European Medicines Agency
Evaluation of Medicines for Human Use

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ASSESSMENT REPORT ON *ECHINACEA PALLIDA* (NUTT.) NUTT., RADIX

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I. REGULATORY STATUS OVERVIEW[1]

MA: Marketing Authorisation;
TRAD: Traditional Use Registration;
Other TRAD: Other national Traditional systems of registration;
Other: If known, it should be specified or otherwise add 'Not Known'

Member State	Regulatory Status				Comments[2]
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements.
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. product (1).
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Also comb. prod. (1).
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Hungary	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Also comb. prod. (4).
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products.
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. products.

[1] This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.
[2] Not mandatory field.

Member State	Regulatory Status				Comments[2]
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No answer.

II. ASSESSMENT REPORT

BASED ON ARTICLE 10A OF DIRECTIVE 2001/83/EC AS AMENDED

(WELL-ESTABLISHED USE)

BASED ON ARTICLE 16D(1) AND ARTICLE 16F AND 16H OF DIRECTIVE 2001/83/EC AS AMENDED

(TRADITIONAL USE)

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 (4-8:1; ethanol 50% (v/v)) - tincture (1:5; ethanol 50% (v/v))
Pharmaceutical forms	Herbal preparations in solid or liquid dosage forms for oral use.
Rapporteur	Damjan Janeš

II.1 INTRODUCTION

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

II.1.1.1 Herbal substance(s)[3]:

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

Echinaceae pallidae radix (Ph. Eur.)

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, Barnes *et al.* 2007, Bauer & Remiger 1989, Bradley 2006, ESCOP 2003, Liersch & Bauer 1993, WHO 1999, Willuhn 2002, Wolters Kluwer Health 2004):

- 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.

II.1.1.2 Herbal preparation(s):

A) Dry extract (4-8:1), extraction solvent: ethanol 50% (v/v).
B) Tincture (1:5), extraction solvent: ethanol 50% (v/v).

II.1.1.3 Combinations of herbal substance(s) and/or herbal preparation(s)[4]

Not applicable.

II.1.1.4 Vitamin(s)[5]

Not applicable.

II.1.1.5 Mineral(s)

Not applicable.

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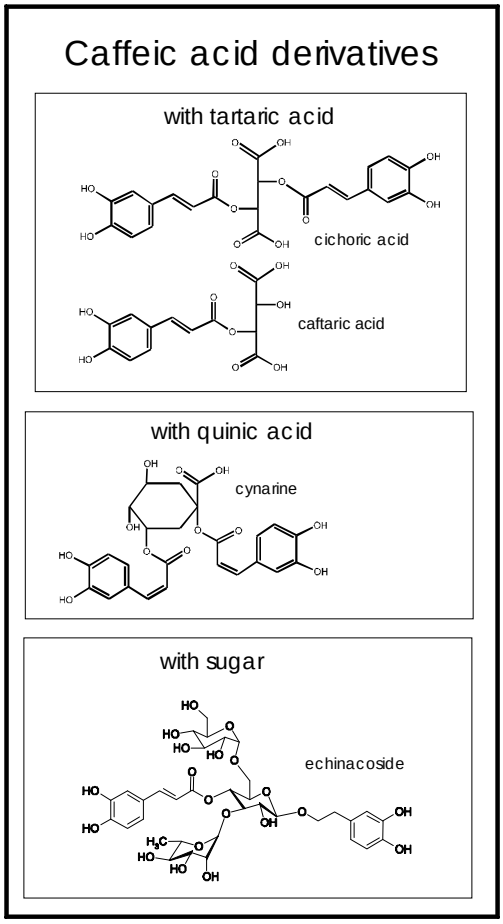
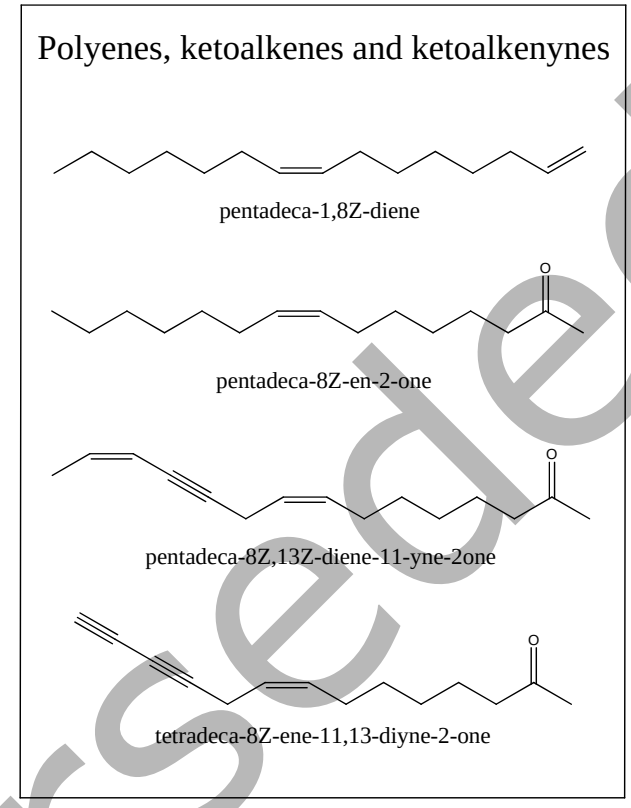
3 According to the 'Procedure for the preparation of Community monographs for traditional herbal medicinal products' (EMEA/HMPC/182320/2005 Rev.2) and the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use (EMEA/HMPC/182352/2005 Rev.2)

