Organoleptic properties *in-vitro* and *in-vivo* pharmacological activities of *Calendula officinalis* Linn: An over review

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**ABSTRACT**

*Calendula officinalis*, 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. It is mainly used because of its various biological activities to treat diseases like analgesic, antidiabetic and anti-inflammatory. It is also used for ingastro-intestinal, gynaecological, eye disease, skin injuries and in some cases of burn. This plant is rich in many pharmaceutical active ingredients like carotenoids, flavonoids, glycosides, steroids and sterols quinines, volatile oil, and amino acid. The extract of this plant as well as pure compound isolated from it, have been demonstrated to possess multiple pharmacological activities such as anti-HIV, cytotoxic, anti-inflammatory, hepatoprotective and spasmolytic amongst others. In this review, we have explored the organoleptic, in-vitro and in-vivo pharmacological activities of *Calendula officinalis* in order to existing information on this plant as well as highlight its multiactivity properties as a medicinal agent.

**Keywords:** *Calendula officinalis*, Organoleptic Properties, Pharmacological activities, phyto-constituents.

**INTRODUCTION**

*Calendula officinalis*, Linn has been widely used in homeopathic medicine for the treatment of many diseases. It has been reported to possess many pharmacological activities, which include antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral. It also possess cytotoxic as well as tumor reducing potential. It is used as analgesic, anthelmintic, anti-bacterial, anti-emetic, anti-fungal, anti-inflammatory, anti-pyretic, antisepetic, anti-spasmodic, anti-viral, astringent, bitter, candidicide, cardiotonic, carminative, chologogue, dermagenic, diaphoretic, diuretic, hemostatic, immunostimulant, lymphatic, uterotonic, and as vasodilator. Generally in cases of external it is used 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. 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. It is also used as a mouthwash after tooth extractions [7].

**Taxonomic description [1, 2, 3, 4]**

Taxonomic classification of *Calendula officinalis*.

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

Traditionally, *C. officinalis* was taken internally to treat fevers, promote menstruation and treat jaundice. 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. *C. officinalis* contains following potentially active chemical constituents; Sesquiterpene and flavonol glycosides, Triterpenoid saponins, Triterpene alcohols, Flavonoids, crotenoides, xanthophylls, Phenolic acids and Other like sterols, mucilage, tocopherols, calendulin, bitters, phytosterols, resin, volatile oil [5].

It suggests that calendula may relieve inflammation and improve immunity by blocking certain inflammatory compounds and limiting the infiltration of white blood cells (an immune system response to inflammation) into tissues. Modern science has also supported the hypothesis of calendula's wound healing properties. Calendula has been found to stimulate the growth of new tissues and blood vessels when applied externally to wounds. Professional homeopaths often recommend ointments containing homeopathic doses of calendula to heal first-degree burns and
sunburns. In fact, some homeopaths consider this remedy the treatment of choice for children. Homeopathic calendula ointments may also be used in the healing stages of second and third degree burns to stimulate regrowth of skin and to diminish scar formation [6].

An annual herb, much branched from the base, very aromatic, up to 0.3–0.6m high; stem angular, hairy and solid. Leaves sessile, light green, with semi amplexicaul base; entire, undulate or remotely denticulate; glandular hairs on both surfaces; lower leaves spatulate, obtuse, sometimes acute at the apex, 10–20cm long and 1–4 cm wide; higher leaves oblong and mucronate, 4–7cm long. Involutural bracts 7–15 mm long, covered with long, glandular hairs; inner involucral bracts with pellucid, scarious margin; marginal flowers in cultivated plants often multi-seriate; corolla oblong-spatulate, bright yellow or orange, 15–25mm long and 3mm wide, 1–3-toothed with 4 or 5 veins, marginally entire, covered at the base with patent, long, thick hairs; corolla of disc flowers rounded, 3-dentate top, 1.5–2.5 cm long and 4–7mm in diameter, 5mm long tube and moderately widened limb. Stigma short, thick, hairy, ovary oblong, 0.5 mm in length, pubescent, shrivelling after anthesis. Achenes narrowly oblong, strongly curved, faintly ribbed, thinly pubescent or glabrous, 10–12mm long, outer achenes warty-ribbed outside, inner achenes prickly-warty, often with broad, thick margins [2,3].

