

Therapeutic Potential of *Calendula officinalis*

Abstract

Calendula officinalis (Calendula), belonging to the family of Asteraceae, commonly known as English Marigold or Pot Marigold is an aromatic herb which is used in Traditional system of medicine for treating wounds, ulcers, herpes, scars, skin damage, frost-bite and blood purification. It is mainly used because of its various biological activities to treat diseases as analgesic, anti-diabetic, anti-ulcer and anti-inflammatory. It is also used for gastro-intestinal diseases, gynecological problems, eye diseases, skin injuries and some cases of burn. Calendula oil is still medicinally used as, an anti-tumor agent, and a remedy for healing wounds. Plant pharmacological studies have suggested that Calendula extracts have antiviral and anti-genotoxic properties *in-vitro*. In herbalism, Calendula in suspension or in tincture is used topically for treating acne, reducing inflammation, controlling bleeding, and soothing irritated tissue. Calendula is used for protection against the plague. In early American Shaker medicine, calendula was a treatment for gangrene. In addition to its first aid uses, calendula also acts as a digestive remedy. An infusion or tincture of the flowers, taken internally, is beneficial in the treatment of yeast infections, and diarrhea. An infusion of *Calendula officinalis* may also be used to treat bee stings, eye inflammations, boils and abscesses, varicose veins, eczema, and as a gargle for mouth sores or to relieve toothache. It improves the circulation of the blood & the lymphatic fluids and aids in elimination of toxins from the body. This plant is rich in many pharmaceutical active ingredients like carotenoids, flavonoids, glycosides, steroids and sterols quinines, volatile oil, and amino acids. The extract of this plant as well as pure compound isolated from it, has been demonstrated to possess multiple pharmacological activities such as anti-cytotoxic, hepato-protective and spasmolytic amongst others. Acute toxicity studies in rats and mice suggest that the extract is relatively nontoxic. Animal tests have demonstrated minimal skin irritation, and no sensitization or photo toxicity. Minimal ocular irritation was seen with one formulation and no irritation with others. Six saponins isolated from *C. officinalis* flowers were not mutagenic in an Ames test, and a tea derived from *C. officinalis* was not genotoxic in *Drosophila melanogaster*. Clinical testing of cosmetic formulations containing the extract elicited little irritation or sensitization. This review has explored the organoleptic, *in-vitro* and *in-vivo* pharmacological activities as well as description, cultivation and active chemical constituents of *Calendula officinalis* in order to existing information on this plant as well as highlighted its multi activity properties as a medicinal agent.

Keywords: *Calendula officinalis*, anti-ulcer, antiviral, anti-genotoxic, anti-inflammatory, hepato-protective, spasmolytic properties

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Introduction

Calendula officinalis belonging to the family Asteraceae is a well known medicinal plant. It is commonly known as English marigold, pot marigold. Chemically, *Calendula officinalis* possesses various biological active constituents such as carotenoids, flavonoids, saponins, sterols, phenolic acids, lipids, etc. Various parts of plant such as leaves, flowers have been reported to possess therapeutic activity.¹ 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. Advanced analytical techniques have been used to isolate novel chemical constituents such as isorhamnetin, rutin, quercetin glucoside, which are biologically active as well as used in food and cosmetic industry.² The plant has yellow or orange coloured flowers which are used as food, dye, spice, tea, ointment or cream in cosmetics. It possesses cytotoxic as well as tumor reducing potential. Traditionally, *Calendula officinalis* was used as anti-inflammatory, diaphoretic, analgesic, antiseptic and in jaundice treatment.³ However, it is pointed out that flowers of the plant have been reported to be most potent therapeutically. The pleiotropic properties of this meritocratic plant include anti-ulcer, anti HIV, immune-stimulant, wound healing.⁴

internally, it is used for mucous membrane inflammations, peptic and duodenal ulcers, spasms of the GI tract, duodenal and intestinal mucosa, dysmenorrhea (painful menstruation) especially in nervous or anemic women, splenic and hepatic inflammations. Generally in cases of external use, it is clinically given for treating skin inflammations, open wounds and laceration wounds with bleeding. It is also used for treating minor diseases like razor burns and wind burns. It is also used as a mouthwash after tooth extractions.⁵

Uses of calendula

Sedative drugs: In early animal studies, high doses of ingested calendula preparations were reported to act as sedatives. Therefore, combination use with sedative agents may lead to additive effects. In rats, calendula was shown to increase hexobarbital induced sleeping time. A systemic effect after topical use of calendula in human is not clear.

