Calendula officinalis

Scientific Name

***Calendula officinalis* L.**

Synonyms

Calendula aurantiaca Kotschy ex Boiss., *Calendula eriocarpa* DC., *Calendula hydruntina* (Fiori) Lanza, *Calendula officinalis* var. *prolifera* Hort., *Calendula prolifera* Hort. ex Steud., *Calendula* × *santamariae* Font Quer, *Calendula sinuata* var. *aurantiaca* (Klotzsch ex Boiss.) Boiss., *Caltha officinalis* (L.) Moench (nom. illeg.)

Family

Asteraceae

Common/English Names

Bull's Eye, Calendula, Common Marigold, Cowbloom, Death Flower, Drunkard Gold, English Marigold, Garden Marigold, Gold Bloom, Golden Flower of Mary, Herb of the Sun, Holligold, Hollygold, Marigold, Husband's Dial, Kingscup, Marybud, Marygold, May Orange, Poet's Marigold, Poor Man's Saffron, Pot Marigold, Ruddles, Scotch Marigold, Scottish Marigold, Shining Herb, Summer's Bride, Sun's Bride, Water Dragon

Vernacular Names

Albanian: Kalendula Mjekësore
Arabic: Ajamir, Djoumaira
Brazil: Calêndula
Chinese: Chin Chan Hua, Jīn Zhǎn Jú
Croatian: Bileć, Ljekoviti Neven, Mesiček, Ognjac, Vridovno Zelje, Zimorod
Czech: Měsíček Lékařský
Danish: Havemorgenfrue, Morgenfrue
Dutch: Goudsbloem, Tuinggoudsbloem
Eastonian: Harilik Saialill
Esperanto: Kalendulo, Kalendulo Kuraca, Orfloro Kuraca
Finnish: Kehäkukka, Tarhakehäkukka
French: Calendule, Fleur De Souci, Fleurs De Tous Les Mois, Gauche-Fer, Soubi, Souci, Souci Des Jardins, Souci Officinal, Yous Les Mois
German: Goldblume, Ringelblume, Ringelrose, Sonnenwende
Hungarian: Kerti Körömvirág, Körömvirág
Iceland: Morgunfrú
India: Genda, Surajmukhee, Zergul (Hindi), Gulsarfi (Punjabi), Sendigai, Sendigai Poo (Tamil), Banti (Telugu), Gul-E-Ashrafi (Urdu)
Italian: Calendola, Calendula, Calta, Fior D'ogni, Fiorrancio, Fiorrancio Coltivato, Fiorrancio Dei Gardine
German: Butterblume, Dotterblume, Echte Ringelblume, Garten-Ringelblume, Gartendotterblume, Goldblume, Ingelblum, Rinderblume, Ringelblume, Ringelrose, Ringula, Sonnenbraut, Sonnenwende,

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Studentenblume, Totenblume, Warzenkraut, Weckbröseln, Wucherblume
Japanese: Kinsenka, To Kinsenka
Korean: Kumjanhwa
Norwegian: Ringblom
Polish: Nagietek Lekarski
Portuguese: Calêndula, Belas-Noites, Boas-Noites, Maravilhas, Margarida
Russian: Kalendula, Nogot'ki, Nogot'ki Lekarstvennye
Slovaščina: Ognjič Vrtni, Vrtni Ognjič, Zdravilni Ognjič
Slovenian: Nechtík Lekársky, Vrtni Ognjič
Spanish: Botón De Oro,Caldo, Calendula, Cempasúchitl, Corona De Rey, Flamenquilla, Flaminquillo, Flor De Difunto, Flor De Merte, Maravilla, Mejorana, Mercadela, Rosa De Muertos, Virreina
Swedish: Ringblomma, Solsicka, Solsocka
Turkish: Tibbi Nergis
Vietnamese: Cúc Kim Tiền, Hoa Xu Xi, Tâm Tư Cúc, Xu Xi
Welsh: Melyn Mari

Origin/Distribution

The species is a native of the Mediterranean area, but now *Calendula* has naturalized in many temperate countries and is cultivated as ornamentals in warm temperate and sub-temperate areas.

Agroecology

The plant grows well in full sun and tolerate most soils—acidic, sandy, loamy and clayey soils with pH 4.5–8.3—but does best on well-drained, moist, loamy soil. In temperate areas, seeds are sown in spring for blooms that last throughout the summer and well into the fall.

Edible Plant Parts and Uses

Flowers and leaves are edible (Hedrick 1972; Facciola 1990; Roberts 2000). Fresh petals are chopped and added to salads or used as garnish in dishes. The petals can be used in omelette, curry and custard (Roberts 2000). Dried petals have a more intense flavour and are used as a seasoning in soups, cakes, drinks and baked products. An edible yellow dye is obtained from the flowers and used as colorant for butter, cheese, drinks, rice, soups, confectionery and baked products and also used as a substitute for saffron. Calendula was once known as 'poor man's saffron' as its extract was fed to hens to make their egg yolks golden. An herbal tea can be prepared from the flowers and petals. The leaves can be eaten raw in salads.

Botany

The plant is usually grown as an annual, erect or procumbent and branched, stipitate-glandular, with a strong tap root. Leaves are sessile or shortly petiolate, elliptic, obovate, oblong, oblanceolate to spatulate, 3–12 (−16 cm) by 2–5 cm, with entire margins, apex acute, base sometimes clasping, sparsely arachnose on both surfaces (Plates 1 and 2). Flower heads (capitula) borne singly; involucre campanulate to hemispheric; phyllaries linear-lanceolate, pubescent and in two series; ray florets 15–50 (>100) in 1–3 or more series, functionally female, with yellow to orange, linear to oblanceolate corolla; central disc florets 20–60 (>100), hermaphrodite but functionally male, tubular with campanulate throat, corolla yellow, orange (Plates 2 and 3), reddish or purplish. Achene curved and tuberculate or transversely ridged.

Nutritive/Medicinal Properties

Flower Phytochemicals

Carbohydrates

The water soluble polysaccharide fraction obtained from *C. officinalis* inflorescences were found to contain 84.58 % pectic substances, 29.25 % ash, 9.25 % moisture, 25.77 % acidic sugars, 31.25 % reducing sugars and 4.92 % proteins and to have the following monosaccharide composition comprising glucose, galactose, arabinose, xylose, rhamnose and galacturonic

Plate 1 Leaves and a flower bud

Plate 2 Yellow flowers in bloom

acid (Chushenko et al. 1988). Three homogeneous polysaccharides were isolated from *Calendula officinalis* flowers (Varljen et al. 1989). All three polysaccharides contained a (1 → 3)-linked β-D-*galactan* backbone with branching points at C-6. The side chains are composedofshortα-Araf(1 → 3)-Araf,α-l-Rhap-(1 → 3)-Araf or simple α-L-Araf units. The carbohydrate composition of *Calendula officinalis* consisted of free glucose and pectin substances with a low degree of esterification (Khodzhaeva and Turakhozhaev 1993). The monosaccharide composition of the stem comprised rhamnose, galactose and galacturonic acid and the inflorescences have rhamnose, galactose, glucose and galacturonic acid. Pectic substances were also found in the stems and inflorescences.

Plate 3 Close view of flower heads

Terpenoids

From *Calendula officinalis* flowers, five glycosides of oleanolic acid were isolated and their structuresestablishedas3-glucuronide;3-(galactosyl-glucuronide); 3-(galactosyl-glucuronide); 17-glucoside; 3-(galactosyl-(glucosyl)-glucuronide); and 3-(galactosyl-(glucosyl)-glucuronide); 17-glucoside (Kasprzyk and Wojciechowski 1967). A number of alcohols representing different types of pentacyclic triterpenes were identified in *Calendula officinalis* flowers (Kasprzyk and Pyrek 1968). In the group of monohydroxy alcohols, the following were identified: α-amyrin, β-amyrin, taraxasterol and lupeol in addition to previously isolated ψ-taraxasterol. In the group of dihydroxyalcohols, the following were identified: brein and calenduladiol (a new diol of lupeol type) in addition to the previously isolated

arnidiol and faradiol. The presence of four other diols of the α-amyrin, β-amyrin and ψ-taraxasterol types was observed. Alcohols of ψ-taraxasterol type, possessing three and four hydroxyl groups, were also isolated as well as a small amount of oleanolic aldehyde. A new triterpene diol, ursodiol, was isolated from dry *Calendula officinalis* flowers (Sliwowski et al. 1973). Its structure was confirmed as 3,21-di-OH-ursa-12-en. Kasprzyk and Wiłkomirski (1973) isolated a triterpene triol of α-amyrin type from the flowers. A triterpene glycoside, calenduloside F, was isolated from the flowers (Vidal Ollivier et al. 1988).

In *Calendula officinalis* flowers, triterpene monols were found mainly in the chromoplast fraction (68 % of total) with smaller amounts in the cell debris, microsomal and supernatant fractions; the mitochondrial fraction was almost devoid of these compounds (Adler and Kasprzyk 1976). Triterpene diols were present exclusively in the chromoplast fraction, 98 % in the form of the 3-monoesters and 2 % in the form of diesters. The data suggested that the hydroxylation of the triterpene monols to the corresponding diols proceeded in the chromoplasts and the esterified form of the monols was probably the substrate for this reaction.

From *Calendula officinalis* flowers, five pentacyclic triterpene trihydroxyalcohols were isolated and identified as olean-12-ene-3β,16β,28-triol, lup-20(29)ene-3β,16β,28-triol, tarax-20-ene-3β,16β,22α-triol, tarax-20-ene-3β,16β,30-triol and ursa-12-ene 3β,16β,21-triol (Wiłkomirski 1985). Gracza (1987) identified the following terpenoids from the flowering heads: menthone, isomenthone, caryophyllene and an epoxide and ketone derivative, pedunculatine, α- and β-ionone, a β-ionone epoxide derivative and dihydroactinidiolide. Triterpenoids found in the flowers included faradiol monoester, monools ψ-taraxasterol, lupeol, taraxasterol and β-amyrin (Della Loggia et al. 1994). Eleven triterpene alcohols helianol, taraxasterol, ψ-taraxasterol, α-amyrin, β-amyrin, lupeol, taraxerol, cycloartenol, 24-methylenecycloartanol, tirucalla-7,24-dienol and dammaradienol were isolated from the tubular flowers of *Calendula officinalis*, *Carthamus tinctorius*, *Cosmos bipinnatus*, *Chrysanthemum morifolium*, *Helianthus annuus* and *Matricaria matricarioides* (Akihisa et al. 1996). All the flowers shared a common characteristic feature by containing helianol as the most predominant component (29–86 %) in the triterpene alcohol fractions.

From the 1-butanol-soluble fraction of the methanol flower extract of *Calendula officinalis*, four new triterpene oligoglycosides, calendasaponins A, B, C and D, were isolated, together with eight known saponins, seven known flavonol glycosides and a known sesquiterpene glucoside (Yoshikawa et al. 2001). They also isolated two new ionone glucosides (officinosides A and B) and two sesquiterpene oligoglycosides (officinosides C and D) from the flowers of Egyptian *Calendula officinalis* (Marukami et al. 2001). One new triterpenoid of oleanane-series, cornulacic acid acetate (1), along with oleanolic acid acetate was isolated from *Calendula officinalis* flowers (Naved et al. 2005). The structure of (1) was established as 3β-acetoxy-olean-12-en-27-oic acid and oleanolic acid acetate was characterized as 3β-acetoxy-olean-12-en-28-oic acid.

Ten oleanane-type triterpene glycosides, 1–10, calendulaglycoside A (1), calendulaglycoside A6′-*O*-*n*-methyl ester (2), calendulaglycoside A 6′-*O*-*n*-butyl ester (3), calendulaglycoside B (4), calendulaglycoside B 6′-*O*-*n*-butyl ester (5), calendulaglycoside C (6), calendulaglycoside C 6′-*O*-*n*-methyl ester (7), calendulaglycoside C 6′-*O*-*n*-butyl ester (8), calenduloside F 6′-*O*-*n*-butyl ester (9) and calenduloside G 6′-*O*-*n*-methyl ester (10), along with five known flavonol glycosides, 11–15 isorhamnetin-3-*O*-neohesperidoside, isorhamnetin-3-*O*-2-rhamnosyl rutinoside, isorhamnetin-3-*O*-rutinoside,quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside, were isolated from the flowers of *Calendula officinalis* (Ukiya et al. 2006).

