Tropical Journal of Pharmaceutical Research, October 2009; 8 (5): 455-465

Available online at http://www.tjpr.org

Review Article

Phytochemical Constituents and Pharmacological Activities of *Calendula officinalis* Linn (Asteraceae): A Review

BP Muley*, SS Khadabadi and NB Banarase
Govt. College of Pharmacy, Kathora Naka, Amaravati, Maharashtra State, India- 444 604.

Abstract

Calendula officinalis Linn. (Asteraceae) is used medicinally in Europe, China and India amongst several places in the world. It is also known as "African marigold" and has been a subject of several chemical and pharmacological studies. It is used in traditional medicine, especially for wound healing, jaundice, blood purification, and as an antispasmodic. Chemical studies have underlined the presence of various classes of compounds, the main being triterpenoids, flavonoids, coumarines, quinones, volatile oil, carotenoids and amino acids. The extract of this plant as well as pure compounds isolated from it, have been demonstrated to possess multiple pharmacological activities such as anti-HIV, cytotoxic, anti-inflammatory, hepatoprotective, spasmolytic and spasmogenic, amongst others. In this review, we have explored the phytochemistry and pharmacological activities of C. officinalis in order to collate existing information on this plant as well as highlight its multi-activity properties as a medicinal agent. This is as a result of the worldwide cultivation of the plant and increasing published reports on it.

Key words: *Calendula officinalis, Asteraceae, Phytochemical constituents, Pharmacological activities, Contraindications.*

Received: 11 April 2009 *Revised accepted: 10 September 2009*

Corresponding author: Email:** muley.b@gmail.com; nbanarase7@gmail.com.*

INTRODUCTION

India is called the botanical garden of the world for its rich natural resources. Over 6,000 plants in India are used in traditional, folklore and herbal medicine[1]. The Indian system of medicine has identified 1500 medicinal plants of which 500 are commonly used[1]. *Calendula officinalis* Linn. is used medicinally in Europe, China, US and India. It belongs to the family, Asteraceae, and is commonly known as Zergul (Hindi), African marigold, Calendula, Common Marigold, Garden Marigold, Marigold, Pot Marigold (English), Butterblume (German), Chin Chan Ts'ao (Chinese), Galbinele (Romanian) and Ringblomma (Swedish)[2,3].

Taxonomic description

The plant is classified as shown in Table 1.

Table 1: Taxonomic classification of *Calendula officinalis* [4]

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Tribe	Calenduleae
Genus	Calendula
Species	*C. officinalis*

Habitat

The plant is native to Central and Southern Europe, Western Asia and the US [5].

Botanical description

Flower and fruit

On the tip of each stem, there is a 5 to 7 cm composite flower head, as shown in Fig 1, 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 pseudohermaphrodites 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.

Leaf, stem and root

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 lanceolate above and are all tomentosae[5-7].

Leaves with stem Flower

Fig 1: The leaf, stem and flower of *C. officinalis*

Traditional uses

In Europe, the leaves are considered resolvent and diaphoretic while the flowers are used as a stimulant, antispasmodic and emmenagogue[2]. In England, the decoction of the flowers was used as a posset drink for the treatment of measles and smallpox, and the fresh juice as a remedy for jaundice, costiveness (constipation) and suppression of menstrual flow [8]. In India, the florets are used in ointments for treating wounds, herpes, ulcers, frostbite, skin damage, scars and blood purification. The leaves, in infusion, are used for treating varicose veins externally [2,8].

PHYTOCHEMISTRY

A number of phytochemical studies have demonstrated the presence of several classes of chemical compounds, the main ones being terpenoids, flavonoids, coumarines, quinones, volatile oil, carotenoids and amino acids.

