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Phytochemical ingredients and pharmacological potential of *Calendula officinalis* Linn.

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ABSTRACT

Calendula officinalis Linn. commonly known as marigold has long history of usage by the folklore system. It has a high economic value as herbal medicine and is widely used in cosmetics, perfumes, dyes, pharmaceutical preparations and food products for centuries. The plant has been approved for food use and appears in the food and drug administration (FDA) list of generally recognized as safe (GRAS) substances. The literature available for the comprehensive review study were taken from the different worldwide accepted scientific database, journals, books for botanical description, pharmacological properties and ethno-botanical uses of *C. officinalis* Linn. Natural products containing calendula either as extract or oil are complex mixtures containing hundreds of biologically active constituents such as carotenoids, flavonoids, saponins, sterols, phenolic acids, lipids, amino acids, carbohydrates, etc. These phytoconstituents have wide applicability in food and cosmetic industries besides their therapeutic applications viz. as antioxidant, antimicrobial, anti-inflammatory, anti-ulcer, anti-proliferative, antiparasitic, hypoglycemic, hypolipidemic and wound healing potential in experimental and clinical trials. Being useful therapeutic agents in their own right, an understanding of these traditional medicines has provided new plant derived drug leads to modern medicine for therapeutic application.

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Introduction

Plants have been used for a thousand of years as medicines for treating different diseases and medical complaints by most of the civilizations. The herbal compounds have made their importance felt in the last few decades whose prevalence is continuously increasing in both developing and developed countries because of their natural origin and negligible side effects (1). About 7500 medicinal plants have been scientifically validated for the treatment of various ailments in human and animals. Traditional medicine has also provided new plant derived drug leads to modern medicine (2, 3). According to analysis, up to 60% of all prescriptions in Eastern Europe and 25% in OECD (Organization for Economic Co-operation and Development) countries prove to consist of unmodified or slightly altered higher plant products (4). Resurgence of interest in herbal medicine has largely been outcome of the realization of ill effects which other systems are inflicting on human being. Therefore, efforts are going all over the globe in exploring and documenting the ethnopharmacological data and scientific research on medicinal plants for the safe and effective herbal formulations (5).

Calendula officinalis Linn. belongs to family Asteraceae and has a long history of usage by the folklore system, because of rich ethnomedicinal values. Calendula flowers are often used in skin care products, wound healing, reducing inflammation, soothing and softening the skin due to its unique cell rejuvenation properties (6). The flowers have also been used as a source of medicinal ingredients, widely used in homeopathic medicine for the treatment of many diseases for centuries (7). *C. officinalis* has high economic value as herbal medicine and has been approved recently for food use in USA and appears in the Food and Drug Administration (FDA) list of generally recognized as safe (GRAS) substances (8). Studies had shown various pharmacological activities viz. nephroprotective, hepatoprotective, hypoglycemic, hypolipidemic, antioxidant potential of *C. officinalis* in experimental and clinical models. Therefore, correlation between phytochemical ingredients associated with pharmacological activities needs to establish to maximize their therapeutic applications in mammals.

Methodology

Literature available for the comprehensive study were taken from different worldwide accepted scientific database Science Direct (http://www.sciencedirect.com), PubMed (http://ncbi.nih.gov/pubmed), Springerlink (http://www.springer.co.in), Google Scholar (http://www.onlinelibrary.wiley.com) and abstracts, journals account for botanical description, pharmacological properties and ethno-botanical uses of different parts of *C. officinalis* Linn. The present review highlights the botanical description, traditional uses,

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phytochemical constituents present in different part of *C. officinalis* Linn. and their pharmacological properties in a comprehensive manner.

Botanical Description

Calendula, a fast growing annual herb is easy to germinate and simple to care. The genus name originated from the Latin calendae which means "first day of the month." Because the flowers follow the sun, it was linked to the astrological sign of summer, Leo, and to treating the heart and conditions caused by heat (9). In English Calendula was known as "gold" and was associated with Virgin Mary and then with Queen Mary; hence "Mary's Gold." Literature suggests that it's an important ingredient in a variety of ayurvedic and homeopathic medicine systems, still efforts are needed to verify its efficacy through scientific screenings in experimental animal models and clinical trials.