Lipids

Sterol esters as well triterpene monol and diol esters isolated *Calendula officinalis* flowers were found to contain as alcohol components all the types of sterols and triterpenic alcohols present in the plant (Wojciechowski et al. 1972). Sterols and triterpene monols were esterified with acetic, lauric, myristic and palmitic acids. Triterpene

diols were esterified with lauric, myristic and palmitic acids. The main diol esters were 3-monoesters; diesters were present only in very small amount.

In *C. officinalis* ligulate flowers, it was shown that all free and ester-bound sterols and triterpene monols in both forms occurred in the chromoplast fraction and in the chromoplast-free fraction, whereas all diols were localized only in the chromoplast fraction (Wilkomirski and Kasprzyk 1979). The compositions of the fatty acids esterifying monols and sterols were similar to those esterifying diols in the chromoplasts. However, the fatty acids esterifying extra-chromoplast monols and sterols were different. This result indicated triterpene monol esters to be substrates for the biosynthesis of 3-monoesters of diols. Faradiol esters, namely, faradiol-3-myristic acid ester, faradiol-3-palmitic acid ester and ψ-taraxasterol, were isolated from *C. officinalis* flower heads (Zitterl-Eglseer et al. 1997).

The main compounds of lipophilic extracts of flower heads of *Calendula officinalis* comprised triterpendiol esters, mainly faradiol laurate, faradiol myristate and faradiol palmitate (Zitterl-Eglseer et al. 2001). These faradiol-3-*O*-monoesters were quantified in different parts of *C. officinalis* plants, namely, ray florets, disc florets, involucral bracts, receptacles, leaves and seeds. The contents of the esters were highest in ray florets, approximately ten times lower in disc florets than in the ray florets, and approximately ten times lower in involucral bracts than in the disc florets. In the leaves only traces of the esters could be detected, and in the receptacles no esters could be detected at all. Quantification in the seed was not possible using this method because of interfering fatty compounds.

Dichloromethane extract of dried flowers of *Calendula officinalis* was found to contain eight known bioactive pentacyclic terpenoids and triterpendiol monoesters, namely, faradiol-3-*O*-palmitate, faradiol-3-*O*-myristate, faradiol-3-*O*-laurate, arnidiol-3-*O*-palmitate, arnidiol-3-*O*-myristate,arnidiol-3-*O*-laurate,calenduladiol-3-*O*-palmitate and calenduladiol-3-*O*-myristate (Neukirch et al. 2004). Of the ten varieties of *C. officinalis* investigated, calypso orange florensis produced the highest amounts of the bioactive monoesters, followed by Fiesta Gitana Gelb and may orange florensis. The lipophilic extract from the flowers of calypso orange florensis variety also contained low levels of the newly characterized calenduladiol-3-*O*-laurate.

Marigold also has oleoresins. The solubility of Marigold (*Calendula officinalis*) oleoresin in supercritical CO_2 (SC-CO_2) varied from 4.74×10^{-4} to 17.04×10^{-4} g oleoresin/g CO_2 (Danielski et al. 2007). The use of palm oil as cosolvent for SC-CO_2 extraction of Marigold flower was found to enhance the yield of lutein fatty acid esters by approximately 16 %, with the most suitable concentration being 10 % w/v palm oil (Palumpitag et al. 2011). Under this condition, approximately 87.2 % recovery of lutein fatty acid esters was obtained after 4 hours extraction at 60 °C and 40 MPa. Furthermore, saponification of the Marigold oleoresin for 3 hours with 2 ml of 40 % (w/v) KOH solution per 1 g of oleoresin resulted in the maximum conversion of lutein esters, giving approximately 157.24.4 mg of free lutein/g oleoresin.

Carotenoids

The carotenoid content was higher in orange varieties *of C. officinalis*: 276 mg/100 g fresh flowers for Double Esterel Orange variety and 111 mg/100 g fresh flowers for Radio Extra variety (Pintea et al. 2003). All varieties contain the same pigments (xanthophylls) but there were significant differences for the ratio between individual pigments. The main pigments identified were flavoxanthin, lutein, rubixanthin, β-carotene, γ-carotene and lycopene. The great majority of xanthophylls present had a β-ε structure: flavoxanthin, lutein and luteoxanthin. Zeaxanthin (3,3′-dihydroxy-β, β-carotene) was present in small amount, as well as their epoxides: antheraxanthin, mutatoxanthin and auroxanthin. Other carotenoids present included neoxanthin, lactucaxanthin and α-carotene. Orange varieties contained higher amounts of hydrocarbons, 44.5 % of total carotenoid in Double Esterel Orange, while yellow varieties contain mostly oxygenated derivatives, 97 % of total carotenoids in Double Esterel Jaune. In the orange varieties, a

preferential biosynthesis of hydrocarbons with ψ-ψ and β-ψ structure and of monoxanthophylls with β-ψ (or α-ψ) structure was noted.

In the petals and pollens of *Calendula officinalis*, the main carotenoids were flavoxanthin and auroxanthin while the stem and leaves mostly contained lutein and β-carotene (Bakó et al. 2002).

Carotenoids present in *C. officinalis* cv Alice Orange flowers in percent of total carotenoids (Kishimoto et al. 2005) and in μg/g fresh weight (Kishimoto et al. 2007) were respectively (8′*R*)-luteoxanthine 11 % 186.6 μg; lutein-5,6-epoxide 1.6 % 27.1 μg; (8*R*)-lutein-5,8-epoxide (flavoxanthin) 28.5 %, 483.4 μg; (8*R*,8′*R*)-auroxanthin 7.1 %, 120.4 μg; (9′*Z*)-lutein-5,6-epoxide 5 %, 84.8 μg; lutein 2 % 33.9 μg; antheraxanthin 1 % 17.0 μg; (9*Z*)-lutein 0.6 %, 10.2 μg; (5′*Z*,9′*Z*)-rubixanthin 4 %, 67.8 μg; α-carotene 0.8 %, 13.6 μg; β-carotene 3.4 %, 57.7 μg; (5′*Z*)-rubixanthin 3 %, 50.9 μg; δ-carotene 1.4 %, 23.7 μg; (5*Z*,9*Z*,5′*Z*,9′*Z*)–lycopene 4.1 %, 69.5 μg; γ-carotene 2 %, 33.9 μg; (5′*Z*)-γ-carotene 4.4 %, 74.6 μg; (5*Z*,9*Z*,5′*Z*)-lycopene 3.5 %, 59.4 μg; (5*Z*,9*Z*)-lycopene 4.1 %, 69.5 μg and (all – *E*)-lycopene 8.7 %, 147.6 μg. The carotenoid composition in Alice Yellow flowers were (8′*R*)-luteoxanthin 15.6 %, 195 μg; lutein-5-6-epoxide 3.2 %, 40 μg; (8*R*)-lutein-5,8-epoxide (flavoxanthin) 42.6 %, 532.5 μg; (8*R*,8′*R*)-auroxanthin 10.7 %, 133.7 μg; (9′*Z*)-lutein-5,6-epoxide 8.5 %, 106.2 μg; lutein 5 %, 62.5 μg; antheraxanthin 2.5 %, 31.2 μg; (9*Z*)-lutein 1.5 %, 18.7 μg; and β-carotene 1.0 %, 12.5 μg. Total carotenoids in *C. officinalis* Alice orange was 1696.2 μg/g FW comprising 963.4 μg of yellowish carotenoids and 668.3 μg of reddish carotenoids and total carotenoids in Alice yellow was 1249.9 μg/g FW comprising 119.9 μg of yellowish carotenoids and 1.2 μg of reddish carotenoids (Kishimoto et al. 2007).

Studies showed that solvent had an influence on the stability of carotenoids in oil extracts of *Calendula officinalis* (Bezbradica et al. 2005). The highest degradation rates were observed in extracts prepared with linoleic acid-rich solvents (sunflower oil, soybean oil and grape seed oil), while the lowest were found in oil with saturated fatty acids (Myritol 312®) and paraffin oil. Studies showed that saline irrigation water decreased the fresh and dry weights of *Calendula officinalis* flower heads and pigment contents (total flavonoids and total carotenoids) but increased essential oil yield and its main components (α-cadinol, γ- and δ-cadinene) (Khalid and da Silva 2010). Fresh and dry weights of flower heads and essential oil increased towards the full bloom stage of flowering while pigment content, such as total flavonoids and total carotenoids, increased.

Flavonoids

Eight flavonoids were isolated from *C. officinalis* inflorescence: two aglycones (quercetin, isorhamnetin) and six glycosides (isoquercetin, isorhamnetin 3-*O*-β-D-glucoside, narcissin, calendoflaside, calendoflavoside and calendoflavobioside) (Komissarenko et al. 1988). Seven flavonol 3-*O*-glycosides were isolated from the flowers of *Calendula officinalis* and elucidated as isorhamnetin 3-*O*-glucoside, rutinoside, neohesperidoside, quercetin glucoside and 2 (G)-rhamnosylrutinoside (Vidal-Ollivier et al. 1989). Bilia et al. (2001) identified narcissin, rutin, isoquercitrin, quercetin-3-*O*-rutinosylrhamnoside, isorhamnetin-3-*O*-rutinosylrhamnoside, isorhamnetin-3-*O*-glucosylglucoside and isorhamnetin-3-*O*-glucoside in *C. officinalis* flowers. The triglycoside isorhamnetin-3-*O*-α-l-rhamnopyranosyl-(1 → 2)-*O*-[α-l-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside together with the already known glycosides isorhamnetin-3-*O*-β-D-glucopyranoside and isorhamnetin-3-*O*-α-l-rhamnopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside (narcissin) was isolated from *Calendula officinalis* flowers (Masterova et al. 1991). The total content of flavonoids in ligulate ray florets and tubular discflorets inclusively involucre was found to be 0.88 and 0.25 %, respectively.

The following flavonoids isorhamnetin (3′-metoxy-4′,3,5,7-tetrahydroxyflavone), isorhamnetin-3-*O*-glucoside, rutin, quercetin glucoside, quercetin-neohesperoside and quercetin-2G-rhamnosil-rutinoside were isolated from flowers and found to have good antioxidant activity (Albulescu et al. 2004). Two flavonoids

were isolated from the flowers and identified as isorhamnetin 3-*O*-rutinoside (narcissin) quercetin 3-*O*-β-D-glucopyranoside (isoquercitrin) (Kurkin and Sharova 2007). Patulitrin (1) and patuletin (2) were found only in Marigold flowers during and after flowering (Guinot et al. 2008). A water-ethanol mixture gave a high extraction efficiency of both flavonoids.

Essential Oil

Crabas et al. (2003) reported that the essential oil of *Calendula officinalis* obtained from dried flowers in Italy contained methyl hexadecanoate (23.8 %), methyl linoleate (18.6 %), methyl 9,12,15-octadecatrienoate (17.2 %), methyl octadecanoate (4.8 %), methyl tetradecanoate (4.6 %), g-cadinene and cubenol (4.0 %), d-cadinene (3.2 %), a-cadinol (1.8 %) and oplopanone (1.3 %). Essential oil constituents of *C. officinalis* flower head (French source) and Slovakian source without fertilizer application, respectively, were β-pinene 0.45 %, 0.38 %, α-pinene 2.47 %, 2.18 %, myrcene 0.19 %, 0.29 %, phellandrene 2.76 %, 0.74 %, *p*-cymene 2.76 %, 2.22 %, limonene 19.16 %, 18.28 %, 19.61 %, terpinene 0.98 %, 1.578 %, caryophyllene 0.96 %, 1.06 %, 1,18cineole 0.47 %, 0.66 %, linalool 20.88 %, linalyl acetate 36.5 %, 38.64 %, camphor 1.98 %, 5.63 %, borneol 0.96 %, 1.84 %, carvone 0.43 %, 2.54 %, geraniol 2.66 %, 1.9 %, geranyl acetate 0.17 %, 0.17 % and caryophyllene oxide 0.44 %, 1.05 % (Naguib et al. 2005). In another study, essential oil constituents from *Calendula officinalis* flowers from steam distillation were identified as α-copaene 0.9 %, α-ionone 1.5 %, α-humulene 1.2 %, geranylacetone 1.6 %, γ-muurolene 2.3 %, β-ionone 3.2 %, ledene 2.3 %, α-muurolene 5.6 %, γ-cadinene 8.9 %, δ-cadinene 22.5 %, α-cadinene 0.9 %, α-calacorene 2.3 %, caryophyllene oxide 0.5 %, copaene-4-α-ol 0.6 %, β-oplopenone 1.7 %, viridiflorol 2.2 %, ledol 1.3 %, 1,10-di-epi-cubenol 1.6 %, epi-α-muurolol 12.9 %, α-cadinol 20.4 % and cadalene 0.8 % (Gazim et al. 2008). Volatile constituents from *Calendula officinalis* flowers from headspace solid-phase microextraction were β-cyclocitral 2.1 %, α-cubebene 1.8 %, α-copaene 15.1 %, β-cubebene 1.8 %, α-gurjunene 2.7 %, β-caryophyllene 2.7 %, α-ionone 2.3 %, α-humulene 3.9 %, γ-muurolene 5.3 %, β-ionone 3.9 %, α-muurolene 6.2 %, γ-cadinene 25.5 %, δ-cadinene 22.1 % and α-cadinene 2.3 % (Gazim et al. 2008). Volatile constituents from *Calendula officinalis* flowers from headspace cold finger extraction were α-copaene 18.4 %, β-cubebene 3.7 %, α-gurjunene 4.2 %, β-caryophyllene 8.6 %, α-humulene 3.9 %, γ-muurolene 4.7 %, α-muurolene 5.8 %, γ-cadinene 24.9 %, δ-cadinene 18.6 % and α-cadinene 2.3 % (Gazim et al. 2008).

