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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Echinacea pallida* (Nutt.) Nutt., radix

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Cut, dried underground parts of *Echinacea pallida* (Nutt.) Nutt.
Herbal preparation(s)	• dry extract (DER 4-8:1); extraction solvent: ethanol 50% V/V • tincture (ratio of herbal substance to extraction solvent 1:5); extraction solvent: ethanol 50% V/V
Pharmaceutical form(s)	Herbal preparations in solid or liquid dosage forms for oral and oromucosal use
Rapporteur(s)	Samo Kreft, Barbara Razinger
Assessor(s)	Damjan Janeš, Maja Udovič
Peer-reviewer	Jacqueline Wiesner

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of Contents

1. Introduction

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

- Herbal substance(s)

Because of confusion regarding the identification of *Echinacea* species, much of the early research conducted in Europe on *Echinacea angustifolia* was probably conducted on *Echinacea pallida* (Bauer *et al.,* 1988a).

Echinaceae pallidae radix (Ph. Eur 01/2008:1822)

Echinaceae pallidae radix consists of the whole or cut, dried underground parts of *Echinacea pallida* (Nutt.) Nutt. It contains not less than 0.2% of echinacoside in the dried drug.

Constituents (Barnes *et al.,* 2005 and 2007; Bauer & Remiger, 1989; Bradley, 2006; ESCOP 2003; Bauer & Liersch, 2008; WHO, 1999; Willuhn, 2002; Wolters Kluwer Health, 2012):

- Alkamides: mainly absent (0.001%).
- Phenylpropanoids: caffeic acid glycosides (echinacoside as the major component, 0.5-1.0%), caffeic acid esters of quinic acid (chlorogenic acid, isochlorogenic acid, cynarin), caffeic acid glycosides of tartaric acid (caftaric acid, cichoric acid).
- Polysaccharides and glycoproteins.
- Volatile oils (0.2-2.0%): mainly polyenes and polyacetylenes (pentadeca-1,8*Z*-diene), ketoalkenes (pentadeca-8*Z*-en-2-one) and ketoalkenynes (pentadeca-8*Z*,13*Z*-diene-11-yne-2-one, tetradeca-8*Z*-ene-11,13-diyne-2-one). These alkenes are unstable and readily oxidise to 8-hydroxy derivatives.
- Other constituents: phytomelanin.

- Herbal preparation(s)

Information about registered/authorised herbal preparations on the European market of *Echinaceae pallida*, radix was provided by the National Competent Authorities and is presented in the overview of the market products, see section 2.1.1.

In Germany the following herbal preparations of *Echinacea pallida*, radix are or were present as monocomponent medicinal products:

a) dry extract (DER 4-8:1), extraction solvent: ethanol 50% V/V.

b) tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent: ethanol 50% V/V.

These herbal preparations are not described in any available pharmacopoeia or handbook. Herbal preparation similar but not identical to herbal preparation B) was phytochemically investigated (Senchina *et al*., 2011). The investigated herbal preparation was extracted from fresh roots of *Echinacea pallida* with 50% V/V ethanol, but the ratio of herbal substance to extraction solvent was 1:9. The analysis of this preparation showed that the concentration of ketone 23 had been 0.002 mg/ml, and the concentration of echinacoside had been 0.009 mg/ml. The following substances were also analysed, but they were not detected: alkamide 2a, alkamide 2b, alkamide 8a, alkamide 8b,

alkamide 10a, alkamide 10b, alkamides 11–14, ketone 24, caftaric acid, chlorogenic acid and cichoric acid.

- 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 database of PubMed was used.

The research in PubMed contained the keywords "*Echinacea* pallida radix treatment" (36 results), "*Echinacea* and pallida root and treatment" (16 results), "*Echinacea* and pallida and radix and treatment" (6 results) and "*Echinacea* pallida radix and Immunomodulatory activity" (2 results). All articles from 01 January 2007 to 18 March 2017 were included and then assessed due to their value for medicinal use of *Echinacea pallida* radix. Articles with any reference to *Echinacea*'s health-related processes were included, whereas articles without any reference to physiological processes (e.g. the development of a new validation method) were excluded.

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.

Search engines used: Google

Scientific databases: PubMed

Medical databases: PubMed

Toxicological databases: Toxline

Pharmacovigilance resources: Eudravigilance

Data from EU and non-EU regulatory authorities: Information received from the national delegates of HMPC

2. Data on medicinal use

2.1. Information about products on the market

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

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status**
Germany			
Dry extract from Echinaceae pallidae radix (5-7:1); extraction solvent: methanol 30% V/V	Supportive treatment of respiratory tract infections	Coated tablet >12 years: 100 mg dry extract 2 times daily Not longer than 8 weeks	1997, WEU
Dry extract from Echinaceae pallidae radix (4-8:1); extraction solvent: ethanol 50% V/V	Supportive treatment of respiratory tract infections	Lozenge >12 years: 30 mg dry extract 3 times daily Not longer than 2 weeks	1976, WEU
Dry extract from Echinaceae pallidae radix (4-8:1); extraction solvent: ethanol 50% V/V	Supportive treatment of respiratory tract infections	Tablet >12 years: 24 mg dry extract 4 times daily Not longer than 2 weeks	1976, WEU
Tincture from Echinaceae pallidae radix (1:5), extraction solvent: ethanol 50% V/V	Supportive treatment of respiratory tract infections	Oral liquid >12 years: 5 times daily 25 drops containing 100% liquid extract Not longer than 2 weeks	1976-2008, WEU

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

Besides the above listed products, also the following product containing liquid extract from Echinaceae pallidae radix is reported to be on the market in **Hungary** from 1993 till 2013 as 'healing product':

liquid extract (1:6.3-7.0), solvent: ethanol 96% V/V: wine 16% V/V, (1:1,5); pharmaceutical form: oral solution; posology: prevention: 3 times 20 drops daily, treatment: 3 times 50 drops daily; indications: to increase the resistance of the body to prevent of recurrent infections of upper respiratory tract (common cold) and adjuvant therapy of them.

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

In many EU countries a combination product of Echinaceae pallidae radix with Thujae herba, Echinaceae purpureae radix and Baptisiae tinctoriae radix is registered or authorised for the supportive treatment of common cold.

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

No other products were reported from the member states.

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

No data available.

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

Medicinal uses of *Echinacea* species among American Indians were many and varied. *Echinacea angustifolia* was universally used as an antidote for snakebite and other venomous bites and stings and poisonous conditions. Before 1968, *Echinacea angustifolia* and *Echinacea pallida* were considered to be different varieties of the same species until a revision of the genus described them as two separate species (WHO, 1999). In comparison to *Echinacea purpurea* the use of *Echinacea pallida* is much less documented in the literature. In many cases, some important information are missing (e.g. detailed information on herbal preparation and posology). In several references (Bräunig & Knick, 1993; Willuhn, 2002) and phytotherapeutic textbooks (Blumenthal *et al.*, 2000; WHO, 1999 and ESCOP, 2003) usage of liquid or dry extracts from *Echinacea pallida* in indications such as therapy of infections of the upper respiratory tract (influenza, "grippaler infect") could be found.

