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# Biology of *Calendula officinalis* Linn.: Focus on Pharmacology, Biological Activities and Agronomic Practices

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## ABSTRACT

*Calendula officinalis* Linn. (*Asteraceae*), a medicinal plant, is used in traditional medicine around the world and is the subject of several chemical and pharmacological studies. Chemical studies have detected various classes of compounds in its organs, primarily volatile oil, carotenoids, flavonoids, terpenoids, coumarins, quinones, carbohydrates, lipids, amino acids, as well as other minor constituents. *C. officinalis* extract and pure compounds isolated from different organs possess multiple pharmacological activity, including anti-inflammatory, antioedematous, antioxidant, immunostimulant, anticancer, lymphocyte and wound healing, hepatoprotective, antibacterial and antifungal, anti-HIV, spasmolytic and spasmogenic, genotoxic and antigenotoxic, inhibition of heart rate, antiviral, *inter alia*. In this review, we explore the phytochemical and pharmacological activity of *C. officinalis* while also covering aspects of its culture and cultivation that would increase the production of its pharmacologically important compounds.

**Keywords:** *Asteraceae*, pharmacological activities phytochemical constituents, pot marigold
**Abbreviations: AE**, aqueous extract; **AEE**, aqueous ethanol extract; **VO**, volatile oil

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## HISTORY, BOTANICAL DESCRIPTION AND TRADITIONAL USES

*Calendula officinalis* Linn. belongs to the *Asteraceae* (*Compositae*) family. It is an annual with bright or yellow orange daisy-like flowers which are used for medicinal or culinary purposes (Bcerentrup and Robbelen 1987; Cromack and Smith 1988), especially in phytotherapy (Von *et al*. 1992) as well as for ornamental purposes. Common names assigned around the world to *C. officinalis* include calendula, field marigold, garden marigold, poet's marigold, gold bloom, holligold, maravilla, marybud, marygold, pot marigold, rudders, Oculus Christi, Fiore d'ogni mese, Solis Sponsa, Ringelblumen. Incidentally, in old English *Calendula* was known as "gold's", and was first associated with the Virgin Mary and then with Queen Mary, hence the name "Mary's gold" or marigold (Grieve 1931; Mills 1991; Kemper 1999).

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Review

The genus *Calendula* includes 15-20 species. Native to Egypt and the Mediterranean, *Calendula* is cultivated in temperate regions around the world. Easily naturalized, it grows readily in sunny locations throughout North America and Europe. It prefers previously cultivated positions (Mills 1991; Chevallier 1996). Cultivated by the Egyptians, Greeks, Hindus and Arabs, *Calendula* was grown in European gardens and has been used medicinally since the 12th century. Its name comes from the Latin word, *calends*, the first day of every month, because of its long flowering period. Because the flowers follow the sun, it was linked to the astrological sign of summer, Leo, and to treating the heart and conditions caused by heat. *Calendula* was taken internally to treat fevers, promote menstruation and treat cancer. Most importantly, the flowers were made into extracts, tinctures, balms and salves and applied directly to the skin to help heal wounds and to soothe inflamed and damaged skin (Krag 1976). In Italian folk medicine *Calendula* is used as an antipyretic and anti-inflammatory. Teas made from *Calendula* are used as eye washes, gargles or compresses to treat conjunctivitis, pharyngitis, aphthous stomatitis and gingivostomatitis, diaper rashes and other inflammatory conditions of the skin and mucus membranes (Mozherenkov and Shubina 1976; Nedelka *et al*. 1988; Fleming 1998). In India, herbal compounds including *Calendula* are used topically to treat hemorrhoids. *Calendula* cream alone or in combination with other remedies is also a favorite homeopathic remedy to treat abrasions and minor burns. Dried *Calendula* petals are used in the spice trade as an inexpensive alternative to saffron and are used in many ointments to enhance their appearance by adding a gold color. Like other members of the daisy family, the dried flowers have also been used as an insect repellent. Some herbalists combine *Calendula*, comfrey, echinacea and St. John's wort in a cream or ointment as an all-purpose skin salve. Traditional medicine holds great promise as a source of easily available and effective therapy for skin diseases to people, particularly in tropical developing countries, including India. In India, the florets are used in ointments for treating wounds, herpes, ulcers, frostbite, skin damage, scars and blood purification while the leaves, in infusion, are used for treating varicose veins externally (Kirtikar and Basu 1993; Khare 2004). It is in this context that the people use several plant-derived preparations to cure skin diseases in Indonesia (Satyavati 1990). In Europe, *Calendula* leaves are considered to be a resolvent and diaphoretic while the flowers are used as a stimulant, antispasmodic and emmenagogue (Kirtikar and Basu 1993). In the UK, the decoction of the flowers was used as a potent drink for the treatment of measles and smallpox, and the fresh juice as a remedy for jaundice, costiveness (constipation) and suppression of menstrual flow (Khare 2004). The usefulness of *C. officinalis* has been reported in the form of decoctions, infusions and tinctures in traditional systems of medicines for treating skin diseases like psoriasis, leprosy, etc. (Horvath and Ferenc 1992; Zahra *et al*. 2000; Cordova *et al*. 2002; Harrison and Dorothy 2003).

Often referred to as pot marigold or garden marigold, *C. officinalis* has been used for medicinal purposes for centuries. Folk medicine healers in Europe used *Calendula* to produce sweat during fevers and cure jaundice. During the 19th century, preparations were also used in the USA to treat stomach ulcers, liver complaints, conjunctivitis and wounds. Recognized for its antiseptic and vulnerary properties, today *Calendula* flowers are used in the form of infusions, tincture, fluid extracts, cold infused oil and ointments to promote the granulation and facilitate healing of skin inflammations, wounds, burns, bruises, and cuts, as well as prevent the spread of infection (Grieve 1931).

*C. officinalis* can broadly be applied as an antiseptic, anti-inflammatory and cicatrizing (Correa Jr. 1994) as well as a light antibacterial (Chiej 1988) and antiviral (Bogdanova and Farmakol 1970) agent. Many *Calendula* species have a characteristic scent or taste caused by mono- and sesquiterpenes within the volatile oil (VO), which in many cases are the reasons for their application in folk medicine (Yoshikawa *et al*. 2001). Many attempts have been made to better characterize their therapeutic properties and to enhance the production of these useful compounds within their VO. These studies are the primary focus of this review.

Mills (1991) and Chevallier (1996) noted that *Calendula* is a self-seeding annual plant that thrives in virtually any kind of soil. It grows to a height of 30-60 cm with multiple branching stems. Its leaves are spatulate or oblanceolate, sessile, with widely spaced tiny teeth on the borders, and the whole leaf is covered with very short fine hairs. *Calendula* has single flower heads situated on a green crown-shaped receptacle. The inner portion of the flower head consists of orange-yellow, tubular florets (often called petals). As the petals fall off, a circular corona of seeds remains in view. The plant is an annual, seldom biennial. It grows to between 30 and 50 cm high, and has about 20 cm long tap root and numerous thin, secondary roots. The stem is erect, angular, downy and branched from the base up or higher. The alternate leaves are almost spatulate at the base, oblong to lanceolateabove and are all tomentosae (Editorial Boards. PDR for Herbal Medicines 2003; Wikipedia 2011). On the tip of each stem, there is a 5-7 cm composite flower head, consisting of an epicalyx of numerous narrow-lanceolate sepals, which are densely covered on both sides with glandular hairs. The inner section of the flower head is made up of orange-yellow tubular florets. The disc florets are pseudo hermaphrodites but the female is sterile. The zygomorphic ray florets at the edge are female, their stamens are completely absent, and their inferior ovaries are much more developed than those of the tubular florets. The fruit forms only in the female ray flowers. The heterocarp achenes are sickle-shaped, curved and ringed (Norman *et al*. 2001).

## PHYTOCHEMISTRY

A number of phytochemical studies have demonstrated the presence of several classes of chemical compounds, the main ones being VO carotenoids, flavonoids, terpenoids, coumarins, quinines, amino acids, carbohydrates, lipids and other constituents.

### Volatile oil and its constituents

Previously the VO of French *C. officinalis* was obtained in low yield (0.3%) by steam distillation of flowers heads, and 66 components were identified by GC-MS, mainly sesquiterpene alcohols; α-cadinol was the main constituent (**Table 1**) (Chalchat *et al*. 1991). *C. officinalis* flowers contain maximum VO at the full flowering stage (0.97%) and minimum during the preflowering stage (0.13%) (Okoh *et al*. 2007). The VO concentrate of *C. officinalis* flower heads extracted by supercritical $CO_2$ (**Table 1**) was found to consist of methylhexanoate, methyllinoleate, methyl 9, 12, 15-octadecatrienoate, methyloctadecanoate, methyltetradecanoate, γ-cadinene and cubenol, δ-cadinene, α-cadinol and oplopanonec (Nicoletta *et al.* 2003). The main constituents of *C. officinalis* VO as detected by GC-MS and cultivated under pre-sowing low temperature were α-cadinol, Δ-cadinene, δ-cadenene and nerolidol (Khalid and El-Ghorab 2006) (**Table 1**). The main constituents (α-cadinol, γ and Δ-cadinene) of VO extracted from whole *C. officinalis* plants and detected by GC-MS increased when organic fertilizers were applied under soil solarization (**Table 2**) or saline irrigation water conditions (Kalid *et al*. 2006; Khalid and Teixeira da Silva 2010). The composition also showed different patterns at different phases of vegetative cycles. Various mono- and sesquiterpenes have been reported in the VO i.e. α-thujene, α-pinene, sabinene, β-pinene, limonene, 1,8-cineol, *p*-cymene, *trans*-β-ocimene, γ-terpenene, δ-3-carene, nonanal, terpene-4-ol, 3-cylohexene-1-ol, α-phellandrene, α-terpeneol, geraniol, carvacrol, bornyl acetate, sabinyl acetate, α-cubebene, α-copaene, α-bourbonene, β-cubebene, α-gurjunene, aromadendrene, β-caryophyllene, α-

**Table 1** Comparative of volatile oil constituents (%) of *Calendula officinalis* Linn. dried flower isolated by supercritical $CO_2$ extraction and GC-MS. (Compiled using data from Chalchat *et al.* 1991; Nicoletta *et al.* 2003; Khalid and El-Ghorab 2006).

| No. | Components | GC- Mass | Supercritical $CO_2$ | No. | Components | GC- Mass | Supercritical $CO_2$ |
|---|---|---|---|---|---|---|---|
| 1 | Hexanoic acid | - | 0.1 | 49 | Muurol-5-enen-4-B-oL (*cis*) | 1.34 | - |
| 2 | Tricyclene | - | T | 50 | β-Calacorene | 3.22 | 0.2 |
| 3 | α-Pinene | 1.78 | T | 51 | Nerolidol (*E*) | 5.71 | - |
| 4 | 6-Metyl-5-hepten-2-one | - | T | 52 | Dodecanoic acid | - | 0.7 |
| 5 | 6-Metyl-5-hepten-2-ol | - | 0.1 | 53 | Germacrene-D-4-ol | - | 0.4 |
| 6 | γ-Terpinene | - | 0.1 | 54 | Viridiflorol | - | 3.4 |
| 7 | *P*-Mentha-2.4-diene | - | T | 55 | Globulol | - | 10.6 |
| 8 | Linalool | - | 0.1 | 56 | Carotol | - | 0.3 |
| 9 | Menthone | - | T | 57 | 1-10-di-epi-Cubenol | - | 0.5 |
| 10 | Terpinene-4-ol | - | 0.4 | 58 | 1-epi-Cubenol | - | 1.5 |
| 11 | Methyl salicylicate | - | T | 59 | *T*-Cadinol | - | - |
| 12 | α-Terpineol | - | T | 60 | Cubenol | - | 2.9 |
| 13 | (*E*)-Anethole | - | T | 61 | *T*-Muurol | - | 2.8 |
| 14 | Methyl dodecanoate | - | 0.2 | 62 | α-Muurol | 0.10 | 0.8 |
| 15 | α-Cubebene | - | 0.2 | 63 | Guaiol | 1.13 | - |
| 16 | α-Copaene | - | 1.0 | 64 | β-Acorenol | 3.59 | - |
| 17 | β-Bourbonene | - | T | 65 | Cadinol (epi-α) | 0.84 | - |
| 18 | β-Cubebene | - | 0.3 | 66 | β-Eudesmol | 9.77 | - |
| 19 | Longifolene | - | T | 67 | α-Cadinol | 32.01 | 8.3 |
| 20 | α-Gurjurene | - | 0.1 | 68 | α-Patchouli alcohol | 1.66 | - |
| 21 | β-Caryphyllene | - | 0.4 | 69 | Bulnesol | 1.24 | - |
| 22 | E-α-Ionone | - | T | 70 | Eudesmol (7-epi-α) | 0.95 | - |
| 23 | β-Gurjurene | - | 0.2 | 71 | Isocedranol (5) | 0.77 | - |
| 24 | γ-Patchoulene | - | 0.4 | 72 | α-Bisabolol | 1.70 | - |
| 25 | Aromadendrene | - | 0.1 | 73 | Bisabolol (epi-α) | 0.25 | - |
| 26 | *cis*-tent-Muurola-3.5-diene | - | 0.5 | 74 | Methyl tetradecanoate | - | 3.2 |
| 27 | Geranyl acetone | - | 0.2 | 75 | oplopanone | - | - |
| 28 | α-Humulene | 0.23 | 0.4 | 76 | Ethyl teteradecanoate | - | T |
| 29 | β-Farnesene | 0.16 | - | 77 | Methyl pentadecanoate | - | 0.3 |
| 30 | allo-Aromadendrene | 1.21 | 0.3 | 78 | 10,14-Trimethyl-2-pentadecanone | - | 0.2 |
| 31 | α-Patchoulene | 0.20 | - | 79 | Musk ambrette | - | T |
| 32 | β-Cadinene | - | 0.7 | 80 | Methyl hexadecanoate | - | 8.1 |
| 33 | γ-Muurolene | 0.41 | 0.9 | 81 | Dolabradiene | - | 0.4 |
| 34 | Germacrene-D+ (E)-β-ionene | - | 0.9 | 82 | Hexadececanoic acid | - | 2.0 |
| 35 | γ-Gurjunene | 2.45 | - | 83 | Ethyl hexadecanoate | - | 0.1 |
| 36 | β-Selinene | - | - | 84 | Methyl heptadecanoate | - | 0.3 |
| 37 | *cis*-β-Guaiene | - | 1.1 | 85 | Methyl linoleate | - | 4.6 |
| 38 | Valencece | - | 6.3 | 86 | Methyl linolenate | - | 4.8 |
| 39 | epi-Cubebol | - | 1.3 | 87 | Methyl octadecanoate | - | 0.9 |
| 40 | α-Muurolene | 0.58 | 1.8 | 88 | Buthyle hexadecanoate | - | - |
| 41 | γ-Cadinene | 2.27 | 5.0 | 89 | 9,12-Octadecadienal | - | - |
| 42 | δ-Cadinene | 17.79 | 8.9 | 90 | 9,12,17-Octadecatrienal | - | - |
| 43 | Methyl-dodecanoate | - | 1.3 | 91 | Isoamyl laurate | - | 0.1 |
| 44 | Cadina-1,4-diene | - | 0.6 | 92 | Methyl 11,14,17-eicosatrienoate | - | - |
| 45 | α-Cadinene | - | 0.9 | 93 | Methyl eicosanoate | - | - |
| 46 | α-Calacorene | 1.76 | t | 94 | Pentacosane | 2.82 | - |
| 47 | Elemol | - | 0.7 | 95 | Methyl docosanoate | - | - |
| 48 | β-Patchouli alcohol | 3.67 | - | 96 | | | |

ylangene, α-humulene, epibicyclo-sequiphellandrene, germacrene D, alloaromadendrene, β-saliene, calarene, muurolene, δ-cadinene, cadina-1,4-diene, α-cadinene, nerolidol, palustron, endobourbonene, oplopenone, α-cadinol, and *t*-muurolol. The VO was found to be rich in α-cadinene, α-cadinol, *t*-muurolol, limonene, and 1,8-cineol with *p*-cymene at lower levels at the post-flowering periods (Okoh *et al*. 2007). Radulescu *et al*. (2000) studied flowers from Romania by headspace and steam distillation, and found δ-cadinene, 1, 3, 5-cadinatriene and α-muurolol to be the major compounds. The main constituents of the VO were: sesquiterpene hydrocarbons (68.0%) and sesquiterpenols (27.0%), δ-cadinene (22.53%), α-cadinol (20.40%) and epi-α-muurolol (12.87%). The analyses were performed by GC and GC-MS as described by Gazim *et al.* (2007). A comparison of VO constituents extracted from *C. officinalis* dried flowers and a whole plant is presented in **Table 3**.