Antihypertensive drugs: In early animal studies, high doses of calendula preparations were reported to possess hypertensive effects. Therefore, combination use with hypertensive agents may lead to additive effects.

Hypoglycemic drugs: *Calendula* may increase the activity of hypoglycemic medications or insulin.

Cholesterol-lowering drugs: *Calendula* may have an additive effect with agents that decrease lipids and triglycerides.

Classification of *Calendula officinalis* 6–9

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

Synonyms

Pot marigold, English marigold, Bride of the Sun, bull flower, butterwort

Description

Calendula officinalis is a short-lived aromatic herbaceous perennial, growing to 80cm (31in) tall, with sparsely branched lax or erect stems. The leaves are oblong-lance. The disc florets are tubular and hermaphrodite, and generally of a more intense orange color, 5–17cm (2–7in) long, hairy on both sides, and with margins entire or occasionally wavy or weakly toothed. The inflorescences are yellow, comprising a thick capitulum or flower head 4–7cm diameter surrounded by two rows of hairy bracts; in the wild plant they have a single ring of ray florets surrounding the central disc florets-yellow colour than the female, tridentate, peripheral ray florets. The flowers may appear all year long where conditions are suitable. The fruit is a thorny curved achene.¹⁰

Organoleptic properties

The odour of *Calendula officinalis* is faint and aromatic.

The taste of *Calendula officinalis* is bitter.

Cultivation

The plant is native to Central and Southern Europe, Western Asia and the US.¹¹ *Calendula officinalis* is widely cultivated and can be grown easily in sunny locations in most kinds of soils. Although perennial, it is commonly treated as an annual, particularly in colder regions where its winter survival is poor and in hot summer locations where it also does not survive. *Calendulas* are considered by many gardening experts as among the easiest and most versatile flowers to grow in a garden, especially because they tolerate most soils. In temperate climates, seeds are sown in spring for blooms that last throughout the summer and well into the fall. In areas of limited winter freezing, seeds are sown in autumn for winter color. Plants will wither in subtropical summer. Seeds will germinate freely in sunny or half-sunny locations, but plants do best if planted in sunny locations with

rich, well-drained soil. Pot marigolds typically bloom quickly from seed (in under two months) in bright yellows, golds, and oranges.

Discussion

Phytochemistry of *Calendula officinalis*

A number of phytochemical studies have well reported about the presence of several classes of chemical compounds, the main ones being terpenoids, flavonoids, coumarin, quinines, volatile oil, carotenoids and amino acids in the plant.

Terpenoids: Various terpenoids have been reported from the petroleum ether extract of *C. officinalis* flowers. They include sitosterols, stigmaterols,¹² diesters of diols,¹³ 3-monoesters of taraxasterol, lupeol,^{14,15} erythrodiol, brein,^{16,17} ursadiol,¹⁸ faradiol-3-O-palmitate, faradiol-3-O-myristate, faradiol-3-O-laurate,¹⁹ arnidiol-3-O-palmitate, arnidiol-3-O-myristate, arnidiol-3-O-laurate, calenduladiol-3-O-palmitate, calenduladiol-3-O-myristate,^{20,21} oleanolic acid saponins: calendulose AH,²²⁻²³ oleanane triterpene glycoside: calendula glycoside A, calendulaglycoside A6-O-n-methylester, calendulaglycoside A6''-O-n-butylester, calendula glycoside B, calendulaglycoside B6-O-n-butylester, calendula glycoside C, calendula glycoside C 6-O-n-methyl ester, calendula glycoside C 6-O-n-butyl ester, calendulose F6-O-n-butyl ester, calendulose G6-O-n-methyl ester, glucoside of oleanolic acid (mainly found in roots of grown and senescing plants) I, II, III, VI, VII,^{24,25} and glucuronides (mainly found in flowers and green parts) F, D, D2, C, B and A.²⁶ One new triterpenic ester of oleanane series has been isolated from flowers was cornulacic acid acetate from flowers.²⁷