Table 2: Overview of historical data

Herbal preparation	**Documented use / traditional use**	**Pharmaceutical form** **Posology** **Duration of use**	**Reference**
Liquid extract from Echinaceae pallidae radix (DER not reported), extraction solvent: water, ethanol (concentration not reported)	Therapy of recurrent infections of the upper respiratory tract (influenza, "grippaler infekt").	Oral solution Adult daily dose: hydroethanolic extract corresponding to 900 mg of crude drug=90 drops Duration of use: 8-10 days	Bräunig & Knick, 1993

Herbal preparation	Documented use / traditional use	Pharmaceutical form Posology Duration of use	Reference
Tincture from Echinaceae pallidae radix (1:5); extraction solvent: ethanol 50% V/V from native dry extract (7-11:1) extraction solvent: ethanol 50% V/V	Adjuvant therapy of flu infections ("grippeartige infekte")	Oral liquid preparations Adult daily dose: tincture equivalent to 900 mg of herbal drug The duration of treatment should not exceed 8 weeks	Blumenthal *et al*., 2000; German Comission E Monograph, 1992.
Dry extract from Echinaceae pallidae radix (5:7.1), extraction solvent: not reported Dry extract from Echinaceae pallidae radix (6.1:7.2), extraction solvent: 30% ethanol (not reported if V/V) Dry extract from Echinaceae pallidae radix (4:7), extraction solvent: 50 % ethanol (not reported if V/V) Liquid extract from Echinaceae pallidae radix (1:6.66), extraction solvent: 48% ethanol (not reported if V/V)	Therapy of recurrent infections of the upper respiratory tract (influenza, "grippeartige infekte")	Tablet, capsule Adult daily dose: equivalent to 900 mg of herbal drug The duration of treatment should not exceed 8 weeks	Willuhn, 2002
Tincture from Echinaceae pallidae radix (1:5) extraction solvent: ethanol 50% V/V from native dry extract, extraction solvent: ethanol 50% V/V	Supportive therapy for colds and infections of the respiratory and urinary tract	Oral preparations Adult daily dose: hydroethanolic extract corresponding to 900 mg of crude drug The use should not exceed the period of 8 successive weeks	WHO, 1999

Herbal preparation	Documented use / traditional use	Pharmaceutical form Posology Duration of use	Reference
Extract from Echinaceae pallidae radix (DER not reported), extraction solvent: water, ethanol (concentration not reported)	Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold)	Oral preparations Adult daily dose: hydroethanolic extract corresponding to 900 mg of crude drug The duration of treatment should not exceed 8 weeks	ESCOP, 2003

2.3. Overall conclusions on medicinal use

There are currently two herbal preparations with 30 years of tradition on medicinal use:

- dry extract from Echinaceae pallidae radix (DER 4-8:1), extraction solvent: ethanol 50% V/V, in Germany on the market at least from 1976
- tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent: ethanol 50% V/V, with marketing authorisation in Germany from 1976 to 2008.

This two herbal preparations fulfil the regulatory requirement for traditional use.

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
Dry extract from Echinaceae pallidae radix (DER 4-8:1), extraction solvent: ethanol 50% V/V	Supportive treatment of respiratory tract infections	Adults and adolescents: 24-30 mg dry extract) 3-4 times daily Daily dosage: 90-96 mg Not use longer than 2 weeks	Germany since 1976, WEU
Tincture from Echinaceae pallidae radix (ratio of herbal substance to extraction solvent 1:5), extraction solvent: ethanol 50% V/V	Herbal medicinal product for the supportive treatment of common cold.	Adults and adolescents: 5 times daily 25 drops containing 100% liquid extract Not use longer than 2 weeks	Germany since 1976 until 2008, WEU

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

3.1.1.1. Immunomodulatory activity

In vitro experiments

Comparable/similar preparations to preparations of the monograph

In vitro immunomodulatory properties of several species of *Echinacea* was measured. Human peripheral blood mononuclear cells were cultured with root tinctures from *Echinacea laevigata*, *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea*. Fresh root material was extracted in 50% ethanol/50% cell culture water at a ratio of 1:9 parts plant:solvent. Cytokine production (tumor necrosis factor [TNF], interleukin [IL]-2, IL-10) and mononuclear cell proliferation were measured. HPLC was used to assay levels of known bioactive compounds from all extracts tested to statistically determine whether there were relationships between extract phytochemical content and observed immune effects. *Echinacea laevigata* extract was most similar to *Echinacea pallida* extract and able to augment IL-10 and mononuclear cell proliferation, but not TNF or IL-2. Echinacoside, a caffeic acid derivative, correlated most strongly with results (Senchina *et al.,* 2011).

Immunomodulatory properties of *Echinacea* tinctures (fresh root material was extracted in 50% ethanol/50% water solvent at a ratio of 1 part plant/9 parts solvent) from seven species after being stored at −20°C for 2 years was investigated. Two experimental techniques were employed using human peripheral blood mononuclear cells (PBMC). In the first set of experiments, PBMCs were stimulated *in vitro* with tinctures alone and assayed for proliferation and production of interleukin-10 (IL-10), IL-12, and tumor necrosis factor-α (TNF-α). In the second set of experiments, subjects were immunized with influenza vaccine. PBMCs from vaccinated individuals were stimulated *in vitro* with *Echinacea* tinctures and influenza virus; cytokine production (IL-2, IL-10, and interferon-γ [IFN-γ]) was compared prevaccination and postvaccination. In the first experiments, (1) tinctures from *E. angustifolia, E. pallida, E. paradoxa*, and *E. tennesseensis* stimulated proliferation and tended to increase IL-10, (2) *E. sanguinea* and *E. simulata* stimulated only proliferation, (3) *E. purpurea* stimulated only IL-10, and (4) none of the extracts influenced IL-12 or TNF-α. In the second experiments, (1) tinctures from *E. pallida, E. paradoxa, E. sanguinea*, and *E. simulata* diminished influenza-specific IL-2, and (2) none of the extracts influenced influenza-specific IL-10 or IFN-γ. For *in vitro* models using *Echinacea*, immune response may vary based on stimulus (*Echinacea* alone vs. *Echinacea*+recall stimulation with virus) (McCann *et al.,* 2007).

The effects of long-term (>1 year) dry storage on the capabilities of *Echinacea* spp. roots from mature individuals to modulate cytokine production are unknown. Using an older human adult model of influenza vaccination, peripheral blood mononuclear cells were collected from subjects 6 months post-vaccination and stimulated them *in vitro* with the two Type A influenza viruses contained in the trivalent 2004-2005 vaccine with a 50% alcohol tincture (ratio of plant parts:solvent 1:9) prepared from the roots of one of seven *Echinacea* species: *E. angustifolia*, *E. pallida*, *E. paradoxa*, *E. purpurea*, *E. sanguinea*, *E. simulata*, and *E. tennesseensis*. Before being processed into extracts, all roots had been stored under dry conditions for sixteen months. Cells were cultured for 48 hours; following incubation, supernatants were collected and assayed for IL-2, IL-10, and INF-gamma production, cytokines important in the immune response to viral infection. Four species (*E. angustifolia*, *E.*

purpurea, *E. simulata*, *E. tennesseensis*) augmented IL-10 production, diminished IL-2 production, and had no effect on IFN-gamma production. *Echinacea pallida* suppressed production of all cytokines; *E. paradoxa* and *E. sanguinea* behaved similarly, although to a lesser extent. The results from these *in vitro* bioactivity assays indicate that dried *Echinacea* roots stored for sixteen months maintain cytokine-modulating capacities (Senchina *et al.,* 2006).

Other preparations

Chemical investigation of the 80% ethanolic extracts from roots of *Echinacea angustifolia*, *Echinacea purpurea*, and *Echinacea pallida* yielded two new alkamides, identified by analysis of spectroscopic data and comparison with reported alkamides. The new compounds were dodeca-2 *Z*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide from *Echinacea angustifolia* and dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide from *Echinacea purpurea* and *Echinacea pallida*. These two components, as well as previously identified alkamides, exerted inhibition on lipopolysaccharide (LPS)-mediated activation of a murine macrophage line, RAW264.7 (Chen *et al.,* 2005).