Terpenoids

Various terpenoids (Table 2) have been reported from the petroleum ether extract of *C.officinalis* flowers. They include sitosterols, stigmasterols[9], diesters of diols[10], 3-monoesters of taraxasterol, ψ-taraxasterol, lupeol[11,12], erythrodiol, brein[13,14], ursadiol[15], faradiol-3-O-palmitate, faradiol-3-O-myristate, faradiol-3-O-laurate[16], arnidiol-3-O-palmitate, arnidiol-3-O-myristate, arnidiol-3-O-laurate, calenduladiol-3-O-palmitate, calenduladiol-3-O-myristate[17,18], oleanolic acid saponins: calenduloside A-H[19-22], 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[18], glucosides of oleanolic acid (mainly found in roots of grown and senescing plants) I, II, III, VI, VII [23,24], and glucuronides (mainly found in flowers and green parts) F, D, D_2, C, B and A[25]. One new triterpenic ester of olanane series has been isolated from flowers was cornulacic acid acetate from flowers [26].

Flavonoids

Various flavonoids (Table 3) have been isolated from the ethanol extract of the inflorescence of *C. officinalis*. They include quercetin, isorhamnetin[27], isoquercetin, isorhamnetin-3-O-β-D-glycoside, narcissin, calendoflaside [28], calendoflavoside, calendoflavobioside, rutin, isoquercitrin, neohesperidoside, isorhamnetin-3-O-neohesperidoside, isorhamnetin-3-O-2^G-rhamnosyl rutinoside, isorhamnetin-3-O-rutinoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside[18].

Coumarins

The ethanol extract of the inflorescence of the *C. officinalis* reported to contain coumarins - scopoletin, umbelliferone and esculetin [29] .

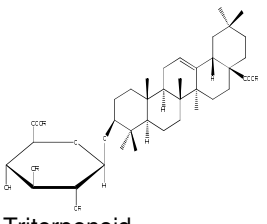
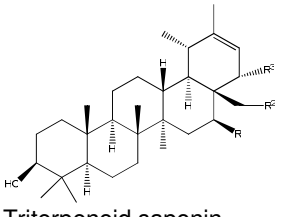
Quinones

Quinones reported from *C. officinalis* were plastoquinone, phylloquinone, α-tocopherol in the chloroplast, ubiquinone, phylloquinone, α-tocopherol in mitochondria, and phylloquinone in the leaves [30].

Volatile oil

C. officinalis flowers contain maximum volatile oil at full flowering stage (0.97 %) and minimum during the preflowering stage (0.13 %) [31]. The composition also showed different patterns at different phases of vegetative cycles. Various monoterpenes and sesquiterpenes have been reported in the volatile oil : α-thujene, α-pienene, sabinene, β-pienene, 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-ylangene, α-humulene, epi-bicyclo-sequiphellandrene, germacrene D, alloaromadendrene, β-saliene, calarene, muurolene, δ-cadinene, cadina 1,4-diene, α-cadinene, nerolidol, palustron, endobourbonene, oplopenone, α-cadinol, T-muurolol. The essential oil 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 [31].

Table 2: Structures and activities of some terpenoids

Structure		Activity
 Triterpenoid	Calendulaglycoside A: R^1=Glc, R^2=Gal, R^3=H, R^4=Glc; Calendulaglycoside A6'-O-methyl ester: R^1=Glc, R^2=Gal, R^3=Me, R^4=Glc; Calendulaglycoside A6'-O-n-butyl ester: R^1=Glc, R^2= Gal, R^3=n-Bu, R^4=Glc; Calendulaglycoside B: R^1=Glc, R^2=Gal, R^3= H, R^4= H; Calendulaglycoside B6'-O-n-butyl ester: R^1=Glc, R^2=Gal, R^3=n-Bu, R^4=H; Calendulaglycoside C: R^1=H, R^2=Gal, R^3=H, R^4=Glc; Calendulaglycoside C6'-O-methyl ester: R^1= H, R^2=Gal, R^3=Me, R^4=Glc; Calendulaglycoside C6'-O-n-butyl ester: R^1=H, R^2=Gal, R^3=n-Bu, R^4=Glc; Calendulaglycoside F6'-O-n-butyl ester: R^1=H, R^2=Gal, R^3=Me, R^4=H	Responsible for antitumor, anti-inflammatory and antioedematous activities.
 Triterpenoid saponin	Faradiol: R^1=OH, R^2=R^3=H ψ-Taraxasterol: R^1=R^2=R^3=H	Responsible for anti-inflammatory and antioedematous activities.

