European Medicines Agency
Evaluation of Medicines for Human Use

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COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

ASSESSMENT REPORT FOR THE DEVELOPMENT OF COMMUNITY HERBAL MONOGRAPHS AND FOR INCLUSION OF HERBAL SUBSTANCE(S), PREPARATION(S) OR COMBINATIONS THEREOF IN THE COMMUNITY LIST

Calendula officinalis L., flos

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)	*Calendula officinalis* L., flos
Herbal preparation(s)	A) Liquid extract (1:1), extraction solvent ethanol 40-50% (v/v) B) Liquid extract (1:1.8-2.2), extraction solvent ethanol 40-50% (v/v) C) Tincture (1:5), extraction solvent ethanol 70-90% (v/v) D) Liquid extract (1:10), extraction solvent fatty vegetable oil e.g. olive oil E) Ointment (1:5 – 1:25), extraction solvent hardened vegetable fat, petroleum jelly[1] F) Comminuted herbal substance
Pharmaceutical forms	Herbal substance or comminuted herbal substance for infusion or other herbal preparations in liquid or semi solid dosage forms for topical use
Rapporteur(s)	Heribert Pittner Reinhard Länger

[1] Calendula ointment is prepared by gentle digestion of the herbal substance in the melted ointment base for up to 16 hours and subsequent filtration and congealment during fall in temperature.

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I. REGULATORY STATUS OVERVIEW[2]

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[3]
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Cosmetics only
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Latvia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Cosmetics
Spain	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

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

II. ASSESSMENT REPORT FOR HERBAL SUBSTANCE(S), HERBAL PREPARATION(S) OR COMBINATIONS THEREOF WITH WELL-ESTABLISHED USE AND/OR TRADITIONAL USE

II.1 INTRODUCTION

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

Herbal substance[4,5]

Calendulae flos (European Pharmacopoeia)

Whole or cut, dried, fully opened flowers, which have been detached from the receptacle, of the cultivated, double-flowered varieties of *Calendula officinalis* L. It contains not less than 0.4% of flavonoids, calculated as hyperoside ($C_{21}H_{20}O_{12}$, M_r 464.4) with reference to the dried herbal substance.

Constituents (Willuhn G (2004), Hänsel R *et al* (1992), Hänsel R *et al* (2007)):

Triterpene saponins: 2-10% derivatives of the oleanolic acid with glucuronic acid on C3

Triterpene alcohols: free and esterified (with fatty acids) mono-, di- and triols of the ψ–taraxene-, taraxene-, lupine- and ursine-type. Approximately 0.8% Monols (α- and β-amyrin, lupeol, taraxasterol, ψ-taraxasterol), approx. 4% Diols, mostly in form of the mono esters (faradiols and arnidiol esters).

Ionon- and sesquiterpeneglycosides: isolated from Calendula grown in Egypt (officinosids, Marukami T *et al* (2001))

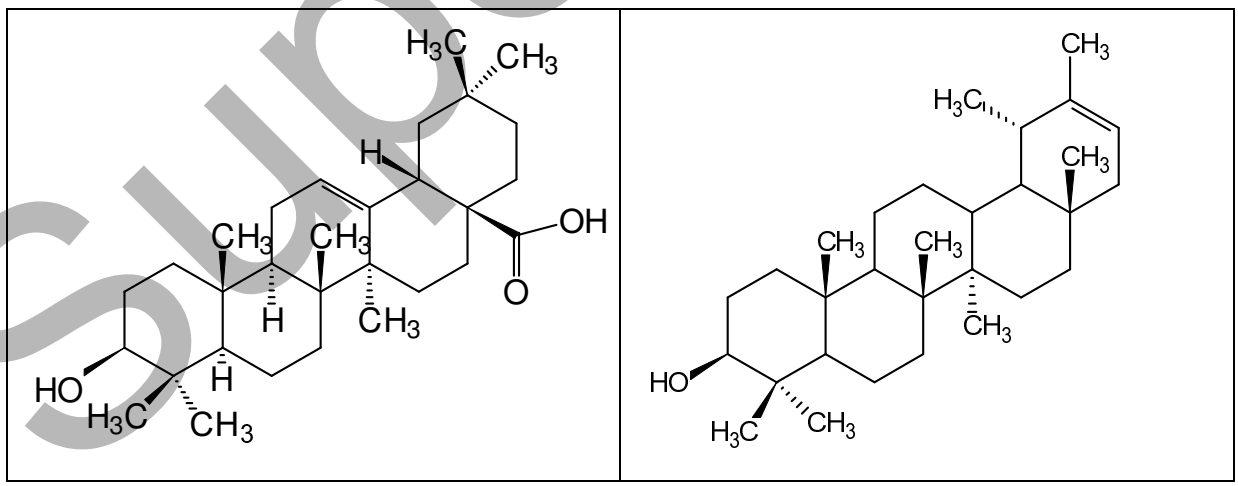
Carotenoids: up to 4.7%; predominately lutein and zeaxanthine (together up to 92% of total carotenoids). The sesquiterpene lactone calendine is not a genuine constituent, the structure is identical with the xanthophyll degredation product loliolide.