Twenty-four compounds were identified in the fresh flower oil of *C. officinalis*, and the yield was 0.09 % (Okoh et al. 2008). Sesquiterpenoids dominated the fresh flower oil. Major components of the fresh flower oil were α-thujene (26.9 %), T-muurolol (24.9 %) and δ-cadinene (13.1 %).

Coumarins

The ethanol extract of the flowers was found to contain coumarins—scopoletin, umbelliferone and esculetin (Derkach et al. 1987).

Terpenoids, Flavonoids and Phenolic Acids

Triterpenoid esters purified from *C. officinalis* flower heads included faradiol-3-*O*-laurate, faradiol-3-*O*-myristate and faradiol-3-*O*-*palmitate* (Hamburguer et al. 2003). Accompanying minor compounds of the triterpene ester fraction purified included maniladiol 3-*O*-laurate, maniladiol-3-*O*-myristate, ψ-taraxasterol and β-amyrin. Oleanolic acid, β-amyrin, β-amyrin acetate, rutin, narcissin, 3-glucoside of isorhamnetin, quercetin, isoquercitrin, vanillic acid, caffeic acid, chlorogenic acid, protocatechuic acid, *p*-coumaric acid and syringic acid were identified in the flower extract (Matysik et al. 2005). Ten oleanane-type triterpene glycosides were isolated from the flowers: calendulaglycoside A (1), calendulaglycoside A6′-*O*-*n*-methyl ester (2), calendulaglycoside A 6′-*O*-*n*-butyl ester (3), calendulaglycoside B (4), calendulaglycoside B 6′-*O*-*n*-butyl ester (5), calendulaglycoside C (6), calendulaglycoside C 6′-*O*-*n*-methyl ester (7), calendulaglycoside C 6′-*O*-*n*-butyl ester (8), calenduloside F 6′-*O*-*n*-butyl ester (9) and

calenduloside G 6′-*O*-*n*-methyl ester (10) (Ukiya et al. 2006).

Miscellaneous Phytochemicals

Eighteen *n*-paraffins ranging from C_{18} to C_{35} were detected in the petals of *Calendula officinalis* (Komae and Hayashi 1971). The flowers also contained the bitter principle loliolide (calendin) (Willuhn and Westhaus (1987) and a tasteless yellow substance calendulin discovered by Geiger in 1819 (Shoemaker 1891).

Seed Phytochemicals

Fatty acid composition of the seed oil revealed the presence of lauric (3.90 %), myristic (3.58 %), palmitic (14.96 %), stearic (10.13 %), palmitoleic (4.55 %), oleic (16.26 %), linoleic (39.45 %) and linolenic (7.15 %) acids (Saleem et al. 1986). In the seed oil, conjugated acid was present to the extent of 4.5 % whereas the percentage of non-conjugated acid (linolenic acid) was only 2.65 %. The residual meal after the extraction of oil was also studied for its proteins (18 %) and amino acids composition.

The amounts (%) of lipids in *C. officinalis* seeds were: neutral lipids, 15.7 %; glycolipids (GLs), 0.9 %; and phospholipids (PhLs), 0.6 % (Ul'chenko et al. 1998). The lipid yield of extracts from the flowers (EF) was 17.1 % and from the leaves (EL) 9.3 %. The following classes of lipids were found (% by weight) hydrocarbons, 0.9 %; esters of sterols and triterpenols with fatty acids, 0.5 %; triacylglycerols (TAGs), 20.0 % comprising TAGs-I, 59.0; TAGs, 2–11.8 %; TAGs, 3–4.3 %; free fatty acids, trace; hydroxy-TAGs, 0.8 %; free sterols and free triterpenols, 0.4 %; diacylglycerols and monoacylglycerols, 1.2 %; and unidentified components, 1.1 %. The phospholipid complex of Marigold seeds consisted of eight classes, which may be arranged in order of content by weight as follows: PCs (phosphatidylcholines) > PIs (phosphatidylinositols) > *N-acyl-PEs* (*N*-acyl phosphatidylethanolamines) > *N*-acyl-lyso-PEs (*N*-acyl-lyso-phosphatidylethanolamines) > lyso-PIs (lyso-phosphatidylinositols) > PSs (phosphatidylserines) > lyso-PCs (lyso-phosphatidylcholines) > PEs (phosphatidylethanolamines). The glycolipids of the seeds were represented by four components forming the following sequence by mass content: SGs (sterylglycosides) > ESGs (ester sterylglycosides) > MGDGs (monogalactosyldiacylglycerols) > DGDGs (digalactosyldiacylglycerols).

The lipid content of seed of 11 genotypes varied between 13.6 and 21.7 g oil/100 g seeds (Dulf et al. 2013). PUFA contents varied between 60.4 and 66.4 %, while saturates comprising mainly palmitic and long chain saturated fatty acids were found in higher amounts in sterol esters (49.3–55.7 % of total fatty acids). Calendic acid [18:3 (8*t*, 10*t*, 12*c*) (*n*−6)] with contents of 51.47–57.63 % of total fatty acids was the predominant polyunsaturated fatty acid (PUFA) followed by linoleic acid [18:2 (*n*−6)] (28.50–31.86 %), oleic acid [18:1 (*n*−9)] (4.44–6.25 %) and palmitic acid (16:0) (3.86–4.55 %). Small and very small (or trace) amounts (<2 %) of stearic (18:0), β-calendic [18:3 (8*t*, 10*t*, 12*t*) (*n*−6)], elaidic [18:1 (9*t*) (*n*−9)], arachidic (20:0), behenic (22:0), gondoic [20:1 (*n*−9)], α-linolenic [18:3 (*n*−3)], linoelaidic [18:2 (9*t*,12*t*) (*n*−6)], *cis-7-hexadecenoic* [16:1 (*n*−9)], palmitoleic [16:1 (*n*−7)], lauric (12:0), myristic (14:0), pentadecanoic (15:0) and margaric (17:0) acids were also present. Cromack and Smith (1998) reported the seed oil content of around 20 %, of which up to 60 % was calendic acid, a useful industrial feedstock. Chisholm and Hopkins (1967) reported that *Calendula* could accumulate more than 40 % of calendic acid. Ozgul-Yucel found that Turkish calendula seed oil was characterized by high concentration of linoleic acid (43.5 %) and low content of CLNAs (calendic acid (18.3 %) + β-calendic (11.2 %). Angelini et al. (1997) reported 16–46 % levels of calendic acid in the Italian Pot Marigold seed oils.

Crombie and Holloway (1985) found that linoleic and oleic acids were precursors in the biosynthesis of calendic acid in Marigold (*C. officinalis*) seeds but not linolenic acid. (9*S*)-Hydroxyoctadeca-(10*E*,12*Z*)-dienoic acid (α-dimorphecolic acid) was isolated and converted into (*R/S*)-hydroxy- and -hydroperoxy-[9-3*H*]

octadeca-(10*E*,l2*Z*)-dienoic acids but neither labelled specimen was converted into calendic acid by Marigold seed homogenate. Also α-dimorphecolic acid, a minor component of Marigold seed oil, was found to be a terminus rather than an intermediate for calendic acid.

Plant and Leaf Phytochemicals

In the flowers, stem and leaves of *C. officinalis*, 15 amino acids were detected in the free state: alanine, arginine, aspartic acid, asparagine, valine, histidine, glutamic acid, leucine, lysine, proline, serine, tyrosine, threonine, methionine and phenylalanine (Absavoa et al. 1994). Among them, six predominated: arginine, proline, glutamic acid, phenylalanine, lysine and leucine. The leaves contained about 5 % of amino acids, the stems 3.5 % and the flowers 4.5 %. Szakiel et al. (2005) found that Marigold (*C. officinalis*) synthesized significant amounts of oleanane saponins, found not only in flowers but also in all organs of plant. These glycosides comprised two series of structurally related compounds, that is, derivatives of 3-*O*-monoglucoside of oleanolic acid (hence named 'glucosides') and derivatives of 3-*O*-monoglucuronide ('glucuronides'), depending on the first sugar moiety linked to the C-3 hydroxyl group of oleanolic acid, which is either glucose or glucuronic acid. The occurrence of both series of oleanolic acid glycosides occurring in *C. officinalis* was earlier reported by Kasprzyk and Wojciechowski (1967) and Wojciechowski et al. (1971). 'Glucuronides', known as the series I, were designated with letters (F, D, D2, C, B, A) and 'glucosides', forming the series II—with Roman numerals from I to VIII. 'Glucuronides' were found in relatively large amounts (up to 2 % of the dry mass) in flowers and in considerably lower quantity in green organs of the plant. Ruszkowski et al. (2003) found 'glucuronides' in roots of young Marigold plants, while 'glucosides' accumulated mainly in roots of grown and senescing plants and also found in green organs of the plant. However, only glycosides I, II, III, VI and VII were found in Marigold shoots. Oleanolic acid and its 3-*O*-glucuronide derivatives and 3-*O*-glucoside derivatives were found in vacuoles prepared from protoplasts and cell walls obtained from leaf cells of *Calendula officinalis* (Szakiel and Kasprzyk 1989). In both cell compartments 37 % of total cellular oleanolic acid accumulated, 0.6 % occurring as free oleanolic acid (only in vacuoles). Glucuronides accounted for 31.1 % (20.7 % in vacuoles and 10.4 % in cell walls) and glucosides for 5.3 % (2.6 % in vacuoles and 2.7 % in cell walls). Szakiel et al. (1995) found that [3-3*H*]oleanolic acid glycosides formed in the cytosol of *C. officinalis* leaf cells were transported to the extracellular space in the form of pentaglucoside VI (44 %), whereas glucuronides derived from [3-3*H*]oleanolic acid 3-*O*-monoglucuronide (29 %) as well as a part of glucosides (24 %) were transported into the cell walls.

Studies confirmed that plastoquinone occurred only in the chloroplasts, ubiquinone only in the mitochondria and α-tocopherol in both these subfractions of *C. officinalis* leaves (Janiszowska et al. 1976). In *Calendula officinalis* leaves the cyclization of squalene to β-amyrin and its further oxidation to oleanolic acid as well as the biosynthesis of all derivatives of oleanolic acid 3-glucoside and some derivatives of oleanolic acid 3-glucuronoside were found to occur in the microsomal fraction (Janiszowska and Kasprzyk 1977). The final metabolites of oleanolic acid 3-glucoside series, that is, pentaglycosides, were translocated from this fraction, one to the cell wall and plasmalemma fraction and the other to the cytosol. The derivatives of oleanolic acid 3-glucoronoside were synthesized partially in other fractions and accumulated in the different membranous structures of the cell.