Habitat and Distribution: *C. officinalis* Linn. commonly known as marigold is cultivated widely in temperate regions around the world. It is an erect, annual herb that grows indigenously to central, eastern and southern Europe, cultivated commonly in North America, Balkans, Eastern Europe, Germany and India (10, 11, 12). Because of cutting and flower arrangements, prolific and durable features make calendula favorite among gardeners. In India, the optimum sowing time is in April, flowering begins in June and seeds ripen in early August. Harvesting is done from early to mid August and continues to flower till autumn frosts.

Morphology: Depending on variety and culture, the plant grows 12-30 inch in height. The leaves are bright green and typically about 4 inch long. The lower leaves are oval with rounded tips (spatulate) and upper are lance shaped with pointed tips. The flowers are typically 2-3 inch in diameter and held on thick sturdy stems. Calendulas are single or double flowered and come in a range of colors from cream to light yellow to orange whereas some have dark brown centre and all are beautiful. The taxonomic features and vernacular names of the calendula are provided in table 1. The growing and flowering *C. officinalis* plant is shown in figure 1.

Traditional uses: In traditional system, different parts (areal part, flower, seed, leaf and stem) as well as whole plant of the *C. officinalis* Linn. are used for the treatment of various disorders in human and animals. Calendula flowers are believed to follow the sun and are linked to sun sign Leo and are used in treating many cardiac disorders and some conditions caused by heat (fever) and cancer (14). Preparations containing calendula are also used effectively for the treatment of skin problems and can be used for hemorrhoids, anal fissure, mastitis, sebaceous cysts, impetigo or other inflammatory cutaneous lesions. Oral administrations of extract are indicated in unresolved infection or erosion of the upper digestive tract, particularly where there is evidence of bleeding into the gut during gastritis and duodenal ulcers (14). Calendula teas are used for gargles, as eye washes and to treat conjunctivitis, diaper rashes, hemorrhoids, stomatitis and inflammatory conditions of mucous membranes (15). Dried petals of the flower are used as an alternative of saffron in spice trade. Because of its golden color, calendula leaves are also used in ointments to impart color (16). Dried flowers are also used in sudorific, blood refiner, hypoglycemic and various insect repellent preparations (17). Flowers of the plant have a

Table 1 The taxonomic features and vernacular names used for the *C. officinalis* Linn.

Taxonomic features	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asrerales
Family	Asteraceae
Subfamily	Calendulaceae
Genus	*Calendula*
Species	*Officinalis*
Vernacular names	
Language	Vernacular names
English	Pot marigold, English marigold, Holligold, Marybud, Gold-bloom
Hindi	Genda
Punjabi	Gulsarfi
Urdu	Gul-e-ashrafi

Figure 1 Growing and mature plant of Herb *Calendula officinalis* Linn. Containing flowers.

potential to be used as an astringent despite not being tannin rich (18). Plant have also been used as an anticonvulsant, energizer, antiseptic, diuretic, blood thinner, antiemetic, antiulcer, antispasmodic, anti-icteric and dewormer (19). In compressed form, it is also used for varicose veins (20), to lower blood cholesterol levels and for curing digestive problems. It is suitable for gum bleeding as it has a vasoconstrictor effect. It is brewed to lower body temperature and perspiration as an effective tranquillizer (21).

Phytochemical ingredients

Whole plant of *C. officinalis* is a rich source of large number of phytochemical ingredients and qualitative and quantitative levels of these ingredients varies in different parts of the plant (22). In addition, amount of these active ingredients also varies with the soil composition, plant maturity and time of harvesting (23, 24, 25). The major active ingredients present in different parts of the pant are enlisted in table 2. The phytochemical ingredients reported are carotene pigments, free and esterified triterpenic alcohols and polyunsaturated fatty acids, such as calendic acid (26, 27, 6), calenduline and oleanolic acid glycosides, α- and β-amyrin, calenduladiol, coflodiol and manilladiol (28, 22). It also contains saponins, flavonoids, carotenes, mucilage, resin and steroidal compounds (29, 30). Marukami et al., (31) reported the structures of new ionone (Officinosides A and B) and sesquiterpenes (Officinosides C and D) glycosides from the flowers of Egyptian *C. officinalis* (31). Other compounds in the extract include proteins, amino acids, saturated hydrocarbons (32, 6), vitamin C (33) and mineral substances (34). Main flavonoids in calendula are isorhamnetin and quercetin as main glycones. Flavonol make up to 0.3-1.5% of calendula flowers (32). Flowers contain pharmacologically active flavonol compounds like flavoxanthin, luteoxanthin, lycopene, auroxanthin, lutein, β-carotene, etc (27).