Flavonoids: 0.3-0.8%; glycosides of isorhamnetin, quercetin

Coumarins: scopoletin, , umbeloiferone, aesculetin

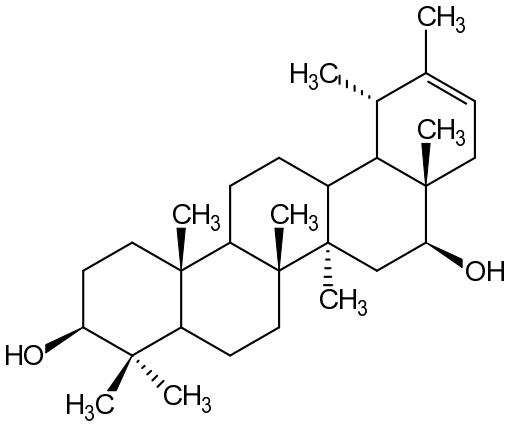
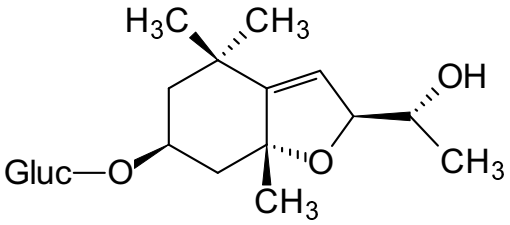
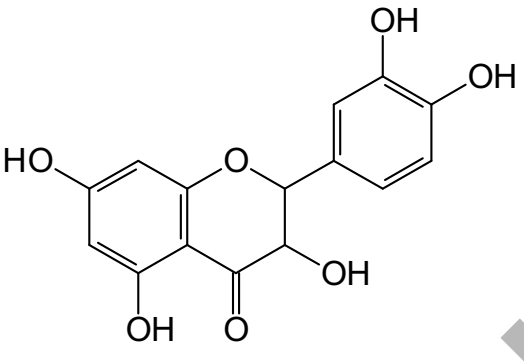
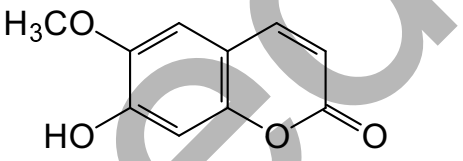
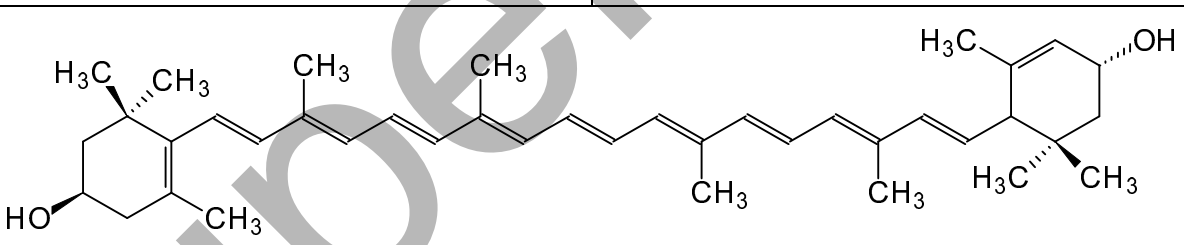
Volatile oil: 0.2-0.3%, mostly sesquiterpenes (e.g., α cadinol)

Water soluble polysaccharides: up to 15%



[4] According to "Guideline on quality of herbal medicinal products/traditional herbal medicinal products" (CPMP/QWP/2819/00 Rev.1, EMEA/CVMP/814/00 Rev.1)

[5] According to "Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products" (CPMP/QWP/2820/00 Rev.1, EMEA/CVMP/815/00 Rev.1)

Oleanolic acid	ψ-Taraxasterol
	
Faradiol	Officinosid A
	
Quercetin	Scopoletin
	
Lutein	

Herbal preparation(s)

A) Liquid extract (DAC 2006, British Herbal Pharm. 1983): DER 1:1, solvent ethanol (40-50% v/v). Liquid extract according to DAC contains not less than 0.4% flavonoids calculated as hyperoside. The content of flavonoids of liquid extracts prepared with different concentrations of ethanol (40%-60%) is very similar, although the qualitative composition is slightly different (Bilia AR et al (2002)).