Carotenoids

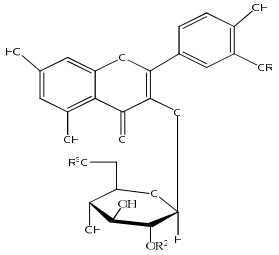
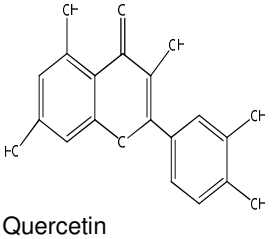
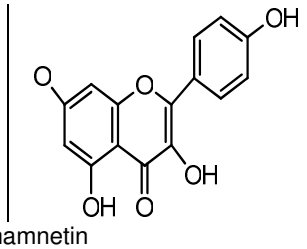
The methanol extract of leaves, petals and pollens of *C. officinalis* flowers showed a number of carotenoids. The carotenoids found in the pollens and petals were neoxanthin, 9Z-neoxanthin, violaxanthin, luteoxanthin, auroxanthin, 9Z-violaxanthin, flavoxanthin, mutatoxanthin, 9Z-anthroxanthin, lutein, 9/9′A-lutein, 13/13′Z-lutein, α-cryptoxanthin, β-cryptoxanthin, z-cryptoxanthin, lycopene, α-carotene, and β-carotene. Total carotenoids (mg/g dry weight) was 7.71 % for petals and1.61 % for pollens.

Reported carotenoid compositions of the leaves and stems reported 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 dry weight) for the leaves is 0.85 % and for stems 0.18 % [32,33].

Amino acids

The ethanol extract of the flowers of the plant is reported to show 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 % [34].

Table 3: Structures and activities of some flavonoids

Structure		Activity
 Isorhamnetin3-O-neohesperidoside	R^1=Me, R^2=Rha, R^3=H; Isorhamnetin3-O-2^G-rhamnosylrutinoside: R^1=Me, R^2=Rha, R^3=Rha; Isorhamnetin3-O-rutinoside: R^1=Me, R^2=H, R^3=Rha; Quercetin-3-O-glucoside: R^1= H, R^2=H, R^3=H; Quercetin-3-O-rutinoside: R^1=H, R^2=H, R^3=Rha	Responsible for antioxidant and wound healing activities
 Quercetin		Responsible for antioxidant activities
 Isorhamnetin		Responsible for antioxidant activities

Carbohydrates

The ethanol extract of the inflorescence of plant 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 alpha-L-rhamnan-(1→3)-araban along with monosaccharides [35,36].

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 [37,38] one oxygenated fatty acid also reported from the seed oil of *C. officinalis* was D-(+)-9-hydroxy-10,12-octadecadienoic acid [39].

Other constituents

Other phytochemicals include the bitter constituent, loliolide (calendin) [40], calendulin [41] and n-paraffins [42].

PHARMACOLOGICAL ACTIVITIES

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

Anti-inflammatory and antioedematous activities

Ethyl acetate soluble fraction of the methanol extract of *C. officinalis* flowers exhibited the most potent inhibition (84 %) of 12-o-tetradecanoyl phorbol-13-acetate (TPA)-induced inflammation (1 µg/ear) in mice with an ID_{50} value of 0.05 - 0.20 mg/ear compared with indomethacin as reference drug. Furthermore, activity-guided isolation showed that its activity was mainly due to oleanane-type triterpene glycoside[18]. A dose of 1200 µg/ear of an aqueous-ethanol 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 [43,44].