Flavonoids: Maximum qualitative as well as quantitative extraction of flavonoids occurs in ethanolic extract of *C. officinalis* and ranges between 0.21-0.68% depending on part of the plant and area of cultivation. But there is no relationship between the total flavonoid content and the color of ligulate and tubular florets of *C. officinalis* (35). They include isorhamnetin, quercetin, isoquercetin, isohamnetin-3-O-β-D-glycoside, narcissin, calendoflaside, calendoflavoside, calendoflavobioside, rutin, neopesperidoside, rutinoside, quercetin-2-rhamnosil-rutinoside quercetin-3-O-rutinoside, etc (36, 37).

Terpenoids: Terpenoids have been reported from petroleum ether extract of flower and areal parts of *C. officinalis*. They include sitosterols, stigmasterols (38), 3-monoesters of traxasterol, lupeol (39, 7), diesters of diols (40), erythrodiol, ursadiol (41), faradiol-3-O-palmitate, faradiol-3-O-myristate, faradiol-3-O-laurate (42), aridiol-3-O-palmitate, arnidiol-3-O-laurate, arnidiol-3-O-myristate, calenduladiol-3-opalmitate, calenduladiol-3-O-myristate (26, 37), oleanane triterpene glycosides: calendulaglycoside A, B and C (37), glucosides of oleanolic acid (43). Flowers also contain triterpene saponins and triterpene alcohols (44, 45). Cornulacic acid acetate was recently isolated from flowers of *C. officinalis* (46).

Carotenoids: The inflorescence of *C. officinalis* has abundant amount of carotenoids that give flowers their yellow orange color and the color shade depends on pigment contents and pigment profile (flavones, anthocyanins and carotenoids). Orange varieties are rich in carotenoids and contain both hydrocarbons and oxygenated derivatives (47). Nineteen carotenoids were identified in extract of petals which provide unique color to the calendula flowers from orange to yellow (27). Moreover, orange color intensity of γ-carotene, calendula is determined by the amount of α-carotene, lycopene and rubixanthin as these pigments are responsible for the orange or even red color of vegetal tissues (47). Its concentrations varies (0.1 to 8.0%) in different part of the plant and highest in petals (7.7%), followed by pollens (1.6%), leaves (0.9%) and stems (0.2%) (48, 49). The carotenoids isolated from the pollen and petals are neoxanthin, violaxanthin, luteoxanthin, auroxanthin, mutatoxanthin, lutein, anthroxanthin, 9Z-neoxanthin, 9Z-violaxanthin, 13/13'Z-lutein, lycopene, α-cryptoxanthin, α-carotene, β-cryptoxanthin etc. Similarly carotenoids present in leaves and stems are neoxanthin, 9Z-neoxanthin, violaxanthin, luteoxanthin, 9Z-violaxanthin, 13Z-violaxanthin, antheraxanthin, mutaxanthin epimer 1 and 2, lutein, β-cryptoxanthin, β-carotene, etc (49).

Coumarins: The ethanolic floral extract of *C. officinalis* contains coumarins like scopoletin, umbelliferone and esculetin (50). Coumarin is the parent molecule of warfarin (a clinically useful anticoagulant), which acts as a vitamin K antagonist (51). Coumarins act as phytoalexins as they are produced by plants for defense against various pathogens (52, 53). Coumarins are leached from the roots into the soil to provide defense against various micro-organisms (51).

Quinones: The quinones in calendula include plastoquinone, phylloquinone, α-tocopherol in chloroplast, ubiquinone, phylloquinone and α-tocopherol in mitochondria and phylloquinone in leaves of the plant (54).

Volatile oils: Extractability of volatile compounds from flowers of the calendula depends on the stage of flower collection viz. maximum (0.97%) during full flowering stage and minimum (0.13%) during pre-flowering stage of flower (55). Plant shows a different pattern of distribution at different vegetative stages. Various monoterpenes and sesquiterpenes have been reported in the volatile oils like α-pienene, α-thujene, sabinene, limonene, 1,8-cimeol, γ-terpenes, trans-β-ocimene, terene-4-ol, geraniol, carvacrol, bornyl acetate, sabinyl acetate, α-cubebene, α-gurjunene, aromadendrene, α-ylangene, α-humulene, α-cadinol, β-saliene,

Table 2 Phytochemical active ingredients present in different parts of *Calendula officinalis* Linn.