B) Liquid extract: DER 1:1.8-2.2, extraction solvent ethanol 40-50% (v/v): ointments containing this liquid extract in a concentration of 10% have been on the Austrian market for more than 30 years, and in a concentration of 4% on the German market.

C) Tincture (DAC 2006, British Herbal Pharm. 1983): DER 1:5, solvent ethanol (70-90% v/v). Tincture according to DAC contains not less than 0.1% flavonoids calculated as hyperoside.

D) Liquid extract: DER 1:10, solvent fatty vegetable oil, e.g. olive oil (Hänsel *et al* (1992)). Peanut oil, which is also mentioned in literature, is not recommended because of the higher probability of adverse reactions.

E) Calendula ointment: DER 1:5 – 1:25, extraction by digestion on the water bath using traditionally lard or hardened vegetable fat (Hänsel *et al* (1992)) or petroleum jelly. The herbal substance may be moistened with ethanol prior to digestion. The ointment base is melted, subsequently the herbal substance is added. The time for extraction is up to 16 hours. After digestion the still liquid mixture is filtrated, the filtrate congeals with falling temperature (Kubelka W *et al* (2007)).

F) Comminuted herbal substance for infusion.

Extracts prepared with supercritical CO_2 and liquid solvents different to water or ethanol (e.g., isopropylmyristate, propyleneglycol, glycerol, diethylenglycol, polyethylenglycol) do not fulfil the requirements for traditional use. The same is true for the so called LACE-extract (laser activated Calendula extract, Jimenez-Medina E *et al* (2006)).

Calendula ointments prepared with liquid extracts or tinctures are not discussed as particular herbal preparations.

Results obtained with homoeopathic preparations prepared from the fresh aerial parts are not considered for this assessment.

Combinations of herbal substance(s) and/or herbal preparation(s)[6]

Calendula flowers and extracts are used in combinations with many other herbal substances / herbal preparations. This monograph refers exclusively to Calendulae flos.

Vitamin(s)[7]

Not applicable

Mineral(s)[8]

Not applicable

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

The therapeutic use of Calendula flowers and ointments goes back at least to Hildegard von Bingen (cited in Mayer JG *et al* (2000)). In fact Calendula flower has been in medical use for many decades. Therefore for Calendula flower a period of at least 30 years in medical use as requested by Directive 2004/24 EC for qualification as a traditional herbal medicinal product is easily fulfilled.

II.1.2.1 Type of tradition, where relevant

European tradition

II.1.2.2 Bibliographic/expert evidence on the medicinal use

II.1.2.2.1 Evidence regarding the indication

Traditional use

The following indications have been reported for Calendula flowers:

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

[7] Only applicable to Community monographs

Inflammations of the skin or the oral mucosa

Symptomatic treatment of minor inflammations of the skin and mucosa;	ESCOP (2003)
Inflammation of the oral and pharyngeal mucosa	Commission E (1998)
Nappy rash	Fintelmann V *et al* (2002)
Atheromas	Hänsel R *et al* (1992)
Perianal eczema, proctitis	Hänsel R *et al* (1992), BHP (1983)
Acne	Hänsel R *et al* (1992)
Dry dermatosis	Hänsel R *et al* (1992)

Wounds

Poorly healing wounds	Commission E (1998)
Healing of minor wounds	ESCOP (2003)
Ulcus cruris	Commission E (1998), BHP (1983), Duran V *et al* (2005)
Purification of contaminated wounds	Schilcher H *et al* (2003), Fintelmann V *et al* (2002)
Inflamed cutaneous lesions	BHP (1983)

Further traditional indications

Enlarged and inflamed lymph nodes	Hänsel R *et al* (1992)
Gastric ulcer, duodenal ulcer	BHP (1983)
Dysmenorrhea, as emmenagogue agent	BHP (1983)

Wording for the traditional use indication

a) Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds.

b) Traditional herbal medicinal product for the symptomatic treatment of minor inflammations in the mouth or the throat.

The product is a traditional herbal medicinal product for use in the specified indication exclusively based upon long-standing use.

