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

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

<table>
<thead>
<tr>
<th>Herbal substance(s) (binomial scientific name of the plant, including plant part)</th>
<th><em>Echinacea purpurea</em> (L.) Moench, radix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbal preparation(s)</td>
<td>Dry extract (DER 5.5-7.5:1), extraction solvent ethanol 45% (V/V)</td>
</tr>
<tr>
<td></td>
<td>Dry extract (DER 4:1); extraction solvent water</td>
</tr>
<tr>
<td>Pharmaceutical form(s)</td>
<td>Herbal preparation in solid dosage forms for oral and oromucosal use</td>
</tr>
<tr>
<td>Rapporteur(s)</td>
<td>S. Kreft, B. Razinger</td>
</tr>
<tr>
<td>Assessor(s)</td>
<td>S. Läer, D. Janeš</td>
</tr>
<tr>
<td>Peer-reviewer</td>
<td>I. Chinou</td>
</tr>
</tbody>
</table>

Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monograph on *Echinacea purpurea* (L.) Moench, radix. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.
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1. Introduction

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

- Herbal substance(s)

Echinacea purpurea radix (European Pharmacopoeia monograph reference 01/2008: 1824)

Echinacea purpurea radix consists of the whole or cut, dried underground parts of *Echinacea purpurea* (L.) Moench. It contains not less than 0.5% for the sum of caftaric acid (C_{13}H_{12}O_{9}; M, 312.2) and cichoric acid (C_{22}H_{18}O_{12}; M, 474.3) in dried drug.

Constituents (Barnes et al., 2005; Barnes et al., 2007; Bauer and Remiger, 1989; Bradley, 2006; ESCOP, 2009; Bauer and Liérsch, 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-2E,4E,8Z,10E/Z-tetraenoic isobutylamide. Undeca-2Z,4E-diene-8,10-diynoic acid isobutylamide is also prominent. Isobutylamides contain mainly 2,4-dienoic units.

- Caffeic acid derivatives (2.0-2.8%): principally cichoric acid (2,3-O-dicaffeoyltartaric acid, 1.7-2.4%) and caftaric acid (2-O-caffeoyltartaric acid, ca. 0.2-0.8%) also echinacoside, verbascoside, caffeoylchinoside, 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,-tetraine, trideca-8,10,12-triene-2,4,6-triine)

- Non toxic pyrrolizidine alkaloids: tussilagine, isolussilagine.

Baiciunaite et al. (2015) evaluated the protein content in dried roots of *E. 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 any reference to Echinacea’s health-related processes were included, whereas articles without any reference to physiological processes (eg. 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

<table>
<thead>
<tr>
<th>Active substance</th>
<th>Indication</th>
<th>Pharmaceutical form</th>
<th>Regulatory Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IRELAND</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dry extract from Echinacea purpurea radix (DER 6-7:1); extraction solvent: Ethanol 30% V/V</td>
<td>A traditional herbal medicinal product used to relieve common cold and flu-like symptoms in adolescents and adults, exclusively based on long-standing use.</td>
<td>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.</td>
<td>Traditional use registration; July 2014</td>
</tr>
<tr>
<td>dry extract from Echinacea purpurea root (DER 6-8:1); extraction solvent: ethanol 75% V/V</td>
<td>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.</td>
<td>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.</td>
<td>Traditional use registration; January 2014</td>
</tr>
<tr>
<td>dry extract) from Echinacea purpurea root (DER 6-8:1); extraction solvent: ethanol 75% V/V</td>
<td>A traditional herbal medicinal product used to relieve common cold and flu-like symptoms in adults and adolescents over 12 years</td>
<td>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.</td>
<td>Traditional use registration; January 2014</td>
</tr>
<tr>
<td><strong>NETHERLANDS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry extract of E. purpurea radix (DER 6-8:1), extraction solvent: ethanol 75% V/V</td>
<td>Traditional medicinal products for supportive treatment of common cold.</td>
<td>Hard capsule per capsule 140 mg dry extract &gt; 12 years: 1 capsule twice daily consult a doctor if symptoms increase or remain after 10 days of use</td>
<td>THMP; 2011</td>
</tr>
<tr>
<td><strong>SWEDEN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dry extract of Echinacea purpurea (purple coneflower)</td>
<td>Traditional herbal medicinal product used for the relief of</td>
<td>Chewable tablet, 1 tablet contains: 40 mg extract</td>
<td>2009, Traditional use (re-classification)</td>
</tr>
<tr>
<td>Active substance</td>
<td>Indication</td>
<td>Pharmaceutical form</td>
<td>Regulatory Status</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>dried root, (DER 6.1-7:1), extraction solvent: ethanol 45% (V/V)</td>
<td>symptoms of common cold. The product is a traditional herbal medicinal product for use in the specified indication exclusively based upon long-standing use.</td>
<td>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.</td>
<td>Since 1978, on the market as a natural remedy under national legislation.</td>
</tr>
<tr>
<td><strong>UNITED KINGDOM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6-8:1); (extraction solvent: ethanol 75% V/V)</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold, and influenza type infections based on traditional use only.</td>
<td>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.</td>
<td>UK; 2012; THR</td>
</tr>
<tr>
<td>dry extract of <em>Echinacea purpurea</em> root (DER 6-8:1); (extraction solvent: ethanol 75% V/V)</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold, and influenza type infections based on traditional use only.</td>
<td>50 mg extract/coated tablet Adults &amp; 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.</td>
<td>UK; 2013; THR</td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6-8:1); Extraction solvent: Ethanol 75% V/V</td>
<td></td>
<td>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.</td>
<td>UK; 2011; THR</td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6-8:1); Extraction solvent: Ethanol 75% V/V</td>
<td></td>
<td>56 mg extract/film coated tablet Adults, elderly &amp; adolescents: 1 - 2 tablets, twice a day. Start at first signs of common cold Do not take for more than 10 days.</td>
<td>UK; 2011; THR</td>
</tr>
<tr>
<td><em>Echinacea purpurea</em> root powder</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold, and influenza type infections based on traditional use only.</td>
<td>250 mg dry root powder/hard capsule Adults, elderly &amp; adolescents: 1-2 capsules, twice a day Start at first signs of common cold. Do not take for more than 10 days.</td>
<td>UK; 2013; THR First approved in UK 1995 under national legislation</td>
</tr>
<tr>
<td>dry extract of <em>Echinacea purpurea</em></td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold, and influenza type infections based on traditional use only.</td>
<td>30 mg extract/film coated tablet</td>
<td>UK; 2010; THR First approved in UK</td>
</tr>
<tr>
<td>Active substance</td>
<td>Indication</td>
<td>Pharmaceutical form</td>
<td>Regulatory Status</td>
</tr>
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</tr>
<tr>
<td>root (DER 8.1-9.3:1); extraction solvent: Ethanol 45% V/V</td>
<td>to relieve the symptoms of common cold and influenza type infections based on traditional use only.</td>
<td>Adults, elderly &amp; 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.</td>
<td>1993 under national legislation</td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6-7:1); extraction solvent: ethanol 30% V/V</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections based on traditional use only.</td>
<td>71.5 mg/film coated tablet Adults, elderly and adolescents: 1-2 tablets, twice a day. Start at first signs of common cold. Do not take for more than 10 days.</td>
<td>UK; 2010; THR</td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6-7:1); extraction solvent: ethanol 30% V/V</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections based on traditional use only.</td>
<td>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.</td>
<td>UK; 2010; THR</td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6-7:1); extraction solvent: ethanol 30% V/V</td>
<td>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.</td>
<td>71.5 mg/film coated tablet Adults, elderly and adolescents: 1 tablet, three times a day. Do not use the product for more than two weeks.</td>
<td>UK; 2010; THR</td>
</tr>
<tr>
<td>dry extract) from <em>Echinacea purpurea</em> root (DER 6-8:1); (extraction solvent: ethanol 75% V/V</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections, based on traditional use only.</td>
<td>140 mg extract/hard capsule Adults, elderly &amp; adolescents: 1 capsules twice a day. Start at first signs of common cold. Do not take for more than 10 days.</td>
<td>UK; 2011; THR</td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6-8:1); extraction solvent: ethanol 75% V/V</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections, based on traditional use only.</td>
<td>105 mg extract/film coated tablet Adults, elderly &amp; adolescents: 1 tablet twice a day. Start at first signs of common cold. Do not take for more than 10 days.</td>
<td>UK; 2011; THR</td>
</tr>
<tr>
<td>dry extract from <em>Echinacea purpurea</em> root (DER 6.6-7.6:1); extraction solvent: ethanol 30% V/V</td>
<td>A traditional herbal medicinal product used to relieve the symptoms of the common cold and influenza type infections, based on traditional use only.</td>
<td>70 mg extract/tablet Adults, elderly &amp; adolescents: 1 tablet three times a day. Start at first signs of common cold.</td>
<td>UK; 2008; THR</td>
</tr>
<tr>
<td>Active substance</td>
<td>Indication</td>
<td>Pharmaceutical form</td>
<td>Regulatory Status</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>-------------------</td>
</tr>
</tbody>
</table>
| dry extract from *Echinacea purpurea* 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; THR |
| dry extract of *Echinacea purpurea* 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 |