Plant part	Groups	Active ingredients	References
Flower	Terpinoids	Lupeol, Ψ-taraxasteol	(7)
		Erythrodiol	(70)
		Calenduloside	(71)
		Calendulaglycoside A, Calendulaglycoside B	(37)
		Cornulacic acid acetate	(46)
	Flavonoids	Isoquercitrin, rutin, calendoflavoside	(37)
		Quercetin, Isorhamnetin	(72)
		Isorhamnetin-3-O-β-D glycoside, Narcissin	(25)
	Coumarins	Esculetin, scopoletin, umbelliferone	(50)
	Volatile oils	Cubenol, α-cadinol, oplopanone, methyllnoleate	(73)
		Sabinene, limonene, α-pinene, p-cymene, nonanal, carvacrol, geraniol, nerolidol, t-muurolol, palustron	(9)
Leaves	Quinones	Phylloquinone, α-tocopherol, ubiquinone, plastoquinone	(54)
Root	Terpenoid	Calenduloside B	(74)

β-caryophyllene, β-caryophyllene, calarene, merolidol, oplopene, t-muurolol. The essential oil is found to be rich in α-cadinene, α-cadinol, t-muurolol, limonene and 1,8-cineol with p-cymene at lower level in the post flowering stages (55).

Amino acids: Total amino acids content is reported to be 4.5% in flowers, 3.5% in stems and 5.0% in leaves of *C. officinalis* (56). The ethanolic floral extract of *C. officinalis* contains more than 15 amino acids in free form like alanine, asparagine, arginine, aspartic acid, histidine, glutamic acid, leucine, lysine, proline, serine, tyrosine, threonine, methionine, valine and phenylalanine (56).

Carbohydrates: The *C. officinalis* inflorescence contain 9.3% moisture, 25.8% acidic sugar, 29.3% ash, 31.3% reducing sugars and 84.6% pectic substances and various monosaccharides including glucose, arabinose, rhamnose, xylose, galactose and galacturonic acid (57). The ethanolic extract of the flowers of calendula consists of polysaccharides, PS-I, -II and -III having a (1>3), D-galactam backbone with short side chains at C-6 comprising α-araban-(1>3)-araban and α-L-rhamnan-(1>3)-araban along with monosaccharide (58, 59).

Lipids: Number of lipids has been isolated from the seeds, leaves and flowers of *C. officinalis* with varying intensity.

A total of nineteen fatty acids were identified in calendula seed oils with calendic acid (51.5 - 57.6%) and linoleic acid (28.5 - 31.9%) being the predominant fatty acids followed by oleic acid (4.4 -6.3%) and palmitic acid (3.9 - 4.6%) (60). Fatty acids of monols, sterol esters, 3-monoesters, 3-monoester diols reported in flowers were lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acid. Amount of neutral lipids in seeds was reported to be 15.7%, glycolipids 0.9% and phospholipids 0.6%. The fatty acids of marigold seeds contain 59% of an 18:3 conjugated trienic acid and 5% of 9-hydroxy- 18:2 acid-dimorphecolic acid (40, 61). One oxygenated fatty acid also reported from seed oil of *C. officinalis* was d-(+)-9-hydroxy-10, 12-octadecadienoic acid (62). Calendula has been reported to accumulate an unusual conjugated C_{18} fatty acid named calendic acid (octadeca-8:10:12-trienoic acid) in its seed (63). Seeds of plant contain approximately 20% oil, and of this oil, up to 60% is calendic acid ($C_{18}H_{30}O_2$). The calendic acid, a conjugated triene containing fatty acid has been shown to be valuable in mammalian physiology, as well as useful to industrial applications. During the seed maturation period, the concentration of calendic acid has been shown to increase steadily and sharply with a decrease in linoleic and oleic acids (64) due to the

presence of a specific conjugase in calendula seeds which converts linoleic acid into calendic acid (65). Recently, many studies are focused on conjugated fatty acids (CFAs), which are the general term for a set of positional and geometric isomers of polyunsaturated fatty acids with conjugated double bonds (66). Due to rapid oxidation, calendic acid has numerous potential industrial applications such as drying agent in paints, varnishes and plastics. It has been estimated that calendic acid could replace a number of hazardous volatile organic compounds with potential market of 3-5 million kilograms annually in Western Europe alone (67).

