echinacea* after cycle 1, subsequent therapy during cycle 2 served as a control to test hypothesis that *Echinacea* (documented in *in vitro* studies to as a cytochrome P450 3A4 inhibitor) interacted with etoposide. As the patient also stopped taking laetrile and his other vitamins after cycle 1, a potential interaction between laetrile and etoposide or cisplatin cannot be fully excluded. Authors of the report concluded that since the exact preparation of *Echinacea* and corresponding plant extract constituents, was unknown, the interaction remains equivocal. Cautions should be exercised in patients receiving chemotherapy including CYP3A4 substrates (antracyclines, etoposide, vinca alkaloids, taxanes) while taking *Echinacea* (Bossauer & Odle, 2012).

Assessor's comment: In a pharmacological study it was found that Echinacea purpurea radix inhibits intestinal CYP3A4 and induces hepatic CYP3A4 (Gorski et al., 2004). Due to unknown formulation and dosages of Echinacea preparation in this case interaction with etoposide could be considered as a signal for interaction between Echinacea purpurea radix preparations and etoposide and other CYP3A4

substrates in spite of the fact that there are no other verifiable reports of drug–herb interactions with any *Echinacea* product. Further pharmacokinetic testing is necessary before conclusive statements can be made about *Echinacea* interaction with other drugs.

5.5.5. Fertility, 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. Using *Echinacea* during lactation is not recommended until further high quality human studies can determine its safety.

The outcome 'pregnancy' in women that used *Echinacea* during pregnancy was studied to evaluate the safety of *Echinacea*. There is no specification which species of *Echinacea* was evaluated. Since at least half of all pregnancies are unplanned, many women inadvertently use *Echinacea* in their first trimester. The study group consisted of 206 women who were prospectively followed up after contacting the Motherisk Program regarding the gestational use of *Echinacea*, 112 women used the herb in the first trimester. This cohort was disease-matched to women exposed to non-teratogenic agents by maternal age, alcohol, and cigarette use. Rates of major and minor malformations between the groups were compared. There were a total of 195 live births, including 3 sets of twins, 13 spontaneous abortions, and 1 therapeutic abortion in the *Echinacea* group. Six major malformations were reported, including one chromosomal abnormality, and 4 of these malformations occurred with *Echinacea* exposure in the first trimester. In the control group, there were 206 women with 198 live births, 7 spontaneous abortions, and 1 therapeutic abortion. Seven major malformations were reported. There were no statistical differences between the study and control groups for any of the endpoints analysed. The authors concluded that gestational use of *Echinacea* during organogenesis is not associated with an increased risk for major malformations (Gallo *et al.*, 2000). The study has several limitations, particularly the small sample size, meaning that the study would have the statistical power only to detect common malformations, and self-report of exposure, since it is possible that misclassification have occurred. In addition participants used a range of different preparations of *Echinacea* at different dosage regimens, so the study does not provide adequate evidence for any specific preparation (Barnes *et al.*, 2007).

In a survey among 400 Norwegian women (Nordeng & Haven 2004) 36% used herbal drugs during pregnancy with an average of 1.7 products per woman. *Echinacea* was used by 23% of pregnant woman and was by far the mostly used herb. No information about the plant species, plant part or type of preparation or duration of intake/trimester is given in the article.

Perri *et al.* (2006) searched 7 electronic databases and compiled data according to the grade of evidence found. Good scientific evidence from a prospective cohort study was found: oral consumption of *Echinacea* during the first trimester did not increase the risk for major malformations (study performed by Gallo *et al.* 2000). Low-level evidence based on expert opinion shows that oral consumption of *Echinacea* in recommended doses is safe for use during pregnancy and lactation. The authors concluded that *Echinacea* is non-teratogenic when used during pregnancy. Caution is advised using *Echinacea* during lactation until further high quality human studies can determine its safety.

Nordeng *et al.* (2011) investigated the use of herbal drugs by pregnant women in relation to concurrent use of conventional drugs, delivery, and pregnancy outcome. Six hundred women at

Stavanger University Hospital Norway were interviewed using a structured questionnaire within five days after delivery. Medical birth charts were reviewed with respect to pregnancy outcome. 39.7% of the women reported the use of herbal drugs during pregnancy, most commonly ginger, iron-rich herbs, *Echinacea* (7.5%) and cranberry. No information about the *Echinacea* species, plant part or type of preparation or duration of intake/trimester is given in the article. Although 86.3% of the women reported to have used conventional drugs during pregnancy there were few potential interactions between herbal drugs and conventional drugs. Except for birth weight, there were no significant differences between users and non-users of herbal drugs in general in any of the pregnancy outcomes investigated. Mean birth weight was higher among the users of herbal drugs during pregnancy (3,663 g vs. 3,508 g). There was a significant association between the use of iron-rich herbs during pregnancy and high birth weight, and use of raspberry leaves and caesarean delivery.