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 *E. 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 *Echinacea purpureae radix* are mentioned in the literature.

Table 2: Overview of historical data

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

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 *Echinacea purpureae radix*.

2.3. Overall conclusions on medicinal use

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

Table 3: Overview of evidence on period of medicinal use

<table>
<thead>
<tr>
<th>Herbal preparation</th>
<th>Indication</th>
<th>Posology, Strength</th>
<th>Period of medicinal use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Echinacea purpurea</em> (purple coneflower) dried root, dry extract (6.1-7:1), extraction solvent: ethanol 45 % V/V</td>
<td>Traditional herbal medicinal product used for the relief of symptoms of common cold.</td>
<td>Chewable tablet, 1 tablet contains: 40 mg extract corresponding to 243-279 mg radix.</td>
<td>Since 1978, on the market as a natural remedy under national legislation.</td>
</tr>
</tbody>
</table>
At the time of the preparation of the first monograph on E. 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 fixed DER (without any range) is not technically possible. Therefore, the Working Party on European Union Monographs and European Union List (MLWP) decided on the meeting on 8-10 March 2010 to extend the DER to 5.5-7.5:1, to cover other comparable preparations that were on the market at that time. HMPC confirmed the MLWP decision.

### 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 *E. 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

Ethanolic extracts

An ethanolic 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 ethanolic 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 diminished influenza-specific IL-2, and did not influenced influenza-specific IL-10 or IFN-γ. (McCann et al., 2007).

The effects of *Echinacea* and several of its phytochemical components on NFκB expression by Jurkat cells (a human T-cell line) were investigated in vitro. In the absence of stimulation, *Echinacea* and its components exerted no significant effect on basal NFκB expression levels. In the presence of endotoxin lipopolysaccharide (LPS), NFκB 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 NFκB expression were mixed. Depending on the concentration, cichoric acid and a 2,4-diene alkylamide significantly induced NFκB levels, whereas a 2-ene alkylamide caused a significant inhibition. In contrast, both the
Echinacea and the mixed alkylamide fraction exerted no effect. The alkylamide results indicate that the two basic forms of these compounds present in Echinacea may have opposing effects. These opposing effects demonstrate the importance of knowledge, not only of the phytochemical make-up of a herbal preparation, but also of the actions of each component and the consequences of differing relative amounts in the preparation being investigated (Matthias et al., 2008).

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.

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 E. angustifolia were the most efficacious in the assays; extracts from E. 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-1β, IL-2, and TNF-α). Phenetic analyses were employed to compare responses across the entire data set, including UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and neighbor-joining methods. In the immune experiments conducted for this investigation, E. angustifolia, E. paradoxa, E. purpurea, E. simulata, and E. tennesseensis extracts prepared from roots significantly augmented IL-1β and TNF-α production, whereas no extracts significantly modulated IL-2.
All phenetic methods produced similar dendrograms, revealing two species pairs (E. angustifolia + E. simulata and E. pallida + E. sanguinea) where both species cluster tightly and have similar immune-response profiles. These two species-pairs are maximally dissimilar from each other. The remaining species (E. paradoxa, E. purpurea, and E. tennesseensis) occupy intermediate positions in the dendrogram. The authors concluded that the results suggest that Echinacea spp. act heterogeneously on immune function (Senchina et al., 2008).

**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 granulocyes- 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 TNFα 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 $6.4 \times 10^6$ to $3.3 \times 10^8$ 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-κB 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 (IFNα/β) in spleen cell cultures, and IL-1, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) in mouse macrophage cultures; increased immunoglobulin M production and the number of antibody-producing cells, and increased nitric oxide (NO) production of macrophages (Beuscher et al., 1995, Bodinet, 1999). Incubation of this fraction with human monocytes also enhanced the production of IL-1, IL-6 and TNF-α (Bodinet, 1999).
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

**Ethanolic extracts**

In contrast with 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 µg/animal) of a purified high molecular weight fraction containing glycoproteins and polysaccharides from purple coneflower root (Bodinet and Beuscher, 1991, Beuscher *et al.*, 1995). Oral administration of this fraction to mice significantly enhanced antibody production in Peyer'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 cca 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-γ) in response to *Echinacea* components, was also apparent in rat’s spleen macrophage, but at higher concentrations. Among the components, alkylamides at the dose level of 12 mg/kg body weight/day significantly increased the phagocytic activity as well as phagocytic index of the alveolar macrophages. None of the components at any concentration had any effect on the release of TNF-α, IFN-γ and IL-2 by the splenocytes. These results suggest that the *Echinacea* preparations containing 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 Uluışık *et al.* (2012) determined the effect of ginseng and echinacea on the mRNA expression of IL-10, TNF-α, and TGF-β1 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-β1 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-β1 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) than, a potentiating synergistic effect of the extract mixture it can be postulated (Wagner and Jurcic, 2002).

El-Ashmawy et al. (2015) describe the effect of *E. 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-diynoic acid isobutylamide, dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide, dodeca-2E,4E-diienoic acid isobutylamide, and undeca-2E-ene-8,10-diynoic acid isobutylamide suppressed production of TNF-α and PGE2 from infected cells. Dodeca-2E,4E-diienoic acid isobutylamide was especially effective at inhibiting production of these mediators and also strongly inhibited production of G-CSF, CCL2/MCP-1, CCL3/MIP-1α and CCL5/RANTES. In contrast, the ethanol extracts (75%), 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**

**Ethanolic 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 E(2) (PGE(2)) 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 µg/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 µM, by Bauer alkamides 8, 12A analogue, and 14, Chen alkamide 2, and Chen alkamide 2 analogue at 25 µM and by Bauer alkamide 14 at 10 µM.