Other constituents: Other phytochemical ingredients include the bitter constituent, loliolide (calendin) (68), calendulin and n-paraffin (69), glucosides (70), calenduloside (71), flavanoids (72), volatile dyes (73), trirerpene glycosides (74) were found in varying quantity in different parts of the *C. officinalis*.

Pharmacological Properties

The experimental and clinical trials on calendula extracts have confirmed that it exhibits a wide range of pharmacological properties. These properties may pave a way for future development for the therapeutic management of different disorders in human and animals. Pharmacological potential of the *C. officinalis* is outlined in the figure 2.

Antioxidant potential: Whole plant of calendula possesses potent antioxidant potential. Out of which, calendula flowers are richest source of flavonoids, especially aglycon and glycosides of flavonol (isorhamnetin, quercetin), saponosides, lipids (sterols and carotenoids), organic acids and saccharrides (75).

Further, extraction of flavonoids and carotenoids from the sample also depends on the type of solvent used for the extraction (76). A residual aqueous extract taken after extraction with 70% methanol extract with ether, chloroform, ethyl acetate and n-butanol showed antioxidant activity by liposomal lipid peroxidation-induced Fe^{2+} and ascorbic acid (76). The biological activity of compounds were measured in extracts of calendula and the antioxidant activity *in-vitro* of an extract containing lipophilic and middle polarity compounds possessed better antioxidant activity than water and 50% ethanolic extracts (77). The *C. officinalis* extract exerted anti-reactive oxygen or nitrogen species (ROS/RNS) activities in a concentration dependant manner, with significant effects being observed even at very low concentration (78). The phytochemical constituents of *C. officinalis* include flavonoids (lupeol, quercetin, proto-catechuic acid), alkaloids and tri-terpenoids (79). The ethanolic floral extract of calendula have high concentrations of phenolic, flavonoids, ß-carotene, lycopene, tannins, chlorophylls contents endowed with high antioxidant potential as compared to aqueous floral extract of *C. officinalis* (30). The results were further substantiated with determining the high *in-vitro* antioxidant capacity, free and superoxide/nitric oxide radicals scavenging potential with high reducing capacity in ethanolic floral extract as compared to aqueous floral extract of *C. officinalis* (30). The median effective concentration (EC_{50}) values for floral extract of *C. officinalis* varies for different types of radicals viz. free radical scavenging activity (1.30 - 1.20 mg/ml), superoxide anion radical (0.53 – 2.00mg/ml), hydroxy radicals (0.79-10.6mg/ml) and nitro radicals (0.68 – 2.0mg/ml) (floral extract much higher as compared to

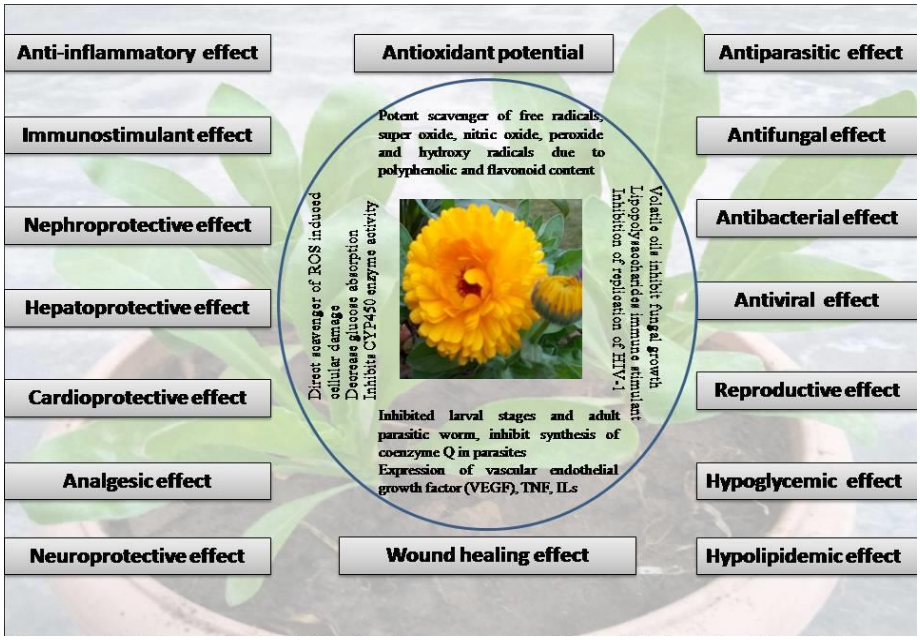

Figure 2 Cellular mechanism(s) for the pharmacological effects of *Calendula officinalis* Linn.