II.1.2.2.2 Evidence regarding the specified posology

Posology for adolescents and adults:

Dosage:

Strength and dosage frequency of preparations for topical use:
Herbal substance and comminuted herbal substance for tea preparation:

	single dose
ESCOP (2003)	1-2 g per 150 ml of water
Commission E	1-2 g per cup
Hänsel R *et al* (1992)	1-2 g
Fintelmann V *et al* (2002)	2 teaspoons (= 2.8 g), several times daily
BHP (1983)	1-4 g thrice daily

The still warm infusion is used as such to rinse or gargle for the treatment of inflammations in the mouth or the throat (wording of the package insert German standard licence).
The infusion is applied to wounds and skin disorders with a compress, which has to be changed several times daily (Bradley P (2006))

A) Liquid extract (DER 1:1):
In semi-solid dosage forms: amount equivalent to 2-10% herbal substance.

B) Liquid extract (DER 1:1.8-2.2)
In semi-solid dosage forms: amount equivalent to 2-5% herbal substance

C) Tincture (DER 1:5):
In compresses diluted at least 1:3 with freshly boiled water; in semi-solid dosage forms: amount equivalent to 2-10% herbal substance
In semi-solid dosage forms: amount equivalent to 2-10% herbal substance (Hänsel R *et al* (1992)).
As a gargle or mouthrinse in a 2% solution (Bradley P (2006)).

D) Liquid extract (DER 1:10):
In semi-solid dosage forms: amount equivalent to 2-8% herbal substance (= ointments contain 20-80% Calendula oil).

E) Calendula ointment:
Semi-solid preparations contain usually 4-20% of the herbal substance (Kubelka W *et al* (2007)).

Dosage frequency: 2 to 4 times daily; duration of treatment with compresses: 30-60 minutes.

Posology for children
In the standard text book on phytotherapy by Weiss (Fintelmann V *et al* (2002)) it is stated that Calendula preparations are superior to Chamomile in the topical treatment of nappy rash.
The Council of Europe published in 1989 a document where the use of certain preparations of Calendula flowers is allowed for cosmetic baby toiletries. Cosmetic products are intended to be used on the intact skin. Therefore the medicinal use of Calendula flowers for minor inflammations of the skin and as an aid in healing of minor wounds cannot be recommended in the same way for babies.
There are no observational data published on the safe use of Calendula preparations in the paediatric population. However, the common use, for example in the treatment of nappy rash, indicates a certain degree of safety. As a compromise the age limits of 6 years (indication a) and 12 years (indication b) are proposed.

Indication a)
The use in children under 6 years of age is not recommended because there is no experience available.

Indication b)
The use in children under 12 years of age is not recommended because there is no experience available.

Special warnings and special precautions for use

If symptoms persist after 1 week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.
If signs of skin infections are observed, medical advice should be sought.

II.1.2.2.3 Evidence regarding the route of administration

The topical administration is the only route of administration for preparations of Calendula flowers in the recommended traditional indication.

II.1.2.2.4 Evidence regarding the duration of use

No restriction on the duration of use has been reported for Calendulae flos. Compresses should be removed after 30-60 minutes.

II.1.2.3 Assessor's overall conclusion on the traditional medicinal use

Preparations from Calendulae flos have been used for the symptomatic treatment of minor inflammations of the skin or the oral mucosa, and as an aid in healing minor wounds. The traditional medicinal use is made plausible by pharmacological data.

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

Wound healing:
The wound healing process involves several distinct phases in which the information of the new blood vessels (angiogenesis) plays an important role. In the chick chorioallantoic membrane (CAM) test using incubated hen eggs, a freeze-dried, cold aqueous infusion of calendula flower proved highly angiogenic, the number of microvessels counted in treated tissue sections being significantly higher than in control CAMs ($p<0,0001$). Hyaluronan, which is known to be involved in the information, alignment and migration of newly formed capillaries, was detected in all calendula flower treated CAMs, while none was found in untreated CAMs. The high level of neovascularisation observed in treated CAMs was attributed to effects of the calendula flower extract, in which the predominant constituents were flavonoids (Patrick KFM *et al* (1996)).
Dry 70%-ethanolic (E) and aqueous (A) extracts of calendula flower, applied topically as 5% ointments, accelerated the healing of surgically inflicted skin wounds in rats; the degree of epithelialisation was 73% (E) and 65% (A) by the 5^{th} day, and 90% (E) and 88% (A) by the 10^{th} day compared to 60% and 79% in control animals treated with vehicle only. In similar experiments, addition of allantoin to the ointment enhanced the effect of the extracts; by the 14^{th} day, compared to 70% in controls and 79% with allantoin alone, the degree of healing was 80% with A + E, and 90% with A + E + allantoin in a 2:2:1 ratio ($p<0.01$) (Klouchek-Popova E *et al* (1982), cited in Hänsel R *et al* (1992)).
A topically applied calendula ointment had better influence on epithelisation of artificially infected wounds (Staphylococcus epidermides) in rats than a combination of Comfrey, propolis and honey (Perri de Carvalho PS *et al* (1991)).
A Calendula ointment (containing 5% dry extract) enhanced the healing of experimental wounds in buffalo calves (Ansari MA *et al* (1997) cited in ESCOP (2003)).
Because of the differences in the structure of the skin between humans and animals these data should be interpreted carefully (Wissinger-Gräfenhahn U (2000)).