### *1. Carotenoids*

**Function of carotenoids:** Carotenoids are isoprenoid compounds (mostly $C_{40}$) with polyene chains that may contain up to 15 conjugated double bonds. More than 700 naturally occurring carotenoids have been identified (Britton *et al*. 1995, 2004). Carotenoids differ from anthocyanins and betalains in that they play essential roles in plant life, for example, photoprotective functions during photosynthesis (Green and Durnford 1996; Niyogi 2000) and provision of substrates for biosynthesis of the plant growth regulator abscisic acid (ABA; Nambara and Marion-Poll 2005) and perhaps other phytohormones as well (Auldridge *et al*. 2006). Carotenoids also play an important role in human nutrition and health, providing provitamin A and having anti-cancer activities (Mayne 1996). Some carotenoids are used as food colorants, cosmetics or pharmaceuticals.

The mechanism(s) involved in this apparently carotenoid-mediated protection against cancer still remain to be elucidated. Some 10% of all known carotenoids are precur-

**Table 2** Comparative of volatile oils constituents extracted from *Calendula officinalis* Linn. (whole plants) growing under chemical fertilization, organic fertilization and organic fertilization + solarization. (Compiled using data from Naguib *et al.* 2005; Khalid *et al.* 2006; Khalid and El-Ghorab 2006; Khalil *et al.* 2007).

| No. | Components | Chemical fertilization | Organic fertilization | Organic fertilization+ solarization |
|---|---|---|---|---|
| 1 | α-Pinene | 0.25 | 0.28 | 0.25 |
| 2 | Camphene | 0.14 | 0.14 | 0.14 |
| 3 | β-Pinene | 0.11 | 0.15 | 0.11 |
| 4 | Sabenene | 0.21 | 0.70 | 0.21 |
| 5 | α-Phellandrene | 0.18 | 0.16 | 0.18 |
| 6 | Myrcene | 0.13 | 0.11 | 0.13 |
| 7 | Terpinolene | 0.14 | 0.12 | 0.14 |
| 8 | Limonene | 0.09 | 0.08 | 0.09 |
| 9 | 1,8-Cineol | 0.1 | 0.10 | 0.1 |
| 10 | β-Cymene | 0.6 | 0.05 | 0.6 |
| 11 | Menthone | 0.02 | 0.03 | 0.02 |
| 12 | β-Farnesene | 0.17 | 0.25 | 0.17 |
| 13 | α-Humulene | 0.4 | 0.22 | 0.4 |
| 14 | α-Patchoulene | 0.13 | 0.23 | 0.13 |
| 15 | Aromadendrene (allo) | 0.6 | 0.20 | 0.6 |
| 16 | Gurjunene | 0.9 | 0.33 | 0.9 |
| 17 | Muurolene | 0.69 | 0.32 | 0.69 |
| 18 | α–Muurolene | 0.50 | 0.50 | 0.50 |
| 19 | γ-Cadinene | 10.2 | 9.10 | 10.2 |
| 20 | α-Cadinene | 27.2 | 25.3 | 27.2 |
| 21 | β-Patchoulene | 0.323 | 2.1 | 0.323 |
| 22 | α-Calcorene | 0.80 | 0.67 | 0.80 |
| 23 | Muurol-5-enen-4-B-ol (*cis*) | 0.50 | 0.60 | 0.50 |
| 24 | β-Calacorene | 0.6 | 0.40 | 0.6 |
| 25 | Nerolidol (*E*) | 3.0 | 0.40 | 3.0 |
| 26 | Guaiol | 0.90 | 0.19 | 0.90 |
| 27 | β-Acorenol | 0.11 | 0.17 | 0.11 |
| 28 | Cadinol (Epi-α) | 1.9 | 1.8 | 1.9 |
| 29 | Muurolol (Epi-α) | 1.71 | 0.90 | 1.71 |
| 30 | α-Eudensmol | 5.8 | 3.00 | 5.8 |
| 31 | α-Patchouli alcohol | 1.6 | 0.60 | 1.6 |
| 32 | Bulnesol | 0.54 | 0.50 | 0.54 |
| 33 | α-Cadinol | 52.5 | 47.0 | 52.5 |
| 34 | Eudesmol (7-epi-α) | 0.50 | 0.09 | 0.50 |
| 35 | Isocedranol (5) | 0.26 | 0.20 | 0.26 |
| 36 | α-Bisabolol | 0.26 | 0.56 | 0.26 |
| 37 | Bisabolol (epi-α) | 0.05 | 0.51 | 0.05 |
| 38 | Pentacosane | 0.13 | 0.52 | 0.13 |

sors of vitamin A (retinol). The anti-cancer properties of retinoids, which can modulate cell proliferation and differentiation, were recognized before those of carotenoids and are the subject of intensive investigation (Mayer *et al*. 1979; Lotan 1980). However, in several studies the anti-cancer effect of carotenoids has been shown not to depend on provitamin A activity (Bendich 1990). Carotenoids are known as very effective quenchers of singlet oxygen and radicals, which are important in cancer etiology (Burton and Ingold 1988; Krinsky 1989). Furthermore, immunomodulatory effects of carotenoids have been reported, such as the enhancement of cell surface activation marker expression on natural killer (NK) cells and lymphocytes, enhancement of NK-cell-mediated cytotoxicity, and an increase in the number of helpedinducer T-lymphocytes in peripheral blood (Alexander 1980, 1985; Leslie and Dubey 1982; Jyonouchi *et al*. 1991). A direct link with cell regulatory functions in the form of specific carotenoid receptors, such as described for retinoids (Brand *et al*. 1988; Prabhala 1989), has not yet been established, however.

**Biosynthesis of carotenoids:** Carotenoid biosynthesis starts from a $C_5$ isoprene unit, IPP. Four IPPs are condensed to form $C_{20}$ geranylgeranylpyrophosphate (GGPP). A head-to-head coupling of two GGPP molecules, catalyzed by phyto-

**Table 3** Combative of volatile oil constituents extracted from C*alendula officinalis* Linn. dried flowers and whole plants. (Compiled using data from Chalchat *et al.* 1991; Khalid *et al.* 2006).

| No. | Components | Whole plant | Flowers |
|---|---|---|---|
| 1 | Butanone | Traces | 0.02 |
| 2 | Pentan-2-one | Traces | 0.04 |
| 3 | α-Pinene | 3.46 | 0.20 |
| 4 | β-Fenchene | 8.52 | 0.65 |
| 5 | Camphene | 0.07 | 0.02 |
| 6 | β-Pinene | 0.33 | 0.24 |
| 7 | Sabinene | 0.63 | 0.11 |
| 8 | $C_{10}H_{24}$ | 0.05 | - |
| 9 | Δ-Carene | 0.09 | 0.21 |
| 10 | α-Phellandrene | 0.16 | 0.02 |
| 11 | Myrcene | 0.54 | 0.17 |
| 12 | α-Terpinene | 0.20 | 0.12 |
| 13 | Limonene | 0.48 | 1.17 |
| 14 | 1,8-Cineole | 0.11 | - |
| 15 | β-Phellandrene | 0.09 | 0.25 |
| 16 | *cis*-β-Ocimene | Traces | - |
| 17 | γ-Terpinene | 0.65 | 0.37 |
| 18 | *trans*-β-Ocimene | 0.26 | 0.40 |
| 19 | *p*-Cymene | 0.20 | 0.59 |
| 20 | Terpinolene | 0.11 | 0.09 |
| 21 | $C_{10}H_{16}O$ | Traces | - |
| 22 | Menthone | Traces | 0.05 |
| 23 | α-Copaene | 0.12 | - |
| 24 | Unknown | 0.13 | 0.12 |
| 25 | β-Ylangene | Traces | 0.13 |
| 26 | Camphor | Traces | - |
| 27 | Unknown | 0.43 | 0.32 |
| 28 | α-Gurjunene | - | 0.58 |
| 29 | β-Cubebene | 0.12 | 0.11 |
| 30 | $C_{10}H_{18}O$ | 0.24 | 0.06 |
| 31 | $C_{10}H_{18}O$ | 0.34 | 0.15 |
| 32 | Bornyl acetate | Traces | 1.37 |
| 33 | Aromadendrene | 0.28 | 2.25 |
| 34 | *trans*-Caryophyllene | 0.49 | 0.82 |
| 35 | Terpinene-4-ol | 0.65 | 0.04 |
| 36 | ε-Cadinene isomers | 0.21 | 0.07 |
| 37 | γ-Cadinene isomers | 0.31 | 0.13 |
| 38 | α-Humulene | 0.56 | 0.94 |
| 39 | γ-Amrophene | 0.11 | 0.10 |
| 40 | β-Farnesene | 0.05 | 0.22 |
| 41 | γ-Muurolene | 1.42 | 0.45 |
| 42 | α-Patchoulene | 1.35 | 2.93 |
| 43 | β-Cubebene | 1.48 | 2.53 |
| 44 | γ-Cadinene | 0.21 | 0.31 |
| 45 | γ-Patchoulene | 0.07 | - |
| 46 | α-Muurolene | 2.87 | 1.54 |
| 47 | $\gamma_2$-Cadinene | - | 0.50 |
| 48 | δ-Cadinene | 17.43 | 5.30 |
| 49 | Cubebenene | 0.34 | 0.17 |
| 50 | α-Cadinene | 1.03 | 0.46 |
| 51 | Calamenene | 0.10 | 0.10 |
| 52 | $C_{13}H_{26}O$ | 0.74 | 0.28 |
| 53 | Calacorene | 0.15 | - |
| 54 | Unknown | - | 1.00 |
| 55 | Unknown | 0.05 | - |
| 56 | Unknown | 0.69 | 0.78 |
| 57 | Unknown | 0.15 | 0.13 |
| 58 | $C_{20}H_{38}$ | - | 0.65 |
| 59 | Unknown | - | 0.111 |
| 60 | Unknown | 0.63 | - |
| 61 | Unknown | 0.86 | 2.76 |
| 62 | Nerolidol | 3.04 | 8.14 |
| 63 | Cubenol | 0.90 | 0.59 |
| 64 | Epicubenol | 0.88 | 7.26 |
| 65 | Unknown | 0.07 | 0.04 |
| 66 | Unknown | Traces | 0.06 |
| 67 | Bisabolol oxide | Traces | 0.12 |
| 68 | $C_{21}H_{44}$ | 0.12 | - |
| 69 | *T*-Cadinol | 5.22 | 3.21 |
| 70 | α-Muurlol | 6.97 | 5.67 |

**Table 3 (cont.)**

| No. | Components | Whole plant | Flowers |
|---|---|---|---|
| 71 | *T*-Muurlol | 1.69 | 1.06 |
| 72 | Eudesmol | 0.30 | 7.83 |
| 73 | α-Cadinol | 24.76 | 20.09 |
| 74 | $C_{23}H_{48}$ | 0.18 | 0.14 |
| 75 | $nC_{25}H_{52}$ | 0.63 | 0.10 |
| 76 | $nC_{27}H_{56}$ | 0.88 | 0.16 |

ene synthase (PSY), yields the first $C_{40}$ carotenoid, phytoene. In tomato, two different types of PSYs (Psy-1 and Psy-2) are expressed in an organ-specific manner (Fraser *et al*. 1999). *Psy-1* encodes a fruit- and flower-specific isoform and is responsible for carotenogenesis in chromoplasts. In green tissues, *Psy-2*, which is homologous to *Psy-1* but highly divergent from it, is predominantly expressed, and makes a major contribution to carotenogenesis in chloroplasts. Conjugated double bonds are subsequently added by two structurally similar enzymes, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS). These desaturation reactions yield the intermediates phytofluene, ζ-carotene, neurosporene and lycopene, containing 5, 7, 9 and 11 conjugated double bonds, respectively. Increasing the number of conjugated double bonds shifts the absorption towards longer wavelengths, resulting in colorless phytoene and phytofluene, pale-yellow ζ-carotene, orange–yellow neurosporene and red lycopene. During desaturation, several reaction intermediates with a *cis*-configuration are produced. Conversion of a *cis*- to a *trans*-configuration to form all-*trans*-lycopene is carried out by carotenoid isomerase (CRTISO), which has been identified in tomato (Isaacson *et al*. 2002) and *Arabidopsis* (Park *et al*. 2002). CRTISO is specific for adjacent double bonds at the 7,9 and 7',9'-positions, and converts 7,9,9'-tri-*cis*-neurosporene and 7',9'-di-*cis*-lycopene, the products of ZDS, to 9'-*cis*-neurosporene and all-*trans*-lycopene, respectively. Li *et al*. (2007) reported a second carotenoid isomerase (termed *Z*-ISO) in maize that converts the 15-*cis*-bond in 9,15,9'-tri-*cis*-ζ-carotene, the product of PDS, to 9,9'-di-*cis*-ζ-carotene, the substrate of ZDS. The cyclization of lycopene is a branch point in the pathway, catalyzed by lycopene b-cyclase (LCYB) and lycopene β-cyclase (LCYE). Because LCYE in most plants adds only one ε-ring to lycopene (Cunningham *et al*. 1996; Cunningham and Gantt 2001), the pathway in plants typically proceeds only along branches, leading to carotenoids with one β- and one ε-ring (α-carotene and its derivatives) or two β-rings (β-carotene and its derivatives). Lycopene ε-cyclase in romaine lettuce (*Lactuca sativa*) has the ability to add two ε-rings to lycopene and yields a bicyclic ε-carotene, lactucaxanthin (Cunningham and Gantt 2001). A single amino acid residue (457th Histidine) is important to form bicyclic ε-carotene. β- and α-carotenes are further modified by hydroxylation or epoxidation, providing a variety of structural features. The oxygenated derivatives of carotene are called xanthophylls. Hydroxylation of the β- and ε-rings is catalyzed by b-hydroxylase (CHYB) and ε-hydroxylase (CHYE), respectively. CHYB is a non-heme di-iron monooxygenase, while CHYE is a P450, CYP97C1 (Tian *et al*. 2004). CHYB is a well studied enzyme that has been cloned and characterized from many organisms, while CHYE has been identified only in *Arabidopsis*. A flower-specific CHYB (CrtR-$b_2$) was recently identified in tomato (Galpaz *et al*. 2006). Taken together with the existence of flower- and fruit-specific PSY, GGPS and LCYB, this finding supports the hypothesis that there is a chromoplast-specific carotenoid biosynthesis pathway. Epoxidation at positions C5, 6 and C5', 6' of the β-ring of zeaxanthin, catalyzed by zeaxanthin epoxidase (ZEP), yields violaxanthin. Violaxanthin is converted to neoxanthin by neoxanthin synthase (NSY). Both 9-*cis*-violaxanthin and 9-*cis*-neoxanthin are cleaved to xanthoxin (C15) by 9-*cis*-epoxycarotenoid dioxygenase (NCED), and then converted to ABA via the ABA aldehyde intermediate (Nambara and Marion-Poll 2005).