ascorbic acid (0.022 mg/ml) (30). Oral administration of alcoholic extract of calendula inhibits 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, respectively. Oral administration of *C. officinalis* to mice for one month significantly increased catalase activity in addition to increased levels of reduced glutathione (GSH) level in blood and liver. Increased activities of glutathione reductase (GR) with decreased activities glutathione peroxidase (GPx) was found after administration of calendula extract (80). The butanolic fraction of plant extract protects rat liver microsomes from the lipid peroxidation due to significant free radical scavenging and antioxidant activity (81). Phytochemical ingredients present in calendula extract shown high *in-vitro* potent antioxidant capacity, 1,1-diphenyl l-2-picrylhydrazyl (DPPH) induced free radical, superoxide, hydroxy and nitric oxide radicals scavenging property along with high reducing power (30). 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 (82). Thus, the dietary supplementation of floral extract of *C. officinalis* may provide protection against degenerative changes associated with free radicals induced cellular damage (aging, cancer, etc) besides improve the food quality by retarding oxidative degeneration of food lipids by acting as a natural antioxidant.

Anti-inflammatory and analgesic effect

The floral extract of *C. officinalis* showed anti-inflammatory activity in both acute and chronic experimental models. The floral extract produce anti-inflammatory potential in both carrageenin (histamine, kinins, and prostaglandins) and dextran (mast cell degranulation) induce inflammatory models despite both produce inflammation through different mechanisms (83). Similarly, extract also showed protective effect in chronic inflammatory model (formalin induced) in rats (83). The ethyl acetate fraction of the methanol extract of *C. officinalis* flowers exhibited the most potent inhibition of 12-alpha-tetradecanoyl phorbol-13-acetate (TPA) - induced inflammation (1μg/ear) in mice with an ID_{50} value of 0.05-0.20mg/ear compared with indomethacin used as reference drug. The results of different studies showed anti-inflammatory activity of *C. officinalis* extract irrespective of induction of inflammation (83).

The acute inflammation causes release of interferon-γ (IFN-γ), histamine, prostaglandins, etc. into the circulation which mediates host inflammatory response. Treatments with calendula extract lowered the IFN-γ level and reduce expression of cyclooxygenase 2 (COX-2) which may be contributing the anti-inflammatory effect in different inflammatory models (83). Further, calendula extract administrations also declined the production of reactive oxygen/nitrogen species (ROS/RNS) and suppression inflammatory cells such as neutrophils, eosinophils and macrophages which produces cellular damage during the chronic inflammation (83, 84). Flowers of *C. officinalis* contain high concentration of flavonoids, carotenoids and lycopene which possess potent antioxidant potential at very low concentrations. The chemo-preventive properties of flavonoids like lutein are generally believed to reflect their ability to scavenge endogenous ROS (85). By inhibiting or stimulating various signaling pathways, flavonoids at low concentration could affect cellular functions (86). Carotenoids have been reported to quench ROS whereas lycopene reducing the transcription levels of inflammatory cytokines (88). Hence the combined action of these active ingredients present in the *C. officinalis* through their free radical scavenging and inhibition of mediators of inflammation especially cytokines, prostaglandins may be exerting the anti-inflammatory activity.

Study showed that anti-inflammatory activity of *C. officinalis* was mainly due to presence of oleanane type triterpene glycoside in the extract (37). The dichloromethane extract of the plant's flower heads inhibited croton oil-induced oedema and further isolation showed that the esters of faradiol-myristic acid, faradiol-palmitic acid and taraxasterol had anti-edematous activity with an oedema inhibition of nearly 50% at a dose of 240 μg/cm. When the doses of these two faradiol esters were doubled, oedema inhibition increased to 65 and 66%, respectively, without any synergism between them (7). A dose of 1200μg/ear of hydroalcoholic extract showed 20% inhibition in croton oil-induced mouse oedema. The activity was attributed due to the presence tri-terpenoids, the three most active compounds are the esters of fradiol-3-myristic acid, faradiol-3-palmitic acid and 4-taraxasterol (88, 89). Also, a cream containing calendula extract has been reported to be effective in dextran and burn edemas as well as in acute lymphoedema in rats. Activity against lymphoedema was primarily attributed to enhanced macrophage proteolytic activity (90).