Cytotoxicity did not play a role in the noted reduction of PGE2 production in either the *Echinacea* extracts or synthesized alkamides. HPLC analysis identified individual alkamides present at concentrations below 2.8 µM in the extracts from the six *Echinacea* species (15 µg/ml crude extract). Because active extracts contained <2.8 µM of specific alkamide and the results showed that synthetic alkamides must have a minimum concentration of 10 µM to inhibit PGE2, it is likely that alkamides may contribute toward the anti-inflammatory activity of *Echinacea* in a synergistic or additive manner (LaLone *et al.*, 2007).

Alcohol extracts from 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-1β. Only *E. angustifolia* - and *E. pallida*-treated mice demonstrated significantly higher production of IL-4 and increased IL-10 production. Taken together, these findings demonstrated that *Echinacea* is a wide-spectrum immunomodulator that modulates both innate and adaptive immune responses. In particular, *E. angustifolia* or *E. pallida* may have more anti-inflammatory potential (Zhai *et al.*, 2007a).
The effect of the same alcohol extracts as in previous paragraph on the production of inflammatory mediators (nitric oxide (NO), TNF-α and IL-1β) 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 µM (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)-1α, IL-6, MCP-1, MIP-1β] 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

<table>
<thead>
<tr>
<th>Herbal preparation tested</th>
<th>Posology</th>
<th>Experimental model</th>
<th>Reference</th>
<th>Main non-clinical conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparable/similar preparations to preparations of the monograph</strong></td>
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<tr>
<td>50% ethanol tincture of <em>E. purpurea</em> roots, drug solvent ratio 1:9 w/v</td>
<td>50 µg/ml</td>
<td><em>In vitro</em></td>
<td>Sharma et al., 2006</td>
<td>Immunomodulatory activity though cytokine stimulation</td>
</tr>
<tr>
<td>50% ethanol tincture of <em>E. purpurea</em> roots, drug solvent ratio 1:9</td>
<td>4 µl of extract per 50 µl of medium</td>
<td><em>In vitro</em></td>
<td>Senchina et al., 2006</td>
<td>Immunomodulatory activity though cytokine stimulation</td>
</tr>
<tr>
<td>50% ethanol tincture of <em>E. purpurea</em> roots, drug solvent ratio 1:9</td>
<td>4 µl of extract per 50 µl of medium</td>
<td><em>In vitro</em></td>
<td>McCann et al., 2007</td>
<td>Immunomodulatory activity though cytokine stimulation only partially confirmed</td>
</tr>
<tr>
<td><strong>Other preparations</strong></td>
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<tr>
<td>Purified polysaccharides prepared from the <em>E. purpurea</em> herb and root (alkaline-water extract)</td>
<td>100 µg/ml</td>
<td><em>In vitro</em></td>
<td>Stimpel et al., 1984</td>
<td>Polysaccharides activate in vivo cells of the macrophage system to cytotoxicity.</td>
</tr>
<tr>
<td>Ethanolic <em>E. purpurea</em> root extract</td>
<td>Various concentrations, 10⁻⁴ mg/ml showed effects</td>
<td><em>In vitro</em></td>
<td>Bauer et al., 1989</td>
<td>Aqueous and lipophilic fractions showed immunostimulatory activity (enhancement of phagocytosis)</td>
</tr>
<tr>
<td>Polysaccharides and glycoproteins from <em>E. purpurea</em> radix</td>
<td>High molecular weight fraction (Mr &gt;10,000 D) of polysaccharides and glycoproteins</td>
<td><em>In vitro</em></td>
<td>Beuscher et al., 1995, Bodinet et al., 1999</td>
<td>Stimulation of cytokines, IL-1, IL-6, TNFα/β, IgM, number of antibody-producing cells, NO production of macrophages</td>
</tr>
<tr>
<td><em>E. purpurea</em> root powders and various extracts, <em>E. purpurea</em> herb (whole plant)</td>
<td>Extract concentration 5-320 µg/ml; LPS 0.1 µg/ml; various media/solvents</td>
<td><em>In vitro</em></td>
<td>Rininger et al., 2000</td>
<td>Macrophage activation, LPS showed most effect</td>
</tr>
<tr>
<td><em>E. purpurea</em> extracts</td>
<td>Various concentrations</td>
<td><em>In vitro</em></td>
<td>Rininger et al., 2000</td>
<td>No induction of macrophage cytokine production, but anti-inflammatory and antioxidant effect</td>
</tr>
<tr>
<td>Herbal preparation tested</td>
<td>Posology</td>
<td>Experimental model</td>
<td>Reference</td>
<td>Main non-clinical conclusions</td>
</tr>
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<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Root extract, prepared from both <em>E. angustifolia</em> and <em>E. purpurea</em>, 1:2 extraction with ethanol 60%, 300 mg/ml E. purpurea</td>
<td>Alkylamide fraction and cichoric acid fraction tested in different concentrations</td>
<td><em>In vitro</em></td>
<td>Matthias et al., 2008</td>
<td>Opposing effects of compounds on NFxkB: cichoric acid and a 2,4-diene alkylamide significantly induced levels, whereas a 2-ene alkylamide caused a significant inhibition. Echinacea and the mixed alkylamide fraction exerted no effect.</td>
</tr>
<tr>
<td>Aqueous extraction of <em>E. purpurea</em> root</td>
<td>150 or 450 µg/ml</td>
<td><em>In vitro</em></td>
<td>Benson et al., 2010</td>
<td>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</td>
</tr>
<tr>
<td><em>E. purpurea</em> samples (aerial and root plant parts), 95% ethanolic extraction, further extracted with 4% SDS</td>
<td>EC$_{50}$ values see article</td>
<td><em>In vitro</em></td>
<td>Pugh et al., 2013</td>
<td>Differences in total bacterial load within Echinacea samples correlate with the activity (NF-κB activation in THP-1 cells) and content of bacterial lipopolysaccharides within extracts</td>
</tr>
<tr>
<td><em>Echinacea purpurea</em> powder suspension (5%, w/v) in distilled water, containing 0.4% (w/v) yeast extract</td>
<td>Various concentrations</td>
<td><em>In vitro</em></td>
<td>Rizzello et al., 2013</td>
<td>Echinacea suspension exerted immune-modulatory effects on Caco-2 cells</td>
</tr>
<tr>
<td>Water extract of <em>E. purpurea</em> root</td>
<td>Various concentrations, IC$_{50}$ see article</td>
<td><em>In vitro</em></td>
<td>Fast et al., 2015</td>
<td>Non-alkylamide/non-polyphenolic phytochemicals contribute to the anti-inflammatory effects</td>
</tr>
<tr>
<td>75 % ethanol root extract of <em>E. purpurea</em></td>
<td>Various concentrations, IC$_{50}$ see article</td>
<td><em>In vitro</em></td>
<td>Todd et al., 2015</td>
<td>Macrophage-stimulating activity in <em>E. purpurea</em> extracts can originate from endophytic bacteria</td>
</tr>
<tr>
<td>Purified high molecular weight fraction containing glycoproteins and polysaccharides from <em>E. purpurea</em> root</td>
<td>Oral administration</td>
<td><em>In vivo</em></td>
<td>Bodinet, 1999</td>
<td>Enhancement of antibody production in Peyer’s plaque cells</td>
</tr>
<tr>
<td>75 % ethanolic extract of <em>E. purpurea</em> root</td>
<td>Tested in 6 rats/group via cracker, 50-250 mg/kg body weight</td>
<td><em>In vivo</em></td>
<td>South and Exon, 2001</td>
<td>No evidence of NK cell activity or antibody formation</td>
</tr>
<tr>
<td><em>E. purpurea</em> root extract (solvent not available/found)</td>
<td>Oral administration of 0.45 mg/day to 7-week-old mice</td>
<td><em>In vivo</em></td>
<td>Sun et al., 1999</td>
<td>Doubling of the number of NK cells and monocytes in bone marrow and spleen</td>
</tr>
<tr>
<td><em>E. purpurea</em> root extract (solvent not available/found)</td>
<td>Oral administration of 0.45 mg/day to ageing mice</td>