4 According to the 'Guideline on the clinical assessment of fixed combinations of herbal substances/herbal preparations' (EMEA/HMPC/166326/2005)

5 Only applicable to traditional use





II.1.2 Information on period of medicinal use in the Community regarding the specified indication

II.1.2.1 Specified products on the market in the European Union Member States

Dry extracts

Germany:
Preparations:
1) dry extract (5-7:1), extraction solvent: methanol 30% (v/v)
2, 3) dry extract (4-8:1), extraction solvent: ethanol 50% (v/v)

Preparation are on the market:
1) since 1997
2, 3) at least since 1976

Pharmaceutical form:
1) coated tablet
2) lozenge
3) tablet

Liquid extracts

Germany
Preparations:
1) tincture (1:5), extraction solvent: ethanol 50% (v/v)

Preparation are on the market:
1) at least since 1976

Pharmaceutical form:
1) oral liquid

Hungary
Preparations:
1) liquid extract (1:6.3-7.0), solvent: ethanol 96% (v/v): wine 16% (v/v), (1:1,5)

Preparation are on the market:
1) since 2002

Pharmaceutical form:
1) oral solution

II.1.2.2 Specified strength/posology/route of administration of use for relevant preparations and indications

Dry extracts

All for oral use and all for use in adults and adolescents over 12 years.

1) dry extract (5-7:1), extraction solvent: methanol 30% (v/v): 3 x daily 1 coated tablet containing 100 mg dry extract

2) dry extract (4-8:1), extraction solvent: ethanol 50% (v/v): 3 x daily 1 tablet containing 30 mg dry extract or 4 x daily 2 tablets containing 12 mg dry extract

Indications:

1, 2) Herbal medicinal product for the supportive treatment of common cold.

Risks:

1, 2) Hypersensitive reactions (rash, urticaria, angioedema of the skin, bronchospasm with obstruction and anaphylactic reactions) may occur.
A list of the same adverse drug effects as in the draft monograph of E. purpurea herba is proposed:
Hypersensitive reactions (rash, urticaria, Stevens-Johnson Syndrome, angioedema of the skin, Quincke edema, bronchospasm with obstruction, asthma and anaphylactic shock) may occur.
Echinacea can trigger allergic reactions in atopic patients. Association with autoimmune diseases (encephalitis disseminata, erythema nodosum, immunothrombocytopenia, Evans Syndrome, Sjögren syndrome with renal tubular dysfunction) has been reported.