**Regulation of carotenoid biosynthesis and accumulation of carotenoids:** Plant tissues, in particular flower petals and fruits, have a wide variety of carotenoid contents, ranging from little or none to large amounts even within the same plant species. There is increasing evidence that carotenegenesis in plant tissues is predominantly regulated at the transcriptional level (Sandmann *et al*. 2006). In marigold, the differences in petal color from pale-yellow to orange–red are caused by the different levels of accumulation of the yellow carotenoid lutein. Moehs *et al*. (2001) demonstrated that a higher level of PSY and 1-deoxy-D-xylulose-5-phosphate synthase might be responsible for the color development from pale-yellow to orange. It has also been demonstrated that PSY is a rate-limiting enzyme of carotenoid biosynthesis in canola (*Brassica napus*) seeds (Shewmaker *et al*. 1999) and tomato fruits (Fraser *et al*. 1994).

The successful isolation of genes for carotenoid biosynthesis will allow identification of the key regulatory steps of carotenoid biosynthesis. Nevertheless, knowledge on the molecular aspects that regulate the pathway is still limited. Recently, the genes responsible for *hp1* and *hp2* (mutations conferring a high level of carotenoids) have been shown to encode the proteins UV-DAMAGED DNA-BINDING PROTEIN 1 (DDB1) and DEETIOLATED 1 (DET1), components that are involved in the light-signal transduction pathway (Liu *et al*. 2004). In addition, other light-signaling components, such as HY5 and COP1, have been shown to antagonistically regulate the carotenoid level in tomato fruits (Liu *et al*. 2004; Davuluri *et al*. 2005). In petals of the *Mimulus* species, a single QTL at the YUP locus controls the presence and absence of carotenoids (Bradshaw and Schemske 2003). It is interesting to note that the observed changes of pollinator preference associated with YUP alleles in *Mimulus* are comparable to those associated with AN2 alleles in petunia (Hoballah *et al*. 2007) as described above, although the identity of the YUP locus remains to be elucidated.

The amount of carotenoids in the tissues is not attributed solely to the ability to synthesize carotenoids. Some plant tissues have the capacity to synthesize carotenoids but contain only a trace amount of carotenoids. The mechanism that controls carotenoid accumulation is largely unknown. Recently, two different regulatory mechanisms were postulated. One is focused on carotenoid degradation, and the other is focused on sink capacity. In the case of chrysanthemum petals, there was no significant difference in the expression levels of the carotenogenic genes between the white and yellow petals of chrysanthemums (Kishimoto and Ohmiya 2006). However, a gene encoding carotenoid cleavage dioxygenase (CmCCD4a) was specifically expressed in white petals (Kishimoto and Ohmiya 2006). Suppression of CmCCD4a expression resulted in a change in the petal color from white to yellow, indicating that normally white petals synthesize carotenoids but immediately degrade them into colorless compounds. The importance of sink capacity for carotenoid accumulation was first demonstrated in cauliflower (*Brassica oleracea* var. *botrytis*) Orange (*OR*) mutant. *OR* is a gain-of-function mutation, and single-locus *OR* mutation confers a high level of β-carotene accumulation in tissues where accumulation of carotenoid is normally repressed (Li *et al*. 2001). The *Or* gene encodes a plastid-associated protein with a cysteine-rich domain similar to that found in DnaJ-like molecular chaperones (Lu *et al*. 2006). This protein plays an important role in triggering differentiation of proplastids and/or other non-colored plastids into chromoplasts, which in turn act as a metabolic sink for carotenoids. Transformation of the *OR* gene into wild-type cauliflower (*OR*) converts the white colour curd tissue into an orange color with increased levels of β-carotene.

**Carotenoids in marigold:** *C. officinalis* accumulates large amounts of carotenoids in its inflorescences (**Table 4**). The yellow-to-orange color of inflorescences is mostly due to carotenoids and the shade is dependent on pigments content and profile. The carotenoid content and profile in four sel-

**Table 4** Carotenoid composition of *Calendula officinalis* Linn. petals. (Compiled using data from Pintea *et al*. 2003; Kishimoto *et al*. 2005).

| Carotenoid constituents | % of total carotenoids |
|---|---|
| (8'*R*)-Luteoxanthin | 11.0 |
| Lutein-5,6-epoxide | 1.6 |
| Flavoxanthin | 28.5 |
| (8*R*,8'*R*)-Auroxanthin | 7.1 |
| (9'*Z*)-Lutein-5,6-epoxide | 5.0 |
| Lutein | 2.0 |
| Antheraxanthin | 1.0 |
| (9*Z*)-Lutein | 0.6 |
| (5'*Z*,9'*Z*)-Rubixanthin (5) | 4.0 |
| α-Carotene | 0.8 |
| β-Carotene | 3.4 |
| (5'*Z*)-Rubixanthin (6) | 3.0 |
| δ-Carotene | 1.4 |
| (5*Z*,9*Z*, 5'*Z*,9'*Z*)-Lycopene (3) | 4.1 |
| γ-Carotene | 2.0 |
| (5'*Z*)-γ-Carotene (4) | 4.4 |
| (5*Z*,9*Z*, 5'*Z*)-Lycopene (3) | 3.5 |
| (5*Z*,9*Z*)-Lycopene (1) | 4.1 |
| (all-*E*)-Lycopene | 8.7 |

ected varieties of *Calendula* ('Double Esterel Orange', 'Radio Extra Selected', 'Bonbon Abricot' and 'Double Esterel Jaune') were investigated (**Table 5**). The total carotenoid content was evaluated spectrophotometrically and pigments were separated using chromatographic methods (CC, TLC, and HPLC). An HPLC gradient system with a Nucleosil $C_{18}$ column and a Waters PDA detector was used for the separation and identification of carotenoids. The carotenoid content was higher in orange varieties: 276 mg/100 g fresh flowers for 'Double Esterel Orange' and 111 mg/100 g fresh flowers for 'Radio'. All varieties contained the same pigments but there were significant differences in the ratio between individual pigments. 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'. The main pigments identified were flavoxanthin, lutein, rubixanthin, β-carotene, γ-carotene and lycopene (Pintea 2003). The methanolic extract of leaves, petals and pollen of *C. officinalis* flowers showed different types of carotenoids (Bako *et al*. 2002). The carotenoids found in the pollen and petals were neoxanthin, 9Z-neoxanthin, violaxanthin, luteoxanthin, auroxanthin, 9Z-violaxanthin, flavoxanthin, mutatoxanthin, 9Z-anthroxanthin, lutein, 9/9A-lutein, 13/13Z-lutein, α-cryptoxanthin, β-cryptoxanthin, z-cryptoxanthin, lycopene, α-carotene, and β-carotene. Total carotenoids (mg/g DW) were 7.71% for petals and 1.61% for pollen. Reported carotenoid composition of the leaves and stems were neoxanthin, 9Z-neoxanthin, violaxanthin, luteoxanthin, 9Z-violaxanthin, 13Z-violaxanthin, antheraxanthin, mutatoxanthin epimer 1, mutatoxanthin epimer 2, lutein, 9/9-2-lutein, α-cryptoxanthin, β-cryptoxanthin, β-carotene. Total carotenoids (mg/g DW) for the leaves was 0.85% and for stems 0.18% (Goodwin 1954; Bako *et al*. 2002). In the petals and pollen, the main carotenoids were flavoxanthin and auroxanthin while the stem and leaves mostly contained lutein and hcarotene (Bako *et al*. 2002). Nineteen carotenoids were identified in the petal extracts of orange and yellow-flowered *Calendula*. Ten carotenoids were unique to orange flowered cultivars. The UV-vis absorption maxima of these 10 carotenoids were at a longer wavelength than that of flavoxanthin, the main carotenoids of *Calendula* petals; these carotenoids are responsible for the orange color of the petals. Six carotenoids had a *cis* structure at C5, and it is conceivable that these (5Z)-carotenoids are enzymaticaly isomerized at C-5 in a pathway that diverges from the main carotenoid biosynthesis pathway. Among them, (5Z, 9Z)-lycopene (1), (5Z, 9Z, 5'Z, 9'Z)-lycopene (3), (5Z)-γ-lycopene (4), and (5Z, 9Z)-rubixanthin (5) had never before been identified. Additionally, (5Z, 9Z, 5'Z)-lycopene (2) had only been reported as a synthesized compound (Kishimoto *et al*. 2005).

### *2. Flavonoids*

Various flavonoids have been isolated from the ethanolic extract of the inflorescences of *C. officinalis* include quercetin, isorhamnetin (Kurkin and Sharova 2007), isoquercetin, isorhamnetin-3-*O*-β-D-glycoside, narcissin, calendoflaside (Vidal-Ollivier 1989), calendoflavoside, calendoflavobioside, rutin, isoquercitrin, neohesperidoside, isorhamnetin-3-oneohesperidoside, isorhamnetin-3-*O*-$2^G$-rhamnosyl rutinoside, isorhamnetin-3-orutinoside, quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside (Ukiya 2006). Isorhamnetin (3'-metoxi-4', 3, 5, 7-tetrahydroxyflavone), isorhamnetin-3-*O*-glucoside, rutin, quercetin-glucoside, quercetin-neohesperoside, quercetin-$2^G$-rhamnosil-rutinoside) (Albulescu *et al*. 2004). Total flavonoid contents in the inflorescences of different varieties of *C. officinalis* were investigated. The commercial seeds (20 samples) of varieties of *C. officinalis* originating from eight European countries were cultivated in home gardens in two different counties of Estonia. Total flavonoid contents, determined spectrophotometrically (λ = 370 nm), varied from each other by more than three times (0.21-0.68%) in the investigated varieties. The variety with the highest flavonoid content was 'Kablouna', was produced by the Finnish company Siemen (0.68%). Other varieties with high flavonoid content, such as 'Touch of Red' produced by the Latvian company Kurzemes Seklas (0.55%), 'Golden Emperor' produced by the Finnish company Suvipiha (0.50%), 'Pomyk' from Poland (0.50%), etc., may also be preferred for cultivation as natural sources, as they are also rich in flavonoids.

**Table 5** Comparative of colors and carotenoid composition (%) in inflorescences of *Calendula officinalis* Linn. (Compiled using data from Pintea *et al.* 2003; Kishimoto *et al.* 2005).

| Characters | | Varieties | | | |
|---|---|---|---|---|---|
| | | **Bonbon Abricot** | **Double Esterel Jaune** | **Radio Extra Selected** | **Double Esterel Orange** |
| Color | | Yellow-orange | Lemon yellow | Orange | Dark orange |
| Carotenoid (mg/100 g fresh flowers) | | 48.2 | 97.0 | 111.8 | 276.0 |
| Carotenoid constituents | Neoxanthin | 2.84 | 1.74 | 1.71 | 0.92 |
| | Luteoxanthin + Auro | 15.43 | 18.97 | 11.3 | 8.90 |
| | Antheraxanthin | 4.56 | 6.83 | 4.31 | 2.09 |
| | Flavoxanthin | 35.42 | 42.05 | 17.4 | 14.1 |
| | Mutatoxanthin | 2.17 | - | - | 0.38 |
| | Lactucaxanthin | - | 11.31 | 8.02 | 4.49 |
| | Lutein | 8.27 | 12.29 | 11.38 | 9.18 |
| | Zeaxanthin | - | 0.15 | 0.23 | 0.11 |
| | Rubixanthin | 4.58 | - | 7.27 | 14.36 |
| | Lycopene | 0.57 | - | 5.00 | 14.03 |
| | γ-Carotene | 5.11 | - | 6.15 | 12.15 |
| | α-Carotene | 1.89 | 0.2 | 1.15 | 0.98 |
| | β-Carotene | 10.31 | 2.37 | 17.51 | 16.68 |

The amount of total flavonoids depends on the variety and/or the place and time of cultivation. There appeared to be no conclusive relationship between the total flavonoid content and the colour of ligulate and tubular florets of *C. officinalis* (Raal and Kirsipuu 2011).

### *3. Terpenoids*

Various terpenoids have been reported from the petroleum ether extract of *C. officinalis* flowers. They include sitosterols, stigmasterols (Adler *et al*. 1975), diesters of diols (Wilkomirski and Kasprzyk 1979), 3-monoesters of taraxasterol, Ψ-taraxasterol, lupeol (Wilkomirski and Pentacyclic 1985; Zittwel-Eglseer *et al*. 1985), erythrodiol, brein (Wojciechowski *et al*. 1972; Kasprzyk and Wilkomirski 1973), ursadiol (Sliwowski *et al*. 1973), faradiol-3-*O*-palmitate, faradiol-3-*O*-myristate, faradiol-3-*O*-laurate (Eitterl-Eglseer 2001), arnidiol-3-*O*-palmitate, arnidiol-3-*O*-myristate, arnidiol-3-*O*-laurate, calenduladiol-3-opalmitate, calenduladiol-3-*O*-myristate (Neukiron *et al*. 2004; Ukiya *et al*. 2006), oleanolic acid saponins: calenduloside AH (Vecherko *et al*. 1969, 1971, 1974, 1975), oleanane triterpene glycoside: calendulaglycoside A, calendulaglycoside A6'-*O*-*n*-methyl ester, calendulaglycoside A6'- *O*-*n*-butyl ester, calendulaglycoside B, calendulaglycoside B 6'*O*-*n*-butyl ester, calendulaglycoside C, calendulaglycoside C 6'-*O*-*n*-methyl ester, calendulaglycoside C 6' -*O*-*n*-butyl ester, calenduloside F6'-*O*-*n*-butyl ester, calnduloside G6'-*O*-*n*-methyl ester (Ukiya *et al*. 2006), glucosides of oleanolic acid (mainly found in roots of grown and senescing plants) (Wojciechowski *et al*. 1971; Ruszkowski *et al*. 2003), and glucuronides (mainly found in flowers and green parts) (Vidal-Ollivier and Balansard 1989). Faradiol 3-*O*-laurate, palmitate and myristate, are the major triterpenoid esters in the flower heads of *C. officinalis* (Hamburge *et al*. 2003). The ligulate flowers contain triterpene saponins, and triterpene alcohols (Hansel *et al*. 1992; Isaac 1992). Cornulacic acid acetate, new triterpenic ester of olanane series, was isolated from flowers (Naved 2005).

### *4. Coumarins*

The ethanolic extract of the inflorescence of *C. officinalis* contain the coumarins, scopoletin, umbelliferone and esculetin (Kerkach 1986).

### *5. Quinones*

The quinones reported from *C. officinalis* were plastoquinone, phylloquinone, α-tocopherol in the chloroplast, ubiquinone, phylloquinone, α-tocopherol in mitochondria, and phylloquinone in the leaves (Janiszowska 1976).

### *6. Amino acids*

The ethanolic extract of the flowers of the plants showed the presence of 15 amino acids in free form: alanine, arginine, aspartic acid, aspargine, valine, histidine, glutamic acid, leucine, lysine, proline, serine, tyrosine, threonine, methionine and phenylalanine. Amino acid content of the leaves is about 5%, stems 3.5% and flowers 4.5% (Abajova 1994).

### *7. Carbohydrates*

The ethanolic extract of the inflorescences showed the presence of polysaccharides, PS-I, -II, and -III having a (1→3)-β-D-galactam backbone with short side chains at C-6 comprising α-araban-(1→3)-araban and α-L-rhamnan-(1-→3)-araban along with monosaccharides (Wagner *et al*. 1985; Varlijen 1989).