A 90% ethanolic extract (1:10) of *Echinacea pallida* root at concentration of 10^{-2} mg/ml enhanced the phagocytosis index of human granulocytes by 23%; no effect was observed at concentations of 10^{-6} mg/ml or lower. The chloroform soluble fraction from the ethanolic extract increased phagocytosis by 39% at 10^{-4} mg/ml, while the water-soluble fraction stimulated phagocytosis by a maximum of only at 10^{-3} mg/ml (Bauer *et al*., 1988b).

Fractions of extracts, isolated compounds

A high molecular weight fraction (M_r>10000 D) containing polysaccharides and glycoproteins from pale coneflower root (30% ethanolic extract of *Echinacea pallida* roots) enhanced the proliferation of mouse spleen cells, and stimulated the production of IFN-/and IgM as well as the number of antibody-producing cells in spleen cell cultures. It also increased the production of cytokines and nitric oxide in mouse macrophage cultures (Beuscher *et al.,* 1995, Bodinet 1999). Incubation of this fraction with human monocytes enhanced the production of IL-1, IL-6 and TNF (Bodinet *et. al*., 1999).

In contrast to *Echinacea purpurea* alkamides, *Echinacea pallida* compounds have not been synthesized and studied for immunostimulatory effects (Egger *et al.,* 2008). A synthetic approach to the ketoalkenes using palladium-catalysed cross-coupling reactions and the pharmaceutical results at the human cannabinoid receptors was investigated. The synthetic route developed provides overall good yields for the ketoalkenes and is applicable to other natural products with similar 1,4-diene motifs. No significant activity was observed at either receptor, indicating that the ketoalkenes from *Echinacea pallida* are not responsible for immunomodulatory effects mediated via the cannabinergic system. However, newly synthesized non-natural analogues showed micro-molar potency at both cannabinoid receptors.

The influences of different arabinogalactan-proteins (AGPs) on proliferation and immunoglobulin (Ig)M-production of mouse lymphocytes as well as nitrite-and IL6-production of mouse macrophages were investigated *in vitro*. AGPs have been isolated and purified from roots of *Baptisia tinctoria* and *Echinacea pallida* and suspension culture of *Echinacea purpurea*. Precipitation with absolute ethanol and Yariv's reagent yielded a purified AGP. Comparing the AGPs, there are differences with regard to fine structure as well as to activities. AGPs from roots of *B. tinctoria* and *Echinacea pallida* show high activity in all test systems. AGP from cell culture of *Echinacea purpurea* shows no influence on proliferation of mouse lymphocytes, only weak influence on the IgM-production of mouse lymphocytes and weak stimulation of nitrite-and IL6-production in alveolar mouse macrophage culture (Classen *et al.,* 2006).

***In vivo* experiments**

Other preparations

Extracts from roots of *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea*, were investigated for immunomodulating properties. Alcohol extracts from ground root powder of three widely used *Echinacea* species was refluxed with 250 ml of different organic solvents, 100% ethanol, 95% ethanol, chloroform, and hexane, for 6 hours using a Soxhlet extraction device. The extract was evaporated to dryness. Before animal treatment, the extracts were dissolved in 95% ethanol and then diluted in water to a final suspension containing 14.7 mg/ml extracts in 5% ethanol. The endotoxin level was evaluated in the three *Echinacea* preparations and was below the limit of detection (0.1 EU/ml). 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 herb extracts induced similar, but differential, changes in the percentage of immune cell populations and their biological functions, including increased percentages of $CD49^+$ and $CD19^+$ lymphocytes in spleen and natural killer cell cytotoxicity. Antibody response to sRBC was significantly increased equally by extracts of all three *Echinacea* species. Concanavalin A-stimulated splenocytes from *Echinacea angustifolia*- and *Echinacea pallida*-treated mice demonstrated significantly higher T- cell proliferation. In addition, the *Echinacea* treatment significantly altered the cytokine production by mitogen-stimulated splenic cells. The three herbal extracts significantly increased interferon (IFN)-alpha production, but inhibited the release of tumor necrosis factor (TNF)-gamma and interleukin (IL)-1beta. Only *Echinacea angustifolia*- and *Echinacea pallida*-treated mice demonstrated significantly higher production of IL-4 and increased IL-10 production. Taken together, these findings demonstrated that *Echinacea* is a wide-spectrum immunomodulator that modulates both innate and adaptive immune responses (Zhai *et al.,* 2007a).

In the carbon clearence test in mice, oral administration of a 90%-ethanolic extract (1:10) of pale coneflower root daily for 2 days at 0.5 ml/kg body weight increased phagocytosis 2.2-fold. When chloroform and water soluble fractions of the ethanol extract were administered separately at concentrations corresponding to their content in the original extract, the lipophilic fraction (2.6-fold increase) proved more active than the hydrophilic (1.3-fold increase) (Bauer *et al.,* 1988b).

Fractions of extracts, isolated compounds

Intravenous injection of 50, 100 or 500 l of a high molecular weight fraction (M_r>10000 D) containing polysaccharides and glycoproteins from pale coneflower root significantly increased the concentration of the cytokine IL-1 in the serum of mice ($p<0.05$) (Beuscher *et al.,* 1995). A single oral administration of this fraction to mice at 3.7 mg per animal significantly enhanced antibody production in Peyer's plaque cells (it is not known how fraction was prepared) (Bodinet, 1999).

3.1.1.2. *In vitro* antimicrobial activity

Other preparations

The antibacterial activity of echinacoside isolated from roots of *Echinacea pallida* were tested (0.4%-1.4 mg/g dry weight). against *Staphylococcus aureus.* Its antimicrobial activity (at 8 times 10^{-3} M) corresponds to approximately 10 Oxford units of penicillin, one potent unit of penicillin is 6 mg (Stoll *et al.,* 1950).

3.1.1.3. In vitro antiviral activity

Other preparations

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

Extracts prepared with 20%, 40%, 60%, or 80% aqueous ethanol (V/V) and pressed juice from aerial parts of *Echinacea pallida* were characterised by HPLC-MS analyses (Schneider *et al.,* 2010). Ferulic and caffeic acid derivatives were identified as major constituents. All tested extracts and pressed juice exhibited a low cytotoxic activity on monkey kidney cells *in vitro*. The inhibitory activity of against *Herpes simplex* virus types 1 and 2 (HSV-1, HSV-2) was analysed with plaque reduction assays. All hydroalcoholic extracts exhibited high levels of antiviral activity against both types of herpesvirus in a dose-dependent manner. Plaque formation was significantly reduced by more than 99% or completely absent. Pressed juice revealed the highest antiviral activity against HSV-1 and HSV-2 when compared to hydroalcoholic extracts and even highly diluted pressed juice still inhibited viral infectivity. Hydroalcoholic extracts were quite active against herpetic infection when HSV-1 or HSV-2 were pretreated with the extracts. In contrast, pressed juice revealed antiviral activity during all phases of the viral replication cycle. Additionally, pressed juice demonstrated protection of cells against viral infection. In conclusion, hydroalcoholic *Echinacea pallida* extracts interfere with free herpesvirus but pressed juice is able to interact with herpesvirus inside and outside the cell as well as to protect cells against viral infection, probably by interfering with virus attachment.