<td><em>In vivo</em></td>
<td>Currier and Miller, 2000</td>
<td>Increase of NK cells</td>
</tr>
<tr>
<td>Herbal preparation tested</td>
<td>Posology</td>
<td>Experimental model</td>
<td>Reference</td>
<td>Main non-clinical conclusions</td>
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</tr>
<tr>
<td>Powdered <em>E. purpurea</em> root</td>
<td>Oral administration to mice injected with leukaemia cells</td>
<td><em>In vivo</em></td>
<td>Currier and Miller 2001</td>
<td>Increase of survival time compared to controls</td>
</tr>
<tr>
<td>Powdered <em>E. purpurea</em> root</td>
<td>0.45 mg/day (dose per body weight) via chow</td>
<td><em>In vivo</em></td>
<td>Currier and Miller 2002</td>
<td>Strong adjuvant effect on vaccination with inactivated leukaemia cells</td>
</tr>
<tr>
<td>Dry root powder of <em>E. purpurea</em> root</td>
<td>30 or 100 mg/kg in 7 mice/group</td>
<td><em>In vivo</em></td>
<td>Di Carlo et al., 2003</td>
<td>Increase of resistance of splenic lymphocytes to apoptosis</td>
</tr>
<tr>
<td>Commercial <em>E. purpurea</em> root extract</td>
<td>Tested in mice, 2 mg <em>E. purpurea</em>/6 g chow/day</td>
<td><em>In vivo</em></td>
<td>Brousseau and Miller, 2005</td>
<td>Regular intake lead to immunosurveillance against spontaneous-developing tumors</td>
</tr>
<tr>
<td><em>E. purpurea</em> root powder</td>
<td>0.75 g/kg in 6-week-old Fischer rats</td>
<td><em>In vivo</em></td>
<td>Uluşık et al., 2012</td>
<td>Increase in expression of TNF-α mRNA</td>
</tr>
<tr>
<td><em>E. purpurea</em> (EP) dried roots</td>
<td>Assessment of the acquired immunity in 24 Awassi ewes to the vaccine and/or 250 mg dried roots tablet/daily, orally</td>
<td><em>In vivo</em></td>
<td>Barbour et al., 2015</td>
<td>vaccination by the experimental <em>Salmonella enteritidis</em> bacterin with daily oral intake of 250 mg of <em>Echinacea purpurea</em> dried roots for obtaining a significant seroconversion</td>
</tr>
<tr>
<td>Methanolic and aqueous extract of <em>Echinacea purpurea</em> root</td>
<td>Not available/found</td>
<td><em>In vitro</em></td>
<td>Wacker and Hilbig, 1978</td>
<td>Resistance to influenza A2, herpes, and vesicular stomatitis virus infection for 24 hours in mouse fibroblasts</td>
</tr>
<tr>
<td><em>E. purpurea</em> 75% ethanol extracts</td>
<td>Various concentrations</td>
<td><em>In vitro</em></td>
<td>Cech et al., 2010</td>
<td>Range of effects from suppression to stimulation of mediator production</td>
</tr>
<tr>
<td><em>E. purpurea</em> dry root extract</td>
<td>Oral administration to mice at 30 and 100 mg/kg, twice daily</td>
<td><em>In vivo</em></td>
<td>Raso et al., 2005</td>
<td>Anti-inflammatory effect, down-regulation of COX-2 expression</td>
</tr>
<tr>
<td><em>E. purpurea</em> root extract</td>
<td>Topical application of gel containing 100 mg/ml in rats</td>
<td><em>In vivo</em></td>
<td>Speroni et al., 2002</td>
<td>Less anti-inflammatory activity than <em>E. pallida</em></td>
</tr>
<tr>
<td><em>E. purpurea</em> 95 % ethanol root extract</td>
<td>Extract usually evaporated to 0.5 g/mL in stimulated rat basophilic cells</td>
<td><em>In vivo</em></td>
<td>Merali et al., 2003</td>
<td>S-lipoxygenase-inhibiting activity</td>
</tr>
<tr>
<td>Crude extract of <em>E. purpurea</em>, (not specified)</td>
<td>15 µg/ml (various solvents) in mouse macrophages</td>
<td><em>In vivo</em></td>
<td>Lalone et al., 2007</td>
<td>Alkamides contribute to anti-inflammatory activity of Echinacea in a synergistic or additive manner</td>
</tr>
<tr>
<td><em>E. purpurea</em> alcohol extract, (not further specified)</td>
<td>130 mg/kg/daily, administered orally in mice</td>
<td><em>In vivo</em></td>
<td>Zhai et al., 2007a</td>
<td>Modulation of innate and adaptive immune responses, less anti-inflammatory potential than <em>E. angustifolia</em> and <em>E. pallida</em></td>
</tr>
<tr>
<td>Herbal preparation tested</td>
<td>Posology</td>
<td>Experimental model</td>
<td>Reference</td>
<td>Main non-clinical conclusions</td>
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<tr>
<td><em>E. purpurea</em> alcohol extract, (not specified)</td>
<td>Administered orally at 130 mg/kg per day both in macrophages in vitro and in murine peritoneal exudate in vivo (bacterial-infected)</td>
<td><em>In vitro and in vivo</em></td>
<td>Zhai <em>et al.</em>, 2007b</td>
<td>Anti-inflammatory (inhibition of NO production and TNFα in vitro, weaker effect in vivo)</td>
</tr>
<tr>
<td><em>E. purpurea</em> root 100% methanol extract</td>
<td>Topic treatment on mouse skin (200 µl per site)</td>
<td><em>In vivo</em></td>
<td>Hou <em>et al.</em>, 2010</td>
<td>Anti-inflammatory (especially alkamides, not cichoric acid)</td>
</tr>
<tr>
<td><strong>Single substances</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>isolated cichoric acid, polysaccharides and alkylamides</td>
<td>Various concentrations tested in 6 rats/group</td>
<td><em>In vivo</em></td>
<td>Goel <em>et al.</em>, 2002a, Goel <em>et al.</em>, 2002b</td>
<td>Cichoric acid, polysaccharides and alkylamides effective in stimulating in vivo, non-specific immune response; alkylamides at a dose level of approximately 12 mg/kg body weight/day stimulate alveolar macrophage function</td>
</tr>
<tr>
<td>Dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides, trienoic and dienoic acid derivatives from standardized tincture made from aerial parts and roots of <em>E. purpurea</em></td>
<td>Various concentrations</td>
<td><em>In vitro</em></td>
<td>Gertsch <em>et al.</em>, 2004</td>
<td>Alkylamids = immunomodulators and potential ligands for CB2 receptors</td>
</tr>
<tr>
<td>Undec-2Z,4E-diene-8,10-diyenic acid isobutylamide, dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide, dodeca-2E,4E-dienoic acid isobutylamide, and undeca-2E-ene-8,10-diyenoic acid isobutylamide</td>
<td>Various concentrations</td>
<td><em>In vitro</em></td>
<td>Cech <em>et al.</em>, 2010</td>
<td>Suppression of production of TNF-α and PGE₂ from influenza A infected cells</td>
</tr>
<tr>
<td>Xanthienopyran from 75% ethanolic root extract of <em>E. purpurea</em></td>
<td>Various concentrations</td>
<td><em>In vitro</em></td>
<td>Todd <em>et al.</em>, 2015</td>
<td>Suppression of production of TNF-α, IC₅₀ = 3.4 µg/ml</td>
</tr>
<tr>
<td>Alkamides from <em>E. purpurea</em> root dichloromethane extract</td>
<td>Concentration in final DMSO assay: 10 and 100 µg/ml</td>
<td><em>In vivo</em></td>
<td>Clifford <em>et al.</em>, 2002</td>
<td>Inhibition of COX-1 and, lesser, of COX-2</td>
</tr>
<tr>
<td>Dodeca-2E,4E,8Z,10Z(E)-tetraenoic acid isobutylamides, isolated from <em>E. purpurea</em> root</td>
<td>Various concentrations tested in mice</td>
<td><em>In vivo</em></td>
<td>Hou <em>et al.</em>, 2011</td>
<td>Anti-inflammatory</td>
</tr>
</tbody>
</table>
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 (EC50 = 6.6 µM), while caftaric acid had the lowest (EC50 = 20.5 µM). The average EC50 values for *E. purpurea*, *E. pallida* and *E. angustifolia* were 134, 167 and 231 µg/ml, respectively. The radical scavenging activity of *Echinacea* root extracts reflected their phenolic composition (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 a water extract containing the high molecular weight compounds. This contributes to the hypothesis that the physiologically beneficial effects of *Echinacea* are exerted by the multitude of constituents present in the preparations (Dalby-Brown et al., 2005).