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 ψ-taraxasterol had antioedematous activity with an oedema inhibition of nearly 50 % at a dose of 240 µg/cm^2. Furthermore, when the doses of these two faradiol esters were doubled, oedema inhibition increased to 65 and 66 %, respectively, without any synergism between them [45]. 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 enhancement of macrophage proteolytic activity [46].

Anti-HIV activity

Dichloromethane-methanol (1:1) extract of *C. officinalis* flowers exhibited potent anti-HIV activity in *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 the HIV-mediated fusion at 500 µg/mL [47].

Antibacterial and antifungal activities

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*. The results showed marked inhibition against all tested microorganisms with MIC ≥2048 mg/L [48].
When the essential oil of the flowers was tested (using 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 potential antifungal activity (at 15 µl/disc) [49].

Anticancer and lymphocyte activation dual activities

The ethyal acetate soluble fraction of the methanol extract of *C. officinalis* flowers has shown cytotoxic activity in *vitro* [18]. Further activity-guided isolation of that fraction showed that the active compounds were: 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; and calenduloside G6'-O-methyl ester, which is active against all the cancer cell lines mentioned above with GI_{50}≤ 20 µmole except for ovarian cancer (IGROVI, GI_{50} = 20.1 µmole) and renal cancer (VO-31, 33.3 µmole) cell lines[18]. Aqueous laser-activated calendula flower extract (LACE) 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} concentration of 60 µg/mL. The mechanisms of the inhibition were identified as cell cycle arrest in G0/G1 phase and 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 B-lymphocytes, $CD4^+$, T lymphocytes and NKT lymphocyte[50].

Various extracts of the leaf, flower and whole plant have also been found to be cytotoxic to MRC5, HeP2, ascetic cells from Ehrlich carcinoma. The saponin rich fraction of these extracts displayed antitumoural activity *in vivo* in the Ehrlich mouse carcinoma model [51].

Hepatoprotective activity

The hydroalcohol extract of the flowers, when given to CCl_4-intoxicated liver in albino male Wistar rats at a dose of 10 mL/kg, resulted in a reduction of hepatocytolysis by 28.5 % due to reduction in glutamo-oxalate-transaminase (GOT) and glutamo-pyruvate-transaminase (GPT). However, histoenzymology showed reduction of steatosis of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), cytochromoxidase (Cyox) and Mg^{2+}-dependant adenosine triphosphatase (ATPase) [52]. The hot water extract of *C. officinalis* flowers exhibited antihepatoma 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 [53].

Immunostimulant activity

The polysaccharide fraction of *C. officinalis* extract showed immunostimulant activity, based on *in vitro* granulocyte test. Polysaccharide 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 [35,36].

Antioxidant activity

A 70 % methanol 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. The ether, butanol and water extracts, containing flavonoids, showed antioxidant activity [54]. Propylene glycol extracts of the petals and flower heads, assayed for antioxidant activity by lipid peroxidation, indicate that the extract of the petals was more potent than the flower head extract, based on analysis of plasma and urine malondialdehyde (MDA) and urine isoprostane inventrations (ipf2α-VI)[55].

Wound healing activity

The ethanol 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 of wounds as indicated by increase in collagen-hydroxyproline and hexosamine contents. The level of acute phase proteins (heptaglobin, orosomycid and tissue damage marker enzymes - alkaline phosphatase), alanine and aspartate transaminase decreased significantly. The decrease in lipid peroxidation might be due to its antioxidant property [56].

The daily application of 2% calendula gel resulted in a greater number of wound healing due to its antimicrobial and antioxidant property [57].

Spasmolytic and spasmogenic dual activity

The aqueous-ethanol 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 ten times more potent than the parent crude extract, and spasmolytic activity was found to be due to calcium channel blockade (CCB)[58]. On the other hand, the aqueous fraction of the parent extract exhibited spasmogenic activity in a dose range of 1 - 10 mg/mL [58].