Fractions of extracts, isolated compounds

A high molecular weight fraction (Mr>10000 D) containing polysaccharides and glycoproteins from pale coneflower root exhibited antiviral activity against HSV type 1 in a plaque-reduction assay (Beuscher *et al*., 1995). Antiviral activity of ecinacoside against vesicular stomatitis virus in L-929 mouse cells was demonstrated in a plaque reduction assay. In the article it is not described how the extract was prepared (Cheminat *et al.*, 1988).

3.1.1.4. In vivo anti-inflammatory activity

Other preparations

It was found in a comparative metabolomics study, integrating supercritical fluid extraction, gas chromatography/mass spectrometry and data mining, 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. A mitogen-induced murine skin inflammation study suggested that alkamides were the active anti-inflammatory components present in *Echinacea* plants. Mixed alkamides and the major component, dodeca-2*E*,4*E*,8*Z*,10Z(E)-tetraenoic acid isobutylamides (8/9), were then isolated from *E. purpurea* root extracts (approximately 1.5 kg of fresh E. purpurea roots were extracted by 100% methanol for further bioactivity elucidation. GC/MS analyses: *E. purpurea* and *E. angustifolia* root extracts contained approximately 74.06% and 90.73% alkamides, respectively, while *E. pallida* root extract mainly composed of polyacetylenes (80.81%) and only 6.12% alkamides. 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 (isolated from *E. purpurea*) however, exhibited little or no effect (Hou *et. al.,* 2010).

Antiinflammatory activity was found to be different in different *Echinacea* species, with *E. sanguinea* having the greatest activity and *E. angustifolia*, *E. pallida*, and *E. simulata* having somewhat less. Fractionation and studies with pure compounds indicate that this activity is explained, at least in part, by the alkamide constituents. 70% ethanol extracts from *Echinacea* roots (no further information given) had potent activity as novel agonists of TRPV1, a mammalian pain receptor reported as an integrator of inflammatory pain and hyperalgesia and a prime therapeutic target for analgesic and antiinflammatory drugs. One fraction from *E. purpurea* ethanol extract was bioactive in this system. Interestingly, the antiinflammatory compounds identified to inhibit prostaglandin E(2) production differed from those involved in TRPV1 receptor activation. They tested the effect of 6 purified *Echinacea* alkamides on TRPV1 with use of the frog oocyte model. Interestingly, no alkamide tested activated the TRPV1 channel (Birt *et. al.,* 2008).

The effect of alcohol extracts (alcohol extracts from the dried roots were prepared as previously described (Zhai *et al*., 2007a) followed by evaporation until dry. These dry residues were subsequently dissolved in ethanol and phosphate buffed saline (PBS) to desired concentrations for *in vitro* assays or dissolved in ethanol and Nanopure water for animal studies.) from roots of *Echinacea angustifolia* (EA), *Echinacea pallida* (EPA) and *Echinacea purpurea* (EP) on the production of inflammatory mediators (nitric oxide (NO), TNF-alpha, and IL-1beta) in both LPS-stimulated RAW 264.7 macrophages *in vitro* and murine peritoneal exudate cells (PECs) *in vivo* were investigated. As macrophages produce these inflammatory mediators in response to pathogenic infection, parallel cultures of macrophages were studied for phagocytosis and intracellular killing of *Salmonella enterica*. EPA and EP *in vitro* inhibited NO production and TNF-alpha release in a dose-dependent manner. RAW 264.7 cells treated with EA or EP showed decreased killing over 24 hours, although EA enhanced bacterial phagocytosis. Upon bacterial infection, RAW 264.7 cells produce high levels of NO; however, an *Echinacea*-mediated decrease in NO production was observed. *Echinacea* alcohol extracts administered orally at 130 mg/kg per day for seven days had a weak effect on NO production and phagocytosis by LPS-stimulated PECs. The results indicated that all *Echinacea* species significantly decreased inflammatory mediators *in vitro*, however, only EA and EP reduced bacterial killing. Oral administration of *Echinacea* alcohol extracts did not adversely affect the development and anti-bacterial function of inflammatory PECs *in vivo*; however, NO production was decreased during bacterial infection of PECs (Zhai *et al.,* 2007b). The posology used in this study (130 mg/kg of extract per day) was much higher than posology recommended for human, which is 90 mg per day (=1.8 mg/kg for a 50 kg adult), therefore the results of the study can not be correlated to the effects in human patients.

Inhibition of prostaglandin E_2 (PGE_2) production in LPS-stimulated RAW264.7 mouse macrophage cells was assessed with an enzyme immunoassay following treatments with *Echinacea* extracts or synthesized alkamides. Results indicated that 95% ethanol extracts from roots diluted in media to a concentration of 15 µg/ml from *E. angustifolia*, *E. pallida*, *E. simulata*, and *E. sanguine* significantly inhibited PGE_2 production. In further studies, PGE_2 production was significantly reduced by all synthesized alkamides assayed at 50 M, by Bauer alkamide 8, Bauer alkamide 12A analogue, and Bauer alkamide 14, Chen alkamide 2, and Chen alkamide 2 analogue at 25 M and by Bauer alkamide 14 at 10 M. Cytotoxicity did not play a role in the noted reduction of PGE_2 production in either the *Echinacea* extracts or synthesized alkamides. High-performance liquid chromatography analysis identified individual alkamides present at concentrations below 2.8 M in the extracts from the six *Echinacea* species. Because active extracts contained<2.8 M of specific alkamide and the results showed that synthetic alkamides must have a minimum concentration of 10 M to inhibit PGE_2, it was concluded by the authors that alkamides may contribute toward the anti-inflammatory activity of *Echinacea* in a synergistic or additive manner (LaLone *et al.,* 2007).

5-lipoxygenase (5-LOX)-inhibiting activity of extracts of five wild and three commercially used species of the genus *Echinacea* were investigated to characterise anti-inflammatory activity of *Echinacea*. The inhibition of the 5-LOX enzyme of the arachidonic acid pathway was determined by HPLC detection of a direct metabolic product (LTB4) of 5-LOX derived from stimulated rat basophilic cells. 95% ethanol extracts from roots 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).

The anti-inflammatory and wound healing activities of echinacoside, compared with the ones of the total dry ethanolic root extract of *Echinacea purpurea* and *Echinacea angustifolia*, 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 h treatment and excised for histological observation at the end of the experiment. Results confirm the good anti-inflammatory and wound healing properties of *Echinacea pallida* and of its constituent echinacoside, with respect to *Echinacea purpurea* and control (ethanolic extract (1:10) of *Echinacea pallida* and *Echinacea purpurea,* concentration of ethanol not reported). The authors concluded that this activity probably resides in the antihyaluronidase activity of echinacoside (Speroni *et al.,* 2002).