Polyphenolic compounds (g/kg dry matter) in the aerial Marigold plant parts were determined as follows: chlorogenic acid 0.55 g, 3,5-DCQA (dicaffeoylquinic acid) 0.78, total caffeoyl derivatives 1.33 g, total dihydroxycinnamic acid derivatives 7.54 g, total flavonoids 5.12 g, total dihydroxycinnamic acid derivatives + flavonoids 12.66 g and total polyphenolic compounds 24.97 g (Fraisse et al. 2011).

Fatty acids C12–C22 were found to be components of acylated steryl glucosides in *Calendula officinalis* (Zdzislaw et al. 1975). As a source of acyl groups for the synthesis of steryl acylglucosides, various phospholipids obtained from the same plant were utilized in the following sequence: phosphatidylinositol greater than phosphatidylethanolamine greater than phosphatidylcholine. It does not utilize triacylglycerols and monogalactosyldiacyl glycerols.

In 3-day and 14-day-old seedlings and leaves of *Calendula officinalis*, the following sterols were identified: cholestanol, campestanol, stigmastanol, cholest-7-en-3-β-ol, 24-methylcholest-7-en-3β-ol, stigmast-7-en-3β-ol, cholesterol, campesterol, sitosterol, 24-methylcholesta-5,22-dien-3β-ol, 24-methylenecholesterol, stigmasterol and clerosterol (Adler and Kasprzyk 1975). Sitosterol was predominant in young and stigmasterol in old tissues. Young tissues contained relatively more campesterol, but in old tissues a $C_{28}\Delta^{5,22}$ diene was present suggesting transformation of campesterol to its $\Delta^{5,22}$ analog, similar to that of sitosterol to stigmasterol. All the identified sterols were present as free compounds and also in the steryl esters, glucosides, acylated glucosides and water-soluble complexes.

Petroleum ether extract of *Calendula officinalis* leaf showed the presence of fatty acids, and chloroform extracts showed the presence of triterpenes and sterols (Chakraborthy 2010). Flavonoids, carbohydrates, amino acids, and saponins were present in methanol extract, and saponins, phenolic substances, and tannins were present in the water extract of *Calendula officinalis*.

Thirty and twenty-one compounds were identified in the fresh leaf and dry leaf oils of *C. officinalis* and the yield was 0.06 and 0.03 %, respectively (Okoh et al. 2008). Sesquiterpenoids dominated the fresh leaves (59.5 %) while the monoterpenes dominated the oil in the dry leaves (70.3 %). The fresh leaf oil was dominated by T-muurolol (40.9 %), α-thujene (19.2 %) and δ-cadinene (11.4 %), while the dry leaves oil was found to be rich in 1,8-cineole (29.4 %), γ-terpinene (11.6 %), δ-cadinene (9.0 %), β-pinene (6.9 %) and α-thujene (6.3 %).

The yield in *Calendula officinalis* leaf essential oil showed a maximum at the full flowering stage (0.97 %) and a minimum during the pre-flowering stage (0.13 %) (Okoh et al. 2007). The compositions also showed different patterns at different phases of the vegetative cycle. Sesquiterpenes (α-cadinene, α-cadinol, T-muurolol and epi-bicyclosesquiphellandrene) and monoterpenes (limonene, 1,8-cineole and *trans*-β-ocimene) showed the highest correlations with the age of the plant. Aiming the use of essential oil as a food ingredient, the most interesting stage is the post-flowering period, the essential oil at this time being rich in α-cadinene, α-cadinol, t-muurolol, limonene and 1,8-cineole, with *p*-cymene present at lower levels. The total yields of the essential oils at the different stages of the vegetative cycle increased with the age of the plant and ranged between 0.13 % (3rd week) and 0.97 % (12th week, flowering); α-cadinene is an important flavouring agent in baked foods, candy and chewing gum and also used as a fragrance in cosmetics and detergents. T-muurolol and α-cadinol are important antimicrobial agents. The essential oil at 12 week was dominated by geraniol (44.5 %), α-cadinol (24.20 %), δ-cadinene (23.8 %), T-muurolol (22.50 %), 1,8-cineole (22.10 %), cadina-1,4-diene (12.20 %), germacrene D (11.50 %), α-cadinene (10.7 %) and calarene (5.70 %). Other minor components included α-pinene (2.90 %), limonene (2.6 %), α-cubebene (1.7 %), α-humulene (1.7 %), β-pinene (1.4 %), nerolidol (1.3 %), sabinene (0.9 %), endobourbonene (1.0 %), β-caryophyllene (0.9 %), α-ylangene (0.8 %), palustron (0.7 %), epi-bicyclosesquiphellandrene (0.5 %), β-selinene (0.3 %), alloaromadendrene (0.2 %), α-bourbonene (0.2 %), muurolene (0.10 %), terpene-4-ol (0.10 %), *p*-cymene (0.10 %), carvacrol (0.10 %), α-thujene (0.10 %) and α-gurjunene (0.10 %). These compounds occurred in trace amounts: δ-3-carene, nonanal, 3-cyclohexene-1-ol, α-phellandrene, bornyl acetate, sabinyl acetate, α-copaene, β-cubebene, aromadendrene and oplopenone.

Root Phytochemicals

A new series of glycosides of oleanolic acid was found in the roots of old *Calendula officinalis* plants (Wojciechowski et al. 1971). These compounds were derivatives of 3-glucoside of oleanolic acid and were different, from those previously isolated from the flowers, derivatives of 3-glucuronoside of oleanolic acid. The sugar components of eight representatives of the new series are glucose and galactose in following ratios: in glucoside I, 1:0; in II, 1:1; in III, 1:2; in IV, 2:1; in V, 3:1; in VI, 3:2; in VII, 4:1; and in VIII, 4:1. The sugars in I–VII were attached only in position 3 of oleanolic acid, but in VIII one glucose molecule was joined to 28-carboxyl of oleanolic acid. Glucoside I was identified as 3-monoglucoside and II as 3-(4′-galactosyl)-glucoside of oleanolic acid. Besides these compounds, 6′-methyl ester of 3-glucuronoside of oleanolic acid was also found in the roots.

A monoside 3-*O*-β-D-glucopyranoside of oleanic acid and a bioside (calenduloside A) with the structure 3-galactosylglucosyloleanolic acid (Vecherko et al. 1969, 1971b) and calenduloside B with the structure *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 3)-oleanoloyl-(28 → 1)-α-D-glucopyranoside (Vecherko et al. 1971a); oleanolic acid 3-{[galactopyranosido-(1 → 3)] [glucopyranosido–(1 → 2)]-β-D-glucopyranoside} (calenduloside C) and 28-acyl-β-D-glucopyranoside of calenduloside C (calendulososide D) (Vecherko et al. 1975); glucopyranosyl oleanolate 3-*O*-β-D-glucuronopyranoside (calenduloside F) (Vecherko et al. 1973), oleanolic acid 3-*O*-β-D-galactopyranosyl-(1 → 3)-β-D-glucuronoside (calenduloside G) and 28-acyl-β-D-glucopyranoside of calenduloside G (calenduloside H) (Vecherko et al. 1974) were isolated from the roots of *C. officinalis*. Calenduloside B, trioside of oleanolic acid, was isolated from the roots (Iatsyno et al. 1978).

Chemical studies have revealed the presence of various classes of compounds, the main being triterpenoids, flavonoids, coumarins, quinones, volatile oil, carotenoids and amino acids in *C. officinalis* plant parts (Muley et al. 2009; Khalid and da Silva 2012). The extract of this plant as well as pure compounds isolated from it had been demonstrated to possess multifold pharmacological activities: antioxidant, antiinflammatory, antiedematous, immunostimulant, anticancer, lymphocyte and wound healing, hepatoprotective, antibacterial and antifungal, anti-HIV, spasmolytic and spasmogenic, cytotoxic, genotoxic and antigenotoxic, inhibition of heart rate and antiviral, among others.

Antioxidant Activity

Superoxide radicals and hydroxyl radicals were observed in decreasing concentrations in the presence of increasing concentrations of *C. officinalis* butanolic fraction with IC_{50} values of 1 and 0.5 mg/ml, respectively, suggesting a possible free radical scavenging effect (Cordova et al. 2002). Lipid peroxidation in liver microsomes induced by Fe^{2+}/ascorbate was 100 % inhibited by 0.5 mg/ml of the fraction (IC_{50} = 0.15 mg/ml). Its total reactive antioxidant potential (TRAP) (in μM Trolox equivalents) was 368.14 and its total antioxidant reactivity (TAR) was calculated to be 249.19 μM. The results suggested the butanolic fraction of *C. officinalis* to have significant free radical scavenging and antioxidant activity.

The methanol and water extracts of wild Marigold, *Calendula arvensis* (GWM) and cultivated Marigold, *Calendula officinalis* (CM), in a concentration range of 0.10–0.90 mg/ml, scavenged all types of investigated radicals in dependence on their applied concentrations (Ćetković et al. 2004). Generally, CM extracts possessed higher scavenging and antioxidant activity than GMW extracts, while methanol extracts exhibited lower activities than water extracts. Water extracts of CM had the best antioxidant properties; 0.75 mg/ml extracts completely eliminated hydroxyl radical, which was generated in the Fenton system. The same concentration of this extract scavenged 92 % DPPH and 95 % peroxyl radical during lipid peroxidation. Antioxidant properties were in correlation with the contents of total phenolic compounds (14.49–57.47 mg/g) and flavonoids (5.26–18.62 mg/g) in extracts. The electron spin

resonance (ESR) spectroscopy data demonstrated that methanol and water extracts of CM possessed similar free radicals scavenging and antioxidative activity as synthetic antioxidants BHA. Total antioxidant capacity (%) (DPPH scavenging activity) of *C. officinalis* aerial plant parts was 1.52 % and contribution from the main caffeoyl derivatives was as follows: chlorogenic acid 3.15 %, 3,5-DCQA (dicaffeoylquinic acid) 6.90 % and total caffeoyl derivatives 10.05 % (Fraisse et al. 2011).

Calendula officinalis flower top extract was found to scavenge superoxide radicals generated by photoreduction of riboflavin and hydroxyl radicals generated by Fenton reaction and inhibited in-vitro lipid peroxidation (Preethi et al. 2006). Concentrations needed for 50 % inhibition (IC_{50}) were 500, 480 and 2,000 mg/ml, respectively. The extract scavenged ABTS radicals and DPPH radicals with IC_{50} of 6.5 and 100 mg/ml, respectively. The extract also scavenged nitric oxide (IC_{50} = 575 mg/ml). Oral administration of *Calendula* extract inhibited superoxide generation in macrophages in vivo by 12.6 and 38.7 % at doses of 100 and 250 mg/kg body weight. Oral administration of the extract to mice for 1 month significantly increased catalase activity and produced significant increase in glutathione levels in the blood and liver. Glutathione reductase was found to be increased, whereas glutathione peroxidase was found to be decreased after extract administration. The results indicated *Calendula officinalis* possessed significant antioxidant activity in-vitro and in-vivo.

The propylene glycol extract of *Calendula officinalis* exerted its anti-ROS (reactive oxygen species) and anti-RNS (reactive nitrogen species) activity in a concentration-dependent manner during polymorphonuclear leukocytes burst, with significant effects being observed at even very low concentrations: 0.20 μg/ml without L-arginine, 0.10 μg/ml when L-arginine was added to the test with phorbol 12-myristate 13-acetate and 0.05 μg/ml when it was added to the test with *N*-formyl-methionyl-leucyl-phenylalanine (Braga et al. 2009). Electron paramagnetic resonance (EPR) spectroscopy confirmed these findings, 0.20 μg/ml being the lowest concentration of *C. officinalis* extract that significantly reduced 2,2-diphenyl-1-picrylhydrazyl (DPPH). *Calendula officinalis* propylene glycol extracts were found to have protective effect against oxidative DNA damage and lipid peroxidation induced by high polyunsaturated fatty acid (PUFA) intake in young growing pigs (Frankic et al. 2009). It elicited a numerical trend towards the reduction of plasma malondialdehyde and urinary isoprostane (iPF2α-VI) excretion. Its effect was comparable with that of vitamin E. The extract from flower tops showed less antioxidant potential than the extract from petals. They concluded that the amount of *C. officinalis* extracts proposed for internal use by traditional medicine protected the organism against DNA damage induced by high PUFA intake.