Organoleptic Properties
Odour: faint, pleasantly aromatic
Taste: bitter.

Microscopic Characteristics
Inner epidermal cells of ray floret elongated, rectangular and almost straightwalled, cuticle faintly striated; stomata absent; outer epidermal cells similar, but with 3 or 4 anomocytic stomata; trichomes very numerous on the tube, biseriate; stigma epidermal cells straight-walled, polygonal. In disc floret, outer epidermal cells elongated, straight or slightly sinuous-walled, stomata absent; abundant trichomes on area below point of insertion of the stamens, mainly glandular, uniseriate or biseriate. Within the upper part of the anthers, a layer of isodiametric to elongated, moderately thick-walled, lignified and pitted cells; pollen grains spherical, up to 45mm in diameter, with 3 germinal pores, exine finely granular with numerous short spines; apex of stigma covered by short, bulbous papillae[2,3].

Flower
Calyx represented by 2 or 3 scales, superior; Corolla 2–5, gamopetalous, ligulate, superior, yellow or orange; Androecium 5, syngenesious, epipetalous, alternate to petals, anthers bicelled, introrse, superior; Gynoecium 2, bicarpellary, ovary inferior, unilocular, stigma bifid[2,8].

Powdered Plant Material
Yellow-green; fragments of corollas containing light yellow oil droplets; some corollas with fairly large anomocytic stomata, others containing prismatic and very small clusters of calcium oxalate crystals. Covering trichomes biseriate, multicellular and conical; glandular trichomes with a uniseriate or biseriate, multicellular stalk and a large, ovoid, biseriate, multicellular head. Spherical pollen grains up to 45mm in diameter, exine finely granular with numerous short spines and with 3 germinal pores; occasional fragments of stigmas with short, bulbous papillae[2].

Physical Character
For moisture contents 1.9738 gm of fresh flowers of C. officinalis was spread in watch glass for drying in hot air oven and then flowers were again weighted. The percentage of moisture content
18.79% per gram. Similarly for Ash 3.6741 gm of air dried flowers of *C. officinalis* was ignited until white obtained. Total ash content was 0.09874 gm or 98.74 mg per gram of air dried flowers. So from these results it was seen that Calenda flowers mainly composed of organic compounds [8].

**In-Vivo Pharmacology Activities**  
**Antidiabetic and Antihyperlipidaemic Activities**

The antidiabetic and antihyperlipidaemic effect of hydroalcoholic extract of *calendula officinalis* in alloxan induced diabetic rats. Diabetes was inducing by single intraperitoneal injection of alloxan (150 mg/kg) of body weight. The blood glucose and urine sugar were significant elevated in diabetic rats compared to normal rats. Upon oral administration of hydroalcoholic extract of *calendula officinalis* in to diabetic rats at dose 25 and 50 mg/kg body weight significantly lowered the blood glucose and urine sugar as they compared with group of diabetics rats. Hydroalcoholic extract of *calendula officinalis* in to diabetic rats at a dose of 100 mg/kg body weight 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 haemoglobin lever. The extract was similar to that of insulin. Thus, the investigation clearly show that hydroalcoholic extract of *calendula officinalis* has both antidiabetic and antihyperlipidaemic effect [9].

The structures of the officinosides were elucidated on the basis of chemical and physicochemical evidence. The inhibitory activities of the principle saponins from the flowers of *C. officinalis* on the increase of serum glucose levels in oral glucose-loaded rats, on gastric emptying in carboxymethyl 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 these activities [10].