Insecticidal activity

The acetone: methanol (2:1 v/v) extract of the flowers showed insecticidal activity when it was tested on milkweedbug[59].

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 0.3mg/L [60].

Genotoxic and antigenotoxic dual activities

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 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 genotoxic effect [61].
The propylene glycol extract of *C. officinalis* also showed 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) [55].

Antiviral activity

A tincture of the flowers suppressed the replication of herpes simplex, influenza A2 and influenza APR-8 viruses *in vitro* [62].

TOXOCOLOGICAL STUDY

The hydroalcohol 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 didn't 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 [63].

CONTRAINDICATION

The extract was found to cause allergy in 9 patients out of 443 (2.03 %) when assessed by patch testing method[64]. Therefore, it is advisable that the persons who have an established allergy to the Asteraceae (daisy) family should use it with caution [65,66] .

CONCLUSION

In this review, we have presented information on the botanical description, traditional uses, phytochemistry and pharmacology of *C. officinalis* Linn. (Asteraceae), a medicinal plant found in central and southern Europe, western Asia and the United States, amongst others. A variety of phytochemicals such as terpenoids, flavonoids, coumarins, quinones, volatile oil, carotenoids and others have been reported to be present in this plant. It exhibits several pharmacological activities such anti-HIV, anti-cancer (dual activity), anti-inflammatory, hepatoprotective, spasmolytic and spasmogenic (dual activity), amongst others. It is potentially an important medicinal plant for mankind.

ACKNOWLEDGEMENT

The authors wish to thank Professor UA Deokate for the photographs used in this review, BA Baviskar for assistance with software used in drawing the chemical structures, and Professor IA Farooqui for financial assistance.