### *8. Lipids*

The lipids in the petroleum ether extract of the seeds, leaves and flowers of *C. officinalis* have been analyzed. The amount of neutral lipids in the seeds was 15.7%, phospholipids 0.6% and glycolipids 0.9%. Fatty acids of monols, sterol esters, 3-monoesters, 3- monoester diols reported in flowers were lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acid. The fatty acids of marigold seeds contain about 59% of an 18:3 conjugated trienic (*trans*-8, *trans*-10, *cis*-12) acid and about 5% of 9-hydroxy-18:2 (*trans*- 9, *cis*-11) acid - dimorphecolic acid (Vlchenko 1998; Wilkomirski and Kasprzyk 1979). One oxygenated fatty acid also reported from the seed oil of *C. officinalis* was D-(+)-9-hydroxy-10, 12-octadecadienoic acid (Badami and Morris 1965).

### *9. Other constituents*

Other phytochemicals include the bitter constituent, loliolide (calendin) (Willuhn and Westhaus 1987), calendulin (Fleisonner 1985) and *n*-paraffins (Fleisonne 1971).

## BIOLOGICAL ACTIVITIES

Trends in research on *C. officinalis* L. saponins performed in the Department of Plant Biochemistry at Warsaw University were reviewed by Szakiel *et al*. (2005). *C. officinalis* contains significant amounts of oleanane saponins, which form two distinct series of related compounds, the "glucosides" and "glucuronides", according to the structure of the respective precursor. Both series differ in the pathway of their biosynthesis and further metabolism, i.e. the rate of formation and stages of possible degradation; distribution in the single cell and in the whole plant, including accumulation sites; and the possible physiological role played in the plant according to appropriate biological activities (Szakiel *et al*. 2005). *C. officinalis* extracts have protective and cytotoxic effects. Barajas-Farias *et al*. (2005) reported the dual activity of *C. officinalis* in primary rat hepatocyte cultures treated with *N*-nitrosodiethylamine. At nM concentrations it was anti-genotoxic while at μM concentrations it exhibited genotoxic effects. In those studies, Barajas-Farias *et al*. (2005) tested the activity of *C. officinalis in vivo* in male Fischer 344 rats initiated with *N*-nitrosodiethylamine, promoted with 2-acetylaminofluorene, and 70% partially heaptectomized. Liver γ-glutamyltranspeptidase positively altered hepatocyte foci 25 days after initiation. The protective effect of *C. officinalis* started at 0.1 mg/kg, increased at 0.5 mg/kg and reached a 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. At 5 mg/kg the number and area of altered hepatocyte foci were still lower, but almost reached the figures of carcinogen-treated rats. Extract at 10 and 20 mg/kg produced a notorious increment in the area and number of altered hepatic foci, and at 40 mg/kg the increment was 40 and 53%, respectively. Additionally, when 2-acetylaminofluorene was substituted by 40 mg/kg of *C. officinalis* extract, a promoting effect was observed with 175 and 266% increments in area and number of altered hepatocyte foci, respectively with respect to controls. When *N*-nitrosodiethylamine was substituted by 40 mg/kg of extract, the latter did not show initiator activity. In summary, the authors showed a protecting activity of *C. officinalis* at low doses, but doses above 10 mg/kg increased altered hepatocyte foci. This dual effect is an example of the phenomenon of hormesis. Furthermore, 40 mg/kg of dry weight of extract administered instead of 2-acetylaminofluorene induced a clear promoting activity. These *in vivo* results are similar and consistent with those reported in primary rat liver cell cultures (Barajas-Farias *et al*. 2005).

Pharmacological studies have confirmed that *C. officinalis* exhibits a broad range of biological effects, some of which are very interesting for possible future development.

## Anti-inflammatory and antioedematous

The ethyl acetate soluble fraction of the methanolic extract of *C. officinalis* flowers exhibited the most potent inhibition (84%) of 12-otetradecanoyl phorbol-13-acetate (TPA)-induced inflammation (1 μg $ear^{-1}$) in mice with an $ID_{50}$ value of 0.05-0.20 mg/ear compared with indomethacin as the reference drug. Furthermore, activity-guided isolation showed that its activity was mainly due to oleanane-type triterpene glycoside (Ukiya *et al*. 2006). A dose of 1200 μg/ear of an aqueous-ethanolic extract showed 20% inhibition in croton oil-induced mouse oedema. The activity was attributed to the presence of triterpenoids, the three most active compounds of which were the esters of faradiol-3-myristic acid, faradiol-3-palmitic acid and 4-taraxasterol (Della 1990; Della *et al*. 1994). The dichloromethane extract of the plant's flower heads inhibited croton oil-induced oedema, and further isolation showed that the esters of faradiol-myristic acid, faradiol-palmitic acid and taraxasterol had antioedematous activity with an oedema inhibition of nearly 50% at a dose of 240 μg/cm. Furthermore, when the doses of these two faradiol esters were doubled, oedema inhibition increased to 65 and 66%, respectively, without any synergism between them (Zitterl-Eglseer 1997). A cream containing calendula extract has been reported to be effective in dextran and burn oedemas as well as in acute lymphoedema in rats. Activity against lymphoedema was primarily attributed to enhanced macrophage proteolytic activity (Casley-Smith 1983).

## Antioxidant

The contents of biological activity compounds were measured in extracts of *C. officinalis* and this antioxidant activity *in vitro* of an extract containing lipophylic and middle polarity compounds possessed better antioxidant activity than water and 50% ethanolic extracts (Lubsandorzhieva 2009). *C. officinalis* plants are rich in flavonoids, especially aglycons and glycosides of flavonols (isorhamnetin, quercetin), saponosides, lipids (including sterols and carotenoids), organic acids and saccharides. Thus, the extraction solvent is very important to obtain extracts rich in powerful antioxidant compounds, particularly in flavonoids (Saleem and Zaka 1986) since the solvent influences the extract composition and content of the compounds in the extract. A 70% methanolic extract of the plant was successively extracted with ether, chloroform, ethyl acetate and *n*-butanol leaving a residual aqueous extract which was assayed for antioxidant activity by liposomal lipid peroxidation-induced $Fe^{2+}$ and ascorbic acid. Ether, butanolic and aqueous extracts, all containing flavonoids, showed antioxidant activity (Popovic *et al*. 1999). Propylene glycol extracts of the petals and flower heads, assayed for antioxidant activity by lipid peroxidation, indicate that the petal extract was more potent than the flower head extract, based on analysis of plasma and urine malondialdehyde (Frankic *et al*. 2008). The *C. officinalis* extract exerted anti-ROS and anti-RNS activity in a concentration-dependent manner, with significant effects being observed even at 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). Another study confirmed these findings: 0.20 μg/ml, being the lowest concentration of *C. officinalis* extract, significantly reduced 2, 2-diphenyl-1-picrylhydrazyl. These findings are interesting for improving the antioxidant network and restoring the redox balance in human cells with plant-derived molecules as well as extending the possibility of antagonizing the oxidative stress generated in living organisms when the balance is in favor of free radicals as a result of the depletion of cell antioxidants (Braga *et al*. 2009). The phytochemical constituents of *C. officinalis* include flavonoids like lupeol, quercetin, protocatechuic acid, etc., many alkaloids and triterpenoids (Matysik *et al*. 2005).

**Table 6** Comparison of growth characters, phenolic compounds and antioxidant activity of *Calendula officinalis* Linn. cultivated under organic farming and chemical fertilizers and solarization. (Compiled using data from Khalil *et al*. 2006, 2007).

| Characters | | Chemical fertilizers | Organic farming |
|---|---|---|---|
| Growth characters | Plant height (cm) | 75.1 | 90.0 |
| | Fresh weight (g $plant^{-1}$) | 101.3 | 133.7 |
| | Dry weight (g $plant^{-1}$) | 32.4 | 36.5 |
| Phenolic compounds (mg/l00 g D.W.) | Pyrogallic acid | 6.3 | 11.88 |
| | Hydroquinone | 42.5 | 63.55 |
| | Resorcinol | 35.7 | 55.48 |
| | Protochatechenic acid | 0.65 | 1.16 |
| | Catechol | - | - |
| | Hydroxyl benzoic acid | 2.63 | 5.01 |
| | Chlorogenic acid | 3.25 | 6.25 |
| | Phenol | 22.6 | 34.01 |
| | Vanillin | - | - |
| | *p*-Coumaric acid | 7.65 | 11.88 |
| | Ferulic acid | 4.69 | 10.58 |
| | Salicylic acid | 122.8 | 181.43 |
| | *O*-Coumaric acid | 22.9 | 38.55 |
| | Coumarin | 1.2 | 3.53 |
| | Cinnamic acid | 0.95 | 2.00 |
| Antioxidant activity of ethanol extracted | Inhibition of peroxidation (%) | 46.8 | 63.11 |

Flavoxanthin, luteoxanthin, lycopene, auroxanthin, lutein, β-carotene and phenolic compounds (Khalil *et al*. 2006) (**Table 6**) are the major carotenoids present in this flower (Kishimoto *et al*. 2005; Preethi and Ramadasan 2008). Most of these constituents are reported as free radical scavengers and enhance wound healing by producing artificial cross linkage (Kuppast and Nayak 2006).

## Immunostimulant

The polysaccharide (PS) fraction of *C. officinalis* showed immunostimulant activity, based on the *in vitro* granulocyte test. PS-III showed the highest phagocytosis (54-100%) at a concentration of $10^{-5}$-$10^{-6}$ mg/mL, while PS-I and PS-II exhibited 40-57 and 20-30% phagocytosis, respectively (Wagner *et al*. 1985; Varlijen 1989).

## Anticancer, lymphocyte and dual

The ethyl acetate-soluble fraction of the methanolic extract of *C. officinalis* flowers showed cytotoxic activity *in vitro* (Ukiya *et al*. 2006). Further activity-guided isolation of that fraction showed two main active compounds. Firstly, calenduloside F6'-*O*-*n*-butyl ester, which is active against leukaemia (MOLT-4 and RPMI 8226), colon cancer (HCC-2998) and melanoma (LOXIMVI, SK-MEL-5 and UACC- 62), cell lines with $GI_{50}$ values of 0.77-0.99 μmole, except for leukaemia (CCRF-CEM, $GI_{50}$ = 23.1 μmole), renal cancer (AK-1, 17.2 μmole; UO-31, 12.7 μmole) and breast cancer (NCI/ADR-RES, > 50 μmole) cell lines. The second active compound is calenduloside G6'-*O*-methyl ester, which is active against all the cancer cell lines mentioned for compound 1 with a $GI_{50}$ of 20 μmole except for ovarian cancer (IGROVI, $GI_{50}$ = 20.1 μmole) and renal cancer (VO-31, 33.3 μmole) cell lines (Ukiya *et al*. 2006). Aqueous laser-activated calendula extract (LACE) from flowers showed potent *in vitro* inhibition of tumour cell proliferation when assayed against a wide variety of human and murine tumour cell lines. The inhibition ranged from 70-100% with an $IC_{50}$ of 60 μg/mL. The inhibition mechanisms were identified as cell cycle arrest in the G0/G1 phase and as caspase-3-induced apoptosis. On the other hand, when LACE was assayed against human peripheral blood lymphocyte (PBLs) and human natural killer cell lines (NKL) it showed *in vitro* induction of proliferation and activation of these cells, mainly blymphocytes, CD4+, T lymphocytes and NKT lymphocyte (Medina *et al*. 2006). Various extracts of the leaves,

**Table 7** Antifungal activities of the essential oil of flowers of *Calendula officinalis* Linn. (Compiled using data from Gazim *et al.* 2008; Roopashree *et al.* 2008).

| Isolate | Microorganisms | Origin* | Mean zone of inhibition a (mm) | |
|---|---|---|---|---|
| | | | Calendula oil1 (5 µl/disc) | Nystatin (20 µg/disc) |
| 1 | *Candida albicans* | ATCC 64548 | 16 | 12 |
| 2 | *C. albicans* | Orotracheal tube | 11 | 13 |
| 3 | *C. albicans* | OC – HIV | 26 | 11 |
| 4 | *C. albicans* | VVC | 18 | 12 |
| 5 | *C. albicans* | VVC | 15 | 12 |
| 6 | *C. albicans* | VVC | 15 | 12 |
| 7 | *C. albicans* | Urine | 27 | 11 |
| 8 | *C. dubliniensis* | ATCC 777 | 24 | 11 |
| 9 | *C. parapsilosis* | ATCC 22019 | 20 | 12 |
| 10 | *C. parapsilosis* | Onychomycosis | 14 | 13 |
| 11 | *C. parapsilosis* | Paronychia | 30 | 11 |
| 12 | *C. parapsilosis* | Blood | 30 | 11 |
| 13 | *C. glabrata* | ATCC 90030 | 15 | 12 |
| 14 | *C. glabrata* | Hands colonization | 23 | 11 |
| 15 | *C. glabrata* | Hands colonization | 28 | 11 |
| 16 | *C. tropicalis* | Urine | 11 | 13 |
| 17 | *C. tropicalis* | Granulomatous lesion | 15 | 12 |
| 18 | *C. tropicalis* | Urine | 21 | 12 |
| 19 | *C. tropicalis* | Urine | 22 | 11 |
| 20 | *C. guilliermondii* | Hands colonization | 25 | 11 |
| 21 | *C. guilliermondii* | Hands colonization | 24 | 11 |
| 22 | *C. krusei* | ATCC 6258 | 15 | 12 |
| 23 | *Rhodotorulla* sp. | Hands colonization | 30 | 11 |

* Except to ATCC microorganisms all of others are human clinical isolates OC – HIV: oral candidiasis; VVC: vulvovaginal candidiasis. Mean of inhibition zone by oil of flowers of *Calendula officinalis:* Good activity (11 -18 mm); high activity (20-27 mm); highest activity (28-30 mm).

flowers and whole plant were cytotoxic to MRC5, HeP2, and ascetic cells from Ehrlich carcinoma. The saponin-rich fraction of these extracts displayed antitumoural activity *in vivo* in the Ehrlich mouse carcinoma model (Boucard-Maitre 1988).

LACE extract demonstrated *in vitro* growth inhibition of various tumor cell lines caused by the induction of cell cycle arrest and apoptosis. In contrast, it induced proliferation and activation of PBL cells. In addition, LACE showed anti-tumor activity *in vivo* in nude mice (Medina *et al*. 2006).

## Wound healing

The ethanolic extract of the plant's flowers was investigated against experimentally induced thermal burns in rats. Among the various extract doses (20, 100, and 200 mg/kg of body weight), the 200 mg/kg dose showed significant improvement in healing wounds as indicated by an increase in collagenhydroxyproline and hexosamine contents. The level of acute phase proteins (heptaglobin, orosomycid) and tis-sue damage marker enzymes (alkaline phosphatase, alanine and aspartate transaminase) decreased significantly. The decrease in lipid peroxidation might be due to its antioxidant property (Chandran and Kutton 2008). The daily application of 2% calendula gel resulted in greater wound healing due to its antimicrobial and antioxidant properties (Leach 2008).

## Hepatoprotective

The hydroalcoholic extract of the flowers, when administered to $CCl_4$-intoxicated livers in albino male Waster rats at a dose of 10 mL/kg, resulted in a reduction of hepatocytolysis by 28.5% due to a reduction in glutamo-oxalate-transaminase (GOT) and glutamo-pyruvate-transaminase (GPT). However, histoenzymology showed a reduction of steatosis of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), cytochromoxidase (Cyox) and $Mg^{2+}$-dependant adenosine triphosphatase (ATPase) (Rasu *et al*. 2005). The hot water extract of *C. officinalis* flowers exhibited anti-hepatoma activity against five human liver cancer cells – HepG2/C3A, SK-HEP-1, HA22T/VGH, Hep3B and PLC/PRF/5 – with an inhibitory effect of 25-26% at a dose of 2000 µg/mL (Lin *et al*. 2002).