The study of Cuzzolin *et al.* (2010) explored the use of herbal products among Italian pregnant women and the possible influence of herbal consumption on pregnancy outcome. It was conducted over a 10-month period (2 days a week, from January to October 2009) at the Maternity wards of Padua and Rovereto Hospital. Data were collected through a face-to-face interview on the basis of a prestructured questionnaire including socio-demographic characteristics of the enrolled subjects, specific questions on herbal use, information about pregnancy and newborn. In total, 392 interviews were considered. 109 out of 392 women (27.8%) reported to have been taking one or more herbal products during pregnancy, in the 36.7% of cases throughout all pregnancy. The most frequently herbs were chamomile, liquorice, fennel, aloe, valerian, *Echinacea* (9.2%), almond oil, propolis, and cranberry. No information about the *Echinacea* species, plant part or type of preparation or duration of intake/trimester is given in the article. Four out of 109 women (3.7%) reported side-effects: constipation after a tisane containing a mix of herbs, rash and itching after local application of aloe or almond oil. Users were more often affected by pregnancy-related morbidities and their neonates were more frequently small for their gestational age. A higher incidence of threatening miscarriages and preterm labours was observed among regular users of chamomile and liquorice.

Holst *et al.* (2011) performed a survey at the antenatal clinic at Norfolk and Norwich University Hospital between November 2007 and February 2008 among 578 expectant mothers at least 20-weeks pregnant. 57.8% of them used one or more herbal remedies. The most commonly used herbal preparations during pregnancy were ginger, cranberry, raspberry leaf, chamomile, peppermint and *Echinacea*. No information about the *Echinacea* species, plant part or type of preparation is given in the article.

In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

No fertility data are available.

5.5.6. Overdose

No case of overdose has been reported.

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

No data available.

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

The oral administration of *Echinacea purpurea* root extracts can be regarded as safe for intended use, taking into account recommended contraindications, warnings and precautions for use.

Hypersensitivity reactions e.g. skin reactions were observed. Even though the frequency is not known individuals with allergic tendencies particularly those with known allergy to other members of the *Asteraceae* (*Compositae*) family should avoid *Echinacea purpurea* preparations. Atopic patients and those with asthma should be cautious since rare allergic reactions have been reported. They should consult their doctor before using *Echinacea*.

Based on the assumption that *Echinacea purpurea* has immunomodulatory effects, its use is not recommended in cases of progressive systemic disorders, autoimmune diseases, immunodeficiencies, immunosuppression and diseases of the white blood cell system.

There are no sufficient data on safety of purple coneflower root preparations in children under 12 years of age; therefore the use of *Echinacea purpurea* root and preparations thereof is not recommended.

Due to unreliable studies the use during pregnancy and lactation is not recommended in accordance with general medical practice.

Herbal remedies made from *Echinacea purpurea* appear to have a low potential to generate cytochrome P450 (CYP450) drug–herb interactions including CYP 450 1A2 (CYP1A2) and CYP 450 3A4 (CYP3A4). Currently there are no verifiable reports of drug–herb interactions with any *Echinacea* product.

6. Overall conclusions (benefit-risk assessment)

Well-established use of *Echinacea purpurea* root preparations for the relief of symptoms of common cold is not possible, due to insufficient clinical data. Well-established use of *Echinacea purpurea* root preparations is also not possible for the relief of spots and pimples due to mild acne, due to the absence of clinical data.

Traditional use of dry extract of *Echinacea purpurea* root (5.5-7.5:1), extraction solvent ethanol 45% (V/V) for the relief of symptoms of common cold is possible based on its longstanding safe use. Traditional use of dry extract of *Echinacea purpurea* root (4:1), extraction solvent water for the relief of spots and pimples due to mild acne is possible based on its longstanding safe use.

There are data on pharmacological effects of *Echinacea purpurea* root preparations on immune system of adults but the pharmacological mechanisms and active compounds still remain mainly unclear. So far the only compounds for which the oral availability has been established are alkamides.

Safety and plausible efficacy in children under 12 years of age has not established, therefore the use in this age group is not recommended.

Toxicological data are limited. However a certain level of safety could be expected due to the long-standing use of *Echinacea purpurea* root preparations with no serious side effects reported.

If patients with known hypersensitivity to *Echinacea*, or other plants of the *Asteraceae* (*Compositae*) family are excluded (contraindication), a traditional use is possible if administration follows the instructions as specified in the monograph.

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed. An European Union list entry is not supported due to lack of adequate data.

Therapeutic areas for browse /search at the EMA website:

Indication 1: Cough and cold

Indication 2: Skin disorders and minor wounds

Annex

List of references