REFERENCES

1. *Agrawal OP, Raju PS. Global market of herbal products: Opportunities for Indian Traditional System of Medicine. New Delhi, India, Narcosa Publishing House, 2006, pp 5-10.*
2. *Lt. Colonel Kirtikar KR, Major Basu BD. Indian Medicinal Plants. Vol II, Deharadun, India, International Book Distributor, 1993, pp 1413-1414.*
3. *The Wealth of India, Raw Materials, A Dictionary of Indian Raw Material & Industrial Products. Vol 3, New Delhi, Publications & Information Directorate CSIR, 1992, pp 55-58.*
4. *Calendula officinalis. From Wikipedia, the free encyclopedia. [Cited 2009 January 30} http://en.wikipedia.org/wiki/Calendula_officinalis.*
5. *Editorial Boards. PDR for Herbal Medicines. 2nd edn, Montvale, Thomson-Medical Economics, 2003; pp 497-500.*
6. *Norman GB, Max W. Herbal Drugs & Phytopharmaceuticals. 2nd edn, Germany, MedPharm GmbH Scientific Publishers, 2001; pp 118-120.*
7. *Ben-Erik VW, Michael W. Medicinal Plants of the Worlds. Times Edition, 2004, pp 74.*
8. *Khare CP. Encyclopedia of Indian Medicinal Plants. Germany, Springer-Verlag Publisher, 2004, pp 116-117.*
9. *Adler G, Kasprzyk Z. Free sterols, steryl esters, glycosides, acelyted glycosides and water-soluble complexes in Calendula officinalis. Phytochemistry, 1975; 14: 627-631.*
10. *Wilkomirski B, Kasprzyk Z. Free and ester-bound triterpene alcohols and sterols in cellular subfractions of Calendula officinalis. Phytochemistry, 1979; 18: 253-255.*
11. *Wilkomirski B. Pentacyclic triterpene triols from calendula officinalis flowers. Phytochemistry, 1985; 24(12): 3066-3067.*
12. *Zittwel-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Loggia RD, Tubaro A, Bertoldi M, Franz C. Antioedematous activities of the main triterpenoid esters of marigold (Calendula officinalis L.). J Ethnopharmacol, 1997; 57: 139-144.*
13. *Wojciechowski Z, Bochenska-Hryniewicz M, Kurcharezak B, Kasprzyk Z. Sterol and triterpene alcohol esters from calendula officinalis. Phytochemistry, 1972; 11: 1165-1168.*
14. *Kasprzyk Z, Wilkomirski B. Structure of a new triterpene triol from calendula officinalis flowers. Phytochemistry, 1973; 12: 2299-2300.*
15. *Sliwowski J, Dziewanowska K, Kasprzyk E. Ursadiol: a new triterpene diol from calendula officinalis flowers. Khim Prir Soed, 1973; 12: 157-160.*
16. *Eitterl-Eglseer K, Reznicek G, Jurenitsch J , Novak J, Zitterl W, Franz C. Morphogenetic variability of faradiol monoesters in marigold Calendula officinalis L. Phytochem Anal, 2001; 12: 199-201.*
17. *Neukiron H, D'Ambrosio M, Dovia J, Guerriero A. Simultaneous Quantitative Determination of Eight Triterpenoid Monoesters from Flowers of 10 Varieties of Calendula officinalis L. and Characterisation of A New Triterpenoid Monoester. Phytochem Anal, 2004; 15: 30-35.*
18. *Ukiya M, Akihisa T, Yasukava K, Tokuda H, Suzuki T, Kimura Y. Antiinflammatory, anti-Tumor-Promoting and Cytotoxic Activities of Constituents of Marigold (Calendula officinalis) Flowers. J Nat Prod, 2006; 69: 1692-1696.*
19. *Vecherko LP, Sviridov AF, Zinkevich EP, Kogan LM. Structures of calenduloside g and h from the roots of calendula officinalis. Khim Prir Soed, 1974; 4:532-534.*
20. *Vecherko LP, Sviridov AF, Zinkevich EP, Kogan LM. The structure of calenduloside C and D from the roots of Calendula officinalis. Khim Prir Soed, 1975; 3:366-73.*
21. *Vecherko LP, Kabanov US, Zinkevich EP, Kogan LM. The structure of calenduloside B from the roots of Calendula officinalis. Khim Prir Soed, 1971; 4: 533-533.*
22. *Vecherko LP, Zinkevich EP, Libizov NI, Ban'kooskii AI. Calenduloside A from Calendula officinalis. Khim Prir Soed, 1969; 5(1): 58-59.*