Methanol enabled more efficient extraction of flavonoids from *C. officinalis* flowers than the other solvents isopranol and ethanol tested (Butnariu and Coradini 2012). From cv Petran and cv Plamen flowers, methanol extracted, respectively, 2.04 and 2.142 mg/100 g FW of carotene pigments (lycopene, lutein), 10.358 and 5.222 mg/100 g FW of total photosynthetic pigments (chlorophyll a, b), total flavonoid content 96.17 and 90.37 mg quercetin equivalent (QE)/100 ml and total phenolic content of 134 and 153.23 mg GAE/100 ml. The highest antioxidant activity correlated to the polyphenol content was obtained for extracts prepared using methanol. For the methanol extract of cv Petran and cv Plamen, the DPPH radical scavenging activity was2.64 and 2.97 mmol Trolox/g and the ferric reducing antioxidant power (FRAP) was 0.29 and 1.55 mmol Fe^{2+}/g.

Studies by Ozkol et al. (2012) showed that administration of *Calendula officinalis* extract protected rats against subacute cigarette smoke-induced cell injury. Marigold extract decreased the elevated levels of malondialdehyde, reduced glutathione and protein carbonyl caused by cigarette smoke. Further, Marigold extract increased the diminished levels of glutathione peroxidase (GPx), superoxide dismutase activities and β-carotene and vitamins A and C caused by cigarette smoke.

Bernatoniene et al. (2011) confirmed dry *Calendula officinalis* extract to be an effective scavenger of H_2O_2 radicals in in-vitro studies with the mitochondria of rat cardiac muscles. *Calendula* extract incorporated into hydrophilic cream containing complex emulsifier provided significant antioxidant effect due to the content of carotenoids, polyphenols and flavonoids and good emulsion quality. Cream with the best properties (0.9 % of *Calendula* extract) contained 0.73 mg/100 g of total carotenoids expressed as β-carotene.

Anticancer/Antiproliferative Activities

Laser Activated Calendula Extract (LACE) showed a potent in-vitro inhibition (70–100 %) of tumour cell proliferation when tested on a wide variety of human (leukaemias, melanomas, fibrosarcomas and cancers of the breast, prostate, cervix, lung, pancreas and colorectal) and murine tumour cell lines (Jiménez-Medina et al. 2006). Mechanisms of inhibition were identified as cell cycle arrest in G0/G1 phase and caspase-3-induced apoptosis. In contrast, the same extract showed an opposite effect when tested on human peripheral blood lymphocyte (PBL) and NKL (natural killer) cell line, in which in-vitro induction of proliferation and activation of these cells was observed. The intraperitoneal injection or oral administration of LACE in nude mice inhibited in-vivo tumour growth of Ando-2 melanoma cells and prolonged the survival day of the mice. Two triterpene glycosides from the flowers, calenduloside F 6′-*O*-*n*-butyl ester (9) and calenduloside G 6′-*O*-*n*-methyl ester, exhibited potent cytotoxic effects against colon cancer, leukaemia and melanoma cells (Ukiya et al. 2006).

Simultaneous administration of *C. officinalis* flower extract to tumour-bearing male C57BL/6 mice reduced the lung B16F-10 melanoma tumour nodules by 74 % with 43.3 % increase in life span (Preethi et al. 2010). Elevated levels of hydroxyproline, uronic acid, hexosamine, serum sialic acid and γ-glutamyl transpeptidase in the metastatic controls were significantly lowered in the *C. officinalis*-treated animals. The extract also inhibited expression of MMP-2, MMP-9, prolyl hydroxylase and lysyl oxidase and activated TIMP-1 and TIMP-2 and downregulated proinflammatory cytokines. The results indicated antimetastatic effects of *Calendula officinalis* flowers through the inhibition of key enzymes were involved in processes of metastasis.

Studies demonstrated that chamomile and Marigold (*Calendula officinalis*) tea exerted selective dose-dependent cytotoxic action against target cancer cells; cytotoxicity of Marigold tea was higher than chamomile (Matić et al. 2013). However, the cytotoxic effect of chamomile tea was very weak to healthy peripheral blood mononuclear cells (PBMC), while the effect of Marigold tea on PBMC was more pronounced. Marigold tea exerted highly selective antitumor effect especially to melanoma Fem-x cells in comparison to the action to normal healthy PBMC. Chemical analyses showed that dominant phenolic compounds in examined infusions and decoctions were flavonoid glycosides and hydroxycinnamic acid derivatives. Ethanol extracts of *C. officinalis* and other Asteraceae species were found to have antileukemic properties and to induce J-45.01 human acute T leukaemia cell death via apoptosis. The correlation between antileukemic activity and total polyphenol content was determined (Wegiera et al. 2012).

Three major flavonoid fractions separated from *C. officinalis* flower methanol extract did not exhibit inhibitory effect on the parent and tamoxifen-resistant T47D human breast cancer (Ostad et al. 2004). Quercetin increased cell proliferation of the resistant T47D cells in the presence of tamoxifen but no effect was detected by using quercetin itself. Also it was found that isorhamnetin did not have any proliferative or antiproliferative activity on the both cell lines.

Wound Healing Activity

Studies on surgically induced skin wound in Wistar albino rats showed that application of % unguentum containing fractions C1 and C5, isolated from *Calendula officinalis* flowers of in combination with allantoin, significantly stimulated

physiological skin regeneration and epithelialization (Klouchek-Popova et al. 1982).

In-vitro studies showed that chick chorioallantoic membrane (CAM) treated with a freeze-dried aqueous extract of *C. officinalis* flowers were positive for hyaluronan, a tissue glycosaminoglycan associated with neovascularization; no hyaluronan was found in control CAMs (Patrick et al. 1996). The numbers of microvessels in calendula-treated CAMs were statistically and significantly higher than in the control CAMs. Although the extract contained water-soluble compounds such as flavonoids, the exact nature of the active angiogenic component(s) was yet to be identified.

Studies showed that rats with experimentally induced burns treated with *Calendula officinalis* flower extract showed significant improvement in healing when compared with the control untreated animals (Preethi and Kuttan 2008). The indicators of the wound healing such as collagen hydroxyproline and hexosamine contents were significantly augmented in the treated group indicating accelerated wound healing in the treated animals. The acute phase proteins haptoglobin and orosomucoid which were elevated due to burn injury were lowered significantly in 200 mg/kg body weight extract-treated animals. The antioxidant defense mechanism, which was decreased in the liver during burn injury, was enhanced in treated animals. The lipid peroxidation was significantly lowered in the treated group when compared to control animals. Tissue damage marker enzymes alkaline phosphatase, alanine and aspartate transaminases were significantly lowered in the treated groups in a dose-dependent manner. The histopathological analyses of skin tissue also confirmed the increased healing potential of the extract after burn injury. Another study reported that rats with excision wounds treated with *Calendula officinalis* flower extract had 90 % wound closure compared with 51.1 % in the control group on the eighth day of wounding (Preethi and Kuttan 2009b). The days needed for reepithelialization were 17.7 for the control animals; extract treatment at a dose of 20 or 100 mg/kg body weight reduced the period to 14 and 13 days, respectively. A significant increase was observed in the hydroxyproline and hexosamine content in the extract-treated group compared with the untreated animals. *Calendula* extracts were found to have wound healing effect using the scratch assay (Fronza et al. 2009). The hexane and ethanol extracts of *Calendula officinalis* stimulated proliferation and migration of Swiss 3 T3 albino mouse fibroblasts at low concentrations, for example, 10 μg/ml enhanced cell numbers by 64.35 and 70.53 %, respectively. Inhibition of proliferation showed that this effect was mainly due to stimulation of migration. The triterpenoids, faradiol myristate and palmitate gave comparable stimulation rates at an almost 50 μg/ml concentration, indicating that they contributed partially but not most significantly to the wound healing effects of *Calendula* preparations.

Studies by Parente et al. (2012) found that the ethanol extract, the dichloromethane and hexanic fractions of *C. officinalis* flowers presented anti-inflammatory and antibacterial activities as well as angiogenic and fibroplastic properties acting in a positive way on the inflammatory and proliferative phases of the healing process as through the chorioallantoic membrane and cutaneous wounds in rat models. The angiogenic activity of *C. officinalis* flower ethanol extract and dichloromethane and hexanic fractions was evidenced in both experimental models using 36 rats and 90 embryonated eggs to evaluate healing and angiogenic activities through the induction of skin wounds and the chorioallantoic membrane, respectively (Parente et al. 2011). They verified that this effect was not directly related to the expression of vascular endothelial growth factor (VEGF) and it could be associated to other pro-angiogenic factors. Their data suggested the healing activity of *C. officinalis* could be related, among other factors, to its positive effect on angiogenesis, characterized by the induction of neovascularization. In a prospective nonrandomized pilot study of 25 patients (10 men and 15 women) with venous ulcers, 7 week treatment with the herbal-based ointment Herbadermal® comprising extracts of garlic, St. John's wort and calendula elicited 99.1 % epithelialization without significant effects on the microbial flora

(Kundaković et al. 2012). Based on this, they recommended the herbal-based ointment as topical treatment for wound healing because of its epithelializing, anti-erythematous and antiedematous properties.

Hepatoprotective and Nephroprotective Activities

Animal studies showed that the flower extract of *C. officinalis* had a protective effect against CCl_4-induced acute hepatotoxicity and cisplatin-induced nephrotoxicity (Preethi and Kuttan 2009a). The activities of serum marker enzymes of liver injury like glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) which were elevated by CCl_4 injection were found to be significantly reduced by the pretreatment of the flower extract at 100 and 250 mg/kg body weight. The lipid peroxidation in liver, the marker of membrane damage and the total bilirubin content in serum were also found to be at significantly low level in the extract pretreated group. The elevated kidney urea and creatinine levels in cisplatin-treated animals were significantly lower in the flower extract pretreated groups (100 and 250 mg/kg body weight). Moreover, cisplatin-induced myelosuppression was ameliorated by the flower extract pretreatment. Treatment with the extract produced enhancement of antioxidant enzymes—superoxide dismutase and catalase and glutathione.

Antidermatitic/Radioprotective/Skin Therapeutic Activities

In a phase III randomized trial of *Calendula officinalis* (126 patients) compared with trolamine (128 patients) Calendula treatment was found to reduce acute dermatitis during irradiation for breast cancer (Pommier et al. 2004). The occurrence of acute dermatitis of grade 2 or higher was significantly lower with the use of calendula (41 %) than with trolamine (63 %). Moreover, patients receiving calendula had less frequent interruption of radiotherapy and significantly reduced radiation-induced pain. *Calendula* was considered to be more difficult to apply, but self-assessed satisfaction was greater. Body mass index and adjuvant chemotherapy before radiotherapy after lumpectomy were significant prognostic factors for acute dermatitis. Marigold (*Calendula officinalis*) and rosemary cream preparations were found to be effective against experimentally induced irritant contact dermatitis in healthy volunteers (Fuchs et al. 2005). In a prospective assessment phase III study, *Calendula officinalis* was shown to be superior to trolamine for the prevention of radio-epithelitis (Chargari et al. 2009). In another study of topical products used in the prevention of radiodermatitis, to support care delivery to women with breast cancer during teletherapy, de Andrade et al. (2012) found that *Calendula*, corticosteroids and Xclair have shown significant protective effects against radiodermatitis. Data from a randomized and double-blind study of 66 infants with diaper dermatitis suggested that topical application of aloe vera cream and *Calendula officinalis* ointment could serve as safe and effective treatment for the treatment of diaper dermatitis in infants (Panahi et al. 2012).

The hydroalcoholic extract of *C. officinalis* was found to contain polyphenol, flavonoid, rutin and narcissin contents of 28.6, 18.8, 1.6, and 12.2 mg/g, respectively (Fonseca et al. 2010). The extract exhibited in-vitro dose-dependent antioxidant activity against different radicals. Cytotoxicity experiments demonstrated that the extract was not cytotoxic for L929 and HepG2 cells at concentrations less than or equal to of 15 mg/ml. However, in concentrations greater than or equal to 30 mg/ml, toxic effects were observed. Further, oral treatment of hairless mice with 150 and 300 mg/kg of the extract maintained GSH levels close to nonirradiated control mice. In addition, this extract affected the activity/secretion of matrix metalloproteinases 2 and 9 (MMP-2 and -9) stimulated by exposure to UVB irradiation. The gel formulation [Formulation 3 (F3)] found to be the most effective for the topical delivery of *Calendula officinalis* extract, which was detected as 0.21 μg/cm(2) of narcissin and as 0.07 μg/cm(2) of the rutin in the viable

epidermis of hairless mice exposed to ultraviolet (UV) B irradiation (Fonseca et al. 2011). This formulation was able to maintain glutathione reduced levels close to those of nonirradiated animals but did not affect the gelatinase-9 and myeloperoxidase activities increased by exposure to UVB irradiation. In addition, F3 reduced the histological skin changes induced by UVB irradiation that appeared as modifications of collagen fibrils.