The antioxidant activity of extracts of the stems, leaves, and roots of *Echinacea purpurea* was compared with the antioxidant activity of purified cichoric acid and alkamides, both constituents of *E. purpurea*. The 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 (IC50 > 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 Ca2+ inCB2-positive but not in CB2-negative promyelocytic HL60 cells, an effect that was inhibited by the CB2 antagonist SR144528. At 50 nM A1, A2, and the endogenous cannabinoid anandamide (CB2 Ki >200 nM) up-regulated constitutive IL-6 expression in human whole blood in a seemingly CB2-dependent manner. A1, A2, anandamide, the CB2 antagonist SR144528 (Ki <10 nM), and also the non-CB2-binding alkylamide undeca-2E-ene-8,10-diynoic acid isobutylamide all significantly inhibited LPS-induced TNF-α, IL-1β, and IL-12p70 expression (5–500
nM) in a CB2-independent manner. Alkylamides and anandamide also showed weak differential effects on anti-CD3- versus anti-CD28-stimulated cytokine expression in human whole blood. Overall, alkylamides, anandamide, and SR144528 potently inhibited LPS-induced inflammation in human whole blood and exerted modulatory effects on cytokine expression, but these effects are not exclusively related to CB2 binding (Raduner et al., 2006).

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 curative efficacy of an Echinacea extract (Echinacin®; Madaus AG, Germany) in γ-irradiated male mice was studied. Significant changes were observed in the microenvironment of the major constituents, including tyrosine and protein secondary structures. E. purpurea administration significantly ameliorated all estimated parameters. The radio-protection effectiveness was similar to the radio-recovery curativeness in comparison to the control group in most of the tested parameters. The radio-protection efficiency was greater than the radio-recovery in haemoglobin level during the first two weeks, in lymphoid cell count and thiobarbituric acid-reactive substances (TBARs) level at the fourth week and in superoxide dismutase (SOD) activity during the first two weeks, as compared to the levels of these parameters in the control group (Abouelella et al., 2007).

Effect of three products: Product A containing dry ethanol-water extract of herb, Product B containing thick extract of herb and Product C combination product containing extracts of Thuja occidentalis, Baptisia tinctoria, Echinacea purpurea and Echinacea pallida on mice fetuses was studied to establish whether pharmaceuticals containing alcoholic extracts of E. purpurea given to pregnant mice influence angiogenic activity and tissue VEGF and bFGF production of their fetuses. The results show that there is some possibility that pharmaceuticals containing E. purpurea might influence fetal development in human also, because they may interfere with embryonal angiogenesis, and should not be recommended for pregnant women (Barcz et al. 2007).

The 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 colon cancer 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 E. 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 mediated 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 E. 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 E. 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 *E. 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 *E. purpurea*. The expressions of PPARγ and C/EBPα in adipocytes treated with *E. 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 *E. 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-isobutylundeca-2-ene-8,10-dynamide (1) metabolised to only a tenth the extent of (2E,4E,8Z,10Z)-N-isobutylldodeca-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 Cmax, 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 dextrorphan (product of dextromethorphan O-demethylation). Ethanol showed a biphasic effect on CYP2D6 metabolism, increasing initially the CYP2D6 activity with 175% of control up to a concentration of 1.1%, where after ethanol linearly inhibited the CYP2D6 activity. All the investigated herbs inhibited CYP2D6 activity to some extent, but only St. John's wort, common sage and common valerian were considered possible candidates for in vivo clinically significant effects. They showed IC50 values of 0.07 +/- 7 x 10(-3) mg/ml, 0.8 +/- 0.05 mg/ml and 1.6 +/- 0.2 mg/ml, respectively. St. John's wort inhibited CYP2D6-mediated metabolism in an uncompetitive manner, while common valerian and common sage in a non-competitive manner demonstrated interherb differences in inhibition patterns and differences when compared to the more homogenous competitive inhibitor quinidine. Common valerian was the only herb that showed a mechanistic inhibition of CYP2D6 activity and attention should be paid to a possible toxicity of this herb (Hellum and Nilsen, 2007).