Anti-inflammatory effect:
The anti-inflammatory effect of Calendulae flos has been reported in the croton oil test in mice in which a CO_2 extract showed a more pronounced oedema inhibition than a 70% hydroalcoholic extract (Della Loggia R *et al* (1994)). The most active substance in the croton oil test is faradiol, its molar activity is comparable to indometacin. The esters of faradiol are about 50% less active, the free Monols (like taraxasterol, lupeol) are less active than the Diols (Zitterl-Eglseer K *et al* (1997)).

Isorhamnetin 3-glycosides isolated from calendula flower inhibited lipoxygenase (a key enzyme in the synthesis of leukotrienes) from rat lung cytosol at a concentration of 1.5×10^{-5} M (Bezakova L *et al* (1996)).

Ukiya M *et al* (2006) tested ten oleanane-type triterpene glycosides along with five flavonol glycosides from the flowers of Calendula officinalis. 8 triterpenes exhibited a marked anti-inflammatory activity in the TPA-induced inflammation in the mouse ear.

Antimicrobial activity:
The essential oil inhibited the growth of *Bacillus subtilus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The activity is attributed to the terpene alcohols and terpene lactones (Janssen AM *et al* (1986)).
The essential oil exhibits also a weak fungicide activity against dermal fungi like *Trichophyton mentagrophytes* var. *interdigitale*, *Trichophyton rubrum*, *Trichophytum concentricum* and *Epidermophyton floccosum* (Hänsel R *et al* (1992)).
A fraction containing flavonoids isolated from the leaves inhibited the growth of *Sarcina lutea*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae* and *Candida monosa*, the saponins were not effective (Tarle D *et al* (1989)). The water soluble components of ethanolic extracts are active against *Staphylococcus aureus* (Dumenil D *et al* (1989)). An antimicrobial activity against several bacteria is also documented for infusions with DER 1:10 (Gasiorowska I (1983), cited in Hänsel R *et al* (1992)).
The methanol extract exhibited a weak activity against periodontopathic bacteria, a decoction showed even less potential (Iauk L *et al* (2003)). Compared to a solution of NaF and sodium lauryl sulphate an extract of Calendula flowers had no antimicrobial effect on biofilms and oral microorganisms from children (Modesto A (2000)).

Antiviral activity:
A tincture of calendula flower suppressed the replication of herpes simplex, influenza A2 and influenza APR-8 viruses in vitro (Bogdanova NS *et al* (1970)), however, an aqueous extract was not active (May G *et al* (1978)). A chloroform extract inhibited the replication of HIV Type I in acutely infected lymphocytic Molt-4 cells in vitro. A chloroform extract also inhibited the HIV-I reverse transcriptase activity in a dose dependent manner (Kalvatchev Z *et al* (1997)).

Further activities:
Immunostimulation:
Polysaccharide fractions from Calendula (molecular weight in the range of 25000-500000) showed significant immunostimulating activity in the granulocytes – and carbon clearance tests (Wagner H *et al* (1985)).
Isolated polysaccharides from Calendula flower were found to stimulate phagocytosis of human granulocytes (Varljen J. *et al* (1989)).
Amirghofran Z *et al* (2000) found that extracts of Calendula flowers do not show a direct mitogenic effect on human lymphocytes and thymocytes.

Antitumoral activities:
The monodesmosides Arvenoside B and D exhibit an in vitro cytotoxic effect on HeLa-cells, B 16-melanoma cells, 3T3 fibroblasts and human 2002-cells (Quetin-Leclerque J *et al* (1992)). Triterpenes like

faradiol and taraxasterol inhibit experimental tumor promotion and are therefore considered as inhibitors of tumor growth (Yasukawa K *et al* (1996)).
Dietary lutein from Calendula flowers increased tumor latency and inhibited mammary tumor growth in mice (Chew BP *et al* (1996), Park JS *et al* (1998)).
Two triterpenes of Calendula flowers showed cytotoxic effects against colon cancer, leukemia, and melanoma cells (Ukiya M *et al* (2006)).