Results of studies by Mishra et al. (2012a) suggested that calendula oil cream can be used to protect the skin from UV radiations in form of sunscreen cream and to maintain the natural pigmentation of the skin. The essential oil of *Calendula officinalis* flowers in cream formulation exhibited good activity sun protection factor (SPF) (SPF = 14.84). In another paper they reported that treatment with creams containing 4 and 5 % of *Calendula officinalis* essential oil caused a significant decrease in the malonyldialdehyde level, whereas the levels of catalase, glutathione, superoxide dismutase, ascorbic acid and the total protein level were significantly increased after 1 month of daily UVB irradiation treatment when compared to untreated control groups (Mishra et al. 2012b). The results suggested that the cutaneous application of the essential oil of *Calendula* prevented UVB-induced alterations in the level of antioxidants in skin tissue.

In a multicentre, controlled, parallel-group study, menopausal women with vaginal dystrophy were randomized to vaginal gel EG (containing isoflavones, *Lactobacillus sporogenes*, *Calendula officinalis* extract and lactic acid) (103 women) or no topical treatment (NT, 83 women) for 4 weeks (Tedeschi et al. 2012). The severity of itching, burning, vulvovaginal erythema, vaginal dryness and dyspareunia were significantly reduced during EG treatment compared with the NT group.

Marigold therapy (*Tagetes* and *Calendula* species) had been used for over 30 years in the United Kingdom and had been evaluated by numerous randomized double-blind placebo-controlled studies for various skin problems on the lower extremity (Hadfield et al. 2008; Khan 2008). Various species of Marigold had been reported to be naturally antiviral, keratolytic and antiinflammatory when applied topically to the affected area. In particular, through numerous research, controlled and case studies by M Taufiq Khan and M Tariq Khan specific extracts had been developed that were directly applied by the podiatric physician to the patient: an antiviral paste (for verruca), an antiinflammatory paste (for bursitis and tendonitis), a keratolytic paste (for hyperkeratosis) and an antifungal paste (for nails). Marigold therapy had been reported to provide a noninvasive and gentle treatment for difficult to treat plantar verruca, painful hyperkeratotic lesions and inflamed bursa secondary to hallux abducto valgus.

Calendula officinalis flowers and *C. officinalis* extracts are used as skin conditioning agents in cosmetics. Using the threshold of toxicological concern (TTC) approach, Re et al. (2009) evaluated their safe use in cosmetic and personal care products. For each of its known constituents, the concentration in the plant, the molecular weight and the estimated skin penetration potential were used to calculate a maximal daily systemic exposure which was then compared to its corresponding TTC class value. Owing to the variability of composition of plant extracts, back calculation was used to determine the maximum acceptable concentration of a given constituent in an extract of *C. officinalis*. Akhtar et al. (2011) evaluated the effects of newly formulated topical cream of *Calendula officinalis* extract on the mechanical parameters of the cheek skin in healthy human volunteers by using the cutometer, a device that is designed to measure the mechanical properties of the skin in response to the application of negative pressure. After 8 weeks, the instrumental measurements produced by formulation reflected significant improvements in hydration and firmness of skin.

Antiinflammatory Activity

Russian studies reported that preparations of calendula alleviated the signs of chronic inflammatory conjunctivitis and other chronic ocular inflammatory conditions in laboratory animals (Marinchev et al. 1971; Mozherenkov and Shubina 1976).

The triterpenoids were shown to be the most important antiinflammatory principles of *C. officinalis* flower CO_2 extract (Della Loggia et al. 1994). Among them, the faradiol monoester appeared to be the most relevant principle for the activity of the extract, due to its quantitative prevalence. The unesterified faradiol, not present in the extract, was the most active of the tested compounds and equalled indomethacin in activity, whereas the monools ψ-taraxasterol, lupeol, taraxasterol and β-amyrin were less active than the free diol. Isorhamnetin glycosides isolated from *Calendula officinalis* were found to inhibit the activity of lipoxygenase in vitro (Bezákova et al. 1996). All of the triterpene alcohols helianol, taraxasterol, ψ-taraxasterol, α-amyrin, β-amyrin, lupeol, taraxerol, cycloartenol, 24-methyl-enecycloartanol, tirucalla-7,24-dienol and dammaradienol isolated from the tubular flowers of *Calendula officinalis*, *Carthamus tinctorius*, *Cosmos bipinnatus*, *Chrysanthemum morifolium*, *Helianthus annuus* and *Matricaria matricarioides* exhibited marked antiinflammatory activity against 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation (1 μg/ear) in mice, and their 50 % inhibitory dose was 0.1–0.8 mg/ear (Akihisa et al. 1996). Faradiol esters, namely, faradiol-3-myristic acid ester and faradiol-3-palmitic acid ester, isolated from *C. officinalis* flower heads showed nearly the same dose-dependent anti-oedematous activity in the inhibition of Croton oil-induced oedema of the mouse ear, and no significant synergism appeared with their mixture (Zitterl-Eglseer et al. 1997). The free monol, ψ-taraxasterol, had a slightly lower effect and showed the same effect as an equimolar dose of indomethacin. Furthermore, faradiol was more active than its esters and than ψ-taraxasterol. The major antiinflammatory triterpenoid esters purified from *C. officinalis* flower heads were faradiol-3-*O*-laurate, faradiol-3-*O*-myristate and faradiol-3-*O*-palmitate (Hamburguer et al. 2003). Accompanying minor compounds of the triterpene ester fraction purified included maniladiol 3-*O*-laurate, maniladiol-3-*O*-myristate, ψ-taraxasterol and β-amyrin.

Three new terpenoid derivatives derived by systematic chemical modifications of faradiol from *C. officinalis* flowers, the C(16) benzyl ether 15, the C(30) aldehyde 24 and the C(30) primary alcohol 25 showed significantly improved antiinflammatory potencies in the inhibition of croton oil induced ear oedema in mouse (Neukirch et al. 2005). In evaluation of oleanane-type triterpene glycosides 1–9 isolated from the flowers for inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 μg/ear) in mice, calendulaglycoside A (1), calendulaglycoside A 6′-*O*-*n*-methyl ester (2), calendulaglycoside A 6′-*O*-*n*-butyl ester (3), calendulaglycoside B (4), calendulaglycoside B 6′-*O*-*n*-butyl ester (5), calendulaglycoside C (6), calendulaglycoside C 6′-*O*-*n*-methyl ester (7), calendulaglycoside C 6′-*O*-*n*-butyl ester (8) and calenduloside F 6′-*O*-*n*-butyl ester (9), all except compound (1), exhibited marked antiinflammatory activity, with ID_{50} values of 0.05–0.20 mg/ear (Ukiya et al. 2006).

Oral administration of 250 and 500 mg/kg body weight *Calendula officinalis* flower extract produced significant inhibition (50.6 and 65.9 %, respectively) in paw oedema of animals induced by carrageenan and 41.9 and 42.4 %, respectively, with inflammation produced by dextran (Preethi et al. 2009). In chronic antiinflammatory model using formalin, administration of 250 and 500 mg/kg body weight *Calendula* extract produced an inhibition of 32.9 and 62.3 %, respectively, compared to controls. TNF-alpha production by macrophage culture treated with lipopolysaccharide (LPS) was found to be significantly inhibited by *Calendula* extract. Furthermore, elevated levels of proinflammatory cytokines IL-1beta, IL-6, TNF-alpha and IFN-gamma and acute phase protein, C-reactive protein (CRP), in mice induced by LPS injection were inhibited significantly by the extract. LPS-induced cyclooxygenase-2 (Cox-2) levels in mice spleen were also found to be inhibited by the extract. The results showed that potent antiinflammatory response of *C. officinalis* extract may be mediated by the inhibition of proinflammatory cytokines and Cox-2 and subsequent prostaglandin synthesis.

In a study of 103 children aged 6–18 years diagnosed with otalgia associated with acute otitis media (AOM), Otikon, an ear drop formulation of

naturopathic origin (containing *Allium sativum*, *Verbascum thapsus*, *Calendula flores* and *Hypericum perforatum* in olive oil), was found to be as effective as anesthetic ear drops and was proven appropriate for the management of AOM-associated ear pain (Sarrell et al. 2001). In a subsequent double-blind trial in an outpatient community clinic involving a total of 171 children aged 5–18 years with otalgia and otitis media, they found the herbal formulation Naturopathic Herbal Extract Ear Drops (NHED) (comprising *Allium sativum*, *Verbascum thapsus*, *Calendula flores*, *Hypericum perforatum*, lavender and vitamin E in olive oil) to be beneficial. Results were better in the NHED group than in the controls (Sarrell et al. 2003).

Calendula officinalis, rich in quercetin, carotenoids, lutein, lycopene, rutin, ubiquinone, xanthophylls and other antioxidants, at 2–3 %, completely inhibited human matrix metalloproteinase 2 (MMP-2) activity and human gingival fibroblast-mediated collagen degradation more than the corresponding concentration of quercetin (Saini et al. 2012). They attributed this to additional components in *Calendula* other than quercetin.

Cell Growth Stimulating Activity

The heptane, ethyl acetate and methanol extracts of *Calendula officinalis* flowers, were found to stimulate cell growth when introduced to a human skin fibroblast (HSF) cells culture and a culture of human breast cancer cells (T47D), cell culture collection ECACC number 85102201 (Matysik et al. 2005). The ethyl acetate but not the heptane and methanol extracts in concentrations above 25 μg/ml stimulated cell proliferation and cellular metabolism by increase of mitochondrial dehydrogenase activity. However, concentrations exceeding 75 μg/ml were toxic for cells.

Exfoliative Cheilitis Therapeutic Activity

Roveroni-Favaretto et al. (2009) described a case of recurrent exfoliative cheilitis in an 18-year-old man that responded to treatment with a standardized topical preparation of *Calendula officinalis.*

Hypoglycaemic, Gastric Emptying and Gastroprotective Activities

The methanol extract and its 1-butanol-soluble fraction from *Calendula officinalis* flowers were found to show a hypoglycaemic effect, inhibitory activity of gastric emptying and gastroprotective effect (Yoshikawa et al. 2001). From the 1-butanol-soluble fraction, four new triterpene oligoglycosides, calendasaponins A, B, C and D, were isolated and were shown to exhibit potent inhibitory effects on an increase in serum glucose levels in glucose-loaded rats, gastric emptying in mice and ethanol- and indomethacin-induced gastric lesions in rats. Some structure-activity relationships are discussed. They further isolated two new ionone glucosides (officinosides A and B) and two sesquiterpene oligoglycosides (officinosides C and D), from the flowers of Egyptian *Calendula officinalis* (Marukami et al. 2001).

Hypotensive and Sedative Activity

Animal studies found that high doses of *Calendula* exhibited sedative effect and also reduced blood pressure (Bojadjiev 1964). For this reason, it might not be safe to combine calendula with sedative or blood pressure medications.

Antipyretic and Analgesic Activities

Crude ethanol extract of *C. officinalis* displayed significant antipyretic (74.95 % inhibition at a dose of 300 mg/kg) and analgesic (27.42 % inhibition at a dose of 40 mg/kg) in rat models (Ahmad et al. 2000). The extract not only reversed induced hyperthermia but also affected normothermia in rats. The extract at a dose of 20 mg/kg was as potent in its analgesic properties as acetylsalicylic acid at 40 mg/kg.