Liquid extracts

1) Tincture (1:5), extraction solvent: ethanol 50% (v/v): 5 x daily 25 drops containing 100% liquid extract

Indications:

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

Risks:

1) Hypersensitive reactions (rash, urticaria, angioedema of the skin, bronchospasm with obstruction and anaphylactic reactions) may occur.
A list of the same adverse drug effects as in the draft monograph of E. purpurea herba is proposed:
Hypersensitive reactions (rash, urticaria, Stevens-Johnson Syndrome, angioedema of the skin, Quincke edema, bronchospasm with obstruction, asthma and anaphylactic shock) may occur.
Echinacea can trigger allergic reactions in atopic patients. Association with autoimmune diseases (encephalitis disseminata, erythema nodosum, immunothrombocytopenia, Evans Syndrome, Sjögren syndrome with renal tubular dysfunction) has been reported.

2) Liquid extract (1:6.3-7.0), solvent: ethanol 96% (v/v): wine 16% (v/v), (1:1,5): Prevention: 3 x 20 drops daily. Treatment: 3 x 50 drops daily

Indications:

2) To increase the resistance of the body to prevent of recurrent infections of upper respiratory tract (common cold) and adjuvant therapy of them.

Risks:

2) Very rarely hypersensitivity reactions, e.g. cutaneous eruption, pruritus, oedema of the face, dyspnoea, dizziness, and blood pressure drop. It is not recommended for pregnant women and during lactation, children below 6 years of age.
CI: hypersensitivity, progressive systemic disease (e.g. tuberculosis, multiple sclerosis, leukosis, collagenosis, AIDS, HIV infection, and other auto-immune disorders.)
Duration: Not more than 8 weeks. If the symptoms persist for more than 4-5 days or adverse effects occur, stop taking the preparation and see your physician.

II.1.2.3 Bibliographic/expert evidence on the medicinal use

II.1.2.3.1 Evidence regarding the indication/traditional use

Traditional indications for oral use of liquid extracts

Indication	References
Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold).	ESCOP 2003, Bräunig & Knick 1993, WHO 1999, Willuhn 2002

II.1.2.3.2 Evidence regarding the specified posology

Oral administration

Herbal preparations:

Liquid extract: 0.25-1.0 ml (1:1 in 45% ethanol) three times daily.
Tincture: 0.5-1.0 ml (1:5 in 45% ethanol) or 2-5 ml (1:5 in 45% ethanol) three times daily. (Barnes *et al.* 2007, ESCOP 2003).

The duration of treatement should not exceed eight weeks (Barnes *et al.* 2007, ESCOP 2003, German Commission E Monographs 1999).

II.2 NON-CLINICAL DATA

II.2.1 Pharmacology

II.2.1.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

II.2.1.1.1 Immunomodulatory activity

Alcohol extracts from roots of three widely used *Echinacea* species, *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea*, were investigated for immunomodulating properties. The three *Echinacea* species demonstrated a broad difference in concentrations of individual lipophilic amides and hydrophilic caffeic acid derivatives. Mice were gavaged once a day (for 7 days) with one of the *Echinacea* extracts (130 mg/kg) or vehicle and 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 *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 interferon (IFN)-alpha production, but inhibited the release of tumor necrosis factor (TNF)-gamma and interleukin (IL)-1beta. 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 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*. Comparing the AGPs, there are differences with regard to fine structure as well as to activities. AGPs from roots of *B. tinctoria* and *E. pallida* show high activity in all test systems. AGP from cell culture of *E. 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).

The effects of long-term (>1 year) dry storage on the capabilities of *Echinacea* spp. roots from mature individuals to modulate cytokine production are unknown. Using an older human adult model of influenza vaccination, peripheral blood mononuclear cells were collected from subjects 6 months post-vaccination

and stimulated them *in vitro* with the two Type A influenza viruses contained in the trivalent 2004-2005 vaccine with a 50% alcohol tincture prepared from the roots of one of seven *Echinacea* species: *E. angustifolia*, *E. pallida*, *E. paradoxa*, *E. purpurea*, *E. sanguinea*, *E. simulata*, and *E. tennesseensis*. Before being processed into extracts, all roots had been stored under dry conditions for sixteen months. Cells were cultured for 48 hours; following incubation, supernatants were collected and assayed for IL-2, IL-10, and 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. The data support and extend previous research and indicate that tinctures from different *Echinacea* species have different patterns of immune modulation; further, they indicate that certain species may be efficacious in the immune response to viral infection (Senchina *et al.* 2006).