Flavonoids: Various flavonoids have been isolated from the ethanol extract of the inflorescence of *C. officinalis*. They include quercetin, isorhamnetin,²⁸ isoquercetin, isorhamnetin-3-O-D-glycoside, narcissin, calendoflaside,²⁹ calendoflavoside, calendoflavobioside, rutin, isoquercetin neohesperidoside, isorhamnetin-3-O-neohesperidoside, isorhamnetin-3-O-2G-rhamnosyl rutinoside, isorhamnetin-3-O-rutinoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside.³⁰

Coumarins: The ethanol extract of the inflorescence of the *C. officinalis* was reported to contain coumarins-scopoletin, umbelliferone and esculetin.³¹

Quinones: Quinones reported from *C. officinalis* were plastoquinone, phylloquinone, and tocopherol in the chloroplast, ubiquinone, phylloquinone, tocopherol in mitochondria, and phylloquinone in the leaves.³²

Volatile oil: *C. officinalis* flowers contain maximum volatile oil at full flowering stage (0.97 %) and minimum during the pre-flowering stage (0.13%). 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 -pinenene, sabinene, β -pinenene, limonene, 1,8-cineol, p-cymene, trans- β -ocimene, γ -terpinene, δ -3-carene, nonanal, terpene-4-ol, 3-cyclohexene-1-ol, α -phellandrene, α -terpeneol, geraniol, carvacrol, bornyl acetate, sabinyl acetate, α -cubebene, α -copaene, α -bourbonene, cubebene, α -gurjunene, aromadendrene, β -aryophyllene, α -ylangene, α -humulene, epibicyclosequiphellandrene, germacrene D, allo aromadendrene, β -saliene, calarene, muurolene, δ -cadinene, cadina 1,4-diene, α -cadinene, nerolidol, palustron, endobourbonene, oplophenone, α -cadinol, Tmuurolol. The essential oil was found to be

rich in α -cadinene, α -cadinol, t-murolol, limonene, and 1,8-cineol with p-cymene at lower levels at the post-flowering periods.³³

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, 9Zanthroxanthin, lutein, 9/9''A-lutein, 13/13''Zlutein, α -cryptoxanthin, β -cryptoxanthin, z-cryptoxanthin, lycopene, α -carotene, and β -carotene. Total carotenoid (mg/g dry weight) was 7.71% for petals and 1.61% for pollens. Carotenoid compositions of the leaves and stems were reported as neoxanthin, 9Zneoxanthin, violaxanthin, luteoxanthin, 9Zviolaxanthin, 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%.^{34,35} Glycosides of quercetin and isorhamnetin were the predominant components of the flavonoids, while beta-carotene and lutein were the most abundant carotenoids.³⁶ Analysis of carotenoid composition in petals of *Calendula officinalis* was made. Nineteen carotenoids were identified in extracts of petals of orange and yellow flowered cultivars of calendula.

In addition, ten carotenoids were unique to orange-flowered cultivars. The ultraviolet (UV) visible absorption maxima of these ten carotenoids were at longer wavelengths than that of flavoxanthin, the main carotenoid of calendula petals providing the evidence that these carotenoids are responsible for the orange color of the petals. Six carotenoids had a cis structure at C-5(C-5') and it is conceivable that these (5Z)-carotenoids are enzymatically isomerised at C-5 in a pathway that diverges from the main carotenoid biosynthesis pathway. Among them, (5Z, 9Z)-lycopene, (5Z, 9Z, 5'Z, 9'Z)-lycopene, (5'Z)-gamma-carotene, (5'Z, 9'Z)- rubixanthin and (5Z, 9Z, 5'Z)-lycopene have been identified.³⁷ According to the Research work on specificity of the tonoplast, transport of oleanolic acid monoglycosides in the vacuoles from *Calendula officinalis* leaves, the proper structure of both parts of oleanolic acid monoglycoside, i.e. aglycon and the sugar moiety, are required for binding to a specific tonoplast carrier.³⁸ These two glycosides were isolated from leaf protoplasts of the plant with the use of chemically synthesized analogues. Structures of new ionone and sesquiterpene glycosides were investigated from Egyptian *Calendula officinalis*. Two new ionone glucosides (officinosides A and B) and two sesquiterpene oligoglycosides (officinosides C and D) were isolated from the flowers of Egyptian *Calendula officinalis*, the structures of which were elucidated on the basis of chemical and physicochemical evidences.³⁹