Antiulcerogenic Activity

Calendulozide B, trioside of oleanolic acid, isolated from *C. officinalis* roots was shown to have antiulcerogenic activity (Iatsyno et al. 1978). In oral doses of 5, 10, 20 and 50 mg/kg, it exerted an antiulcerous action in three experimental ulcer models (caffeine-arsenic induced, butadion induced and pylorus ligation induced) and also exhibited antiphlogistic and sedative action. It was devoid of effects on the cardiovascular system, the tone of intestinal smooth muscles, the diuretic renal function, the electrolytes excretion with urine or on the biligenic function of the liver. It had no local irritation properties, manifested a relatively low haemolytic activity and an insignificant toxicity both with its one-time and chronic administration.

In a clinical trial of 24 patients with chronic nonspecific colitis, treatment of an herbal combination of *Taraxacum officinale*, *Hipericum perforatum*, *Melissa officinalis*, *Calendula officinalis* and *Foeniculum vulgare* abrogated spontaneous and palpable pains along the large intestine in 95.83 % of the patients by the 15th day of their admission to the clinic Chakurski et al. 1981). Defecation became daily in the patients with obstipation syndrome. In another clinical trial, 137 patients (78 with duodenal ulcer and 59 with gastroduodenitis) were treated only with the herbal combination of *Symphytum officinalis* and *Calendula officinalis* and 33 (21 with duodenal ulcer and 12 with gastroduodenitis) were treated with the herbal combination together with antacid (Chakŭrski et al. 1981) As a result from the treatment, the spontaneous pains disappeared in 90 % of the patients in the group with antacid, and in the group without antacid, the dyspeptic complaints decreased over 85 %, but in the patients treated with herbs and antacid, these complaints disappeared several days earlier. The palpitation pains, in both groups, disappeared in more than 90 % of the patients within the same time. Gastric acidity, in both groups, showed a statistically insignificant tendency to decrease prior and post treatment. The ulcer niche, in both groups, was healed in almost the same percentage of the patients.

In a clinical study of patients with venous leg ulcers, after 3 weeks of treatment with an ointment of *C. officinalis* extract, of 21 patients treated, total ulcer surface area decreased by 41.7 % and 7 patients had complete epithelialization (Duran et al. 2005). In the control group of 13 patients, treated with saline solution dressings, total ulcer surface area decreased by 14.52 % and four patients had complete epithelialization. The preliminary results suggested the positive effects of the Marigold extract ointment on venous ulcer epithelialization. Studies showed that treatment of dogs with of acetic acid-induced ulcerative colitis resolved the damages of ulcerative colitis (Mehrabani et al. 2011). Loose stools, diarrhoea, gross bleeding and loss of body weight happened after administration of acetic acid, and crypt damage, loss of epithelium, infiltration of inflammatory cells and depletion of goblet cells were observed histologically.

Strychnos nux-vomica (nux vomica) and *Calendula officinalis* are used in highly diluted form in homeopathic medicine to treat patients suffering from gastritis and gastric ulcers. Results of studies suggested both drugs prepared in ethanol solution to be potent inhibitors of *Helicobacter pylori* induced gene expression (Hofbauer et al. 2010). Addition of nux vomica and *Calendula officinalis* in a 43 % ethanol solution produced a significant reduction of *H. pylori*-induced increase in gene expression of heparin-binding epidermal growth factor (HB-EGF) in gastric epithelial cell line KATO-III (reduced to 53.12 and 75.32 % vs. control), respectively. This effect was only observed when the drugs were primarily prepared in ethanol, not in aqueous solutions.

Antigingivitis Activity

Polysorb-immobilized *Calendula* was found to be highly effective in the treatment of chronic catarrhal gingivitis (Krazhan and Garazha 2001). Two patients with a diagnosis of lichen planus presenting as desquamative gingivitis who had undergone previous treatments for this condition with no significant results were treated by a handling gel containing clobetasol, nystatin, *Calendula*

officinalis, and pectin in custom trays (Machado et al. 2010). Both patients had remission of symptoms while using the trays, and after they stopped the treatment, the symptomatic outbreaks were delayed and presented as less severe symptoms in the 2 years follow-up. The treatment is aimed primarily at reducing the length and severity of symptomatic outbreaks of desquamative gingivitis.

Antimicrobial Activity

Hydroacetonic extract from fresh *C. officinalis* plant inhibited the growth of *Staphylococcus aureus* at a concentration of 1 mg/ml in vitro (Dumenil et al. 1980). *Calendula* extract tested on biofilms of infant dentifrices did not demonstrate antimicrobial effects in vitro against *Actinomyces viscosus*, *C. albicans*, *Lactobacillus casei*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus sanguis*, and *Streptococcus sobrinus* (Modesto et al. 2000). *Melissa officinalis* and *C. officinalis* flower extracts exhibited low inhibiting activity (MIC > or = 2,048 mg/l) against all the tested periodontopathic bacteria species with the exception of *Prevotella* sp. (Iauk et al. 2003). Oleanolic acid isolated from Marigold (*Calendula officinalis*) inhibited bacterial growth and survival, influenced cell morphology and enhanced the autolysis of Gram-positive bacteria, suggesting bacterial envelopes were the target of its activity (Szakiel et al. 2008). The sap of *Calendula* racemes demonstrated the most profound antimicrobial effect while that of the roots was the least effective (Radioza and Iurchak 2007). *Calendula* species inhibited all tested pathogenic microorganisms, especially *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Xanthomonas campestris* and *Agrobacterium tumefaciens*. Chloroform, ethanol and water extracts of *C. officinalis* leaves exhibited in-vitro antibacterial activity against Gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, and Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumonia*, whereas significant activity was not observed with petroleum ether extract (Chakraborthy 2008). All the extracts did not show any antifungal activity.

Calendula officinalis flower extract showed high in-vitro inhibitory activity against *Aspergillus niger*, *Rhizopus japonicus*, *Candida albicans*, *Candida tropicalis* and *Rhodotorula glutinis* (Kasiram et al. 2000). The inhibitory effects of extracts were very close and identical in magnitude and were comparable with that of standard antibiotics used. The ethanol, water and n-butanol *C. officinalis* aerial plant parts extracts were found to be effective against most of the human pathogenic microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* sp., coagulase (+) *Staphylococcus* sp., coagulase (−) *Staphylococcus* sp., *Candida albicans* and *Candida parapsilosis* (Goyal and Mathur 2011). The methanol extract of *C. officinalis* petals exhibited better antibacterial activity against most of the clinical bacteria tested than the ethanol extract (Efstratiou et al. 2012). Both methanol and ethanol extracts showed excellent antifungal activity against tested strains of fungi, comparable to fluconazole.

In a trial of 18 patients to compare the antimicrobial effect of mouthwashes (6 patients per mouthwash) containing *Calendula officinalis*, *Camellia sinensis* and 0.12 % chlorhexidine digluconate on the adherence of microorganisms to suture materials after extraction of unerupted third molars, all three mouthwashes exhibited antimicrobial activity against the adherence of microorganisms to sutures (Faria et al. 2011). However, *C. officinalis* and *C. sinensis* were not as efficient as chlorhexidine digluconate. The hydroethanol herbal extract of *C. officinalis* was one of several herbal extracts that showed high growth inhibition of *Campylobacter jejuni*, the most common cause of enteric infections, particularly among children, resulting in severe diarrhoea (Cwikla et al. 2010).

Antiviral Activity

Tinctures of *C. officinalis* flowers inhibited replication of herpes simplex virus, influenza A2 and influenza APR 8 viruses in-vitro (Bogdanova et al. 1970). However, an aqueous extract was

inactive (May and Willhun 1978). A 5 % hot aqueous extract of *Calendula* flowers inhibited replication of tick-borne encephalitis virus after intraperitoneal administration to mice (Fokina et al. 1991).

Studies found that the organic extract from *Calendula officinalis* flowers caused a significant dose- and time-dependent reduction of human immunodeficiency virus type 1 (HIV-1) reverse transcription (RT) activity in the in-vitro MTT/tetrazolium-based assay (Kalvatchev et al. 1997). An 85 % RT inhibition was achieved after 30 minutes. In addition, in the presence of the organic extract (500 µg/ml), the uninfected human lymphocytic Molt-4 cells were completely protected for up to 24 hours from fusion and subsequent death, caused by cocultivation with persistently infected U-937/HIV-1 cells. Ten oleanane-type triterpene glycosides isolated from the flowers, calendulaglycoside A (1), calendulaglycoside A6′-*O*-*n*-methyl ester (2), calendulaglycoside A 6′-*O*-*n*-butyl ester (3), calendulaglycoside B (4), calendulaglycoside B 6′-*O*-*n*-butyl ester (5), calendulaglycoside C (6), calendulaglycoside C 6′-*O*-*n*-methyl ester (7), calendulaglycoside C 6′-*O*-*n*-butyl ester (8), calenduloside F 6′-*O*-*n*-butyl ester (9) and calenduloside G 6′-*O*-*n*-methyl ester (10), exhibited moderate inhibitory effects against the Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), (IC_{50} values of 471–487 mol ratio/32 pmol TPA) (Ukiya et al. 2006).

Calendula officinalis aqueous extract was found to have an immunomodulation effect against three different live viruses in broiler chickens (Barbour et al. 2004). There was a reduction in immune response to IB (infectious bronchitis) virus at 42 days of age, to ND (Newcastle disease) virus at 29 and 42 days of age, and to IBD (infectious bursal disease) virus at 14, 29 and 42 days of age in the *Calendula-treated* birds in comparison with controls. This immune reduction in *Calendulas*-treated birds was associated with insignificant reduction in the bursal weight index at 42 days of age and an improvement in mean weights at 21 and 41 days of age.

Immunomodulatory Activity

Polysaccharide fractions with molecular weights in the range of 25,000–500,000 and higher isolated from *C. officinalis* and several other plants showed significant immunostimulating activities in granulocytes and carbon clearance tests (Wagner et al. 1985). Three polysaccharides isolated from *Calendula officinalis* flowers showed immunostimulating activity in several in-vitro immunological test systems (Varljen et al. 1989). Studies found that the ethanol extract of *C. officinalis* exhibited no direct mitogenic effect on human lymphocytes or thymocytes (Amirghofran et al. 2000). They showed a complete inhibitory effect on the proliferation of lymphocytes in the presence of PHA (SI (simulation index) range 0.01–0.49). Treatment of mixed lymphocytes with 0.1–10 µg/ml of *C. officinalis* (SI range 1.34–1.80) strongly increased the cell proliferation.

Calendula officinalis extract was one of several herbal extracts that was shown to be not inferior to *Echinacea purpurea* tincture in terms of stimulation of humoral immune response, phagocytic and bactericidal activity of peritoneal macrophages in mice but exceeded effect of *E. purpurea* on phagocytic activity of peripheral blood neutrophils (Borsuk et al. 2009).

Antimutagenic Activity

All 13 saponins isolated *Calendula officinalis*, *C. arvensis* and *Hedera helix* were found to be nontoxic and non-mutagenic for doses of 400 µg in the *Salmonella*/microsomal assay (Elias et al. 1990). Chlorophyllin inhibited the mutagenic activities of benzo-[a]pyrene (BaP) (1 µg) and a mutagenic urine concentrate from a smoker (5 µl) in a dose-dependent manner.

Genotoxic/Antigenotoxic Activity

A fluid extract of *Calendula officinalis* exhibited dose-dependent genotoxic properties when assayed for mitotic segregation in the heterozygous diploid

D-30 of *Aspergillus nidulans* (Ramos et al. 1998). Mutagenicity testing with the *Salmonella*/microsome assay in strains TA 1535, TA 1537, TA 98 and TA 100 was negative in a plate incorporation protocol, with concentrations ranging from 50 to 5,000 μg/plate. The extract was also negative in the mouse bone marrow micronucleus test at 1 g/kg per os. Pérez-Carreón et al. (2002) found that in the unscheduled DNA synthesis (UDS) assay in liver cell cultures, diethylnitrosamine (DEN) at 1.25 μM elicited a maximal increase of 40 % (3) H-thymidine ((3)HdTT) incorporation, and both aqueous (AE) and aqueous-ethanol (AEE) extracts of *C. officinalis* flower completely reverted the DEN effect at around 50 ng/ml and between 0.4 and 16 ng/ml, respectively. In the absence of DEN, these two polar extracts induced UDS at concentrations of 25 μg for AE and 3.7 μg/ml for AEE to 100 μg/ml in rat liver cell cultures. Concentrations producing genotoxic damage were three orders of magnitude above concentrations that conferred total protection against the DEN effect. Thus, at the lower end, ng/ml concentrations of the two polar extracts AE and AEE conferred total protection (antigenotoxic) against the DEN effect, and at the higher end, g/ml concentrations produced genotoxic effects.