Spasmogenic and spasmolytic activities:
Activity directed fractionation of an aqueous-ethanol extract of Calendula flowers showed that the spasmolytic activity was concentrated in the organic fraction, while the aqueous fraction exhibited a marked atropine sensitive spasmogenic effect (Bashir S *et al* (2006)).

Hepatoprotective activity:
Calendula extract (liquid extract DER 1:1, solvent ethanol 70%) was examined in CCl_4-intoxicated rat livers. It was able to reduce the hepatocytolysis by 28% compared to control, to reduce histological modifications as well as enzyme and steatosis modifications (Rusu MA *et al* (2005)).

Antioxidative activity:
The butanolic fraction of Calendula flowers possesses a significant free radical scavenging and antioxidant activity (Cordova CA *et al* (2002), Herold A *et al* (2003)).

II.2.1.2 Assessor's overall conclusions on pharmacology

The published data on pharmacological activities support the traditional topical use of preparations containing Calendula flowers in the proposed indications.

II.2.2 Pharmacokinetics

No specific data are available on Calendulae flos.

II.2.3 Toxicology

II.2.3.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Acute toxicity
For an aqueous extract from calendula flower administered to mice, the intravenous LD_{50} was determined as 375 mg/kg body weight and the intraperitoneal LD_{100} as 580 mg/kg (Manolov P *et al* (1964)). For a hydroalcoholic extract (DER 1:1, 30% ethanol) the subcutaneous LD_{50} was 45 mg in mice and the intravenous LD_{50} was 526 mg/100g in rats (Boyadzhiev TSV *et al* (1964)). An ethylene glycol extract (DER 2:1) was non-toxic in albino mice after subcutaneous administration of 10 ml/kg (Russo M (1972)). Calendula oil has a LD_{50} of 20 ml/kg rat p.o. (cited in Blaschek W *et al* (2006)).
Hydroethanolic dry extracts showed no signs of toxicity when administered orally to mice and rats up to a dose of 5 g/kg (Silva EJ *et al* (2007)).

Subchronic toxicity
An aqueous extract was reported to be non toxic in chronic administration to mice (Manolov P *et al* (1964)). No symptoms of toxicity were observed after oral administration of a Calendula flower extract (solvent unspecified) at 0.15 g/kg body weight to hamsters over 18 months and to rats over 21 months (Avramova S *et al* (1988)). No toxic symptoms appeared in rats after daily oral administration of calenduloside B at 200 mg/kg body weight for 2 months (Yatsuno AI *et al* (1978)).
No death of experimental animals was detected during oral administration of a hydroethanolic dry extracts in dose of 1 g/kg for 30 days. The biochemical profile showed no changes for most of the parameters,

however, there was a dose dependent increase of blood urea nitrogen and of the ALAT level in doses up from 0.25 g/kg extract. The authors interpret these findings with a hepatic overload (Silva EJ *et al* (2007)).

Mutagenicity
In the Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100, a fluid extract (60% ethanol) was non mutagenic at concentrations of 50-5000 µg/plate. With *Aspergillus nidulans* diploid strains genotoxic effects with mitotic crossing over and chromosome malsegregation were observed at higher concentrations of 0.1-1.0 mg/ml, at which a concentration-dependent increase of cytotoxicity also occurred.
These findings were not confirmed *in vivo* in the mouse bone marrow micronucleus test; after oral administration of the extract up to 1 g/kg body weight for two days no increase in the number of micronucleated polychromatic erythrocytes was observed (Ramos A *et al* (1998)).

Six saponins from Calendula flower (at 400 µg/plate) were non-mutagenic in the Ames test using *Salmonella typhimurium* TA98 with and without S9 activation (Elias R *et al* (1990)).

An aqueous extract showed no genotoxic effects in the Drosophila Wing Somatic Mutation and Recombination Test (SMART) (Graf UA *et al* (1994)).