Chemical investigation of the roots of *Echinacea angustifolia*, *E. purpurea*, and *E. 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 *E. angustifolia* and dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide from *E. purpurea* and *E. pallida*. These two components, as well as previously identified alkamides, exerted inhibition on Lipopolysaccharide (LPS)-mediated activation of a murine macrophage line, RAW264.7. These data suggest that these alkamides may have antiinflammatory activity (Chen *et al.* 2005).

A 90% ethanolic extract (1:10) of pale coneflower root at concentration of 10^{-2} m/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).

A high molecular weight fraction (M_r > 10000 D) containing polysaccharides and glycoproteins from pale coneflower root enhanced the proliferation of mouse spleen cells, and stimulated the production of IFN-α/β 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 1999).

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 ative than the hydrophilic (1.3-fold increase) (Bauer *et al.* 1988b).

Intravenous injection of 50, 100 or 500 µl 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 (Bodinet 1999).

II.2.1.1.2 Antimicrobial activity

The antibacterial activity of echinacoside (8×10^{-3} M) against *Staphylococcus aureus* corresponds to approx. 10 Oxford units of penicillin (Stoll *et al.* 1950).

Extracts of pale coneflower root 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*. Root extracts of eight *Echinacea* taxa, including *Echinacea pallida var. angustifolia* and *E. pallida var. pallida* showed antifungal activity against most of the pathogenic fungi (Merali *et al.* 2003).

II.2.1.1.3 Antiviral activity

Extracts of 8 taxa of the genus *Echinacea* 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 *E. 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).

A high molecular weight fraction (M_r > 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 (Cheminat *et al.* 1988).

II.2.1.1.4 Anti-inflammatory activity

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

It has been suggested that *Echinacea* has anti-inflammatory activity *in vivo*. Nitric oxide (NO), TNF-alpha, and IL-1beta are important mediators in the inflammatory response. The effect of alcohol extracts from roots of *E. angustifolia* (EA), *E. pallida* (EPA) and *E. purpurea* (EP) on the production of these inflammatory mediators in both LPS-stimulated RAW 264.7 macrophages *in vitro* and murine peritoneal exudate cells (PECs) *in vivo* were investigated. As macrophages produce these inflammatory mediators in response to pathogenic infection, parallel cultures of macrophages were studied for phagocytosis and intracellular killing of *Salmonella enterica*. 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 h, 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 2007b).

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 arachadonic acid pathway was determined by 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).

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. 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 *E. pallida* and of its constituent echinacoside, with respect to *E. purpurea* and control. This activity probably resides in the antihyaluronidase activity of echinacoside (Speroni *et al.* 2002).

II.2.1.1.5 Antioxidant activity

The radical scavenging activity of *Echinacea* methanolic extracts was evaluated *in vitro* with a spectrophotometric method based on the reduction of an alcoholic 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution at 517 nm in the presence of a hydrogen donating antioxidant. As for pure compounds, echinacoside had the highest capacity to quench DPPH radicals (EC50 = 6.6 μM), while caftaric acid had the lowest (EC50 = 20.5 μM). The average EC_{50} values for *Echinacea purpurea*, *E. pallida* and *E. angustifolia* were 134, 167 and 231 μg/ml, respectively. The radical scavenging activity of *Echinacea* root extracts reflected their phenolic composition. The results indicate that *Echinacea* roots and derivatives are a good source of natural antioxidants and could be used to prevent free-radical-induced deleterious effects (Pellati *et al.* 2004).

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 (Sloley *et al.* 2001).

Methanol extracts of freeze-dried *Echinacea* (*E. angustifolia, E. pallida,* and *E. purpurea*) roots were examined for free radical scavenging capacities and antioxidant activities. Root extracts of *E. angustifolia, E. pallida,* and *E. purpurea* were capable of scavenging hydroxyl radical. Similar scavenging activities for each variety were found for both 1,1-diphenyl-2-picrylhydrazyl 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) 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 (Maffei Facino *et al.* 1995).