23. *Ruszkowski D, Szakiel A, Janiszowska W. Metabolism of [3-^{3}H] oleanolic acid in Calendula officinalis L. roots. APP, 2003; 25(4): 311-317.*
24. *Wojciechowski Z, Jelonkiewicz-Konador A, Tomaszewski M, Jankowski J, Kasprzyk Z. The structure of glucosides of oleanolic acid isolated from the roots of calendula officinalis flowers. Phytochemistry, 1971; 10: 1121-24.*
25. *Vidal-Ollivier E, Balansard G. Revised structures of triterpenoid saponins from the flowers of Calendula officinalis. J Nat Prod, 1989; 52(5): 1156-1159.*
26. *Naved T, Ansari SH, Mukhtar HM, Ali M. New triterpenic esters of oleanene-series from the flowers of Calendula officinalis Linn. Org Chem Incl Med Chem, 2005; 44: 1088-1091.*
27. *Kurkin VA and Sharova OV. Flavonoids from calendula officinalis flowers. Khim Prir Soed, 2007; 2: 179-180.*
28. *Vidal-Ollivier E. Flavonol glycosides from Calendula officinalis flowers. Planta Med, 1989; 55: 73-73.*
29. *Kerkach AI, Komissarenko NF and Chernobai VT. Coumarines of the inflorescences of Calendula officinalis and Helichrysum arenarium. Khim Prir Soed, 1986; 6: 777-777.*
30. *Janiszowska W, Michalski W, Kasprzyk Z. Polyprenyl quinones and α-tocopherol in calendula officinalis. Phytochemistry, 1976; 15: 125-127.*
31. *Okoh OO, Sadimenko AA, Afolayan AJ. The effects of age on the yield and composition of the essential oils of Calendula officinalis. J Appl Sci; 2007; 7(23): 3806-3810.*
32. *Bako E, Deli J, Toth G. HPLC study on the carotenoid composition of Calendula products. J Biochem Biophys Methods, 2002; 53: 241-250.*
33. *Goodwin TW. Studies in carotenogenesis: the carotenoids of the flower petals of calendula officinalis. Biochem J, 1954; 58: 90-94.*
34. *Abajova RL, Aslanov SM, Mamedova ME. Amino acids of calendula officinalis. Chemistry of Natural Compounds, 1994; 30(15): 641-641.*
35. *Varlijen J. Structural analysis of rhamnoarabinogalactans and arabinogalactans with immunostimulating activity from Calendula officinalis. Phytochemistry, 1989; 28: 2379-2383.*
36. *Wagner H, Proksch A, Riess-Maurer I, Vollmar A, S. Odenthal, Stuppner H , Jurcic K, Le Turdu M, Fang Jn.. Immunstimulierend wirkende Polysaccharide (Heteroglykane) aus höheren Pflanzen. Arzneimittel-Forschung, 1985; 7: 1069–1075.*
37. *Vlchenko NT, Glushenkova AI and Mukhamedova KS. Lipids of calendula officinalis. Chemistry of Natural Compounds, 1998; 34(3): 272-274.*
38. *Wilkomirski B, Kasprzyk Z. Free and ester-bound triterpene alcohols and sterols in calendula flowers. Phytochemistry, 1979; 18: 253-255.*
39. *Badami RC, Morris LJ. The oxygenated fatty acid of calendula seeds oil. The Journal of the American Oil Chemist's Society, 1965; 42: 1119-1121.*
40. *Willuhn G, Westhaus RG. Loliolide (Calendin) from Calendula officinalis. Planta Med, 1987; 53: 304-304.*
41. *Fleisonner AM. Plant extracts: To accelerate healing and reduce inflammation. Cosmet Toilet, 1985; 45: 100-113.*
42. *Komoe H, Hayashi N. Paraffins of the petals of Calendula officinalis. Phytochemistry, 1971; 10: 1944-1944.*
43. *Della LR. Topical anti-inflammatory activity of Calendula officinalis extracts. Planta Med, 1990; 56: 658-658.*
44. *Della LR, Della LR, Tubaro A, Sosa S, Becker H, Saar S, Isaac O. The role of triterpenoids in topical anti-inflammatory activity of Calendula officinalis flowers. Planta Med, 1994; 60: 516-520.*
45. *Zitterl-Eglseer K. Anti-oedematous activities of the main triterpenoidal esters of marigold (Calendula officinalis L.) J Ethnopharmacol, 1997; 57: 139-144.*
46. *Casley-Smith JR. The effects of 'Unguentum lyphaticum' on acute experimental lymphedema and other high-protein edema. Lymphology, 1983; 16: 150-156.*
47. *Kalvatene Z, Walder R, Gabzaro D. Anti-HIV activity of extracts from Calendula officinalis flowers. Biomed & Pharmacother, 1997; 51: 176-180.*
48. *Iauk L, Lo-Bue AM, Milazzo I, Rapisarda A, Blandino G. Antibacterial Activity of Medicinal Plant Extracts Against Periodontopathic Bacteria. Phytother Res, 2003; 17: 599-604.*
49. *Gazim ZC, Rezende CM, Fraga SR, Svidzinski TE, Cortez DG. Antifungal activity of the essential oil from calendula officinalis l. (asteraceae) growing in brazil. Braz. J. Microbiol 2008; 39: 61-63.*
50. *Medina EJ, Lora AG, Paco L, Algarra I, Collado A, Garrido F. A new extract of the plant Calendula officinalis produces a dual in vitro effect: cytotoxic antitumor activity and lymphocyte activation. BMC Cancer, 2006; 6: 119-132.*
51. *Boucard-Maitre Y, Boucard-Maitre Y, Algernon O, Raynaurd, J. Genotoxic and antitumoral activity of Calendula officinalis extracts. Pharmazie, 1988; 43: 220-221.*
52. *Rasu MA, Tamas M, Puica C, Roman I, Sabadas M. The hepatoprotective action of ten herbal extracts in CCl_4 intoxicated liver. Phytother Res, 2005; 19: 744-749.*
53. *Lin LT, Liu LT, Chiang LC, Lin CC. In vitro anti-hepatoma activity of fifteen natural medicines from Canada. Phytother Res, 2002; 16: 440-444.*
54. *Popovic M, Kaurinovic B, Mimica-Dukic N, Vojinovic-Miloradov M, Cupic V.. Combined effects of plant extracts and xenobiotics on liposomal lipid peroxidation. Part 1. Marigold*