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, asparagines, 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%.⁴⁰

Carbohydrates: The ethanol extract of the inflorescence of plant showed the presence of polysaccharides, PS-I, II, and III having a (1-3)-D-galactam backbone with short side chains at C-6 comprising -araban(1-3)-araban and alpha-L-rhamnan-(1-3)- araban along with monosaccharide's.^{41,42}

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^{43,44} one oxygenated fatty acid also reported from the seed oil of *C. officinalis* was D-(+)-9-hydroxy-10,12-octadecadienoic acid.⁴⁵

Other constituents: Other phytochemicals include the bitter constituent, loliolide (calendin),⁴⁶ calendulin⁴⁷ and paraffins.⁴⁸

Therapeutic potential

In-vivo pharmacology activities of calendula officinalis

Antidiabetic and anti hyperlipidemic activities: Diabetes was induced to the rat by single intraperitoneal injection of alloxan (150mg/kg) of body weight. The blood glucose level and urine sugar level were significantly elevated in diabetic rats compared to normal rats. Upon oral administration of hydro alcoholic extract of *Calendula officinalis* in diabetic rats at dose 25 and 50mg/kg body weight significantly lowered the blood glucose and urine sugar as they compared with group of diabetics rats. Hydro alcoholic extract of *Calendula officinalis* in diabetic rats at a dose of 100mg/kg body weight was found to be highly significant as it restored all the parameters to the normal levels of blood glucose, urine sugar and serum lipid in alloxan diabetic rats. The extract increases the total hemoglobin level. The extract was similar to that of insulin. Thus, the investigation clearly shows that hydro alcoholic extract of *Calendula officinalis* has both antidiabetic and antihyperlipidemic effects.⁴⁹ The structures of the officinosides were elucidated on the basis of chemical and physicochemical evidence. The inhibitory activities of the principal saponins from the flowers of *C. officinalis* was noted on the increase of serum glucose levels in oral glucose-loaded rats, on gastric emptying in carboxy methyl cellulose sodium salt test meal-loaded mice, and on ethanol or indomethacin induced gastric mucosal lesions in rats and also discussed the structure requirements for this activities.⁵⁰

Cardiovascular activities: *Calendula officinalis* could be cardio-protective against ischemic heart disease. Two groups of hearts were used: the treated rat hearts were perfused with *Calendula officinalis* solution at 50mM in KHB buffer (in mM) sodium chloride 118mM, potassium chloride 4.7mM, calcium chloride 1.7mM, sodium bicarbonate 25mM, potassium biphosphate 0.36mM, magnesium sulfate 1.2mM, and glucose 10mM) for 15min prior to subjecting the heart to ischemia, while the control group was perfused with the buffer only. Calendula achieved cardio protection by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocytes apoptosis. Cardio protection appears to be achieved by changing ischemia reperfusion-mediated death signal into a survival signal by modulating antioxidant and anti-inflammatory pathways as evidenced by the activation of Akt and Bcl2 and depression of TNF α . The results further strengthen the concept of using natural products in degeneration diseases like ischemic heart disease.⁵¹

Hepatoprotective activities: The 80% methanolic extract of *Calendula officinalis* leaves was investigated against acetaminophen-induced hepatic damage in 30 male albino rats. Acetaminophen produces 100% mortality at dose of 1gm/kg in mice, while

pretreatment of mice with *Calendula officinalis* (1.0gm/kg) reduced the death to 30%. Pretreatment of mice with leaves extract (500mg/kg orally, four doses at 12 hours interval) prevented ($p < 0.05$) the acetaminophen (640mg/kg induced rise in serum transaminases (SGOT, SGPT), serum bilirubin and serum alkaline phosphatase. Post treatment with three successive doses of leaves extract (500mg/kg, 6 hourly) restricted the hepatic damage induced by acetaminophen ($p < 0.05$).⁵²