## Antibacterial and antifungal

The methanol extract and 10% decoction of the plant's flowers were assessed for their activity against anaerobic and facultative aerobic periodontal bacteria, namely, *Porphyromonos gingivalis*, *Prevotella* spp., *Furobacterium nucleatum*, *Caphocytophaga gingivalis*, *Veilonella parvula*, *Eikenella corrodens*, *Peptostreptococcus micros* and *Actinomyces odontolyticus* (**Tables 7, 8**). The results showed marked inhibition against all tested microorganisms with a MIC ≥ 2048 mg/L (Iauk 2003). When the VO of the flowers was tested (using the disc diffusion technique) against various fungal strains, namely, *Candida albicans* (ATCC64548), *Candida dubliniensis* (ATCC777), *Candida parapsilosis* (ATCC22019), *Candida glabrata* (ATCC90030), *Candida krusei* (ATCC6258), and yeast isolated from humans, viz, *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, *Candida guilliermondii*, *Candida krusei* and *Rhodotorella* spp., it showed good antifungal activity (at 15 µl/disc) (Gazim *et al*. 2008). Aqueous extracts of *C. officinalis* flowers exhibited better antibacterial activity than their petroleum ether, methanolic and ethanolic extracts. Among the organisms tested *S. aureus* was more susceptible to the aqueous extracts of *C. officinalis* flowers (Roopashree *et al*. 2008) (**Table 8**). Seven microorganisms were used to study the antimicrobial effects of *C. officinalis* extracts. The bacterial pathogens used were human pathogenic microorganisms, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* sp., *Cogulase* (+) *Staphylococcus* sp., *Cogulase* (-) *Staphylococcus* sp., *Candida albicans* and *Candida parapsilosis*. Extracts of *C. officinalis* leaves, stems, roots and flowers made in *n*-butanol, ethanol and distilled water were tested for their antimicrobial activity. The extracts of all three solvents were effective against most of the microorganisms. Leaves, stems and flowers of *C. officinalis* showed very effective results against pathogenic microorganisms (Mathur 2011). The effects of *C. officinalis* extract and cephalosporin, a cell wall synthesis inhibitor and antibiotic, on bacteria isolated from patients with cellulites was conducted. This experimental study was done on bacteria isolated from 100 patients. *C officinalis* extract was prepared by perculation with 85% methanol. Solvent was vaporized and lyophilized. Antibacterial effects of the herb extract,

**Table 8** Phytoconstituents, minimum inhibitory concentration and antibacterial activity present in different extracts of *Calendula officinalis* Linn. (Compiled using data from Kishimoto *et al.* 2005; Gazim *et al.* 2008; Roopashree *et al.* 2008).

| **Phytoconstituents** | ***Calendula* extract** | | | |
|---|---|---|---|---|
| | **Petroleum ether** | **Methanol** | **Ethanol** | **Aqueous** |
| Alkaloids | - | - | - | - |
| Carbohydrates | - | + | - | + |
| Glycosides | - | + | + | + |
| Saponins | - | + | + | + |
| Triterpenes | + | - | + | + |
| Fats and oils | + | - | - | - |
| Resins | - | - | - | - |
| Phenols | - | - | - | - |
| Tannins | - | - | - | - |
| Flavonoids | - | + | + | + |
| Proteins | - | - | - | - |
| Diterpenes | - | + | + | + |
| **Microorganisms** | **Minimum inhibitory concentration** | | | |
| | **Petroleum ether** | **Methanol (mg/ml)** | **Ethanol (mg/ml)** | **Aqueous (µg/ml)** |
| *Staphyllococcus aureus* | - | 64 | 32 | 125 |
| *Bacillus subtilis* | - | - | 64 | 200 |
| *Pseudomonas aeruginosa* | - | - | 32 | 250 |
| *Escherichia coli* | - | 64 | 164 | 300 |
| **Microorganisms** | **Antibacterial activity of different extracts (diameter of zone of inhibition in mm)** | | | |
| | **Petroleum ether** | **Methanol (mg/ml)** | **Ethanol (mg/ml)** | **Aqueous (µg/ml)** | **Streptomycin** |
| *Staphyllococcus aureus* | - | - | - | 14 | |
| *Bacillus subtilis* | - | - | - | 13 | 28 |
| *Pseudomonas aeruginosa* | - | - | - | 12 | 23 |
| *Escherichia coli* | - | - | - | 11 | 25 |

cefixim, cephalexin, ceftriaxone, ceftizoxime, and cefazolin were evaluated by two methods: disc diffusion and MIC. *Streptococcus pyogenes*, *Enterococcus fecalis*, *Staphylococcus*, MRSA and *Staphylococcus saprophiticus* were susceptible in dilutions of 1:128 of *C. officinalis* extract. In the disc diffusion method, 100% of *Streptococcus*, *Enterococcus fecalis*, MRSA and 50% of *Staphylococcus saprophiticus* were susceptible to *C. officinalis* extract. Gram-negative bacteria, including *E. coli*, *Proteus*, *Klebsiella*, *Serattia*, and *Pseudomonas* were resistant. The diameter of no growth of bacteria among Gram-positive and -negative bacteria was significantly different in *C. officinalis* extract, which has the possibility of being used in combination with antibiotics for the treatment of cellulitis (Eslami *et al.* 2011). The antibacterial and antiparasitic activities of free oleanolic acid and its glucosides and glucuronides isolated from *C. officinalis* were investigated. The MIC of oleanolic acid and the effect on bacterial growth were estimated by $A_{600}$ measurements. Oleanolic acid's influence on bacterial survival and the ability to induce autolysis were measured by counting the number of colony-forming units. Cell morphology and the presence of endospores were observed under electron and light microscopy, respectively. Oleanolic acid inhibited bacterial growth and survival influenced cell morphology and enhanced the autolysis of Gram-positive bacteria suggesting that bacterial envelopes are the target of its activity. On the other hand, glycosides of oleanolic acid inhibited the development of L3 *Heligmosomoides polygyrus* larvae, the infective stage of this intestinal parasitic nematode. In addition, both oleanolic acid and its glycosides reduced the rate of L3 survival during prolonged storage, but only oleanolic acid glucuronides affected nematode infectivity. Those results suggest that oleanolic acid and its glycosides from *C. officinalis* can be considered as potential therapeutic agents (Szakie *et al*. 2005).

Antifungal assay results showed for the first time that the *C. officinalis* VO has good potential antifungal activity: it was effective against all 23 clinical fungi strains tested (Gazim *et al*. 2008).

## Anti-HIV

The dichloromethane-methanolic (1:1) extract of *C. officinalis* flowers exhibited potent anti-HIV activity in an *in vitro* MTT/tetrazolium-based assay. This activity was attributed to inhibition of HIV1-RT at a concentration of 1,000 µg/mL as well as suppression of HIV-mediated fusion at 500 µg/mL (Kalvatchev *et al*. 1997).

## Spasmolytic and spasmogenic (dual)

The aqueous-ethanolic extract of *C. officinalis* flowers, when assayed in rabbit jejunum, caused a dose-dependant (0.03-3.0 mg/mL) relaxation of spontaneous and $K^+$-induced contraction; further fractionation of the extract with dichloromethane showed inhibition of spontaneous contractions in a dose range of 0.01-0.3 mg/mL. This is 10 times more potent than the parent crude extract, and spasmolytic activity was due to a calcium channel blockade (CCB) (Bashir *et al*. 2006). On the other hand, the aqueous fraction of the parent extract exhibited spasmogenic activity in a dose range of 1-10 mg/mL (Bashir *et al*. 2006).

## Genotoxic and antigenotoxic (dual)

The aqueous (AE), aqueous-ethanol (AEE), ethanol and chloroform extracts of *C. officinalis* flowers were evaluated to determine if they caused induction of unscheduled DNA synthesis (UDS) in rat liver culture and reversal of diethylnitrosamine (DEN)-induced UDS. In the UDS test in liver culture, DEN, at a level of 1.25 µmole, produced a maximum increase of 40% $^3$H-thymidine ($^3$HdTT) incorporation while AE and AEE extracts showed complete reversal of the DEN effect at levels of 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 and 3.7-100 µg/mL for AE and AEE, respectively, in rat liver cell culture. Thus these polar extracts (AE and AEE) at low concentrations (i.e., ng/mL range) showed antigenotoxic effect while at high concentrations (i.e., µg/mL range) they exhibited a genotoxic effect (Perez-Carreon *et al*. 2002). The propylene glycol extract of *C. officinalis* also showed an antigenotoxic effect based on an evaluation in young growing pigs which involved the measurement of the excretion of lymphocyte DNA fragmentation and 24 h urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Frankic *et al*. 2008).

**Table 9** Pests and disease (Modified from Braithwaite and Drost 2009)

| Insect | Identification | Control |
|---|---|---|
| Whiteflies | Small white flies that hide and feed on the underside of leaves. | These insect are easily controlled by a hard stream of water or by regular applications of insecticidal soap. |
| Aphids | Green or black soft bodies insects that feed on the underside of the leaves. Aphids produce honeydew and cause crinkled or curled leaves. | |
| **Disease** | **Symptom** | **Control** |
| Powdery mildew | White fungal patches on leaves that can spread to all the plants. Associated with cool, wet weather conditions. | Insure good air circulation, control irrigation and remove infested plant parts. |

### Insecticidal activity

The acetone: methanolic (2: 1, v/v) extract of the flowers showed insecticidal activity when it was tested on milkweedbug (Alexenizor and Dorn 2007). Despite the wealth of pests and diseases plaguing calendula (**Table 9**), suprisingly there are no agents or biocompounds isolated from *Calendula* that have been used in pest control programmes, either in marigold cultures or any other crop species.

### Inhibition of heart rate

The aqueous extract was tested on the heart of male Wistar rats and found to inhibit heart rate contractility by up to 100% at a dose of 0.3 mg/L (Perez-Carreon *et al*. 2002).

### Antiviral

A tincture of the flowers suppressed the replication of herpes simplex, influenza A2 and influenza APR-8 viruses *in vitro* (Silva *et al*. 2007).

### Toxicological studies

The hydroalcoholic extract of *C. officinalis* flowers, based on assessment in rats and mice, did not show acute toxicity following administration of an oral dose of up to 5.0 g/kg. It did not show haematological alterations at doses of 0.025, 0.25, 0.5 and 1.0 g/kg. However, the biochemical parameters, blood urea nitrogen (BUN) and alanine transaminase (ALT) were elevated due to renal and liver overload (Silva *et al*. 2007).

### Contraindication and allergenic effects

The flower extract caused allergy in 9 patients out of 443 (2.03%) when assessed by the patch testing method (Reider 2001). Therefore, it is advisable that persons who have an established allergy to the Asteraceae (daisy) family should use it with caution (Bone 2003; Braun and Cohen 2005).

## AGRONOMIC APPROACHES TO IMPROVING PHYTOCHEMICAL YIELD AND QUALITY

One of the most effective ways to improve the phytochemical composition of plants, bit quali- and quantitatively, is through agronomic manipulation.

Selected *Calendula* chemotypes growing in soil or *in vitro*, for example, flowers of the cadinol chemotype, are very important in European and western Asian folk medicines and are used to treat inflammatory conditions (Yoshikawa *et al*. 2001). Distinct subspecies of *C. officinalis* have been reported from various countries (Chalchat *et al*. 1991; Nicoletta *et al*. 2003) *C. officinalis* can be used as a colorant because it primarily contains two classes of pigments, flavonoids and carotenoids, which can be used as yellow and orange natural colors, respectively. Natural colors are gaining considerable attention since several synthetic colorants have given rise to allergic, toxic and carcinogenic effects (Lea 1988). Flavonoids have antioxidant activities which play an important role in food preservation and human health by combating damage caused by oxidizing agents (Meda *et al*. 2005). Carotenoids are important to humans and other animals as precursors of vitamin A and retinoids. In addition, they act as antioxidants, immunoenhancers, inhibitors of mutagenesis and transformation, inhibitors of premalignant lesions, screening pigments in primate fovea, and nonphotochemical fluorescence quenchers (Castenmiller and West 1998).

Crnobarac *et al*. (2011) tried to find the best adapted variety and optimal row distance to maximize flower and petals yield and quality. A trial was conducted in 2006-2007 in Serbia. There were 4 cultivars: 'Bački Petrovac', 'Orange King' (originated from Serbia), 'Plamen' and 'Plamen Plus' (from Czech Republic) and 4 row distances: 40, 50, 60 and 70 cm with a constant 10 cm between plants in a row. Year had a significant effect only on dry flower yield. Variety had very significant effect on fresh and dry petal yield: highest in 'Plamen Plus', lowest in 'Bački Petrovac'. Row distance had a very significant effect on fresh and dry flower and petal yield, the values increasing as distance was widened from 40 to 70 cm. The interaction was very significant only between years and varieties for all traits; in 2006, the best was 'Orange King' and in 2007 it was 'Plamen Plus'. The quality of dried flowers and petals was significantly affected only by variety. The essential oil (EO) content in flowers ranged from 0.11-0.33 ml/100 g in petals and was highest in the Czech variety 'Plamen'. The highest content of total flavonoids (11.92%) and total phenol substances (1.37%) in petals was observed in domestic var. 'Bački Petrovac', while the content in flowers was highest in 'Plamen Plus'. Under Egyptian conditions (**Table 10**), vegetative growth and chemical constituents of Slovakian *C. officinalis* was more promising than French *C. officinalis* and *C. stellata*. Moreover, applying foliar fertilizer (containing 6% Mn, Mg and Cu, in chelated forms) at 4 mg/5L increased growth characters, flower yield and chemical constituents (**Table 11**) (Naguib *et al*. 2005). The effects of sowing date and planting density on grain and flower yield of pot marigold were assessed during an experiment conducted in Iran (Eghatoleslami and Mousavi 2009). In that study, three sowing dates (30 March, 14 April and 30 April) and three plant densities (inter-row planting distance = 10, 20 and 30 cm) were compared. Seed and flower yield were significantly different depending on planting date and planting density. Sowing date had significant effects on flower and seed harvest index (HI). The last sowing date resulted in the highest flower and seed HI. Plant density did not have any significant effect on flower HI, but the effect on seed HI was significant. In total, the result showed that the first sowing date with 25 plants/$m^2$ had the highest grain and flower yield.