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

**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<sub>max</sub>, but prolonged the elimination half-life after administration of the pure dodeca-2 E,4 E,8 Z,10 E/ Z-tetraenoic acid isobutyramides 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 *E. 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 pilot study suggests 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 alkamides, 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. These results 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

<table>
<thead>
<tr>
<th>Type</th>
<th>Study</th>
<th>Test Product(s)</th>
<th>Number of subjects</th>
<th>Type of subjects</th>
<th>Outcomes</th>
<th>Statistical analysis</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigation of immunomodulatory activity, Melchart et al., 1995</td>
<td>Placebo-controlled, randomized, double-blinded, duration 9-14 days</td>
<td>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 E. pallida radix and placebo, acid-resistant capsules, one capsule 3x daily for 5 consecutive days, orally,</td>
<td>134 (18 female and 166 male) between 18 and 40 years, 1 drop out because of eosinophilia</td>
<td>Healthy</td>
<td>Primary endpoint: relative phagocytic activity of polymorphonuclear neutrophil granulocytes (PNG) → significant increase, secondary endpoint: number of leukocytes in peripheral venous blood → no significant influence</td>
<td>2: Wilcoxon-Mann-Whitney-U-test; 3: Kruskal-Wallis test; 2: Maximal stimulation 54.0% (95% CI 8.4-99.6%)</td>
<td>Immuno-modulating effect in study 2, no effect in study 3a</td>
</tr>
<tr>
<td>Influence on phagocytosis, Jurcic et al., 1989</td>
<td>Placebo-controlled, double-blinded, duration 5 days</td>
<td>30 drops of an ethanolic E. purpurea root extract, orally</td>
<td>24 male</td>
<td>Healthy</td>
<td>Significant increase in phagocytosis of 120% by day 5 (placebo: 20%)</td>
<td>Not available/found</td>
<td>Small study, only males and healthy, randomization?</td>
</tr>
<tr>
<td>Effects on immune markers, Agnew et al., 2005</td>
<td>Not available/found</td>
<td>tablets containing 675 mg of E. purpurea root extract and 600 mg of E. angustifolia root extract, prepared by ethanol extraction Duration: 14 days</td>
<td>11 (5 male, 6 female), 26-61 years of age,</td>
<td>Healthy</td>
<td>Leucocyte hsp70 expression increase (P=0.029); white cell counts increase (P=0.043); erythrocyte haemolysis increase (P=0.006) → antioxidant</td>
<td>Not available/found</td>
<td>invoke an immune response, and protection of free radical;</td>
</tr>
</tbody>
</table>
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 (concentratin 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. Alkamides were rapidly absorbed and were measurable in plasma 20 min after tablet ingestion and remained detectable for up to 12 h. Concentration-time curves for 2,4-diene and 2-ene alkamides were determined. The maximal concentrations for the sum of alkamides in human plasma were reached within 2.3 hours post ingestion and averaged 336±31 ng eq/ml plasma. No obvious differences were observed in the pharmacokinetics of individual or total alkamides in 2 additional fasted subjects who took the same dose of the *Echinacea* preparation. (Matthias et al., 2005b).

The relative oral bioavailability of alkylamides from two different *Echinacea* dosage forms (liquid and tablet) were compared in a small two-way crossover study in humans (n = 3). The liquid preparation investigated contained a mixture of *E. purpurea* root (300 mg/ml) and *E. angustifolia* root (200 mg/ml) extracted in 60% ethanol. The tablet preparation investigated was also a mixture of *E. purpurea* root (675 mg/tablet) and *E. angustifolia* root (600 mg/tablet), but was prepared from the dried 60% ethanolic extracts of these two *Echinacea* species. Alkylamides were found to be rapidly absorbed and measurable in plasma from both preparations. No significant differences in the tetraene alkylamide pharmacokinetic parameters for T1/2, AUCt-lin and Cmax in the two different preparations were found. Tmax 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/m(2). *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

<table>
<thead>
<tr>
<th>Type</th>
<th>Study</th>
<th>Test Product(s)</th>
<th>Number of subjects</th>
<th>Type of subjects</th>
<th>Outcomes</th>
<th>Statistical analysis</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination of plasma concentration,</td>
<td>Single dose study</td>
<td>4 tablets, each containing dried ethanolic extract equivalent to 675 mg of E. purpurea root plus 600 mg of E. angustifolia root; duration: 12 h</td>
<td>9 (male and female), 18-26 years; drop out not mentioned</td>
<td>Healthy</td>
<td>Cmax 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</td>
<td>Pharmaco-kinetic analysis</td>
<td>Availability of most alkamides after oral intake shown, but no caffeic acid conjugates</td>
</tr>
<tr>
<td>Matthias et al., 2005b</td>
<td>Two-way crossover study</td>
<td>Liquid: mixture of E. purpurea root (300 mg/ml) and E. angustifolia root (200 mg/ml) extracted in 60% ethanol; tablets: mixture of E. purpurea root (675 mg/tablet) and E. angustifolia root (600 mg/tablet), prepared from the dried 60% ethanolic extracts of E. purpurea and E. angustifolia, orally</td>
<td>3 male volunteers (39-62 years), no other medication, no drop out mentioned</td>
<td>Healthy</td>
<td>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</td>
<td>Pharmaco-kinetic analysis</td>
<td>Improvement of knowledge about bioavailability of alkylamides in two different dosage forms</td>
</tr>
</tbody>
</table>
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 revew the same studies, they 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

<table>
<thead>
<tr>
<th>Type</th>
<th>Study</th>
<th>Test Product(s)</th>
<th>Number of subjects</th>
<th>Type of subjects</th>
<th>Outcomes</th>
<th>Statistical analysis</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect on influenza, Bräunig et al., 1992</td>
<td>Placebo-controlled, double-blinded, randomized</td>
<td>Tincture of <em>E. purpurea</em> 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</td>
<td>180 patients (18-60 years),</td>
<td>Patients with influenza</td>
<td>Group taking the 900 mg dose showed a highly significant reduction in symptom score at both time points ($p &lt; 0.0001$), but no difference in symptoms between the group taking the 450 mg dose and the placebo group</td>
<td>Not mentioned</td>
<td>900 mg <em>E. purpurea</em> root tincture with 55% ethanol (V/V) may have some effects on influenza</td>
</tr>
</tbody>
</table>
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 this 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 at the University of Wisconsin, on 148 students with common colds of recent onset was assessed. Each active capsule contained a dried mixture of *E. angustifolia* root (50% [123 mg]), *E. purpurea* root (25% [62 mg]), and *E. purpurea* herb (25% [62 mg]). *Echinacea* capsules also contained thyme (49 mg) and peppermint (31 mg) to disguise taste and flavour, as well as citric acid (3 mg) as a preservative. The placebo capsules contained 333 mg of alfalfa. The patients took 4 capsules 6 times during the first 24 hours of the study, and 4 capsules 3 times each day thereafter until symptoms resolved, for a maximum of 10 days. Severity and duration of self-reported symptoms of upper respiratory tract infection were recorded. No statistically significant differences were detected between the *Echinacea* and placebo groups for any of the measured outcomes. Trajectories of severity over time were nearly identical in the 2 groups. Mean cold duration was 6.01 days in both groups as a whole, 5.75 days in the placebo group, and 6.27 days in the *Echinacea* group (between-group difference, -0.52 day [95% CI, -1.09 to 0.22 days]). After controlling for severity and duration of symptoms before study entry, sex, date of enrolment, and use of nonprotocol medications, researchers found no statistically significant treatment effect (adjusted hazard ratio, 1.24 [CI, 0.86 to 1.78]). Multivariable regression models assessing severity scores over time failed to detect statistically significant differences between the *Echinacea* and placebo groups (Barrett et al., 2002).
A formulation containing alkamides, cichoric acid, and polysaccharides at concentrations of 0.25, 2.5, and 25 mg/ml, respectively, was prepared from freshly harvested Echinacea purpurea herb and root. 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 isobutyramide, 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, standardized 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 about oral solution (KJ) which is 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 for 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 haematological 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 \textit{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 \textit{E. purpurea}-based hotdrink formulation. The hotdrink contained the ethanolic extract (65% V/V) of freshly harvested \textit{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 \textit{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 \textit{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 \textit{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 \textit{E. purpurea}-based hotdrink formulation. In this study it was demonstrated that \textit{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 Echinacea purpureae radix and its combinations