Antioxidant effects: An extract of *Calendula officinalis* Linn., was evaluated for its antioxidant potential by oral administration of Calendula alcoholic extract inhibit superoxide generation in macrophages in female swiss albino mice by 12.6% and 38.7% at doses of 100 and 250 mg/kg body wt. Oral administration of *Calendula officinalis* to mice for 1 month significantly increased catalase activity. The extract produced significant increase in glutathione levels in blood and liver. Glutathione reductase was found to be increased, whereas glutathione peroxidase was found to be decreased after administration of Calendula extract.⁵³

Anthelmintic activities: The dried flowers and leaves of *C. officinalis* have anthelmintic activity. The aqueous extract of dried flowers and leaves of *C. officinalis* were prepared by decoction method. The assay was performed on Indian adult earth worm, due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. *Calendula officinalis* flowers and leaf extracts were also shown to have anthelmintic activity the crude extracts of *C. officinalis* flowers and leaf extracts demonstrated paralysis at 56.5min and death of worms at 111.2minutes. The plants contain saponins and have also shown anthelmintic potential which are in accordance with previous reports which reveals that saponins are known to have anthelmintic activity.⁵⁴

Anti-inflammatory activities: The ethanolic extract of *Calendula officinalis* possessed significant anti-inflammatory activity against carrageenan and dextran-induced paw edema. Oral administration of the 250 and 500mg/kg body weight of *Calendula officinalis* extract produced significant inhibition (50.6 and 65.9% respectively) in paw edema of animal induced by carrageenan and 42.9 and 42.4% respectively with inflammation produced by dextran. In chronic anti-inflammatory model using formalin, administration of 250 and 500mg/kg body weight calendula extract produced an inhibition of 32.9 and 62.3% respectively compared to controls. TNF- α production by macrophage culture treated with lipopolysaccharide (LPS) was found to be significantly inhibited by extract. Moreover, increased levels of proinflammatory cytokines IL-1 β , IL-6, TNF- α and IFN- γ and acute phase protein, C reactive protein (CRP) in mice produced by LPS injection were inhibited significantly by the extract. LPS induced cyclooxygenase-2 (Cox-2) level in mice spleen were also found to be inhibited by extract treatment. The results showed that potent anti-inflammatory response of *C. officinalis* extract may be mediated by the inhibition of pro-inflammatory cytokines and Cox-2 and subsequent prostaglandin synthesis.²⁴ Topical application of a 70% ethanol extract of the flowers to mice at a dose of 1.2mg/ear (corresponding to 4.16mg crude drug) reduced croton oil-induced ear oedema by 20%. External application of a carbon dioxide extract of the flowers (300mg/cm²) suppressed croton oil induced ear oedema in mice.⁵⁵

Wound-healing and angiogenic activities: Angiogenic activity of *Calendula officinalis* L. (Asteraceae) ethanolic extract and

dichloromethane and hexanic fractions were evaluated by using Models 36 rats and 90 embryonated eggs to evaluate healing and angiogenic activities of extracts and fractions of the plant, through the induction of skin wounds and the chorioallantoic membrane, respectively. The effect of vascular proliferation was also tested from the study to verify the intensity of expression of vascular endothelial growth factor (VEGF) in cutaneous wounds in rats. In morphometric evaluation increase of the vascular area and of percentage of red-marked areas was observed in CAM treated as positive control 1% (17 β -estradiol), ethanolic extract 1%, dichloromethane fraction 1% and hexanic fraction 1%, compared to solvent control (ethanol 70%). Digital planimetry by point counting performed on mice derm treated with ethanolic extract 1% revealed an increase in the number of blood vessels compared to solvent control.⁵⁶ They reported a statistically significant difference in reduction of total wound area compared with the control ($p < 0.05$), showing an overall decrease of 41.71% in the experimental group compared with 14.52% in the control group. They conclude that application of Calendula extract significantly increases epithelization in chronic venous ulcerations. Marigold therapy offers a non-invasive and gentle treatment for difficult to treat plantar verruca, painful hyperkeratotic lesions, and inflamed bursa secondary to hallux abducto valgus.⁵⁷

Anticancer activities: The results obtained indicated that none of the extracts had a direct mitogenic effect on human lymphocytes or thymocytes (stimulation index, SI < 0.07). Among the plants studied, *C. officinalis* showed a complete inhibitory effect on the proliferation of lymphocytes in the presence of PHA (SI range 0.01-0.49).⁵⁸