Table 3: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Posology**	Experimental model	**Reference**	**Main non-clinical conclusions**
comparable/similar preparations to preparations of the monograph				
Roots extracts (extraction solvent: 70% ethanol)	MIC=0.78 mg/ml;	*In vitro* inhibiotion of Herpes simplex virus Type I	Binns *et al.,* 2002	*E. pallida* extract and pure cichoric acid inhibited HSV
other preparations				
70% ethanol extract from roots	15 µg/ml	Prostaglandin E production in cell culture; Agonistic activity on TRPV1	Birt *et al.,* 2008	Agonist of TRPV1; inhibition of prostaglandin E production

Herbal preparation tested	**Posology**	Experimental model	**Reference**	**Main non-clinical conclusions**
Alcohol extracts (100% ethanol, 95% ethanol, chloroform, and hexane) from ground root powder *E. pallida*	Orally administered to the animals at 130 mg/kg body weight once daily for seven consecutive days	Production of inflammatory mediators in macrophages (*in vitro*); phagocytosis and intracellular killing of *Salmonella enterica* and murine peritoneal exudate cells (*in vivo*);	Zhai *et al.,* 2007b	Decreased inflammatory mediators *in vitro*, however, bacterial killing was not reduced. No effect on PECs *in vivo*; however, NO production was decreased
95% ethanol extract	15 µg/ml	PGE2 production in mouse macrophage cells	LaLone *et al.,* 2007	Significant inhibition of PGE2 production
90%-ethanolic extract (1:10) of *E. pallida*	Oral administration for 2 days at 0.5 ml/kg body weight	*In vivo*	Bauer *et al.,* 1988b	Increased phagocytosis (2.2-fold): lipophilic fraction (2.6-fold increase) proved to be more active than the hydrophilic fraction (1.3-fold increase)
Ethanolic extract (1:10) of *E. pallida* root	Examined in rats, topical application of gel containing 100 mg/ml	*In vivo*	Speroni *et al*., 2002	Results confirm anti-inflammatory and wound healing properties of *E. pallida* and of its constituent echinacoside. This activity probably resides in the antihyaluronidase activity of echinacoside

3.1.2. Secondary pharmacodynamics

3.1.2.1. *In vitro* antioxidant activity

The radical scavenging activity of *Echinacea* methanolic (MeOH 80%; DER 1:20) extracts was evaluated *in vitro* with a spectrophotometric method based on the reduction of an alcoholic 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution at 517 nm in the presence of a hydrogen donating antioxidant. As for pure compounds, echinacoside had the highest capacity to quench DPPH radicals (EC50=6.6 M), while caftaric acid had the lowest (EC50=20.5 M). The average EC_{50} values for *Echinacea purpurea*, *Echinacea pallida* and *Echinacea angustifolia* were 134, 167 and 231 g/ml, respectively. The radical scavenging activity of *Echinacea* root extracts reflected their phenolic composition (Pellati *et al.,* 2004).

Alcoholic extracts (EtOH 85%; DER 1:10) 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 (Sloley *et al.,* 2001).

Methanol extracts (MeOH 100%; DER not reported) of freeze-dried *Echinacea* (*E. angustifolia*, *E. pallida*, and *E. purpurea*) roots were examined for free radical scavenging capacities and antioxidant activities. Root extracts of *E. angustifolia*, *E. pallida*, and *E. purpurea* were capable of scavenging hydroxyl radical. Similar scavenging activities for each variety were found for both 1,1-diphenyl-2-picrylhydrazyl radical and ABTS radical. Meanwhile, antioxidant activities of all three varieties of *Echinacea* were found to delay the formation of conjugated diene hydroperoxide induced by the thermal decomposition of 2,2'-azobis(2-amidinopropane) dihydrochloride and extend the lag phase of peroxidation of soybean liposomes. *Echinacea* root extracts suppressed the oxidation of human low-density lipoprotein, as evaluated by reduced agarose electrophoretic mobility following oxidative modification by Cu^{2+}. The mechanisms of antioxidant activity of extracts derived from *Echinacea* roots included free radical scavenging and transition metal chelating (Hu & Kitts, 2000).

The protective effect of caffeoyl derivatives (echinacoside, chlorogenic acid, chicoric acid, cynarine, and caffeic acid, typical constituents of *Echinacea* species (plant part not reported)) on the free radical-induced degradation of Type III collagen has been investigated. The macromolecule was exposed to a flux of oxygen radicals (superoxide anion and hydroxyl radical) generated by the xanthine/xanthine oxidase/Fe2+/EDTA system and its degradation assessed qualitatively by SDS-PAGE and quantitatively as the amount of soluble peptides (according to the 4-hydroxyproline method) released from native collagen after oxidative stress. The SDS-PAGE pattern of native collagen is markedly modified by free radical attack, with formation of a great number of peptide fragments with molecular masses below 97 kDa: in the presence of µM concentrations of echinacoside, there is a complete recovery of the native profile. Collagen degradation was, in fact, dose-dependently inhibited by all the compounds, with the following order of potency: echinacoside approximately chicoric acid>cynarine approximately caffeic acid>chlorogenic acid, with IC_{50} ranging from 15 to 90 µM. These results indicate that this representative class of polyphenols of *Echinacea* species protects collagen from free radical damage through a scavenging effect on reactive oxygen species and/or C-, N-, S-centered secondary radicals, and provide an indication for the topical use of extracts from *Echinacea* species for the prevention/treatment of photodamage of the skin by UVA/UVB radiation, in which oxidative stress plays a crucial role (Facino *et al.,* 1995).

3.1.2.2. *In vitro* antifungal activity

Other preparations

Extracts of *Echinacea pallida* root (extraction solvent: 95% ethanol at a ratio of 1 g fresh weight/10 ml (1:10) parts plant:solvent) exhibited near UV-mediated phototoxic and antifungal activity, measured by inhibition of the growth of *Candida shehata*: the activity was attributed primarily to ketoalkenes and ketoalkynes (Binns *et al.,* 2000).

Antifungal activity was tested against *Cryptococcus neoformans*, two *Candida albicans* isolates (D10 and CN1A), *Trychophyton tonsurans*, *T. mentagrophytes*, *Mycrosporum gypseum* and *Pseudallescheria boydii*. 95% ethanol root extracts at a ratio of 1 g fresh weight/10 ml (1:10) parts plant:solvent of eight *Echinacea* taxa, including *Echinacea pallida var. angustifolia* and *Echinacea pallida var. pallida* showed antifungal activity against most of the pathogenic fungi (Merali *et al.,* 2003).

3.1.2.3. Other activities

***In vitro* experiments**

Acetylenic constituent of *Echinacea pallida* roots, namely, pentadeca-(8 Z,13 Z)-dien-11-yn-2-one, showed a concentration-dependent cytotoxicity on several human cancer cell lines, including leukemia (Jurkat and HL-60), breast carcinoma (MCF-7), and melanoma (MeWo) cells (Chicca *et al*., 2010). As part of its mechanism of action, the ability of this constituent to arrest the cell cycle in the G1 phase was demonstrated on HL-60 cells.

The isolation and structure characterization of a dienone from the roots of Echinacea pallida, namely (8Z,11Z)-pentadeca-8,11-dien-2-one, were described (Morandi *et al*., 2008). To assess the configuration of this secondary metabolite, the stereoselective total synthesis of the two isomeric forms, (8Z,11Z) and (8Z,11E)-pentadeca-8,11-dien-2-one, was undertaken and the structure elucidation of the natural compound was unambiguously carried out. The cytotoxic activity of both isomers was also evaluated on a human T cell leukaemia cancer line (Jurkat cells). The results indicated that these compounds exert a dose-dependent cytotoxicity with a medium-level potency on the tested cell line.