Similarly, tribal people use calendula herb in the form of decoction and its aqueous extract as an analgesic agent. The analgesic activity was scientifically validated by intra-peritoneal administrations of *C. officinalis* extract in mice using tail immersion method and results showed that pure extract produces greatest analgesia for longer duration of action with maximum activity after 30 minutes of injection (91, 92). The hydroalcoholic extracts of *C. officinalis* suppressed the cell-free systems activities of 5-lipoxegenase and cyclooxygenase-2, the key enzymes in formation of pro-inflammatory eicosanoids from arachidonic acid (93). The analgesic effect of *C. officinalis* was also evaluated in thermal pain threshold model in male rats. The extract significantly increased tail flick latency compared to the control group,

indicating that the extract reduced pain threshold (94). The crude extract exhibited significant antipyretic and analgesic effect at a dose of 300 mg/kg and 40 mg/kg body weight, respectively as compared to standard i.e. acetyl salicylic acid which exhibited 50.5% and 11.3%, respectively. A dose of 20 mg/kg body weight crude extract was found to be equipotent in its analgesic action to 40 mg/kg of acetyl salicylic acid (95). Local application of the extract in the form of poultice or infusion soaked in a cloth also acts as an effective remedy (96). The oil extracted from calendula, applied externally to ear, alleviates pain and discomfort caused by earache (97). It also aids in relieving fever and menstrual cramps (98).

Antiulcer effect

In traditional system, areal parts, rhizome and flowers of plant have been used for the treatment of gastrointestinal disorders. The ethanolic extract of calendula has been found to possess antiacid and anti-ulcer activity in rats due to its gastro-protective and anti-secretory potential (99). Administrations of calendula extract stimulate mucus secretion and levels of reduced glutathione, while suppressing the pepsin level, this may be possible mechanism of gastro-protection (99). Experimental studied have proved that rhizome of the *C. officinalis* produces antiulcer profile on caffeine arsenic butadiene and pylorus ligated induced ulcer models (100). Yoshikawa *et al*., also showed that calendula could relieve chronic stomach irritation linked to peptic ulcers and other inflammatory conditions (101). In other study, calendulozide B-trioside, isolated from rhizomes of *C. officinalis*, in doses of 5, 10, 20 and 50 mg/kg exerted an anti-ulcerous action in experimentally induced ulcer models of different genesis displayed a anti-phagocytic and sedative action (58). The influence of *C. officinalis* on heparin-binding epidermal growth factor (HB-EGF) like growth factor gene expression in KATO-III cells under the stimulation of *H. pylori* strain N_6 using RT-PCR was investigated with and without addition of *C. officinalis*, where addition led to a significant reduction of *H. pylori* induced increase in gene expression of HB-EGF (102). Moreover, in another study conducted on human patients with duodenal ulcers and/or gastroduodenitis were treated with an herbal combination containing calendula showed improvement of symptoms in 90% population (103). Adults with non-specific colitis treated with herbal tea included calendula, showed improved symptoms in 96% of the patient within two weeks of the therapy (104). Dizaye and Ali (105) studied the hydroalcoholic extract of *C. officinalis* and found to be effective in reduction of gastric acid secretion and preventing development of gastric ulcer in mice induced by aspirin. The gastro-protective effect was comparable to ranitidine and the anti-ulcerogenic activity was supported by histopathology of stomach sections (105). Chandra et al., (99) evaluated the antacid capacity and antiulcer activity of *C. officinalis* in experimental rats. Ethanol extract of *C. officinalis* at the dose rate of 100 and 200 mg/kg, orally produced anti-secretory and antacid potential were demonstrated in pyloric ligation induced ulcer model. The gastro-protective effect was carried out by absolute ethanol induced and indomethacin induced ulcer model. *In-vitro* antacid capacity was evaluated by titration method. Gastric wall mucus level was increased in experimentally induced ulcer models and results are comparable to ranitidine. The antiulcer activity of calendula extract was due to its anti-secretory and gastro-protective effect on experimental rats (99). Yadav et al., (106) studied the whole plant extract of *C. officinalis* at different concentrations against different standard ulcer models like aspirin-induced, ethanol-induced ulcers, cold resistant ulcers and pyloric ligation ulcers. The analysis of the data clearly indicated that the whole plant of *C. officinalis* have significant antioxidant effect on ulcer pathology. Supplementation of whole plant extract ulcer decreased lipid peroxidation and superoxide dismutase with concomitant increase in catalase activity in cold resistant stress induced ulcer (106). The aqueous and ethanolic floral extracts of *C. officinalis* flowers, when assayed in rabbit jejunum, caused a dose-dependent relaxation of spontaneous and K^+ induced contraction; further fractionation of the extract with dichloromethane showed inhibition of spontaneous contractions in a dose range of 0.01-0.03 mg/ml and this is 10 times more potent than the parent crude extract. Study indicated that spasmolytic activity was primarily due to blocking effects on calcium channel (107).