<table>
<thead>
<tr>
<th>Type</th>
<th>Study</th>
<th>Test Product(s)</th>
<th>Number of subjects</th>
<th>Type of subjects</th>
<th>Outcomes</th>
<th>Statistical analysis</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>prevention of upper respiratory tract infections, Melchert et al. 1998</td>
<td>Placebo-controlled, double-blinded, randomized</td>
<td>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 <em>E. purpurea</em> radix (n = 99), group B: ethanolic extract (1: 11, ethanol 30%) of <em>E. angustifolia</em> root (n = 100), group C: ethanolic placebo solution (n = 99); duration: 12 weeks</td>
<td>289, 45 drop out</td>
<td>Not mentioned</td>
<td>Primary endpoint: time until first occurrence of first upper respiratory tract infection (\rightarrow) relative reduction in risk of infection of 20 % for <em>E. purpurea</em> root, but not significant; secondary endpoint: number of participants with at least one infection</td>
<td>Not mentioned</td>
<td>Effects on prevention of upper respiratory tract infections could not be shown</td>
</tr>
<tr>
<td>treatment of common cold, Barrett et al. 2010</td>
<td>Placebo-controlled, randomized; placebo and <em>Echinacea</em> pills blinded, <em>Echinacea</em> pills unblinded (open-label)</td>
<td>4 groups: 1) no pills, 2) placebo pills (blinded), 3) <em>Echinacea</em> pills (blinded), 4) <em>Echinacea</em> pills (unblinded, open-label) <em>Echinacea</em> tablets = equivalent of 675 mg of <em>E. purpurea</em> root and 600 mg of <em>E. angustifolia</em> root, each standardized to 2.1 mg of alkamides;</td>
<td>719 patients (12-80 years), 64 % female, 88 % white; 6 drop out</td>
<td>Patients with new-onset common cold (Jackson and colleagues’ criteria)</td>
<td>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</td>
<td>Mann-Whitney-U-Test, Box-Cox transformation</td>
<td><em>Echinacea</em> cannot substantively change course of common cold</td>
</tr>
</tbody>
</table>
**Safety and efficacy in treatment of common cold, Jawad et al. 2012**

<table>
<thead>
<tr>
<th>Echinacea groups received the equivalent of 10.2 g of dried <em>Echinacea</em> root during the first 24 hours and 5.1 g during each of the next 4 days</th>
<th>755 adults, 82 drop out (Echinaforce: 244 female, 111 male; placebo: 227 female, 135 male)</th>
<th>Healthy, but experience of at least two colds/year</th>
<th>Placebo-controlled, double-blinded, randomized, parallel trial</th>
<th>ITT yes, chi-square test</th>
<th>Reduction of total number of cold episodes, &quot;positive risk to benefit ratio&quot;, largest study up to date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic extract from freshly harvested <em>E. purpurea</em> (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</td>
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</table>

**Prevention of respiratory and other symptoms associated with long-haul flights; Tiralongo et al. 2012**

| Tablets containing 112.5 mg *E. purpurea* 6:1 root extract and 150 mg *E. angustifolia* 4:1 root extract or placebo, orally; duration: 4 weeks; regime: treatment 14 days prior to travel, during travel and 14 days after travel | 175 adults randomized, 5 drop out before allocated to intervention → 170 allocated (113 female, 67 male), on average 43 years old | Long-haul flight from Australia to America, Europe or Africa for a period of 1-5 weeks on commercial flights via economy class | Placebo-controlled, double-blinded, randomized | ITT yes, Non parametric Kolmogorov-Smirnov test, 2 x 2 chi-squared test, t-test | Supplementation with standardised *Echinacea* tablets, if taken before and during travel, may have preventive effects against the |
Evaluation of use of available combinations for treatment of the common cold or flu; Lindenmuth and Lindenmuth 2000

| Placebo-controlled, double-blind, randomized | Echinacea compound herbal tea (E. purpurea herb and extract of E. purpurea root equivalent to 1275 mg dried herb and root per tea bag), placebo: cinnamon, ginger, peppermint; regime: 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 *Echinacea* group and placebo group | Not available/found | Echinacea 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**