The n-hexane extracts of the roots of three medicinally used *Echinacea* species exhibited cytotoxic activity on human cancer cell lines, with *Echinacea pallida* found to be the most cytotoxic. Acetylenes are present in *Echinacea pallida* lipophilic extracts but essentially absent in extracts from the other two species. In the present study, the cytotoxic effects of five compounds, two polyacetylenes (namely, 8-hydroxy-pentadeca-(9E)-ene-11,13-diyn-2-one (1) and pentadeca-(9E)-ene-11,13-diyne-2,8-dione (3)) and three polyenes (namely, 8-hydroxy-pentadeca-(9E,13Z)-dien-11-yn-2-one (2), pentadeca-(9E,13Z)-dien-11-yne-2,8-dione (4) and pentadeca-(8Z,13Z)-dien-11-yn-2-one (5)), isolated from the n-hexane extract of *Echinacea pallida* roots by bioassay-guided fractionation, were investigated and the potential bioavailability of these compounds in the extract was studied. Cytotoxic effects were assessed on human pancreatic MIA PaCa-2 and colonic COLO320 cancer cell lines. Cell viability was evaluated by the WST-1 assay and apoptotic cell death by the cytosolic internucleosomal DNA enrichment and the caspase 3/7 activity tests. Caco-2 cell monolayers were used to assess the potential bioavailability of the acetylenes. The five compounds exhibited concentration-dependent cytotoxicity in both cell types, with a greater potency in the colonic cancer cells. Apoptotic cell death was found to be involved in the cytotoxic effect of the most active, compound 5. Compounds 2 and 5 were found to cross the Caco-2 monolayer with apparent permeabilities above 10 times 10(-6) cm s(-1). Compounds isolated from n-hexane extracts of *Echinacea pallida* roots have a direct cytotoxicity on cancer cells (Chicca *et. al.,* 2008).

The *n*-hexane root extracts from *Echinacea pallida*, *Echinacea angustifolia* and *Echinacea purpurea* were evaluated for inhibition of the multidrug transporter P-glycoprotein (Pgp) activity, the product of the ABCB1 gene, involved in cancer multidrug resistance (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 *Echinacea pallida* was found to be the most active species. For the first time, two polyacetylenes and three polyenes, isolated from the *n*-hexane extract of *Echinacea pallida* roots by a bioassay-guided fractionation, were found to be able to reduce Pgp activity. Pentadeca-(8*Z*,13*Z*)-dien-11-yn-2-one was the most efficient compound, being able to decrease the calcein-AM efflux about three-fold with respect to the control at 30 µg/ml (Romiti *et al.,* 2008).

The *n*-hexane extracts of the roots of three medicinally used *Echinacea* species exhibited cytotoxic activity on human cancer cell lines, with *Echinacea pallida* found to be the most cytotoxic. Acetylenes are present in *Echinacea pallida* lipophilic extracts but essentially absent in extracts from the other two species. In the present study, the cytotoxic effects of five compounds, two polyacetylenes (namely, 8-hydroxy-pentadeca-(9*E*)-ene-11,13-diyn-2-one (1) and pentadeca-(9*E*)-ene-11,13 -diyne-2,8-dione (3)) and three polyenes (namely, 8-hydroxy-pentadeca-(9*E*,13*Z*)-dien-11-yn-2-one (2), pentadeca-(9*E*,13*Z*)-dien-11-yne-2,8-dione (4) and pentadeca-(8*Z*,13*Z*)-dien- 11-yn -2-one (5)), isolated from the *n*-hexane extract of *Echinacea pallida* roots by bioassay-guided fractionation, were investigated and the potential bioavailability of these compounds in the extract was studied. Cytotoxic effects were assessed on human pancreatic MIA PaCa-2 and colonic COLO320 cancer cell lines. Cell viability was evaluated by the WST-1 assay and apoptotic cell death by the cytosolic internucleosomal DNA enrichment and the caspase 3/7 activity tests. Caco-2 cell monolayers were used to assess the potential bioavailability of the acetylenes. The five compounds exhibited concentration-dependent cytotoxicity in both cell types, with a greater potency in the colonic cancer cells. Apoptotic cell death was found to be involved in the cytotoxic effect of the most active, compound 5. Compounds 2 and 5 were found to cross the Caco-2 monolayer with apparent permeabilities above 10 times 10^{-6}cms^{-1}. Compounds isolated from *n*-hexane extracts of *Echinacea pallida* roots have a direct cytotoxicity on cancer cells and good potential for absorption in humans when taken orally (Chicca *et al.,* 2008).

Bioassay-guided fractionation of *n*-hexane extracts of *Echinacea pallida* (Asteraceae) roots led to the isolation and structure elucidation of two polyacetylenes (1, 3) and three polyenes (2, 4, 5). Two of them are known hydroxylated compounds, namely 8-hydroxy-pentadeca-(9E)-ene-11,13-diyn-2-one (1) and 8-hydroxy-pentadeca-(9E,13Z)-dien-11-yn-2-one (2). Two dicarbonylic constituents, namely pentadeca-(9E)-ene-11,13-diyne-2,8-dione (3) and pentadeca-(9E,13Z)-dien-11-yne-2,8-dione (4), were isolated and characterized for the first time. Furthermore, the structure elucidation of pentadeca-(8Z,13Z)-dien-11-yn-2-one (5) is described. The structure of the compounds isolated was determined on the basis of UV, IR, NMR (including 1D and 2D NMR experiments, such as ^{1} H-^{1}H gCOSY, gHSQC-DEPT, gHMBC, gNOESY) and MS spectroscopic data. The cytotoxic activity of the isolated constituents against MIA PaCa-2 human pancreatic adenocarcinoma cells was evaluated in the concentration range 1-100 g/ml. Results show that the hydroxylated compounds (1, 2) have low cytotoxicity, while the more hydrophobic polyacetylenes (3) and polyenes (4, 5) displayed moderate activity (Pellati *et al.,* 2007).

Intake of *Echinacea* preparations is common among patients with advanced malignancies enrolled onto phase I chemotherapy trials; however, no data are available regarding the possible direct effect of *Echinacea* species on human cancer cells. The purpose of the study was to investigate potential *in vitro* cytotoxic and pro-apoptotic properties of hexanic root extract of the three medicinal *Echinacea* (Asteraceae) species (*Echinacea pallida* (Nutt.) Nutt., *Echinacea angustifolia* DC. *var. angustifolia*, *Echinacea purpurea* (L.) Moench.) on the human pancreatic cancer MIA PaCa-2 and colon cancer

COLO320 cell lines. It was demonstrated, for the first time, that all the three species reduced cell viability in a concentration-and time-dependent manner; *Echinacea pallida* was the most active species with IC_{50}s of 46.41+/-0.87 and 10.55+/-0.70 g/ml in MIA PaCa-2 and COLO320 cells, respectively. *Echinacea pallida* extract was able to induce apoptosis by increasing significantly caspase 3/7 activity and promoting nuclear DNA fragmentation (Chicca *et al.,* 2007).

***In vivo* experiments**

An alcohol extract of *Echinacea pallida* (solvent concentration and DER are not reported) was administrated orally to mice for 3 days prior to, and 4 days post wounding with a dermal biopsy on the dorsum (Zhai *et al*., 2009). Concomitantly, mice were exposed to 3 cycles of daily restraint stress prior to, and 4 cycles post wounding. *Echinacea* accelerated wound closure in the stressed mice, but had no apparent wound healing effect for the non-stressed mice when compared to their respective controls. Plasma corticosterone concentrations were measured in unwounded mice treated with restraint stress and the herbal extract for 4 days. Plasma corticosterone in restraint stressed mice gavaged with *Echinacea* was not different from mice treated with restraint only, but was increased compared to the vehicle control. This data suggests that the improved wound healing effect of *Echinacea* in stressed mice is not mediated through modulation of corticosterone signalling.

The effect of ten phytotherapeutic products on CCl_4 intoxicated liver in albino male Wistar rats was investigated. Biochemical parameters, including serum transaminase activity (GPT and GOT), histoenzymological measurements (lactate dehydrogenase, succinate dehydrogenase, cytochromoxidase, Mg^{2+}-dependent adenosine triphosphatase) and histochemical (Sudan black) and histological examinations (haematoxylin-eosin staining) of the liver were investigated. Some positive effects such as the reduction of hepatocytolysis and steatosis, and a return to normal values of the activity of some enzymes in the following plants: *Chrysanthemum balsamita*, *Echinacea pallida*, *Calendula officinalis* and *Corylus avelana* were obtained (plant part not reported) (Rusu *et al.,* 2005).