extract-ciprofloxacin/pyralene. Oxidation Commum, 1999; 22: 487-494.

55. *Frankic T, Salobir K, Salobir J. The comparison of in vivo antigenotoxic antioxidative capacity of two propylene glycol extracts of Calendula officinalis (Marigold) and vitamin E in young growing pigs. J Anim Physiol Anim Nutr, 2008; 41: 1-7.*
56. *Chandran PK, Kutton R. Effect of Calendula officinalis flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns. J Clin Biochem Nutr, 2008; 43: 58-64.*
57. *Leach MJ. Calendula officinalis and wound healing: A systematic review. Wounds, 2008; 20(8): 1-7.*
58. *Bashir S, Janbaz KH, Jabeen Q and Gilani AH. Studies on Spasmogenic and Spasmolytic Activities of Calendula officinalis flowers. Phytother Res, 2006; 20: 906-910.*
59. *Alexenizor M, Dorn A. Screening of medicinal and ornamental plants for insecticidal and growth regulating activity. J Pestic. Sci, 2007; 80: 205-215.*
60. *Perez-Guitierrez S, Vargas-Solis R, Miguel ZS, Perez-G C, Perez-G RM. Inhibitory effect of five plant extracts on heart rates of rats. Phytother Res, 1998; 12: S49-50.*
61. *Perez-Carreon JI, Cryz-Jimener G, Licea-Vega JA, Popoca EA, Fazenda SF, Villa-Trevinos. Genotoxic and antigenotoxic properties of Calendula officinalis extracts in rat liver culture treated with diethylnitrosamine. Toxicology in vitro, 2002; 16: 253-258.*
62. *Bogdanova NS, Nikolaeva IS, Shcherbakova LI, Tolstova TI, Moskalenko NI, Pershin GN. Study of antiviral properties of Calendula officinalis. Farmakol Toksikol (Moscow), 1970, 33: 349.*
63. *Silva ER, Goncalves ES, Aguiar F, Evencio LB, Lyra MA, Coelno MC, Fraga MA, Wanderloy AG. Toxicological studies on hydroalcoholic extract of Calendula officinalis L. Phytother Res, 2007; 21: 332-336.*
64. *Reider N, Comericki P, Hausen BM, Fritsch P, Aberer W. The seamy side of natural medicines: Contact sensitization to arnica (Arnica montana L.) and marigold (Calendula officinalis L.). Contact Dermatitis, 2001; 45: 269-272.*
65. *Braun L, Cohen M. Herbs and natural supplements: an evidence based guide. Sydney, Elsevier, 2005, pp 98-100.*
66. *Bone K. A clinical guide to blending liquid herbs. St.Louis Missouri, Churchill Livingstone, 2003, pp 120-123.*