See below
Table 9: Clinical safety data from clinical trials

<table>
<thead>
<tr>
<th>Type</th>
<th>Study</th>
<th>Test Product(s)</th>
<th>Number of subjects</th>
<th>Type of subjects</th>
<th>Adverse reactions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brinke born et al., 1999</td>
<td>Placebo-controlled, double- blinded, randomized</td>
<td>3 times daily 2 tablets of either (<em>Echinacea purpurea</em>-preparation from 95% herba and 5% radix), <em>Echinacea purpurea</em> concentrate (same preparation at 7 times higher concentration), special <em>Echinacea purpurea</em> radix preparation (dry extract from root, 29.6 mg per tablet) or placebo until they felt healthy again but not longer than 7 days.</td>
<td>246 volunteers</td>
<td>Patients with common cold</td>
<td>&quot;Frequency of adverse events was not significantly higher than in the placebo group&quot;</td>
<td>Safe treatment of common cold</td>
</tr>
</tbody>
</table>
| Barrett et al., 2010        | Placebo-controlled, randomized; placebo and *Echinacea* pills blinded, *Echinacea* pills unblinded (open-label) | 4 groups: 1) no pills, 2) placebo pills (blinded), 3) *Echinacea* pills (blinded), 4) *Echinacea* pills (unblinded, open-label)  
*Echinacea* tablets = equivalent of 675 mg of *E. purpurea* root and 600 mg of *E. angustifolia* root, each standardized to 2.1 mg of alkamides; *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 | 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 opendened questions about possible adverse effects during monitoring showed no patterns of adverse effects attributable to *Echinacea*. | Headache might be a possible adverse event, but no responses to openended questions about adverse events |
<p>| Jawad et al., Placebo-controlled, | Ethanol extract from freshly harvested <em>E. purpurea</em> (95% herba and 5% root) or | 755 adults, 82 Healthy, but experience of 293 adverse events (but no serious) occurred with No serious adverse | 293 adverse events (but no serious) occurred with No serious adverse | | | |</p>
<table>
<thead>
<tr>
<th>Type</th>
<th>Study</th>
<th>Test Product(s)</th>
<th>Number of subjects</th>
<th>Type of subjects</th>
<th>Adverse reactions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>double-blind, randomized, parallel trial</td>
<td>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</td>
<td>drop out (Echinacea: 244 female, 111 male; placebo: 227 female, 135 male)</td>
<td>at least two colds/year</td>
<td>Echinacea and 306 with placebo treatment. Nine and 10% of participants experienced adverse events.</td>
<td>events, Echinacea appears to be safe</td>
</tr>
<tr>
<td>Rauš et al., (2015)</td>
<td>Randomized, double-blind, double-dummy, multicenter, controlled</td>
<td>Group 1: Echinacea hotdrink commercial preparation = a hydro-ethanolic extract (65% V/V) of freshly harvested Echinacea 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 Echinacea hotdrink. Group 2: 5 days oseltamivir followed by 5 days of placebo; duration of treatment: 10 days;</td>
<td>473 patients, 12-70 years, Czech Republic, drop out: 50 patients</td>
<td>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; negative pregnancy test, body weight of &gt; 40 kg</td>
<td>26/229 patients in the Echinacea hotdrink commercial preparation group reported adverse events, 4 were assessed to be related to Echinacea. Fewer adverse events (particularly nausea and vomiting) were observed with Echinacea hotdrink commercial preparation compared to oseltamivir.</td>
<td>Echinacea appears to be a safer treatment than oseltamivir. Adverse events were rare; no serious adverse events were discovered.</td>
</tr>
<tr>
<td>Type</td>
<td>Study</td>
<td><strong>Test Product(s)</strong></td>
<td><strong>Number of subjects</strong></td>
<td><strong>Type of subjects</strong></td>
<td><strong>Adverse reactions</strong></td>
<td><strong>Comments</strong></td>
</tr>
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</table>
|      | Hennecke-von Zepelin et al., 1999 | regime:  
group 1:  
days 1-3: 5 x 5 ml *Echinacea* hotdrink commercial preparation verum syrup, dissolved in 150 ml hot water;  
days 4-10: 3 x 5 ml;  
group 2:  
oseltamivir/placebo days 1-10: 2 x 1 capsule, orally | 263 patients  
(male and female), 18-70 years, drop out: 21, 238 valid cases for secondary efficacy analysis | kg, good general health, signed informed consent | Patients who were attending their family doctor for acute common cold  
2 questionable, 1 possible adverse event (insomnia), no serious events | *Echinacea* can be stated as safe |
|      | Linden muth and Linden muth, 2000 | Ethanolic-aqueous extracts of *Herba thujae occidentalis*, *Radix echinaceae* (*purpureae + pallidae = 1 + 1*) and *Radix baptisiae*, 2, 7.5 and 10 mg per tablet, compared to placebo regimen:  
3 tablets t.i.d. for 7 to 9 days, orally | 95 patients  
(81 female, 14 male), 24-62 years, drop out not available/found | Hospital patients with early symptoms of cold and flu | No adverse reactions reported | Safe treatment |
<table>
<thead>
<tr>
<th>Type</th>
<th>Study</th>
<th>Test Product(s)</th>
<th>Number of subjects</th>
<th>Type of subjects</th>
<th>Adverse reactions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goel et al., 2004</td>
<td>Placebo-controlled, double-blinded, randomized</td>
<td>Formulation containing alkamides, cichoric acid, and polysaccharides at concentrations of 0.25, 2.5, and 25 mg/ml, prepared from freshly harvested <em>E. purpurea</em> herb and root (Echinilin®), placebo; regimen: 10 doses the first day, 4 doses per day on subsequent days for 7 days</td>
<td>282 subjects (18-65 years) randomized, 128 contracted a cold and were subjected to ITT, drop out: 17/128</td>
<td>Subjects with a history of two or more colds in the previous year, but otherwise in good health</td>
<td>&quot;A few adverse event profiles were observed in both groups&quot; (in the article, it is not further specified)</td>
<td>Lack of information about adverse events in the article</td>
</tr>
<tr>
<td>Barth et al., 2015</td>
<td>Placebo-controlled, double-blinded, randomized, parallel-group</td>
<td>Kan Jang® oral solution (KJ) = fixed combination aqueous ethanolic extracts of Justicia adhatoda leaf, <em>E. purpurea</em> root, and <em>Eleutherococcus senticosus</em> root, 30 ml/day, orally; 762 mg genuine extracts with standardized contents of 0.2 mg/ml vasicine, 0.8 mg/ml cichoric acid, and 0.03 mg/ml eleutherosides B and E), compared with placebo and bromhexine (24 mg/30 ml/day); duration: 5 days</td>
<td>177 patients (101 female, 76 male), 18-65 years, drop out not available/found</td>
<td>Patients with non-complicated upper respiratory infections (URI; i.e., common cold)</td>
<td>2 minor adverse events in the KJ group (3.9 %), minor adverse events: pruritus, diarrhea, abdominal pain, skin rash; no serious adverse events</td>
<td>Safe treatment</td>
</tr>
</tbody>
</table>
Table 10: Pharmacovigilance reports from Member States (causality has not been evaluated)

<table>
<thead>
<tr>
<th>Country</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>Literature: Case report in the Finnish Medical Journal (3/2002): A man with chronic alcoholism used for years high-concentration ethanol extract of <em>Echinacea</em> 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. (The applicant has been requested to equip the products with a childproof closure but this variation is pending).</td>
</tr>
<tr>
<td>Ireland</td>
<td>There have been 13 case reports associated with <em>Echinacea</em> containing products with 26 adverse reactions as follows: 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</td>
</tr>
</tbody>
</table>
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).

### 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 toxicity, 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,
The overall herbal reference scores ranged from 2.2 to 0.4 (median 1.1). The Natural Medicines Comprehensive Database received the highest overall score and was the most complete and useful reference source. All of the references, however, lacked sufficient information on management of herbal medicine overdose, and several had incorrect overdose management guidelines that could negatively impact patient care. Current herbal reference texts do not contain sufficient information for the assessment and management of adverse health effects of botanical therapies (Haller et al., 2001).

Reports to poison control centers (PCCs) were characterised involving two widely used herbal dietary supplements (HDSs), *Echinacea*, and St. John's wort (SJW). METHODS: Data were purchased from the American Association of Poison Control Center's (AAPCC) toxic exposure surveillance system (TESS(R)) on reports made to PCCs in 2001 involving Echinacea or SJW. Analyses were limited to those cases in which Echinacea or SJW were the only associated products, and in which these HDSs were deemed primary to observed adverse effects. Descriptive statistics were generated for selected demographic and exposure-related variables. During 2001, PCCs were contacted regarding 406 exposures involving Echinacea and 356 exposures involving SJW. Most of the reported exposures for both HDSs occurred among children 5 years and younger, and the majority of exposures were coded as unintentional. For both HDSs, exposures among patients >/=20 years old were more likely to be associated with adverse effects. Intentional exposures accounted for 21% of SJW cases and 3% of Echinacea cases, with 13% of SJW exposures reported as 'suspected suicidal'. TESS represents a potentially important means of assessing and characterizing HDS-related adverse effects. Detailed studies validating the clinical events and outcomes of a sample of exposures reported to TESS(R) might offer substantial insights into adverse events that could be systematically studied with other, established pharmacoepidemiological study designs (Gryzlak et al., 2007).

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 can be 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 found 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 contraindication were indicated.

5.5.3. Special warnings and precautions for use

Based on the presumption 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 made 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, immunosupression 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. 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 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 (Bossaer & 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/μL, 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* (Bossaer & 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 *Echinacea* purpurea radix preparations and their possible interaction with 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* drug-herb interactions.

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.

Pregnancy outcome in women that used *Echinacea* during pregnancy was studied to evaluate the safety of *Echinacea*. There is no specification which species of *Echinacea* was evaluated. Since at least half of all pregnancies are unplanned, many women inadvertently use *Echinacea* in their first trimester. The study group consisted of 206 women who were prospectively followed up after contacting the Motherisk Program regarding the gestational use of *Echinacea*, 112 women used the herb in the first trimester. This cohort was disease-matched to women exposed to non-teratogenic agents by maternal age, alcohol, and cigarette use. Rates of major and minor malformations between the groups were compared. There were a total of 195 live births, including 3 sets of twins, 13 spontaneous abortions, and 1 therapeutic abortion in the *Echinacea* group. Six major malformations were reported, including 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 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* 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 presumption 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 any 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. A European Union list entry is not supported due to lack of adequate data.

**Annex**

*List of references*