Kandeel (2004) reported that gibberellic acid ($GA_3$) at 100 mg/L improved vegetative growth and flowering of *C. officinalis*. Plant height and dry weight (DW) of both *C. officinalis* leaves and flowers were increased when plants were sprayed with 50 ppm $GA_3$ (Hassan *et al*. 1991). Vegetative parameters, aspects of flowering (flowering date, number of flowers/plant and DW of flowers), anatomical properties (especially stalks) and chemical constituents such as chlorophyll (Chl) *a*, *b* and carotenoid contents of flowers and oleanolic acid were altered after the application of $GA_3$, cycocel and humic acid (Azzaz 2007). Plant counts increased from 9 plants/$m^2$ at 3 kg seed/ha, to 26 at 6, 46 at 12, 101 at 24, 179 at 48, and 332 plant/$m^2$ at 96 kg seed/ha. Total flower yield was not significantly different in populations with > 46 plants/$m^2$, but declined with lower plant population densities. The total fresh weight (FW) of fully

**Table 10** Comparative study of *Calendula* varieties under Egyptian solarization conditions. (Modified from Naguib *et al*. 2005).

| *Calendula* varieties | Plant height (cm) | Branches number (plant$^{-1}$) | Flower number (plant$^{-1}$) | Herb fresh weight (g plant$^{-1}$) | Herb dry weight (g plant$^{-1}$) | Volatile oil (%) |
|---|---|---|---|---|---|---|
| French | 54.67 | 12 | 29.33 | 123.33 | 28.12 | 0.079 |
| Slovakian | 69.33 | 15 | 61 | 394.48 | 94.49 | 0.176 |
| *C. stellata* | 50.23 | 10.2 | 25.6 | 211.1 | 50.6 | 0.088 |

**Table 11** Comparative study of *Calendula* characters under foliar spray conditions and solarization. (Compiled using data from Naguib *et al*. 2005; Milad and Mohammed 2009).

| *Calendula* | Plant height (cm) | Branches number (plant$^{-1}$) | Flower number (plant$^{-1}$) | Herb fresh weight (g plant$^{-1}$) | Herb dry weight (g plant$^{-1}$) | Volatile oil (%) | Hydrocarbons compounds | Oxygenated compounds |
|---|---|---|---|---|---|---|---|---|
| Without foliar spray | 38.02 | 7.67 | 15.67 | 218.32 | 44.74 | 0.065 | 28.61 | 64.33 |
| With foliar spray | 54.67 | 12.0 | 29.33 | 394.48 | 94.49 | 0.079 | 31.88 | 74.73 |

**Table 12** Comparison of *Calendula officinalis* L. plants (growth characters and chemical composition) growing with cattle manure and cattle manure + soil solarization. (Compiled using data from Khalid *et al* 2006, 2007).

| Treatments | Growth characters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Branch no (plant$^{-1}$) | Flower number (plant$^{-1}$) | Flower fresh weight (plant$^{-1}$) | Flower dry weight (plant$^{-1}$) | Whole plant fresh weight (plant$^{-1}$) | Whole plant dry weight (plant$^{-1}$) | Seed weight (plant$^{-1}$) |
| Cattle manure | 59.5 | 14.6 | 126.9 | 245.0 | 40.8 | 270.3 | 41.6 | 10.5 |
| Cattle manure+ soil solarization | 67.2 | 17.4 | 141.2 | 272.6 | 36.8 | 289.1 | 47.1 | 11.6 |
| | Chemical composition | | | | | | | |
| | Essential oil (%) | | Total flavonoids | Total carotenoids | N | P | K | |
| | Flowers | Whole plant | (mg g$^{-1}$) | (mg 100 g$^{-1}$) | (%) | | | |
| Cattle manure | 0.19 | 0.11 | 201 | 15.9 | 2.45 | 2.58 | 0.38 | |
| Cattle manure+ soil solarization | 0.20 | 0.13 | 210 | 17.5 | 0.45 | 1.26 | 1.46 | |

opened flower heads collected off each plot increased from 0.5 to 1.4 kg/m$^2$ (650/m$^2$) with increasing plant population. Dried petals made up between 7 and 9% of flower FW. Yields of dry petals/m$^2$ were 36 g at 9 plants/m$^2$. Seed weight/head was 0.78 g at 9 plants/m$^2$ compared to 0.5 g for other plant populations. Seed yield increased with increasing plant population from 128 to 300 g/m$^2$ at 9 plants/m$^2$ (Martin and Deo 2000). Solarized soil, in which soil temperature is increased by using solar radiation as an energy source, with different levels of cattle manure resulted in a significant increase in growth and yield characters, namely plant height, branch number, flower-head number, fresh and DWs of flower head, FWs and DWs of vegetative parts and seed yield (**Table 12**). The interaction between the levels cattle manure and soil solarization increased the chemical composition (VO, total flavonoids, total carotenoids, N, P, K, Fe, Zn and Mn) (Khalid *et al*. 2006). Rahmani et al. (2009) conducted a study on *C. officinalis* in an experimental field in Iran during 2006. N was applied at 30, 60 and 90 kg N/ha and plants were irrigated after 40, 80 and 120 mm water evaporation occurred. Irrigation had a significant effect on seed yield, 1000-seed weight, head diameter and number of seeds/head such that maximum head diameter (25.67 mm), number of seeds/head (31 seeds/head), 1000-seed weight (15.18 g) and seed yield (3044 kg/ha) occurred under irrigation after 40 mm evaporation. N had a significant effect on all plant characteristics and highest 1000-seed weight (12.66 g), seed yield (1998 kg/ha), head diameter (23.96 mm) and number of seeds/head (29.25 seeds/head) after application of 90 kg N/ha. The results of this experiment showed that N increased seed yield and delayed irrigation-reduced seed yield of calendula significantly. Eid and Kasem (2009) studied the effect of phosphorein (phosphate-solubilizing microorganisms) at 0, 1.5 and 3 g/pot and active dry yeast at 0, 2, 4 g/L on plant height, number of branches, number of flowers head/plant, FW and DW of flowers/plant, caroteniods and P content of *C. officinalis* under Egyptian conditions (i.e. hot, dry, arid). Phosphorein at 3 g/pot and 2 g/L dry yeast caused a significant increase in vegetative growth, carotenoid and P contents.

Milad and Mohammed (2009) investigated the effect of foliar spray of two acids (citric and ascorbic) on the growth and chemical composition of marigold plants under Egyptian conditions during two consecutive seasons. All the treatments received a constant dose of ammonium nitrate (33.5% N) at 1 and 0.5 g/pot of potassium sulfate (48% $K_2O$) [recommended] one month after transplanting. Another dose of ammonium nitrate (33.5% N) at 1 g/pot was added one month after the first dose. The plants had been sprayed with citric and ascorbic acids. The treatments were as follows: Control plants sprayed by tap water and treatments of citric acid and ascorbic acid at 75, 150 and 300 mg/L, each individually and in combination. There was a significant increase in plant height, number of branches/plant, Chl in leaves and carotene contents by spraying 75 mg/L citric acid + 75 mg/L ascorbic acid. However, citric acid alone at 75 or 150 mg/L resulted in the highest stem diameter. Citric acid at 75 mg/L + ascorbic acid at 75 or 150 mg/L resulted in a significant increase in the number of leaves/plant, FW and DW of shoots and roots, number of inflorescences/plant, inflorescence diameter, FW and DW of inflorescences in both seasons while spraying ascorbic acid at 300 mg/L or a combination of citric acid (75 mg/L) + ascorbic acid (75 mg/L) lead to the highest total soluble sugar content in leaves. The latter combination was the best treatment for high vegetative and flowering growth as well as a higher content of Chl and carotenes.

The recommended fertilizer amount of N, $P_2O_5$ and $K_2O$ for pot application was 0.4, 0.2 and 0.3 g/kg (each pot filled with 2 kg substrate) to maximize total flowers, functional leaves, plant height and dry mass (Jiang *et al*. 2008). Growth characters, phenolic compounds and antioxidant activities (**Table 6**) increased when organic fertilizers were added compared with chemical fertilizers (Khalil *et al*. 2007). Mahgoub *et al*. (2006) evaluated the use of paclobutrazol (growth regulator) or glutathione (amino acid) at 0, 50, 100 and 150 mg/L on plant growth, flowering and some chemical composition of *C. officinalis* plants grown under Egyptian conditions. The foliar application of paclobutrazol significantly decreased plant height compared with the control treatment while the number of branches, FW and DW of leaves per plant increased. All growth parameters were significantly promoted by foliar application of glutathione, which, at 150 mg/L, gave the highest significant increase in the number of flowers/plant, FW and DW of flowers (g/plant) followed by 100 mg/L paclobutrazol com-

pared with untreated plants. Foliar application of 150 mg/L glutathione resulted in the highest values of Chl *a* and *b*. However, paclobutrazol and glutathione showed no significant increase in carotenoid content. Maximum total carbohydrates (37.64%) were obtained when 150 mg/L glutathione was applied followed by 100 mg/l paclobutrazol (31.42%); total nitrogen % showed the same trend. Arjenaki *et al*. (2011) investigated the effects of priming on seed germination of *C. officinalis* in Iran. Treatments were (control, PEG: polyethylene glycol 6000 at -3, -6 and -12 bar). Marigold seeds were primed for 24 h in treatment solutions at room temperature and then transferred to Petri dishes for germination. Priming significantly improved germination percentage, radicle and shoot length, seedling weight and germination rate, compared to the control. These parameters of seeds primed with PEG (-3 bar) was higher than those for unprimed seeds but seedling weight was statistically similar. Thus, priming with PEG solution could be used as a simple method for improving seed germination of marigold in the laboratory.

## BIOTECHNOLOGICAL APPROACHES TO IMPROVING PHYTOCHEMICAL YIELD AND QUALITY

The capacity to maintain marigold ermplasm *in vitro*, and to manipulate abiotic factors to increase the phytochemcal composition of plants is a key factor for the pharmaceutical improvement of this medicinal plant through biotechnology.

Callus cultures of marigold were induced on Murashige and Skoog (MS) medium with different concentrations of auxin (dichlorophenoxyacetic acid (2,4-D) or indole-3-acetic acid (IAA) and cytokinin (kinetin or 6-(*γ*,*γ* dimethylallylamino) purine (2iP). Of all hormone combinations used in the medium, two were the most efficient in promoting callus development: 1.81 µM (0.4 mg/L) 2,4-D and 1.85 µM (0.4 mg/L) kinetin (0.4d– 0.4k culture) or 0.45 µM (0.1 mg/L) 2,4-D and 2.02 µM (0.5 mg/L) 2iP (0.1d–0.5p culture). These combinations were selected to induce cell suspension cultures. The suspension cultures were maintained under light or dark conditions. Light stimulated cell aggregation in the cultures. In both cultures cells remained undifferentiated in the dark whereas in the light, rhizogenesis was observed in the 0.1d–0.5p culture. Cell growth as well as protein and oleanolic acid contents were determined. Initially, biomass production was similar under light and dark conditions, but 7–8 months after induction, cell growth was reduced by approximately 30% in the light, whereas the growth of cell cultures maintained in the dark did not reveal any changes. The presence of oleanolic acid was detected in the suspension cultures kept in the dark. This compound reached two quantitative peaks: in the lag and stationary phases – beyond the active growth phase of the culture cycle and its concentration was several times higher in 0.1d–0.5p culture than that in 0.4d–0.4k culture. It was for the first time that callus and suspension cultures were induced from marigold (Grzelak and Janiszowska 2002). Hypocotyl, cotyledon and cotyledonary node explants were cultured on MS medium supplemented with various concentrations of thidiazuron (TDZ), kinetin (Kn), α-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) to induce adventitious shoot regeneration and micropropagation. The highest frequency of adventitious shoot regeneration was achieved from hypocotyl and cotyledon explants on MS media supplemented with 0.75 mg/L TDZ and either 0.25 or 0.50 mg/L IBA. Efficient *in vitro* clonal propagation was also induced from cotyledonary nodes on a range of media supplemented with 0.75 mg/L TDZ and 0.05 mg/L NAA or 2 mg/L KIN and 1 mg/L NAA. Regenerated shoots were excised and rooted in MS medium supplemented with 1 mg/L NAA. The rooted plantlets were finally transferred to pots (Cocu *et al*. 2004). The labeling dynamics of sterols and oleanolic acid (OL) during incubation with [2-$^{14}$C] mevalonic acid were examined in *C. officinalis* suspension cultures: 1.81 µM (0.4 mg/L) auxin (2,4-D) and 1.85 µM (0.4 mg/L) Kn, named 0.4-0.4 culture; and 0.45 µM (0.1 mg/L) 2,4-D and 2.02 µM (0.5 mg/L) 2iP, named the 0.1–0.5 culture, maintained under light or dark conditions. The isoprenoid pathway leading to triterpenoids was operative in all cultures tested. Moreover, it was shown that all OL glucosides and glucuronides, characteristic of marigold plant leaves, were synthesized after supplying the cultures with [3-3H] OL. The biosynthesis of OL glycosides as well as their release in the extracellular medium was dependent mainly on hormonal conditions of the culture, which can be of biotechnological importance. The biosynthesis and excretion of glucosides were most intense in the 0.1–0.5 culture with no significant effect of light conditions, although in the 0.4–0.4 culture light strongly stimulated the efflux of di-, tri- and pentaglucosides. In turn, glucuronides were synthesized and released in the medium most remarkably in the 0.4–0.4 culture kept in the light and in the dark, although light seemed to stimulate the efflux of monoglucuronide. Marigold suspension culture is a plausible model system to investigate the regulation of oleanolic glycosides production and excretion (Szakiel 2003).

## STRESS AND *CALENDULA*

To gain better insight into long-term salt-induced oxidative stress, some physiological parameters in marigold under NaCl stress were investigated (Chaparzadeh *et al*. 2004). Salinity affected most of the considered parameters. High salinity caused a reduction in growth parameters, lipid peroxidation and hydrogen peroxide ($H_2O_2$) accumulation. Under high salinity stress, a decrease in total glutathione and an increase in total ascorbate (AsA + DHA), accompanied with enhanced glutathione reductase (GR, EC 1.6.4.2) and ascorbate peroxidase (APX, EC 1.11.1.11) activities, were observed in leaves. In addition, salinity induced a decrease in superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POX, EC 1.11.1.7) activities. The decrease in dehydroascorbate reductase (DHAR, EC 1.8.5.1) and monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) activities suggests that other mechanisms play a major role in the regeneration of reduced ascorbate. The changes in catalase (CAT, EC 1.11.1.6) activities, both in roots and in leaves, may be important in $H_2O_2$ homeostasis. Another study investigated the effects of saline irrigation water on yield (FW and DW of flower heads), VO yield, chemical constituents of the VO and total flavonoids and carotenoids of flower heads at three flowering stages i.e. initial (21 days after bud formation (DABF), full-flowering (81 DABF) and final (111 DABF) (Khalid and Teixeira da Silva 2010). After plants were treated with different levels of saline irrigation water (0.39, 1.56, 3.13, 4.69, 6.25, 7.81 and 9.38 $dSm^{-1}$) consisting of NaCl, $CaCl_2$ and $MgCl_2$ salts, the flower head yield and pigment (total flavonoids and carotenoids) content were significantly reduced. Irrigation with saline water increased the VO content and its main components (α-cadinol, γ- and Δ-cadinene). Fresh and DWs of flower heads and EO increased near 81 DABF while the content of pigments increased by 111 DABF.

## PERSPECTIVES AND FUTURE RESEARCH FOCUS

In this review, we have explored the phytochemistry and pharmacological activities of *Calendula officinalis* Linn. in order to collate existing information on this plant as well as highlight its multi-activity properties as a medicinal agent. In the future the scientific research on *C. officinalis* will be increased to produce good active principals (quantity and quality) because C. *officinalis* is a good source of a wide range of natural products.