No genotoxic or mutagenic effect was observed in blood and bone marrow samples from mice after 2 weeks of treatment with ethanol (250 or 500 mg/kg) or aqueous (90 mg/kg) extracts of *C. officinalis* prior to treatment with saline or methyl methanesulfonate (Leffa et al. 2012). Additionally, both extracts showed an antigenotoxic effect by Comet assay, repairing the DNA damage caused by methyl methanesulfonate. In the micronucleus test, only aqueous extract of *C. officinalis* revealed a protective effect to genetic material. The results suggested that all the extracts of *C. officinalis* contained protective substances that decreased damage to genetic material.

Hepatoprotective Activity

In a rat hepatocarcinogenesis model, *Calendula officinalis* flower extracts exhibited both protective and cytotoxic effects dependent on the concentration used presenting a phenomenon known as hormesis (Barajas-Farias et al. 2006). The protective effect was observed at 0.1 mg/kg concentration, increased at 0.5 mg/kg and reached its maximum at 2.5 mg/kg, when it decreased the area and number of altered foci by 55 and 49 %, respectively, in comparison with rats treated only with carcinogen, *N*-nitrosodiethylamine. Ten and 20 mg/kg doses produced a notorious increment in the area and number of altered hepatic foci, and at 40 mg/kg of extract the increment was 40 and 53 %, respectively.

Cardioprotective Activity

Animal studies showed that *C. officinalis* ameliorated myocardial ischemic reperfusion injury (Ray et al. 2010). Rat hearts perfused with *C. officinalis* prior to subjecting the heart to ischaemia and achieved cardioprotection by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocyte apoptosis. Cardioprotection appeared to be achieved by changing ischaemia reperfusion-mediated death signal into a survival signal by modulating antioxidant and antiinflammatory pathways as evidenced by the activation of Akt and Bcl2 and depression of TNF-α.

Spasmogenic and Spasmolytic Activities

The crude aqueous-ethanol extract of *Calendula officinalis* flowers was found to contain both spasmolytic and spasmogenic constituents, exhibiting these effects through calcium channel blocking and cholinergic activities (Bashir et al. 2006). In isolated rabbit jejunum, the extract caused a dose-dependent (0.03–3.0 mg/ml) relaxation of spontaneous and K^+-induced contractions, suggestive of calcium channel blockade. In a few preparations, a mild non-reproducible spasmogenic effect was observed at lower doses, followed by relaxation. Activity-directed fractionation revealed that the spasmolytic activity of

the plant was concentrated in its organic fractions. The aqueous fraction exhibited a marked atropine sensitive spasmogenic effect but was found to be devoid of any spasmolytic effect. The study provided a scientific base for its traditional use in abdominal cramps and constipation.

Uterotonic Activity

A crude water extract of *C. officinalis* was found to enhance the uterine tonus in isolated rabbit and guinea pig uterine horn (Shipochliev 1981).

Estrogenic Activity

Two Polish studies in early 1960s reported that calendula flower extracts exhibited some estrogenic activity in ovariectomized mice (Banaszkiewicz and Mrozikiewicz 1962; Banaszkiewicz et al. 1962).

Insecticidal Activity

A study on human volunteers showed that the essential oils of myrtle and Marigold (*Calendula officinalis*) exhibited repellency against *Anopheles stephensi*, vector of malaria disease, but was generally lower than DEET, a synthetic repellent (Tavassoli et al. 2011). The protection time of 50 % essential oils of Marigold and myrtle were respectively 2.15 and 4.36 hours compared to 6.23 hours for DEET 25 %. The median effective dose (ED_{50}) of essential oils was 0.1105 and 0.6034 mg/cm^2, respectively, for myrtle and Marigold.

Antiparasitic Activity

Glycosides of oleanolic acid, isolated from Marigold, inhibited the development of L3 *Heligmosomoides polygyrus* larvae, the infective stage of the intestinal parasitic nematode (Szakiel et al. 2008). Furthermore, both oleanolic acid and its glycosides reduced the rate of L3 survival during prolonged storage, but only oleanolic acid glucuronides affected nematode infectivity.

Molluscicidal Activity

Studies found that the *Calendula micrantha officinalis* flower ethanol extract higher molluscicidal activity against *Lymnaea cailliaudi*, Fascioliasis-transmitting snail, than the leaf extract with LC_{50} of 35 and 52.17 ppm, respectively (Abd-El-Megeed 1999). The mortality rate of exposed snails was increased by prolongation of the exposure time. The molluscicidal effect resulted in enhancing energy utilization and nutrient consumption since glucose, lipids, proteins and triglycerides were greatly reduced. The stomach and digestive gland of the treated *L. cailliaudi* snails were greatly altered. Natural rubber used as a binding matrix for *Calendula officinalis* was found to be a source of molluscicidal saponin (Helaly et al. 1999). The amount of saponin released was affected by the environmental temperature and the type of fillers present in the formulations.

Allergy Problem

Members of the Compositae including *C. officinalis* had been suspected of sensitization or elicitation of Compositae dermatitis (Paulsen 2002). Sesquiterpene lactones were the most important allergens, but there were a few cases of sensitization from a coumarin, a sesquiterpene alcohol and a thiophene.

Toxicity Studies

Animal studies by Lagarto et al. (2011) found that the acute and subchronic toxicities of *C. officinalis* extracts were low. In the subchronic study with doses of 50, 250 and 1,000 mg/kg/day administered in drinking water, several of the blood elements were significantly affected in male and female Wistar rats after 90 days, haemoglobin, erythrocytes, leukocytes and blood

clotting time. For blood chemistry parameters, ALT, AST and alkaline phosphatase were affected. Histopathological examination of tissues showed slight abnormalities in hepatic parenchyma that were consistent with biochemical variations observed. In the acute study (dose of 2,000 mg/kg) there were no mortality and signs of toxicity.

Jeschke et al. (2009) conducted a prospective observational study of prescribing patterns of remedies containing Asteraceae extracts and adverse drug reactions (ADR) in Germany from these remedial extracts. Their study involved herbal medicines, containing extracts of Asteraceae such as *Echinacea* spp., *Arnica montana*, *Matricaria recutita* and *Calendula officinalis* and involved 38 physicians, 55 % of whom were general practitioners and 45 % were specialists. During the study period, a total of 50,115 patients were evaluated and 344 ADRs for conventional and complementary remedies were reported. The most frequently prescribed Asteraceae was *Matricaria recutita* (23 %), followed by *Calendula officinalis* (20 %) and *Arnica montana* (20 %). No serious ADRs for Asteraceae-containing remedies were reported. The majority of reported ADRs for Asteraceae-containing remedies were classified as uncommon. The proportional reporting ratios (PRRs) for Asteraceae-containing remedies with respect to all other prescriptions was 1.7 for the system organ class 'skin and subcutaneous tissue disorders' (six ADRs) and 1.0 for 'gastrointestinal disorders' (three ADRs). Neither result was significant. Their results indicated treatment with Asteraceae-containing remedies was not associated with a high risk of adverse drug reactions (ADRs).

In the acute toxicity test, the hydroalcohol extract of *Calendula officinalis* failed to cause death in the animals after administration of oral doses up to 5.0 g/kg (Silva et al. 2007). Oral treatment with the extract at 0.025, 0.25, 0.5 and 1.0 g/kg did not induce hematological alterations when compared with the control group. In the biochemical parameters, there was an increase in blood urea nitrogen and in alanine transaminase levels. Morphological examination of the brain, kidney and heart did not show any alteration. However, inflammatory sites were found in the lung and liver, which were associated, respectively, with oral gavage and a possible hepatotoxic effect. The extract was nontoxic in rats, although there was evidence of renal and liver overload. Subsequent studies by Silva et al. (2009) showed that the treatment of Wistar rats with hydroalcohol extract of *Calendula officinalis* flowers did not affect male reproductive parameters. Besides, it was nontoxic in the preimplantation and organogenic periods (early and middle periods) of pregnancy. However, the extract induced a decrease in the maternal weight gain when administered during the foetal period.

The Cosmetic Ingredient Review (CIR) Expert Panel in their final report concluded that *Calendula officinalis*-derived cosmetic ingredients were safe for use in cosmetics in the practices of use and concentration given in the amended safety assessment (Andersen et al. 2010). *C. officinalis* extract, *C. officinalis* flower, *C. officinalis* flower extract, *C. officinalis* flower oil, and *C. officinalis* seed oil are cosmetic ingredients derived from *C. officinalis*. These ingredients may contain minerals, carbohydrates, lipids, phenolic acids, flavonoids, tannins, coumarins, sterols and steroids, monoterpenes, sesquiterpenes, triterpenes, tocopherols, quinones, amino acids and resins. These ingredients were not significantly toxic in single-dose oral studies using animals. The absence of reproductive/developmental toxicity was inferred from repeat-dose studies of coriander oil, with a similar composition. Overall, these ingredients were not genotoxic. They also were not irritating, sensitizing or photosensitizing in animal or clinical tests but may be mild ocular irritants.

Traditional Medicinal Uses

Calendula officinalis is used medicinally in Europe, China and India among several other places in the world (Muley et al. 2009) and is one of the most common and versatile herbs in western herbal medicine and popularly used as domestic medicine (Grieve 1971; Chevallier

1996). The whole plant, in particular the flowers and the leaves, are deemed antiphlogistic, antiseptic, antispasmodic, aperient, astringent, cholagogue, diaphoretic, emmenagogue, stimulant and vulnerary (Uphof 1968; Grieve 1971; Chiej 1984; Chevallier 1996). *Calendula officinalis* has been widely used from time immemorial in Indian and Arabic cultures as an antiinflammatory agent to treat minor skin wound and infections, burns, bee stings, bites, sunburn, sprains, wounds, sore eyes, ulcers, varicose veins and cancer (Grieve 1971; Chiej 1984; Ray et al. 2010). Pot Marigold is taken internally in treating fevers and chronic infections (Grieve 1971; Chevallier 1996) and used internally in order to speed the healing of wounds (Castro 1996) and prevent suppuration (Grieve 1971). The leaves, blossoms and buds are used to make a homeopathic remedy (Castro 1996). Leaves eaten as salad were considered useful in the treatment of scrofula in children. The petals are used in herbal tea taken to ease varicose veins. The crushed stems are applied to warts and corns. Pot Marigold is also used as a bactericide, antiseptic and antiinflammatory.

Other Uses

Potted Marigolds are grown all over the world for ornamental purposes and also as medicinal herbs in many cultures. Calendula flowers can be used as cut flowers. In eastern countries, Marigold flowers are used in garlands for social and religious purposes. The flowers also provide a yellow dye used for fabrics, cosmetics and food. Potted Marigold plant extracts are widely used in cosmetics presumably due to presence of compounds such as saponins, flavonoids, resins and essential oils. Potted Marigold is also an important dietary and medicinal source of carotenoids such as lutein, lutein esters, zeaxanthin, auroxanthin and flavoxanthin.

Calendula officinalis can be grown as an intercrop or use as soil amendments to deter insect and soil pests. Studies found that intercropping with pot Marigold (*C. officinalis*) afforded the most effective pest control on white cabbage (Jankowska et al. 2009). Populations and ovipositing activities of cabbage pests, namely, cabbage aphid *Brevicoryne brassicae*, flea beetles *Phyllotreta*, small white butterfly *Pieris rapae*, large white butterfly *Pieris brassicae*, cabbage moth *Mamestra brassicae* and larvae and pupae of the diamondback moth *Plutella xylostella*, were significantly reduced. Studies found that when used as a soil mulch (compost), the plant can significantly reduce root-knot nematode, *Meloidogyne incognita*, population in quito orange (*Solanum quitoense*) plantings (Betancourth García et al. 2011). Pérez et al. (2003) found that amending soil with *C. officinalis* flowers significantly reduce reproduction rate of *Meloidogyne artiellia* on chickpea compared to the non-amended treatment.

Comments

C. officinalis is widely grown in Europe, now mainly as an ornamental. It is widely cultivated in the CIS (Commonwealth of Independent States comprising Belarus, Russian Federation and Ukraine) republics, Holland, and in Germany as an herbal medicine.

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