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

3.1.3. Safety pharmacology

No data is available for *Echinacea pallida*. In general, animal studies with different preparations and fractions of other *Echinacea* species have indicated low toxicity (Barnes *et al.,* 2007). Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed with *Echinacea pallida* or preparations thereof.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Pharmacological tests (*in vitro* and/or *in vivo*) on different issue (e.g. immunomodulatory effects, anti-inflammatory effects) were performed with preparations from 30–100% V/V EtOH. Very few are related to the *Echinacea palida* roots' preparations of the monograph (extracts prepared with 50% ethanol). In some experiments concentrations of the solvent and the concentration of active ingredient are not available in the published reports. Quantitative evaluation of pharmacological studies and

comparison with the concentrations of active compounds achieved in real treatment in human was not possible since the description of methodology is not precise enough to enable such evaluation and the information about concentrations in humans are not available.

Results from relevant experimental studies on *Echinacea pallida*, radix to support the proposed indications are very limited. The reported pharmacological effects are not considered contradictory to the traditional uses.

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

No data available.

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

No data available for *Echinacea pallida*. In general, animal studies with different preparations and fractions of other *Echinacea* species have indicated low toxicity (Barnes *et al.,* 2007).

3.3.1. Single dose toxicity

No data available.

3.3.2. Repeat dose toxicity

No data available.

3.3.3. Genotoxicity

No data available.

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

The effect of *Echinacea pallida* (EPAL) (it is not clear which plant part, root or herb was used) on the reproductive performance, serum biochemistry and haematological parameters of rabbits has been studied (Dabbou *et al*., 2016). A total of 100, 21-week-old Grimaud rabbit adult female, were randomly assigned to two groups. One group was fed a basal diet supplemented with 3 g EPAL/kg diet (*Echinacea* group, E), while the other was fed the basal diet without the supplementation (control group, C). The reproductive performance was not affected by the treatment ($P>0.05$). The haematological parameters of pregnant rabbits showed that there was no interaction between gestation day and treatment. The EPAL supplementation induced a reduction (-47.3%) in the basophil cell rate (0.55% and 0.29%, for the control and treatment groups, respectively; $P=0.049$). No significant effect of treatment was observed on the blood serum chemistry. As far as the immune parameters are concerned, no significant differences were observed between groups. No impacts of *Echinacea pallida* have been observed on the reproductive or haematological parameters.

The same researchers studied also the effects on the growth performances, bacterial community, blood parameters and immunity of growing rabbits (Kovitvadhi *et al*., 2016). From the second parturition, 80

weaned kits (40 from the C does and 40 from the E does) were randomly assigned to four groups of 20 animals each and were fed a growing commercial diet supplemented with or without a 3 g EPAL/kg diet: the CC group (rabbits from the C does fed the control diet), CE group (rabbits from the C does fed the supplemented diet), EC (rabbits from the E does fed the control diet) and EE group (rabbits from the E does fed the supplemented diet). The dietary EPAL treatment did not affect the growth performance. Ten fattening rabbits from each group were selected to evaluate the bacterial community and blood parameters, while the remaining rabbits (n=10/group) were used to study phagocytosis and the humoral immune response. The variability was evaluated from hard faeces at 35, 49 and 89 days, and the caecal content at 89 days. The variability of the bacterial community of the EE group was higher than that of the other groups. The phagocytic activity was higher in the CE and EE groups than in the CC and EC ones (30.9 and 29.7 v. 21.2 and 21.8%; $P<0.05$), whereas no statistically significant difference was observed for the blood parameters or humoral immune response against vaccination (rabbit haemorrhagic disease virus) at 95 days old which the serum was collected at 88, 102, 109, 116 and 123 days old. In conclusion, no impact of *Echinacea pallida* has been observed on the growth performances, bacterial community, blood parameters or humoral immune responses in growing rabbits, except for an increase in phagocytic activities.

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

Preclinical data are very limited. Tests on acute and chronic toxicity, reproductive toxicity, genotoxicity and carcinogenicity have not been performed.

3.4. Overall conclusions on non-clinical data

Results from relevant experimental studies on *Echinacea pallida*, radix to support the proposed indications are very limited. The reported pharmacological effects are not considered contradictory to the traditional uses.

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety of *Echinacea pallida* root is scarce.

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

Tests on 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

No data available.

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

No data available.

4.2. Clinical efficacy

No data available.

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

***Echinacea pallida* as a single ingredient**

In a randomized, placebo controlled, double-blind study, 160 patients with influenza-like infections of the upper respiratory tract were treated for 8-20 days with either a hydroalcoholic liquid extract of pale coneflower root at a daily dose corresponding to 900 mg of dried root (n=80) or placebo (n=80). Further specifications for the extract were not given. Significant improvements in four major symptoms, common cold, weakness, pain in arm and legs, and headache ($p<0.0001$), and in the overall symptom score ($p<0.0004$), were observed in the verum group compared to the placebo group. Also, the duration of illness was significantly shorter in verum patients ($p<0.0001$): in those with putative bacterial infections, 9.8 days compared to 13.0 with placebo; in those with putative viral infections, 9.1 days compared to 12.9 with placebo. Side effects were not reported (Bräunig & Knick, 1993; Dorn *et al.,* 1997; Wolters Kluwer Health, 2012).

Assessors' comment:

Despite reported effectiveness of Echinacea pallida extract as a single ingredient in this study, well established use cannot be supported due to insufficient data on preparation and composition of the extract used and use of a non-validated symptom score.

Echinacea pallida in combination with other herbal drugs

A double-blind randomized placebo controlled trial was performed on 263 patients to evaluate the use of commercially available fixed combination herbal remedy containing ethanolic-aqueous extracts of *Herba thujae occidentalis*, *Radix echinaceae* (*purpurae*+*pallidae*=1+1) and *Radix baptisiae*, 2, 7.5 and 10 mg per tablet, respectively. Three tablets of study medication were applied three times daily for 7 to 9 days. 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. In the subgroup of patients who started therapy at an early phase of their cold, the efficacy

of the herbal remedy was most prominent. 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 (Henneicke-von Zepelin *et al.,* 1999; Wolters Kluwer Health, 2012).

A randomized, double-blind placebo-controlled parallel group clinical trial was performed to investigate the therapeutic effect of Kanjang mixture containing *Echinacea pallida* root (10 g/100 ml) in combination with 4 other active ingredients for the treatment of uncomplicated upper respiratory tract infections (common cold) in 66 patients. Medication was taken three times daily for a minimum of 5 days or a maximum of 10 days in a daily dose of 15 ml. The improvement in symptoms following treatment with Kanjang mixture was significantly better on the day 2 and 4 assessments compared with placebo, while the day 10 scores were not significantly different. Tolerability of both treatments was excellent and no side-effects were reported in either of the two groups. Treatment with the herbal mixture Kangjang significantly eased the symptoms related to uncomplicated upper respiratory tract infections. Side effects were not reported (Thom & Wollan, 1997; Wolters Kluwer Health, 2012).

Table 4: Clinical studies in humans in common cold and flu.