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## REFERENCES


**Abajova RL, Aslanov SM, Mamedova ME** (1994) Amino acids of *Calendula officinalis. Chemestry of Natural Composition* **30 (15)**, 614-641

**Adler G, Kasprzyk Z** (1975) Free sterols, steryl esters, glycosides, acelyted glycosides and watersoluble complexes in *Calendula officinalis. Phytochemistry* **14**, 627-631

**Albulescu M, Alexa N, Cojan C** (2004) *Calendula officinalis* flowers, source of extracts with antioxidant activity. *Serius Chemestry* **13 (2)**, 169-176

**Alexander M, Newmark H, Miller RG** (1985) Oral beta-carotene can increase the number of OKT4+ cells in human blood. *Immunnity* **9**, 221-224

**Alexenizor M, Dorn A** (2007) Screening of medicinal and ornamental plants for insecticidal and growth regulating activity. *Immunnity* **80**, 205-215

**Arjenaki FG, Dehaghi MA, Jabbari R** (2011) Effects of priming on seed germination of marigold (*Calendula officinalis*). *Advanced Environmental Biology* **5 (2)**, 276-280

**Auldridge ME, McCarty DR, Klee HJ** (2006) Plant carotenoid cleavage oxygenase and their apocarotenoid products. *Plant Biology* **9**, 315-321

**Azzaz NAI, Hassan EA, El-Emery FA** (2007) Physiological, anatomical, and biochemical studies on pot marigold (*Calendula officinalis* L.) plants. *African Crop Science Conference Proceedings* **8**, 1727-1738

**Badami RC, Morris LJ** (1965) The oxygenated fatty acid of calendula seeds oil. *Journal of the American Oil Chemists' Society* **42**, 1119-1121

**Bako E, Deli J, To G** (2002) HPLC study on the carotenoid composition of calendula products. *Journal of Biochemistry and Biophysics Methods* **53**, 241-250

**Bashir S, Janbaz KH, Jabeen Q, Gilani AH** (2006) Studies on spasmogenic and spasmolytic activities of *Calendula officinalis* flowers. *Phytotherapy Research* **20**, 906-910

**Barajas-Farias LM, Pérez-Carreón JI, Arce-Popoca E, Fattel-Fazenda S, Alemán-Lazarini L, Hernández-García S, Salcido-Neyoy M, Cruz-Jiménez FG, Camacho J, Villa-Treviño S** (2005) A dual and opposite effect of *Calendula officinalis* flower extract: Chemoprotector and promoter in a rat hepatocarcinogenesis model. *Planta Medica* **72 (3)**, 217-221

**Bcerentrup H, Robbelen G** (1987) *Calendula* and *Coriandrum* – new potential oil crops for industrial use. *Fat Sciences Technology* **89**, 227-230

**Bendich A** (1990) Carotenoids and the immune system In: Krinsky NI, Mathews-Roth MM, Taylor RF (Eds) *Carotenoids: Chemistry and Biology*, Plenum Press, New York, pp 323-333

**Bone KA** (2003) *Clinical Guide to Blending Liquid Herbs*, Livingstone Churchill, St Louis, Missouri, pp 120-123

**Boucard-Maitre Y, Algernon O, Raynaurd J** (1988) Genotoxic and antitumoral activity of *Calendula officinalis* extracts. *Pharmazie* **43**, 220-221

**Bradshaw HD, Schemske DW** (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkey flowers. *Nature* **426**, 176-178

**Braga PC, Sasso MD, Culici M, Spallino A, Falchi M, Bertelli A, Morelli R, Scalzo LR (**2009) Antioxidant activity of *Calendula officinalis* extract: Inhibitory effects on chemiluminescence of human neutrophil bursts and electron paramagnetic. *Research Pharmacy* **83 (6)**, 348-355

**Braithwaite L, Drost D** (2009) *Calendula in the Garden*, Utah State University, 2 pp

**Brand N, Petkovich M, Krust A, Chambon P, De TH, Marchio A, Tiollais P, Dejean A** (1988) Identification of a second human retinoic acid receptor. *Nature* **332**, 850-853

**Braun L, Cohen M** (2005) *Herbs and Natural Supplements: An Evidence-Based Guide*, Elsevier, Sydney, pp 98-100

**Britton G, Liaaen-Jensen S, Pfander H** (1995) Carotenoids. In: Britton G, Liaaen-Jensen S, Pfander H (Eds) *Isolation and Analysis* (Vol 1A) Birkhauser, Basel, Switzerland, pp 332-337

**Burton GW, Ingold UK** (1984) p-Carotene: An unusual type of lipid antioxidant. *Science* **224**, 569-573

**Casley-Smith JR** (1983) The effects of *Unguentum lyphaticum* on acute experimental lymphedema and other high-protein edema. *Lymphedema* **16**, 150-156

**Castenmiller JJM, West CE** (1998) Bioavailability and bioconversion of carotenoids. *Annual Review Nutrition* **18**, 19-38

**Chakrab-Orthy GS, Ghorpade PM** (2010) Determination of quercetin by HPTLC in "*Calendula officinalis*" extract. *Planta Medica* **1**, 1-9

**Chalchat JC, Ph R, Mechet AG** (1991) Chemical composition of EO of *Calendula officinalis* L (pot marigold). *Flavour and Fragrance Journal* **6**, 189-192

**Chandran PK, Kutton R** (2008) Effect of *Calendula officinalis* flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns. *Journal of Clinic Biochemical Nutrition* **43**, 58-64

**Chevallier A** (1996) *Encyclopedia of Medicinal Plants*, Dorling Kindersley Pty. Ltd., St. Leonards, New South Wales, 478 pp

**Chiej R** (1988) *The MacDonald Encycopedia of Medicinal Plants*, MacDonald and Co., London, 586 pp

**Cocu S, Urabey S, İpek A, Khawar KM, Sarihan EO, Kaya MD, Parmaksiz İ, Özcan S** (2004) Adventitious shoot regeneration and micropropagation in *Calendula officinalis* L. *Biological Plantarum* **48 (3)**, 449-451

**Colonel Kirtikar KR, Major Basu BD** (1993) *Indian Medicinal Plants* (Vol II), Deharadun, India, pp 1413-1414

**Cordova CA, Siqueira IR, Netto CA, Yunes RA, Volpato AM, Cechinel S, Filho V, Curi-Pedrosa R, Creszynski-Pasa T** (2002) Protective properties of butanolic extract of the *Calendula officinalis* (marigold) against lipid peroxidation of rat liver microsomes and action as free radical scavenger. *Redox Reports* **7**, 95-102

**Crnobarac J, Jacimovic G, Marinkovic B, Latkovic DI, Balijagic J** (2011) The effect of variety and row distance on yield and quality of pot marigold. *Acta Horticulturae* **925**, 141-146

**Cromack HTH, Smith JM** (1998) *Calendula officinalis* production potential and crop agronomy in southern England. *Industrial Crops and Products* **7**, 223-229

**Cunningham FXJ, Gantt E** (1998) Genes and enzymes of carotenoid biosynthesis in plants. *Annual Review of Plant Physiolology* **49**, 557-583

**Cunningham FXJ, Gantt E** (2001) One ring or two? Determination of ring number in carotenoids by lycopene cyclases. *Proceedings of the National Academy of Sciences USA* **98**, 2905-2910

**Cunningham FXJ, Pogson B, Sun ZR, McDonal KA, DellaPenna D, Gantt E** (1996) Functional analysis of the beta and epsilon lycopene cyclase enzymes of *Arabidopsis reveals* a mechanism for control of cyclic carotenoid formation. *Plant Cell* **8**, 1613-1626

**Davuluri GR, Tuinen A, Fraser PD** (2005) Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. *Nature Biotechnology* **23**, 890-895

**Della LR** (1990) Topical anti-inflammatory activity of *Calendula officinalis* extracts. *Planta Medica* **56**, 658-658

**Della LR, Tubaro A, Sosa S, Becker H, Saar S, Isaac O** (1994) The role of triterpenoids in topical anti-inflammatory activity of *Calendula officinalis* flowers. *Planta Medica* **60**, 516-520

**Editorial Boards** (2003) *PDR for Herbal Medicines* (2nd Edn), Montvale, Thomson-Medical Economics, pp 497-500

**Eghatoleslami MJ, Mousavi GR** (2009) The effects of sowing date and plant density on seed and flower yield of pot marigold (*Calendula officinalis* L.). *Acta Horticulureae* **826**, 371-376

**Eid ME, Kasem AH** (2009) Effect of phosphorein and active dry yeast on *Calendula officinalis* L. *Journal of Production Development* **14 (2)**, 112-117

**Eitterl-Eglseer K, Reznicek G, Jurenitsch J, Novak J, Zitter W, Franz C** (2001) Morphogenetic variability of faradiol monoesters in marigold (*Calendula officinalis* L). *Phytochemistry* **12**, 199-201

**Fleisonner AM** (1985) Plant extracts: To accelerate healing and reduce inflammation. *Cosmet Toil* **45**, 100-113

**Fleming T** (1998) *PDR for Herbal Medicines Montvale*, Medical Economics Co., Inc., NJ, 567 pp

**Frankic T, Salobir K, Salobir J** (2008) The comparison of *in vivo* antigenotoxic antioxidative capacity of two propylene glycol extracts of *Calendula officinalis* (marigold) and vitamin E in young growing pigs. *Journal of Animal Physiology and Animal Nutrition* **41**, 1-7

**Fraser PD, Kiano JW, Truesdale MR, Schuch W, Bramley PM** (1999) Phytoene synthase-2 enzyme activity in tomato does not contribute to carotenoid synthesis in ripening fruit. *Plant Molecular Biology* **40**, 687-698

**Fraser PD, Truesdale MR, Bird CR, Schuch W, Bramley PM** (1994) Carotenoid biosynthesis during tomato fruit development. *Plant Physiology* **105**, 405-413

**Galpaz N, Ronen G, Khalfa Z, Zamir D, Hirschberg J** (2006) A chromoplast-specific carotenoid biosynthesis pathway is revealed by cloning of the tomato white-flower locus. *Plant Cell* **18**, 1947-1960

**Gazim ZC, Ferreira GA, Rezende CM, Nakamura CV, Dias-Filho BP, Cortez DAG,** (2007) Identificação dos constituintes químicos da fração volátil da *Calendula officinalis Paraná*. *Horticultura Brasileira* **25**, 118-121

**Gazim ZC, Rezende CM, Fraga SR, Svidzinski TE, Cortez DG** (2008) Antifungal activity of the essential oil from *Calendula officinalis* L. (Asteraceae) growing in Brazil. *Braziloan Journal of Microbiology* **39**, 61-63

**Goodwin TW** (1954) Studies in carotenogenesis: The carotenoids of the flower petals of *Calendula officinalis*. *Biochemistry Journal* **58**, 90-94

**Goyal M, Mathur R** (2011) Antimicrobial effects of *Calendula officinalis* against human pathogenic microorganisms. *Journal of Herbs and Medical Toxicity* **5 (1)**, 97-101

**Green BR, Durnford DG** (1996) The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annual Review of Plant Physiolology and Plant Molecular Biology* **47**, 685-714

**Grieve M** (1931) *A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folklore of Herbs, Grasses, Fungi, Shrubs and Trees with All Their Modern Scientific Uses*, Jonathan Cape Ltd, London, 456 pp

**Grzelak A, Janiszowska W** (2002) Initiation and growth characteristics of suspension cultures of *Calendula officinalis* cells. *Plant Cell, Tissue and Organ Culture* **71**, 29-40

**Hamburger M, Adler S, Baumann D, Forg A, Weinreich B** (2003) Preparative purification of the major anti-inflammatory triterpenoid esters from marigold (*Calendula officinalis*). *Fitoterapia* **74**, 328-338

**Hansel R, Keller K, Rimpler H, Schneider G** (1992) *Hagers Handbuch Derpharmazeutischen Praxis* (Vol 4, 5th Edn), Springer, Berlin, 597 pp

**Harrison D** (inventor) (2003) *Natural Therapeutic Composition for the Treatment of Wounds and Sores*. Canadian Application Publication, 675 pp

**Hassan HA, Waly AA, Zaghloul MA** (1991) Physiological studies on *Calendula officinalis* L. 1. Effect of nitrogen fertilization and $GA_3$ on the growth, flowering and chemical composition of the plant. *Journal of Agriculture Research Tanta Univversity,* **17 (3)**, 437-446

**Hirschberg J** (2001) Carotenoid biosynthesis in flowering plants. *Plant Biology* **4**, 210-218

**Hoballah ME, Gubitz T, Stuurman J, Broger L, Barone M, Mandel T, Dell'Olivo A, Arnold M, Kuhkemeier C** (2007) Single gene-mediated shift in pollinator attraction in petunia. *Plant Cell* **19**, 779-790

**Horvath FI** (1992) Therapeutical compositions against psoriasis. US Patent 5165932, Nov 24, 454 pp

**Howitt CA, Pogson BJ** (2006) Carotenoid accumulation and function in seeds and non-green tissues. *Plant Cell Environment* **29**, 435-445

**Iauk L, Lo-Bue AM, Milazzo I, Rapisarda A, Blandino G** (2003) Antibacterial activity of medicinal plant extracts against periodontopathic bacteria. *Phytotherapy Research* **17**, 599-604

**Isaac O** (1992) *Die Ringelblume. Botanik, Chemie, Pharmakologie, Toxikologie, Pharmazie und Therapeutische Verwendung.* Wissenschaftliche Verlagsgesellschaft, Stuttgart, 787 pp

**Isaacson T, Ronen G, Zamir D, Hirschberg J** (2002) Cloning of tangerine from tomato reveals a carotenoid isomerase essential for the production of β-carotene and xanthophylls in plants. *The Plant Cell* **14**, 333-342

**Janiszowska W, Michalski W, Kasprzyk Z** (1976) Polyprenyl quinones and α-tocopherol in *Calendula officinalis. Phytochemistry* **15**, 125-127

**Jiang Z-P, Lu J-W, Li W-X, Zou J** (2008) Studies on fertilization effect and fertilizer formula screen for *Calendula officinalis* L. *Acta Horticulturae Sinica* **2**, 125-132

**Jyonouchi H, Hill RJ, Tomita Y, Good RA** (1991) Studies of Immunomodulating actions of carotenoids. I. Effects of p-carotene and astaxanthin on murine lymphocyte functions and cell surface marker expression in *in vitro* culture system. *Nutrition Cancer* **16 (2)**, 93-105

**Kalvatchev Z, Walder R, Garzaro D** (1997) Anti-HIV activity of extracts from *Calendula officinalis* flowers. *Biomedical Pharmacology* **51 (4)**, 176-180

**Kandeel YMR** (2004) Effect of NPK fertilization treatments and $GA_3$ on growth, flowering and chemical composition of marigold (*Calendula officinalis* L). *Journal of Agricultural Research Tanta Univesity* **30 (4)**, 925-943

**Kasprzyk Z, Wilkomirski B** (1973) Structure of a new triterpene triol from *Calendula officinalis* flowers. *Phytochemistry* **12**, 2299-2300

**Kemper KJ** (1999) Calendula (*Calendula officinalis*). The Longwood Herbal Task Force and the Centre for Holistic Pediatric Education and Research, 767 pp

**Kennet GL, Gerald D, Weinstein L** (1999) Psoriasis: current perspectives with an emphasis on treatment. *American Journal of Medical Sciences* **107**, 595-605

**Kerkach AI, Komissarenko NF, Chernobai VT** (1986) Coumarines of the inflorescences of *Calendula officinalis* and *Helichrysum arenarium. Khim Prir Soed* **6**, 777-778

**Khalid AK, Teixeira da Silva JA** (2010) Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions. *Scientia Horticulturae* **126**, 297-305

**Khalid KA, El-Ghorabm AH** (2006) The effect of presowing low temperature on essential oil and chemical composition of *Calendula officinalis* L. *Journal of Essential Oil-Bearing Plants* **9 (1)**, 32-41

**Khalid KA, Yassen AA, Zaghloul SM** (2006) Effect of soil solarization and cattle manure on the growth, essential oil and chemical composition of *Calendula officinalis* L plants. *Journal of Applied Sciences Research* **2 (3)**, 142-152

**Khalil MY, Moustafa AA, Naguib NY** (2007) Growth, phenolic compounds and antioxidant activities of some medicinal plants grown under organic farming condition. *World Journal of Agricultural Sciences* **3 (4)**, 451-457

**Khare CP** (2004) *Encyclopedia of Indian Medicinal Plants*, Springer-Verlag, Germany, pp 116-117

**Kishimoto A, Maoka T, Sumitomo K, Ohmiya A** (2005) Analysis of carotenoids composition in pestles of calendula (*Calendula officinalis* L.). *Biotechnology and Biochemistry* **69 (11)**, 2122-2118

**Kishimoto S, Ohmiya A** (2006) Regulation of carotenoid biosynthesis in petals and leaves of chrysanthemum (*Chrysanthemum morifolium* Ramat). *Physiologia Plantarum* **128**, 437-447

**Komoe H, Hayashi N** (1971) Paraffins of the petals of *Calendula officinalis. Phytochemistry* **10**, 1944-1948

**Krag K** (1976) Plants used as contraceptives by the North American Indians: an ethnobotanical study. Botanical Museum, Cambridge, Thesis BS, Harvard University, 196 pp

**Krinsky NI** (1989) Antioxidant functions of carotenoids. *Free Radical Biology and Medicine* **7**, 617-635

**Kuppast IJ, Nayak PV** (2006) Wound-healing activity of *Cordia dichotoma.* Forst f. fruits. *Natural Product Radiance* **5**, 99-102