II.2.1.1.6 Other activities

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 *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-(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 *E. 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 *E. 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 *E. 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 µg/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 µg/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. These results represent the starting point to establish viable scientific evidence on the possible role of *Echinacea* species in medical oncology (Chicca *et al.* 2007).

The 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 (Rusu *et al.* 2005).

A constituent of the root oil of *Echinacea angustifolia* DC. and *E. 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).

II.2.1.2 Assessor's overall conclusions on pharmacology

For the extracts of pale coneflower root, immunomodulatory, antimicrobial, anti-inflammatory, antioxidant and antitumor effects were proven in several *in vitro* and *in vivo* tests. In comparison to other *Echinacea* species, *E. pallida* was frequently the most active; however the pharmacological mechanisms still remain to be elucidated.

II.2.2 Pharmacokinetics

II.2.2.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

No data available.

II.2.2.2 Assessor's overall conclusions on pharmacokinetics

No conclusions due to absence of data.

II.2.3 Toxicology

II.2.3.1 Overview of available 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). Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed with *Echinacea pallida* or preparations thereof.

II.2.3.2 Assessor's overall conclusions on toxicology

Toxicological data are only available for *Echinacea purpurea*; however a certain level of safety could be expected due to the long-time use of *Echinacea pallida* preparations with no serious side effects reported.

II.3 CLINICAL DATA

II.3.1 Clinical Pharmacology

II.3.1.1 Pharmacodynamics

II.3.1.1.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

No data available.

II.3.1.1.2 Assessor's overall conclusions on Pharmacodynamics

No conclusions due to absence of data.

II.3.1.2 Pharmacokinetics

II.3.1.2.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

No data available.

II.3.1.2.2 Assessor's overall conclusions on pharmacokinetics

No conclusions due to absence of data.

II.3.2 Clinical Efficacy[6]

II.3.2.1 Dose response studies

No data available.

[6] In case of traditional use the long-standing use and experience should be assessed.

II.3.2.2 Clinical studies (case studies and clinical trials)

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

In a randomized, placebo controlled, double-blind study, 160 patients with influnza-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 (Bräunig & Knick 1993).

A double-blind randomized placebo controlled trial was performed to evaluate the use of *Echinaceae pallidae* radix in flu-like upper respiratory symptoms in 160 patients. Liquid extract of pale coneflower root was administered for 8 to 10 days at a daily dose corresponding to 900 mg of dried root. Further specifications for the extract were not given. Study demonstrated a highly significant result for the herbal remedy *Echinaceae*, as compared to placebo. The length of illness ($P < 0.0001$), overall symptom scores ($P < 0.0004$) and whole clinical scores ($P < 0.001$) all demonstrates a significant result for real treatment as compared to placebo. Side effects were not reported (Dorn *et al.* 1997, Wolters Kluwer Health 2004).

***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 H *et al.* 1999, Wolters Kluwer Health 2004).

A randomized, double-blind placebo-controlled parallel group clinical trial was performed to investigate the therapeutic effect of Kanjang mixture containing *Echinacea pallida* root (10g/100ml) 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 *et al.* 1997, Wolters Kluwer Health 2004).

II.3.2.3 Clinical studies in special populations (e.g. elderly and children)

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

II.3.2.4 Assessor's overall conclusions on clinical efficacy

The efficacy of pale coneflower root preparations in improving the symptoms of common cold and shortening the time of disease was reported in two randomized, placebo controlled, double-blind studies, 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 data on effectiveness of *Echinacea pallida* for the prevention of infections. The effectiveness of combination with other herbal drugs was reported. Despite reported effectiveness of *Echinacea pallida* as a single ingredient, well established use can not be supported, due to highly insufficient data on preparation and composition of the extracts used in clinical studies.
The available published controlled clinical studies indicate that medicines containing *Echinacea pallida* may be effective immunomodulators. However, for clear therapeutic recommendations as to which preparation to use and which dose to apply, the evidence is not yet sufficient.
Clinical efficacy of *Echinacea pallida* in children is not certain, because only data on combination products are available.
Since *Echinacea*-containing medicinal products can have different contents of active constituents depending on the plant material used, the method of preparation or the addition of other components - an extrapolation of results from one preparation to another is not possible without proof of the chemical-pharmaceutical equivalence. For future investigations it should therefore be required that the preparation is chemically defined and standardised with regard to the composition of the main components. (Melchart *et al.* 1994).

