



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CALENDULAE FLOS

SUMMARY REPORT

1. *Calendulae flos* is the dried ligulate florets or the dried composite flowers of *Calendula officinalis*. *Calendulae flos* contains the following constituents:
 - Flavonoids: flavonol (isorhamnetin, quercetin) glycosides including isoquercitrin, narcissin, neohesperoside and rutin. The total content is 0.3 to 0.8%;
 - Volatile oil, total content 0.2%, containing menthone, isomenthone, caryophyllene, pedunculatine, α - and β -ionone, a β -ionone epoxide derivative, dihydroactinidiolide. Main components: sesquiterpenes: cadinol (27% of total content) and T-cadinol (13% of total);
 - Triterpenoids: saponins with oleanolic acid as an aglycone (i.e. glycosides A-F) in a total amount of 2 to 10% (dry weight). A large number of acylated pentacyclic mono-, di-, and tri-hydroxy-triterpenes derived from ψ -taraxen, taraxen, lupen, oleanen and ursen. The triterpene alcohols occur both in free form and as esters;
 - Sterols: present as free alcohols, esters and glycosides in a total amount of 0.06 to 0.08%;
 - Coumarines: scopoletin, umbelliferon and aesculetin;
 - Carotenoids: a great number of carotene- and xanthophyll-derivatives;
 - Polysaccharides: 14.8% water-soluble polysaccharides consisting of acidic, branched heteroglycanes (rhamno-arabino-galactanes and arabino-galactanes).

Calendulae flos is used in four different veterinary combination products. These products are: two solutions for use in all food producing species, one of which contains 17.6% (w/w) of an ethanolic 1:1 extract and the other one 65% (w/w) of a tincture prepared with 70% isopropanol, corresponding to 6 g *Calendulae flos*, and two ointments, one containing 2.5% (w/w) of the ethanolic 1:1 extract, the other one containing 55% (w/w) of a 70% isopropanol tincture, corresponding to 6 g *Calendulae flos*.

The solutions and ointments are for topical administration (no doses and treatment regimes specified) to all mammalian food producing species, with the following indications: treatment of inflammations, eczema, wounds of skin and mucous membranes and disinfection of wounds on the hooves/feet.

In humans infusions or decoctions of *Calendulae flos* (1 to 4 g to one cup) are used for rinsing the mouth and throat to treat infections. Aqueous extracts are also used for wound-treatment. *Calendulae flos* is also used internally as a diaphoretic (15 to 25 drops of the tincture/corresponding to about 0.5 g of the crude drug 3 to 4 times a day). Traditionally *Calendulae flos* has been used to treat gastric and duodenal ulcers, amenorrhoea, dysmenorrhoea and epistaxis, crural ulcers, varicose veins, haemorrhoids, anal eczema and others. The dose ranges between 1 to 4 g three times a day.

2. Anti-inflammatory, antibacterial and antiviral activities have been reported.

An ethanol extract at an oral dose of 100 mg/kg bw gave 11% reduction of carragenin-induced oedema in the rat paw. Thurfylnicotinate-induced hyperaemia was inhibited by an intraperitoneal dose of 0.45 mg/kg bw of an ethanolic extract of *Calendulae flos* to the same degree as by 5 mg/kg bw of phenylbutazon. A 20% inhibition of croton oil-induced oedema in the ear of the mouse was obtained by doses of 1.2 mg/ear of an ethanol extract or 0.1 mg/ear of a CO₂-extract, indicating the active principles to be lipophilic. A dose of 1.2 mg of the CO₂-extract gave 70% inhibition.

Several publications report antibacterial and antifungal activities of extracts of *Calendulae flos*. The activity is related to the volatile oil and to the flavonoids. *Calendulae flos* tincture has virucidal activity *in vitro* against influenza virus of type A and A₂ as well as against Herpes simplex.

Experimentally induced wounds in rats were treated with an ointment containing aqueous and ethanolic extracts of *Calendulae flos* (5%, 1:1). Regeneration and formation of epithelium were stimulated. Phagocytose-activity and differentiation of macrophages was increased, probably by a more intense metabolism of glycoproteins, nucleoproteins and collagen. Also formation of fibrin is stimulated. These effects have been attributed to the content of carotenoids and manganese.

The saponin fraction has no immunostimulating effect, but such effects have been demonstrated for the polysaccharide fraction.

Extracts of *Calendulae flos* are cytotoxic against diploid MRC5 cells, HEP-2 cells (cell lines not further specified) and Ehrlich-Ascites tumour cells *in vitro*. Daily oral doses of 20 mg/kg bw of *Calendulae flos* extract has antitumour activity against Ehrlich-Ascites tumours in mice.

Effects on blood pressure and the central nervous system are described but are difficult to interpret because of insufficient experimental data.

An *in vitro* uterotonic effect has been described for *Calendulae flos* extract on rabbit and Guinea pig preparations.

The saponin fraction has a comparatively low haemolytic activity.

3. No information on the pharmacokinetics of *Calendulae flos* was available.
4. The intravenous and intraperitoneal LD₅₀ in mice of aqueous extracts is reported to be 300 to 375 mg/kg bw. For an aqueous-ethanolic extract (30% ethanol) the LD₅₀ is reported to be 45 mg/mouse, subcutaneously and for the rat it was reported to be 5260 mg/kg bw, intravenously. No side effects in man have been reported.
5. No toxic symptoms were observed in the hamster orally given 0.15 g/kg bw/day for a period of 18 months of an extract of *Calendulae flos* (solvent not reported).
6. No information was provided on reproductive toxicity or on teratogenicity.
7. No information was provided on the mutagenicity of *Calendulae flos*.
8. No carcinogenic effects were observed in rats and hamsters that were treated orally with an extract of *Calendulae flos* (solvent not reported) for 18 to 22 months at a dose of 0.15 g/kg bw/day.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for Veterinary Medicinal Products for the inclusion of substances in Annex II of Council Regulation (EEC) No. 2377/90 and in particular that:

- *Calendulae flos* has low acute and long term oral toxicity,
- *Calendulae flos* is used only topically in a small number of individual animals, for infrequent or non-regular treatments,
- the animals are unlikely to be sent for slaughter during or immediately after treatment;

The Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for *Calendulae flos* and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
<i>Calendulae flos</i>	All food producing species	For topical use only