**Cardiovascular Activities**

Calenda could be cardioprotective against ischemic heart disease. Two groups of hearts were used: the treated rat hearts were perfused with calenda solution at 50 mM in KHB buffer (in mM: sodium chloride 118, potassium chloride 4.7, calcium chloride 1.7, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulfate 1.2, and glucose 10) for 15 min prior to subjecting the heart to ischemia, while the control group was perfused with the buffer only. Calenda achieved cardioprotection by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocyte apoptosis. Cardioprotection 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 TNFα. The results further strengthen the concept of using natural products in degeneration diseases like ischemic heart disease [11].

**Hepatoprotective Activities**

The effect 80% methanolic extract of leaves Calenda officinalis was investigated against acetaminophen- induce hepatic damage in 30 male albino rats. Acetaminophen produce 100 % mortality at dose of 1 gm/kg in mice, while pretreatment of mice with calenda officinalis (1.0 gm/kg) reduced the death to 30%. Pretreatment of mice with leaves extract (500 mg/kg orally, four doses at 12 hours interval) prevented (p< 0.05) the acetaminophen (640mg/kg induce rise in serum transaminase (GOT, GPT), serum bilirubin and serum alkaline phosphatase. Post treatment with three successive doses of leaves extract (500mg/kg, 6 hourly)) restricted the hepatic damage induce by acetaminophen (p< 0.05) [12].
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, *Pheretima posthuma* 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 56.5 min and death of worms at 111.2 minutes. 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 [13].

Antioxidant Activities
An extract of *Calendula officinalis* Linn. was evaluated for its antioxidant potential by oral administration of *Calendula* alcoholic extract inhibited superoxide generation in macrophages in female swiss albino mice by 12.6% and 38.7% at doses of 100 and 250 mg/kg b.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 [14].

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 500 mg/ kg body weight of calendula 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 500 mg / 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-1β, IL-6, TNF-α and IFN-γ and acute phase protein, C reactive protein (CRP) in mice produced by LPS injection were inhibited significantly 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 [15].

Topical application of a 70% ethanol extract of the flowers to mice at a dose of 1.2 mg/ear (corresponding to 4.16 mg 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 [16].

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 trated with ethanolic extract 1% revealed an increase in the number of blood vessels compared to solvent control [4]. 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 [17].

Calendula \(\text{(Calendula officinalis)}\) infused oil is considered beneficial for the reversal of numerous skin and tissue conditions. It is used only after the threat of infection has passed. It is not used on deep wounds since it is felt that calendula may seal the wound too quickly preventing drainage [18].

**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, \(\text{C. officinalis}\) showed a complete inhibitory effect on the proliferation of lymphocytes in the presence of PHA (SI range 0.01-0.49). A dose dependent inhibitory effect was obtained in the case of D. Kotschyi [19].

**In-Vitro Pharmacology Activities**

**Hepatoprotective Activities**
The dose response effect of different concentrations of \(\text{Calendula Officinalis}\) and \(\text{morus Alba}\) extracts (1, 10, 100 and 1000 \(\mu\text{g/ml}\)) on \(\text{CCl}_4\) induced decrease in the viability% of isolated rat hepatocytes. The results revealed that \(\text{CCL}_4\) (5 mM) 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. The potential hepatoprotective effects of \(\text{Calendula Officinalis}\) and \(\text{morus Alba}\) extracts against cytotoxicity and oxidative stress induced by carbon tetrachloride (\(\text{CCl}_4\)) in isolated primary 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 \(\text{CCL}_4\) caused cytotoxicity and oxidative injury, manifested by loss of cell viability and significant increase in ALT, AST and LDH leakages. As well as, \(\text{CCl}_4\) caused progressive depletion of intracellular GSH content and significant enhancement of TBARS accumulation. Pre-incubation of hepatocytes with either \(\text{Calendula Officinalis}\) and \(\text{morus alba}\) extracts ameliorated the hepatotoxicity and oxidative stress induced by \(\text{CCl}_4\), as indicated by significant improvement in cell viability and enzymes leakages (ALT, AST and LDH). Also, significant improvement of GSH content and significant decrease in TBARS formation as compared to \(\text{CCL}_4\) treated cells. The dose response effect of different concentrations of \(\text{Calendula Officinalis}\) and \(\text{morus alba}\) extracts (1, 10, 100 and 1000 \(\mu\text{g/ml}\)) on \(\text{CCL}_4\) induced decrease in the viability% of isolated rat hepatocytes. The results revealed that \(\text{CCL}_4\) (5 mM) 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 [20].
**Antibacterial Activities**

In case of *Calendula officinalis*, ethanolic and aqueous extracts inhibited 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 [21]. 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 standards. 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 [22].