**Kurkin VA, Sharova OV** (2007) Flavonoids from *calendula officinalis* flowers. *Chemistry of Natural Products* **43 (2)** 216-217

**Lea AGH** (1988) *HPLC in Food Analysis*, Academic Press, London, 277 pp

**Leach MJ** (2008) *Calendula officinalis* and wound healing: A systematic review. *Wounds* **20 (8)**, 1-7

**Leslie CA, Dubey DP** (1982) Carotene and natural killer cell activity. *Food Processing* **41**, 331-331

**Li F, Murillo C, Wurtzel ET** (2007) Maize Y9 encodes a product essential for 15-*cis*-f-carotene isomerization. *Plant Physiology* **144**, 1188-1189

**Li L, Paolillo DJ, Parthasarathy MV, DiMuzio EM, Garvin D** (2001) A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). *Plant Journal* **26**, 59-67

**Lin LT, Liu LT, Chiang LC, Lin CC** (2002) *In vitro* antihepatoma activity of fifteen natural medicines from Canada. *Phytotherapy Research* **16**, 440-444

**Liu Y, Roof S, Ye Z, Barry C, Tuinen A, Vrebalov J, Bowler C, Giovannoni J,** (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proceedings of the National Academy of Sciences USA* **101**, 9897-9902

**Lotan R** (1980) Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. *Biochimistry and Biophysics Acta* **605**, 33-91

**Lu S, Van Eck J, Zhou X** (2006) The cauliflower or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. *Plant Cell* **18**, 3594-3605

**Lubsandorzhieva PB** (2009) *Antioxidant activity of Calendula officinalis* L. *Chemistry of Plant Raw Material* **10**, 123-126

**Mahgoub MH, Abd El Aziz NG, Youssef AA** (2006) Influence of foliar spray with paclobutrazol or glutathione on growth, flowering and chemical composition of *Calendula officinalis* L. plant. *Journal of Appied Sciences Research* **2 (11)**, 879-883

**Martinm RJ, Deo B** (2000) Effect of plant population on calendula (*Calendula officinalis* L.) flower production. *New Zealand Journal of Crop and Horticultural Science* **28**, 37-44

**Matysik G, Wojciak-Kosior M, Paduch R** (2005) The influence of *Calendula officinalis* flos extract on cell cultures and the chromatographic analysis of extracts. *Journal of Pharmaceutical and Biomedical Annals* **38 (2)**, 285-292

**Mayer H, Bollag W, Hanni R, Ruegg R** (1979) Retinoids, a new class of compounds with prophylactic and therapeutic activities in oncology and dermatology. *Experientia* **34**, 1105-1119

**Mayne ST** (1996) β-Carotene, carotenoids and disease prevention in humans. *FASEB Journal* **10**, 690-701

**Meda A, Lamien CE, Romito M, Millogo J, Nacoulma GO** (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry* **91**, 571-577

**Medina EJ, Angel LG, Paco L, Algarra I, Collado A, Garrido F** (2006) A new extract of the plant *Calendula officinalis* produces a dual *in vitro* effect: cytotoxic anti-tumor activity and lymphocyte activation. *BMC Cancer* **6 (19)**, 1-14

**Milad SMN, Mohammed MAA** (2009) Effects of foliar spray of citric and ascorbic acids on growth and chemical composition of marigold (*Calendula officinalis* L). *Journal of Production and Development* **14 (2)**, 18-29

**Mills SY** (1999) *The Essential Book of Herbal Medicine*, Penguin Books Ltd, Harmondsworth, Middlesex, 765 pp

**Moehs CP, Tian L, Osteryoung KW, DellaPenna D** (2001) Analysis of carotenoid biosynthetic gene expression during marigold petal development. *Plant Molecular Biology* **45**, 281-293

**Mozherenkov VP, Shubina LF** (1976) Treatment of chronic conjunctivitis with calendula. *Medica Sestra* **35**, 33-34

**Naguib MY, Khalil MY, El-Sherbeny SE** (2005) A comparative study on the productivity and chemical constituents of various sources and species of calendula plants as affected by two foliar fertilizers. *Journal of Appied Sciences Research* **1 (2)**, 176-189

**Nambara E, Marion-Poll A** (2005) Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56**, 165-185

**Naved T, Ansari SH, Mukhtar HM, Ali M** (2005) New triterpenic esters of oleanene-series from the flowers of *Calendula officinalis* Linn. *Medical Chemistry* **44**, 1088-1091

**Nedelka A, Golovkin V, Solovieva V, Nedelka V** (1988) Experimental study of the effectivity of eye ointments. *Oftalmologicheskii Zhurnal* **43**, 429-431

**Neukiron H, D'Ambrosi M, Dovia J, Guerriero A** (2004) Simultaneous quantitative determination of eight triterpenoid monoesters from flowers of varieties of *Calendula officinalis* L and characterisation of a new triterpenoid monoester. *Phytochemistry* **15**, 30-35

**Nicoletta CB, Marongiu PA, Pivetta T, Procedda S** (2003) Extraction, separation, and isolation of volatiles and dyes from *Calendula officinalis* L. and *Aloysia tryphylla* (L'Her) britton by supercritical $CO_2$. *Journal of Essential Oil Research* **15**, 272-277

**Niyogi KK** (2000) Safety valves for photosynthesis. *Plant Biology* **3**, 455-460

**Norman GB, Max W** (2001) *Herbal Drugs and Phytopharmaceuticals* (2nd Edn), MedPharm GmbH Scientific Publishers, Germany, pp 118-120

**Okoh OO, Sadimenko AA, Afolayan AJ** (2007) The effects of age on the yield and composition of the essential oils of *Calendula officinalis. Journal of Applied Sciences* **7 (23)**, 3806-3810

**Park H, Kreunen SS, Cuttriss AJ, DellaPenna D, Pogson BJ** (2002) Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar formation, and photomorphogenesis. *Plant Cell* **14**, 321-332

**Perez-Carreon JI, Cryz-Jimener G, Licea-Vega JA, Popoca EA, Fazenda SF,**

**Villa T** (2002) Genotoxic and antigenotoxic properties of *Calendula officinalis* extracts in rat liver culture treated with diethylnitrosamine. *Toxic In Vitro* **16**, 253-258

**Petkovich M, Brand NJ, Krust A, Chambon P** (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* **330**, 444-450

**Pintea A, Bele C, Andrei S, Socaciu C** (2003) HPLC analysis of carotenoids in four varieties of *Calendula officinalis* L. flowers. *Acta Biologica Szegediensis* **47 (1-4)**, 37-40

**Popovic M, Kaurinovic B, Mimica-Dukic N, Vojinovic-Miloradov M, Cupic V** (1999) Combined effects of plant extracts and xenobiotics on liposomal lipid peroxidation. Part 1. Marigold extract-ciprofloxacin/pyralene. *Oxidation Communications* **22**, 487-494

**Prabhala RH, Maxey V, Hicks MJ, Watson RJ** (1989) Enhancement of the expression of activation markers on human peripheral blood mononuclear cells by *in vitro* culture with retinoids and carotenoids. *Journal of Leukocyte Biology* **45**, 249-254

**Preethi KC, Ramadasan K** (2008) Effect of *Calendula officinalis* flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns. *Journal of Clinical Biochemistry* **43 (2)**, 58-64

**Raal A, Kirsipuu K** (2011) Total flavonoid content in varieties of *Calendula officinalis* L originating from different countries and cultivated in Estonia. *Natural Product Research* **25 (6)**, 658-62

**Radulescu V, Doneanu C, Loloiu TCGC** (2000) Investigation of chemical composition of *Calendula officinalis. Revue Roumaine de Chimie* **45**, 271-275

**Rahmani N, Daneshian J, Farahani HA** (2009) Effects of nitrogen fertilizer and irrigation regimes on seed yield of calendula (*Calendula officinalis* L). *Journal of Agricultural Biotechnology and Sustinable Development* **1 (1)**, 24-28

**Rasu MA, Tamas M, Puica C, Roman I, Sabadas M** (2005) The hepatoprotective action of ten herbal extracts in $CCl_4$-intoxicated liver. *Phytotheraby Research* **19**, 744-749

**Reider N, Comericki P, Hausen BM, Fritsch P, Aberer W** (2001) The seamy side of natural medicines: Contact sensitization to arnica (*Arnica montana* L.) and marigold (*Calendula officinalis* L). *Contact Dermatitis* **45**, 269-272

**Roopashree TS, Raman Dang, Shobha Rani RH, Narendra C** (2008) Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis. International Journal of Applied Research and Natural Products* **1 (3)**, 20-28

**Ruszkowski D, Szakiel A, Janiszowska W** (2003) Metabolism of [3-3H] oleanolic acid in *Calendula officinalis* L. roots. *Journal of Applied sciences* **25 (4)**, 311-317

**Saleem M, Zaka S** (1986) Studies on marigold (*Calendula officinalis*) seed oil and seed meal. *Fette Seifen Anstrichmittel* **88 (5)**, 178-180

**Sandmann G, Romer S, Fraser PD** (2006) Understanding carotenoid metabolism as a necessity for genetic engineering of crop plants. *Metabolic Engineering* **8**, 291-302

**Satyavati GV** (1990) In: Farnsworth NR, Wagner H (Eds) *Economic and Medicinal Plant Research*, Academic Press Ltd., London, pp 163-198

**Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY** (1999) Seed specific overexpression of phytoene synthase increase in carotenoids and other metabolic effects. *Plant Journal* **20**, 401-412

**Silva ER, Goncalves ES, Aguiar F, Evencio LB, Lyra MA, Coelno MC, Fraga MA, Wanderloy AG** (2007) Toxicological studies on hydroalcoholic extract of *Calendula officinalis* L. *Phytotherapy Research* **21**, 332-336 S

**Sliwowski J, Dziewanowska K, Kasprzyk E** (1973) Ursadiol: A new triterpene diol from *Calendula officinalis* flowers. *Khim Prir Soed* **12**, 157-160

**Szakiel A, Grzelak A, Dudek P, Janiszowska W** (2003) Biosynthesis of oleanolic acid and its glycosides in *Calendula officinalis* suspension culture. *Plant Physiology and Biochemistry* **41**, 271-275

**Szakiel A, Ruszkowski D, Janiszowska W** (2005) Saponins in *Calendula officinalis* L. – Structure, biosynthesis, transport and biological activity. *Phytochemistry Reviews* **4 (2-3)**, 151-158

**Szakiel A, Ruszkowski D, Grudniak A, Kurek A, Wolska IK, Doligalska M, Janiszowska W** (2008) Antibacterial and antiparasitic activity of oleanolic acid and its glycosides isolated from marigold (*Calendula officinalis*). *Planta Medica* **74 (14)**, 1709-1715

**Taheri G, Soudabeh E, Yatollahi SA, Raziyeh MZ** (2011) Effects of *Calendula officinalis* extract on bacteria isolated. *Journal of the Faculty of Medicine* **34 (4)**, 1-12

**Tian L, Musetti V, Kim J, Magallanes-Lundback M, DellaPenna D** (2004) The *Arabidopsis* LUT1 locus encodes a member of the cytochrome P450 family that is required for carotenoid-ring hydroxylation activity. *Proceedings of the National Academy of Sciences USA* **101**, 402-407

**Ukiya M, Akihisa T, Yasukava K, Tokuda H, Suzuki T, Kimura Y** (2006) Antiinflammatory, anti-tumor-promoting and cytotoxic activities of constituents of marigold (*Calendula officinalis*) flowers. *Journal of Natural Products* **69**, 1692-1696

**Varlijen J, Lipták A, Wagner H** (1989) Structural analysis of rhamnoarabinogalactans and arabinogalactans with immunostimulating activity from *Calendula officinalis. Phytochemistry* **28**, 2379-2383

**Vecherko LP, Kabanov US, Zinkevich EP, Kogan LM** (1971) The structure of calenduloside B from the roots of *Calendula officinalis. Khim Prir Soed* **4**, 533-533

**Vecherko LP, Sviridov AF, Zinkevich EP, Kogan LM** (1974) Structures of calenduloside g and h from the roots of *Calendula officinalis. Khim Prir Soed* **4**, 532-534

**Vecherko LP, Sviridov AF, Zinkevich EP, Kogan LM** (1975) The structure of calenduloside C and D from the roots of *Calendula officinalis. Khim Prir Soed* **3**, 366-373

**Vecherko LP, Zinkevich EP, Libizov NI, Ban'kooskii AI** (1969) Calenduloside A from *Calendula officinalis. Khim Prir Soed* **5 (1)**, 58-59

**Vidal-Ollivier E, Elias R, Faure F, Babadjamian A, Crespin F, Balansard G, Boudon G** (1989) Flavonol glycosides from *Calendula officinalis* flowers. *Planta Medica* **55**, 73-74

**Vidal-Ollivier E, Balansard G, Faure A (**1989) Revised structures of triterpenoid saponins from the flowers of *Calendula officinalis. Journal of Natural Products* **52 (5)**, 1156-1159

**Vlchenko NT, Glushenkova AI, Mukhamedova KS** (1998) Lipids of *Calendula officinalis. Chemistry of Naural Compounds* **34 (3)**, 272-274

**Von O, Die O** (1992) *Die Ringelblume Botanik, Chemie*, *Pharmakologie, Toxikologie, Pharmazie und Therapeutische Verwendung*. Wissenschftliche Verlagsgellschaft mbH, Stuttgart, Bundesrepublik Deutschland, 786 pp

**Wagner H, Proksch A, Riess-Maurer I, Vollmar A, Odenthal S, Stuppner H, Jurcic K, Le-Turdu M, Fang JN** (1985) Immunstimulierend wirkende Polysaccharide (Heteroglykane) aus höheren. *Pflanzen Arzneimittel-Forschung* **7**, 1069-1075

**Wilkomirski B** (1985) Pentacyclic triterpene triols from *Calendula officinalis* flowers. *Phytochemistry* **24 (12)**, 3066-3067

**Wilkomirski B, Kasprzyk Z** (1979) Free and ester-bound triterpene alcohols and sterols in cellular subfractions of *Calendula officinalis. Phytochemistry* **18**, 253-255

**Willuhn G, Westhaus RG** (1987) Loliolide (calendin) from *Calendula officinalis. Planta Medica* **53**, 304-309

**Wojciechowski Z, Bochenska-Hryniewicz M, Kurcharezak B, Kasprzyk Z** (1972) Sterol and triterpene alcohol esters from *Calendula officinalis. Phytochemistry* **11**, 1165-1168

**Wojciechowski Z, Jelonkiewicz-Konador A, Tomaszewski M, Jankowski J, Kasprzyk Z** (1971) The structure of glucosides of oleanolic acid isolated from the roots of *Calendula officinalis* flowers. *Phytochemistry* **10**, 1121-24

**Yoshikawa M, Murakami T, Kishi A, Kageura T, Matsuda H** (2001) Medicinal flowers III Marigold (1): Hypoglycemic, gastric emptying inhibitory and gastroprotective principles and new oleanane-type triterpene oligoglycoside, calendasaponins A, B, C, and D, from Egyptian *Calendula officinalis. Chemical and Pharmaceutical Bulletin* **49**, 863-870

**Zahra A, Mohammed A, Mohammed HK** (2000) Evaluation of immunomodulatory effects of five herbal plants. *Journal of Ethanopharmacology* **72**, 167-172

**Zitterl-Eglseer, Sosa S, Jurenitsch J, Schubert-Zsilavecz,¥ M, Della Loggia R, Tubaro A, Bertoldi M, Franz C** (1997) Anti-oedematous activities of the main triterpenoidal esters of marigold (*Calendula officinalis* L). *Journal of Ethanopharmacolology* **57**, 139-144