Type **Reference**	**Study Design**	**Test Product(s)**	**Number of subjects**	**Type of subjects**	**Outcomes**	**Statistical analysis**	**Clinical relevance**
Treatment of influnza-like infections of the upper respiratory tract; Comparative study versus placebo (Bräunig & Knick 1993; Dorn *et al.*, 1997)	Randomized placebo controlled, double-blind study Duration: 8-10 days	Verum: *E. pallida* root hydroalcoholic liquid extract at a daily dose corresponding to 900 mg of dried root Placebo: coloured aqueous alcoholic solution	160 patients (83 males; 77 females) verum: 41 males; 39 females placebo: 42 males; 38 females	Influenza-like infection of the upper respiratory tract. The patients should not have been ill for>3 days. A raised differential lymphocyte count indicated viral infection and raised differential neutrophil count indicated bacterial infection.	Improvements in four major symptoms (common cold, weakness, pain in arm and legs, and headache ($p<0.0001$)) and in the overall symptom score ($p<0.0004$) in the verum group compared to placebo. Duration of illness significantly shorter in verum ($p<0.0001$): • patients with putative bacterial infections 9.8 days (verum) compared to 13.0 (placebo) • patients with putative viral infections 9.1 days (verum) compared to 12.9 (placebo)	Chi^2 test	Insufficient data on preparation and composition of the extract and non-validated symptom score used (WEU can not be supported)

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

For the treatment in children only studies with combination products are available (Linde *et al.,* 2006, Barnes *et al.,* 2007).

4.4. Overall conclusions on clinical pharmacology and efficacy

The efficacy of pale coneflower root preparations in improving the symptoms of common cold and shortening the time of disease was reported in one randomized, placebo controlled, double-blind study, however, specifications for the extracts are not given. The treatment with *Echinacea pallida* preparations should start at the first signs of cold. There are no clinical data on effectiveness of *Echinacea pallida* for the prevention of infections. Despite reported effectiveness of *Echinacea pallida* as a single ingredient, well established use cannot be supported, due to insufficient data on preparation and composition of the extract and non-validated symptom score used in this clinical study.

Clinical efficacy of *Echinacea pallida* in children was not proven.

The clinical evidence of efficacy is not sufficient for *Echinacea pallida* 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.

5. Clinical Safety/Pharmacovigilance

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

Table 5: Clinical safety data from clinical trials

Type Reference	Study Design	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
E. pallida hydroalcoholic liquid extract on influenza-like infections of the upper respiratory tract; (Bräunig & Knick 1993; Dorn *et al.,* 1997)	Randomized placebo controlled, double-blind study Duration: 8-10 days	Verum: *E. pallida* root hydroalcoholic liquid extract at a daily dose corresponding to 900 mg of dried root Placebo: coloured aqueous alcoholic solution	160 patients (83 males; 77 females) Verum: 41 males; 39 females Placebo: 42 males; 38 females	Influenza-like infections of the upper respiratory tract. The patients should not have been ill for>3 days.	No adverse reactions reported	Adverse reactions/ safety was not evaluated in this study

5.2. Patient exposure

No data available.

5.3. Adverse events, serious adverse events and deaths

There are no published case reports about adverse events or reports on adverse events from clinical trials on *Echinacea pallida*, but there are reports which include hypersensitivity reactions on *Echinacea* where details of specific products, species of *Echinacea*, type of extract and other details are not available. This is the reason that the hypersensitivity is kept in the monograph as an adverse effect.

Individuals with allergic tendencies, particularly those with known allergy to other members of the Asteraceae family are advised to avoid *Echinacea* as a general precaution measure (Barnes *et al.,* 2007).

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

No data available.

5.5.2. Contraindications

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

5.5.3. Special Warnings and precautions for use

Based on the assumption that *Echinacea pallida* 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 *and* 2007; ESCOP 2003; Bauer & Liersch, 2008; German Comission E Monograph, 1992). None of them, however, had made a thorough assessment on this issue.

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

In accordance with the 'Guideline on the Summary of Product Characteristics' dated September 2009, the statement that *Echinacea pallida 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 pallidae radix.

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

If the symptoms worsen or high fever occurs during the use of the medicinal product or if symptoms persist longer than 10 days during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

The review by Freeman & Spelman (2008) assessed the occurrence of non-clinical pharmacokinetic drug interactions with *Echinacea* including *E. angustifolia*, *E. pallida*, and *E. purpurea*. Only eight papers containing primary data relating to drug interactions were identified, but all dealing with *E. purpurea. 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) and there are no reports on *E. pallida*. Currently there are no verifiable reports of drug–herb interactions with any *Echinacea* product (Freeman & 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 times 10(3)/l, requiring platelet transfusion support. Upon admission, it was discovered that he was taking vitamin B12, vitamin E, vitamin D, vitamin C, *Echinacea* and 'vitamin B17' (laetrile-apricots kernel), which were discontinued. He received his next cycle of chemotherapy without taking herbal products and vitamins and with the addition of pegfilgrastim. His platelet count decreased to a nadir of 44 times 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) influenced the kinetic of 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. The 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 alcaloids, taxanes) while taking *Echinacea* (Bossaer & Odle, 2012).

Assessor's comment:

Due to unknown formulation and dosages of Echinacea preparation in this case it can only be taken as a signal for an influence of CYP3A4 activity influencing also other CYP3A4-substrates in spite of the fact that there are no other verifiable reports with any Echinacea product. Further pharmacokinetic testing is necessary before conclusive statements can be made about Echinacea interactions with cytochrome P450.

5.5.5. Fertility, pregnancy and lactation

Perri *et al.* (2006) prepared a review on safety of *Echinacea* during pregnancy and lactation. There is no specification which species of *Echinacea* was evaluated. 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 (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. They concluded that *Echinacea* is non-teratogenic when used during pregnancy. Using *Echinacea* during lactation is not recommended until further 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 *Echinacea* group. Six major malformations were reported, including 1 chromosomal abnormality, and 4 of these malformations occurred with *Echinacea* exposure in the first trimester. In the control group, there were 206 women with 198 live births, 7 spontaneous abortions, and 1 therapeutic abortion. Seven major malformations were reported. There were no statistical differences between the study and control groups for any of the end points analysed. The authors concluded that gestational use of *Echinacea* during organogenesis is not associated with an increased risk for major malformations (Gallo *et al*., 2000). 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.

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

Hypersensitivity reactions e.g. skin reactions were observed in rare cases with the use of *Echinacea*; therefore individuals with allergic tendencies should avoid *Echinacea pallida* preparations. Atopic patients should consult their doctor before using *Echinacea*. Those with known allergy to active substance or other members of the Asteraceae family should not use *Echinacea pallida* preparations.

Based on the assumption that *Echinacea pallida* 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 data on safety and efficacy of pale coneflower preparations in children under 12 years of age; therefore the use of *Echinacea pallida* root and preparations thereof is not recommended in this population.

Due to the uncertainty administration during pregnancy and lactation is not recommended in accordance with general medical practice.

The information on its traditional use proves that *Echinacea pallida* is not harmful in the specified conditions of use.

6. Overall conclusions (benefit-risk assessment)

Well-established use of *Echinacea purpurea* root preparations for the relief of symptoms of common cold in accordance with Article 10a of Directive 2001/83/EC is considered not fulfilled due to insufficient clinical data.

Traditional use of *Echinacea pallida* root dry extract (4-8:1) and tincture (1:5) for the relief of symptoms of common cold is acceptable based on their longstanding safe use, taking into account specified conditions for use.

There are no non-clinical toxicological data of the *Echinacea purpurea* root preparations covered by the monograph. However certain level of safety of these preparations could be expected because of the longstanding use and because no serious side effects have been reported. *Echinacea pallida* was often confused with *Echinacea angustifolia* and it is not always clear which species was used for evaluation.

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

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

The therapeutic area for browse search on the EMA website: 'Cough and cold'.

Annex

List of references