II.3.3 Clinical Safety/Pharmacovigilance

II.3.3.1 Patient exposure

No data available.

II.3.3.2 Adverse events

In rare cases hypersensitivity reactions e.g. skin reactions may occur (Liersch & Bauer 1993). Individuals with allergic tendecies, particulary those with known allergy to other members of the Asteraceae family should be advised to avoid *Echinacea* (Barnes *et al.* 2007).

II.3.3.3 Serious adverse events and deaths

None reported.

II.3.3.4 Laboratory findings

No data available.

II.3.3.5 Safety in special populations and situations

II.3.3.5.1 Intrinsic (including elderly and children) /extrinsic factors

As with all imunostimulants not recommended in progressive systemic diseases such as: tuberculosis, diseases of the white blood cells system, collagenoses, multiple sclerosis, AIDS, HIV infections, and other immune diseases (Barnes *et al.* 2007, German Commission E Monographs 1999, Liersch & Bauer. 1993).

II.3.3.5.2 Drug interactions

None reported.

II.3.3.5.3 Use in pregnancy and lactation

A review on safety of *Echinacea* during pregnancy and lactation was published recently (Perri *et al.* 2006). 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. 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 *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).

II.3.3.5.4 Overdose

No case of overdose has been reported.

II.3.3.5.5 Drug abuse

No case of drug abuse has been reported.

II.3.3.5.6 Withdrawal and rebound

No data available.

II.3.3.5.7 Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

II.3.3.6 Assessor's overall conclusions on clinical safety

Hypersensitivity reactions e.g. skin reactions were observed in rare cases; therefore individuals with allergic tendencies particularly those with known allergy to other members of the Asteraceae family should avoid *Echinacea pallida* preparations.
There are no data on safety of pale coneflower preparations in children; therefore the use of herbal drug and preparations is not recommended.

Echinacea pallida should not be used in progressive systemic diseases such as: tuberculosis, diseases of the white blood cells system, collagenoses, multiple sclerosis, AIDS, HIV infections, and other immune diseases.
Due to the uncertainty which *Echinacea* was evalueted in studies, administration during pregnancy and lactation is not generally recommended in accordance with general medical practice.
The duration of use should be restricted to 10 days according to the clinical studies and usual duration of common cold.

II.4 ASSESSOR'S OVERALL CONCLUSIONS

Immunomodulatory effects of *Echinacea pallida* preparations on cells of human immune system were demonstrated *in vitro*, but there are no data on clinical pharmacology. Specifications for herbal preparations used in clinical studies are not given. The available published controlled clinical studies indicate that medicines containing *Echinacea pallida* may be effective immunomodulators; however they lack sufficient data on preparation and composition of the extracts used. According to these studies, common cold was treated as a viral or a bacterial infection. There are no data on clinical efficacy in children. Nothing is known about the toxicity of pale conflower preparations (no data on acute toxicity, genotoxicity and carcinogenicity) although pale coneflower has been used for decades. However, a certain level of safety could be expected, 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.

Despite reported effectiveness of *Echinacea pallida* as a single ingredient, **well-established** use of ***Echinacea pallida*** for the supportive treatment of common cold is **not possible,** because there are highly insufficient data on preparation and composition of the extracts used in clinical studies.

Traditional use of *Echinacea pallida* in this indication is **possible**, although toxicological data are not available. However, a certain level of safety can be assumed due to the long-time use of *Echinacea pallida* preparations with no serious side effects reported.

III. ANNEXES

III.1 COMMUNITY HERBAL MONOGRAPH ON *ECHINACEA PALLIDA* (NUTT.) NUTT., RADIX

III.2 LITERATURE REFERENCES