In-vitro pharmacology activities of calendula officinalis

Hepatoprotective activities: The potential hepatoprotective effects of *Calendula officinalis* and morus Alba extracts was noted against cytotoxicity and oxidative stress induced by carbon tetrachloride (CCl₄) in isolated primary rat hepatocytes. The dose response effect of different concentrations of *Calendula officinalis* and Morus Alba extracts (1, 10, 100 and 1000 μ g/ml) on CCl₄ induced decrease in the viability% of isolated rat hepatocytes. Hepatocytes were isolated by collagenase perfusion two steps technique. Cytotoxicity was determined by assessing cell viability and leakage of cytosolic enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). Oxidative stress was assessed by determining reduced glutathione (GSH) level and lipid peroxidation as indicated by thiobarbituric acid reactive substances (TBARS) production. Exposure of isolated rat hepatocytes to CCl₄ caused cytotoxicity and oxidative injury, manifested by loss of cell viability and significant increase in ALT, AST and LDH leakages. As well as, CCl₄ caused progressive depletion of intracellular GSH content and significant enhancement of TBARS accumulation. Pre-incubation of hepatocytes with either *Calendula officinalis* or morus alba extracts ameliorated the hepatotoxicity and oxidative stress induced by CCl₄, as indicated by significant improvement in cell viability and enzymes leakages (ALT, AST and LDH) and also, significant improvement of GSH content and significant decrease in TBARS formation as compared to CCl₄ treated cells. The dose response effect of different concentrations of *Calendula officinalis* and morus alba extracts (1, 10, 100 and 1000 μ g/ml) on CCl₄ induced decrease in the viability% of isolated rat hepatocytes. The results revealed that CCl₄ (5mM) induced significant decrease in the viability% of isolated rat hepatocytes after 30 min of incubation period. This decrease in the viability was a time dependant compared to a control group.⁵⁹

Antibacterial activities: Ethanolic and aqueous extracts of *Calendula officinalis* inhibit the growth of the bacteria used in the study, at concentrations ranging from 125µg/ml to 64mg/ml. Methanolic extract inhibited the growth of both *S. aureus* and *E. coli* at 64mg/ml. Aqueous extract of *Calendula officinalis* exhibited highest antibacterial activity against all the bacteria tested. *S. aureus* was found to be more susceptible as compared to other bacteria.⁶⁰ The antibacterial activities of *Calendula officinalis* Linn. Dried leaf powder of *Calendula officinalis* was successively extracted with petroleum ether, chloroform and ethanol using Soxhlet and macerated to form water extract. All extracts were screened for its antibacterial and antifungal activity using agar well diffusion method. The microorganisms used for antibacterial and antifungal were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans* and *Aspergillus niger*. Gentamicin 5µg/ml was used as standard. The extracts showed antimicrobial activity were subjected to minimum inhibitory concentration assay by two fold dilutions method. Petroleum ether, chloroform, ethanol and water extract exhibited *in-vitro* antibacterial activity.⁶¹ Antimicrobial activity of ethanolic, methanolic, acetone and chloroform extract of *C. officinalis* was studied against the gram-positive bacterial strains were *Escherichia coli*, *Staphylococcus aureus*, and the gram-negative strains were *Salmonella typhae* and *Vibrio cholera*. The fungal strain used was *Candida albicans*. Ethanolic extract gave activity against *E. coli*, *Vibrio cholera* and *Candida albicans*. Methanolic extract gave only against *Candida albicans*. Chloroform gave antimicrobial activity against all microbes while acetone gave only against *E. coli*.⁶²

Anticancer activities: The flavonoid extracts (0.05-50µg/ml) did not cause significant effect on the proliferation of two cell lines. It may be related to the attached sugar molecule at the position 3 of flavones that can reduce its ability to bind aromatase and other enzymes.⁶³ Thirteen saponins were isolated and identified from *Calendula officinalis*, *C. arvensis* and *Hedera helix*. Mutagenic and antimutagenic activities of these products were investigated using a modified liquid incubation technique of the Salmonella/microsomal assay. The Salmonella tester strain TA98±S9 mix was used. Screening of the antimutagenic activity was performed with a known promutagen: benzo-[a] pyrene and mutagenic urine concentrate from a smoker. Antimutagenic activities were also compared with the activity of chlorophyllin. All the saponins were found to be non-toxic and non-mutagenic for dose of 400µg/kg.⁶⁴