Antimicrobial activity of, ethanolic, methanolic, acetone and chloroform extract of *C. officinalis* was studied against the gram-positive besterial strains were *Escherichia coli*, *Staphylococcus anrens*, and the gram-negative strains were *Salmonella typhae* and *Vibrio cholera*. The fungal strain used was *Candida albicans*. Ethanol extract gave activity against *E. coli*, *Vibrio cholera* and *Candida albicans*. Methanol extract gave only against *Candida albicans*. Chloroform gave antimicrobial activity against all microbes while acetone gave only against *E. Coli* [23].

**Anti-HIV Activities**

A chloroform extract of the flowers inhibited the replication of HIV-1 in acutely infected lymphocytic MOLT-4 cells in vitro (IC$_{50}$ 0.4 mg/ml). A chloroform extract also inhibited HIV-1 reverse transcriptase activity in a dose-dependent manner (ED50 51.0mg/ml) [24].

**Antioxidant Activities**

Antioxidant activity of aqueous extract of the leaves and petals of *Calendula officinalis* Linn. were evaluated for the antioxidant activity by the FTC and TBA methods. 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 [25]. An alcoholic extract of *Calendula officinalis* Linn. (Compositae) was evaluated for its antioxidant potential in vitro. *Calendula officinalis* extract was found to scavenge superoxide radicals generated by photoreduction of riboflavin and hydroxyl radicals generated by Fenton reaction and inhibited in vitro lipid peroxidation. Concentrations needed for 50% inhibition (IC$_{50}$) were 500, 480, and 2000 mg=mL, respectively. Extract scavenged ABTS radicals and DPPH radicals and IC$_{50}$ were 6.5 and 100 mg=mL, respectively [14]. For *Calendula Officinalis* extract of 7.5 mg/ml concentration, the LPO decreased slowly with dose from 68% to 40% at 20 kGy. The LPO for 3.75 mg/ml concentration decreased suddenly from 56% to 28% at 1 kGy dose. Then, it decreased slowly with dose [26].

**Anti-Inflammatory Activities**

The standardized hydroalcoholic plant extracts such as *Calendula officinalis*, *Hypericum perforatum*, Plantago lanceolata and Glycyrrhiza glabra can suppress in cell-free systems the activities of 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2), key enzymes in the formation of proinflammatory eicosanoids from arachidonic acid (AA) [27]. 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 [28].

Anticancer Activities
In the present study, we investigated the effects of flavonoid fractions extracted from C. officinalis and their aglycones -quercetin and isorhamnetin- on the proliferation of the Parent and the tamoxifen-resistant T47D cells at the presence and absence of tamoxifen. The flavonoid extracts (0.05-50 µg/ml) did not cause significant effect on the proliferation of The two cell lines. It may be related to the attached sugar molecule at the position 3 of flavone that can reduce its ability to bind aromatase and other enzymes [29]. 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 Salmononella/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 (BaP) and mutagenic urine concentrate from a smoker (SU). Antimutagenic activities were also compared with the activity of chlorophyllin. All the saponins were found to be non-toxic and non-mutagenic for doses of 400 / µg [30].

CONCLUSION

In this review, we have explored the organoleptic, in-vitro and in-vivo pharmacological activities of Calendula officinalis Linn. (Asteraceae), a medicinal plant found in central and southern Europe, western Asia and united states, amongst others. It exhibits several pharmacological activities such as anti-HIV, cytotoxic, anti-inflammatory, hepatoprotective and spamolytic amongst others. It is potentially an important medicinal plant for mankind.

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