Perez-Carreon JI *et al* (2002) investigated whether dry extracts prepared from Calendula flowers by several solvents are able to induce unscheduled DNA synthesis in rat liver cell cultures and whether they can reverse diethylnitrosamine induced unscheduled DNA synthesis. Polar extracts (solvents water and water/ethanol) completely reversed the effect of diethylnitrosamine in very low concentrations (50 ng/ml and 0.4-16 ng/ml, respectively). In the absence of diethylnitrosamine these two extracts induced at higher concentrations (three orders of magnitude above total protection) unscheduled DNA synthesis. Lipophilic extracts showed no or only slight effects in this model. The authors conclude that flavonoids may act in lower concentrations as radical scavenger, while in higher concentrations their oxidizing potential is dominating. In a model using diethylnitrosamine treated rats the protecting effect could be demonstrated up to a dose of 10 mg/kg, at higher concentrations increased altered hepatocyte foci could be detected (Barajas-Farias LM *et al* (2006)).

Bakkali F *et al* (2005) found that the essential oil of *Helichrysum italicum* (the authors declare this name as synonym to *Calendula officinalis*) exhibits only a weak cytotoxicity, in contrast to other essential oils it did not induce cytoplasmatic petite mutations indicating damage to mitochondrial DNA.

Assessor's comment: the value of this paper is limited, because in the botanical literature *Helichrysum italicum* is not mentioned as synonym of *Calendula officinalis*, therefore the plant source for the tested essential oil remains unclear.

Carcinogenicity
Carcinogenicity studies with Calendula flower extract (solvent not mentioned) have been performed in rats over a period of 22 months and in hamsters over a period of 18 months with a daily oral dose of 0.15 g/kg body weight. The extract was not carcinogenic in either species (Avramova S *et al* (1988)).

Reproduction toxicity:
No tests on reproduction toxicity are published.

II.2.3.2 Assessor's overall conclusions on toxicology

Reliable data from tests on genotoxicity are only available for hydroethanolic liquid extracts. Therefore a community list entry is proposed for this type of extracts only.

The topical administration of preparations of Calendula flowers can be regarded as safe, especially at therapeutic doses.

II.3 CLINICAL DATA

II.3.1 Clinical Pharmacology

II.3.1.1 Pharmacodynamics

No specific data are available on Calendulae flos.

II.3.1.2 Pharmacokinetics

No specific data are available on Calendulae flos.

II.3.2 Clinical Efficacy[8]

II.3.2.1 Dose response studies

No specific data are available on Calendulae flos.

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

Observational studies
Protective effects of different cream preparations containing Calendula extract against sodium-lauryl-sulfate induced irritant contact dermatitis (Fuchs SM *et al* (2005)):
The extract (prepared with supercritical CO_2) in a base cream according to DAC was tested in 20 healthy volunteers with experimentally induced irritant contact dermatitis in a 4-day repetitive irritation test. A statistically significant protective effect was observed; the sequential treatment (postirritation) was without any effect (Fuchs SM *et al* (2005)).

Assessor's comment:
The extract which has been used in this study does not fulfil the criteria for traditional use.

Pilot study with a Calendula jelly containing 10% of a homoeopathic mother tincture in 30 patients with first- and second-degree burns (Baranov AP (1999)).

Assessor's comment:
The lack of a control group makes the evaluation of the efficacy impossible. However, the study medication was well tolerated and no adverse effects have been observed. This study supports the traditional use of Calendula for the treatment of minor inflammations of the skin because of the chemical similarity of homoeopathic mother tinctures and phytotherapeutic tinctures.

Observational study of an ointment containing Calendula in the treatment of venous leg ulcers (Duran V *et al* (2005)):
34 patients were divided into an experimental group (21 patients with 33 venous ulcers) receiving a Calendula extract prepared with absolute ethanol in a neutral base and a placebo group (13 patients with 22 venous ulcers) receiving saline solution dressing. The therapy was applied twice daily for 3 weeks. In the experimental group a significant acceleration of wound healing (expressed as the surface of the ulcers) could be observed.

Assessor's comment:
This observational study does not fulfil the criteria for classification of this type of treatment in the category 'well established use'. The indication 'topical treatment of venous leg ulcer' is not considered as suitable for traditional use without medical supervision.

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

Phase III clinical studies

Controlled study of three ointments for the local management of 2nd and 3rd degree burns (Lievre M *et al* (1992))
Randomized, controlled, open study with parallel groups (only adults > 18 years of age; 53 patients treated with Pommade au Calendula par digestion, 53 patients treated with Elase (proteolytic ointment), 50 patients treated with vaseline (control treatment)). A marginally significant difference in favour of Calendula over vaseline was observed. Calendula was significantly better tolerated than the other treatments.

Assessor's comment:
The study medication not only contained the flowers but also stems and leaves of *Calendula officinalis*. Therefore the results of this study are of limited relevance for the assessment of preparations containing the ligulate florets only. However, the results support the safe use of preparations containing Calendula.