Anti-inflammatory activities: The hydro alcoholic extract of *Calendula officinalis* can suppress the activities of 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2) (key enzymes) in the formation of pro inflammatory eicosanoids from arachidonic acid.⁶⁵ The occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% v 63%; p<.001) with the use of calendula than with trolamine. Moreover, patients receiving calendula had less frequent interruption of radiotherapy and significantly reduced radiation-induced pain. Calendula is highly effective for the prevention of acute dermatitis of grade 2 or higher and should be proposed for patients undergoing postoperative irradiation for breast cancer.⁶⁶

Antioxidant activities: Aqueous extract of petals showed higher antioxidant activity than the leaves. The results obtained in the present study indicate that the leaves and petals of *Calendula officinalis* are a potential source of natural antioxidants.⁶⁷ An alcoholic extract of *Calendula officinalis* Linn (Composite) was evaluated for its

antioxidant potential *in vitro*. *Calendula officinalis* extract was found to scavenge superoxide radicals generated by photo reduction of riboflavin and hydroxyl radicals generated by Fenton reaction and inhibited *in vitro* lipid peroxidation. Concentrations needed for 50% inhibition (IC50) were 500, 480, and 2000 mg/ml, respectively. Extract scavenged ABTS radicals and DPPH radicals and IC50 were 6.5 and 100 mg/ml, respectively.⁶⁸ For *Calendula officinalis* extract of 7.5mg/ml concentration, the LPO decreased slowly with dose from 68% to 40% at 20kGy. The LPO for 3.75mg/ml concentration decreased suddenly from 56% to 28% at 1kGy dose. Then, it decreased slowly with dose.⁶⁹

Anti-HIV activities: Chloroform extract of the flowers inhibited the replication of HIV-1 in acutely infected lymphocytic MOLT-4 cells *in vitro* (IC50 0.4mg/ml). A chloroform extract also inhibited HIV-1 reverse transcriptase activity in a dose-dependent manner (ED50 51.0mg/ml).⁷⁰

Conclusion

The various pharmacological properties have been attributed in preclinical research to various constituents, including anti-inflammatory, immune-stimulating, antibacterial, antiviral, antiprotozoal and antineoplastic properties. The juice from the fresh flowers or stem is said to help remove warts and help to heal mucous membranes and skin. An infusion or tincture of the herb is also helpful in cases of painful or delayed menstruation, and the herb is a beneficial ally in the transition to menopause. The tincture also has many other uses, such as a topical wash for diaper rash in infants, a mouth gargle for sores, a vaginal douche for yeast, an internal soother for inflamed lungs, a topical for hemorrhoids. A LD₅₀ of 375mg/kg and a LD₁₀₀ of 580mg/kg has been reported in mice by intravenous and intraperitoneal administration of aqueous extract of *Calendula officinalis*. In hydro-alcoholic extracts a LD₅₀ of 45mg/mouse (subcutaneous) and LD₅₀ of 526mg/100 g in rats (intravenous) have been reported. *Calendula officinalis* extract is reported to be used in almost 200 cosmetic formulations, over a wide range of product categories. Two homologous cDNAs, CoFad2 and CoFac2, were isolated from a *Calendula officinalis* developing seed by a polymerase chain reaction-based cloning strategy. Both sequences share similarity to FAD2 desaturases and FAD2-related enzymes. In *C. officinalis* plants CoFad2 was expressed in all tissues tested, whereas CoFac2 expression was specific to developing seeds. Expression of CoFad2 cDNA in yeast (*Saccharomyces cerevisiae*) indicated it encodes a Delta12 desaturase that introduces a double bond at the 12 position of 16:1(9Z) and 18:1(9Z). Expression of CoFac2 in yeast revealed that the encoded enzyme acts as a fatty acid conjugate converting 18:2(9Z, 12Z) to calenic acid 18:3(8E, 10E, 12Z). The enzyme also has weak activity on the mono-unsaturated 16:1(9Z) and 18:1(9Z) producing compounds with the properties of 8, 10 conjugated dienes.

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None.

Conflict of interest

None.

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