Phase III randomized single blinded trial of Calendula officinalis compared with Trolamine for the prevention of acute dermatitis during irradiation for breast cancer (Pommier P *et al* (2004)).
254 patients who had been operated on for breast cancer and who were to receive postoperative radiation therapy were randomly allocated to application of either trolamine (128 patients) or calendula (126 patients) on the irradiated fields after each session. The calendula ointment (Pommade au Calendula par digestion) contained 20% of fresh calendula aerial parts in petroleum jelly. The primary end point was the occurrence of acute dermatitis of grade 2 or higher. Secondary end points were the occurrence of pain, the quantity of the topical agent used and patient satisfaction.
The occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% v 63%; P<.001) with the use of calendula than with trolamine. Moreover, patients receiving calendula had less frequent interruption of radiotherapy and significantly reduced radiation-induced pain.

Assessor's comment:
The study medication not only contained the flowers but also stems and leaves of Calendula officinalis. Therefore the outcome of the study does not justify the well-established use of Calendula flower in the prevention of acute dermatitis under postoperative irradiation.
This may be the reason why in an overview and practice guideline for prevention and management of acute skin reactions related to radiation therapy the authors conclude that there is insufficient evidence to support or refute topical agents (Bolderston A *et al* (2006)).

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

No specific data are available on Calendulae flos.

II.3.2.4 Assessor's overall conclusions on clinical efficacy

There are no data available from controlled clinical studies, where herbal preparations have been investigated containing the herbal substance Calendulae flos as defined in the European Pharmacopoeia.

Therefore the medicinal use of Calendula flowers has to be regarded as traditional.

II.3.3 Clinical Safety/Pharmacovigilance

II.3.3.1 Patient exposure

No exact data on patient exposure are available.

II.3.3.2 Adverse events

A cross sensitivity with other members of the Asteraceae cannot be excluded but has not been reported to date.
The data concerning the risk of skin irritation or allergic reactions are controversial. In a review Paulsen concludes that experimentally the plant extract is only weakly sensitizing which is supported by the scarcity of case reports (Paulsen E *et al* (1993), Paulsen E (2002)). This might be explained by the lack of sesquiterpene lactones in Calendula flowers.
In contrast to this Reider N *et al* (2001) report that 2% of 443 patients reacted positively to Calendula, while only 1% reacted to Arnica.
In the assessor's opinion the latter publication is of limited value for the assessment of the safety of Calendula preparations defined in the monograph, because the authors tested the whole aerial parts of Calendula and not the ray florets alone.
During the clinical trial by Pommier P *et al* (2004) no allergic reactions occurred in the group given Calendula (126 patients).
There are no reports in literature that the topical application of preparations of Calendula flowers bear a higher risk for skin irritation for atopic persons.

There is no evidence for phototoxic activities.

Proposed wording:

Undesirable effects:
Skin sensitization. The frequency is not known.

Contraindications:
Hypersensitivity to members of the Asteraceae family (Compositae family).

II.3.3.3 Serious adverse events and deaths

None known

In the literature 1 case report of an anaphylactic shock after gargling with Calendula tincture is cited (Hänsel R *et al* (1992)); no details are reported.

II.3.3.4 Laboratory findings

No specific data are available on Calendulae flos.

II.3.3.5 Safety in special populations and situations

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

None known

II.3.3.5.2 Drug interactions

None reported

II.3.3.5.3 Use in pregnancy and lactation

No data available. However, there are no objections to external use during pregnancy and lactation (ESCOP (2003)).

Since safety during pregnancy and lactation has not been established, the use during pregnancy and lactation is not recommended.

II.3.3.5.4 Overdose

None reported

II.3.3.5.5 Drug abuse

None known

II.3.3.5.6 Withdrawal and rebound

None known

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

None known

II.3.3.6 Assessor's overall conclusions on clinical safety

The topical administration of preparations of Calendula flowers can be regarded as safe, especially at therapeutic doses.

II.4 ASSESSOR'S OVERALL CONCLUSIONS

The positive effects of Calendulae flos preparations on healing of minor wounds and for the treatment of minor inflammations of the skin have long been recognised empirically. The use is made plausible by pharmacological data (level of evidence 4). There are no data available from controlled clinical studies using herbal preparations, containing the herbal substance Calendulae flos, as defined in the European Pharmacopoeia.

In conclusion, Calendulae flos preparations can be regarded as traditional herbal medicinal products.

Pharmaco-therapeutic group: Preparations for treatment of wounds
ATC code: D03A

III. ANNEXES