Immunostimulant activity

Numbers of studies have shown that polysaccharides (PS) fractions of *C. officinalis* extract exhibits potent immune stimulant activity. Polysaccharides such as PS-I and PS-II showed 40-57% and 20-30% of phagocytosis, respectively, whereas PS-III exhibited the highest phagocytosis rate of 54-100% (58, 59). The extract of *C. officinalis* showed complete inhibitory effect on the proliferation of lymphocytes in the presence of phytohemagglutinin (PHA) and mixed lymphocyte reaction (MLR) (108). The *in-vitro* cytotoxic anti-tumor and immunomodulatory activities and *in-vivo* anti-tumor effect of Laser Activated Calendula Extract (LACE) were evaluated. Effect of LACE on human peripheral blood lymphocyte (PBL) proliferation *in-vitro* induces apoptosis in LACE-treated cells. *In-vivo* anti-tumor activity was evaluated in nude mice bearing subcutaneously human Ando-2 melanoma cells. The results indicated that LACE aqueous extract showed cytotoxic tumor cell activity and lymphocyte activation activities of the extract. Moreover, the LACE extract presented *in-vivo* anti-tumor activity in nude mice against tumor growth of Ando-2 melanoma cells that further confirmed its dual effect (109).

Wound healing activity

For centuries, calendula is used as an important ingredient in the preparations used for anti-

inflammatory and antiseptic actions. In Ayurveda, calendula has been shown to possess scar reducing and emollient property by increasing the collagen content in wound areas (110). Hill *et al*., conducted a research on scar reducing and massage emollient properties by addition of calendula flowers extract produce good results (111). Different fractions viz. ethanolic, hexane and dichloromethane of *C. officinalis* were shown healing and angiogenic activities in experimental models. 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. The ethanolic extract increase in the number of blood vessels and reduction in wound area compared to control (12). The study concluded that application of calendula extract significantly increases epithelization in chronic venous ulcerations. The therapy offers a non-invasive and gentle treatment for difficult to treat plantar verruca, painful hyperkeratotic lesions and inflamed bursa (112). Ethanolic floral extract of the calendula showed wound healing potential on the thermal induced burns in rats. Among different concentrations, 200 mg/kg body weight showed significant improvement in healing wounds, indicated by an increase in collagen hydroxyproline and glucosamine contents. Moreover, decreased levels of tissue damage markers (alkaline phosphatase, alanine and aspartate aminotransferase), acute phase proteins (heptaglobulin, orosomycid) and lipid peroxidation may be due to its antioxidant property (113). It not only improves wound healing but also increases blood flow to the damaged area and increasing collagen synthesis (114). Leach, (115) also studied 2% calendula gel and daily application have better wound healing potential due to its antimicrobial and antioxidant properties (115).

Calendula extract heals wound as well as internal and external ulcers due to its antiseptic, anti-inflammatory properties in addition application also improve blood flow to the affected area. The ointment base of calendula in hydrophobic vehicle is more suitable for the therapy because it has good stability, penetrability and ease of application (113, 116, 117). Calendula ointment 10% for ad libitum use has been prescribed for the potential therapy in cases of cheilitis exfoliative (118).

Anti-proliferative effect

C. officinalis extracts showed *in-vitro* anti-cancerous activity on various tumor cell lines derived from leukemias, fibrosacomas, melanomas, breast, cervix, prostate, pancreas and lung (119). Many active ingredients especially carotenoids isolated from *C. officinalis* have been effective against *in-vitro* and *in-vivo* tumor cells models (120, 121). Lin *et al*., have shown that the hot water extract of *C. officinalis* flowers exhibited anti-hepatoma activity against human liver cancer cell lines viz. HepG2/C3A, SK-HEP-1, HA22T/VGH, Hep3B and PLC/PRF/5 with an inhibitory effect of 25-26% at a dose of 2000µg/ml (122). The ethyl-acetate fraction of the methanolic extract of the flowers of plant has shown to possess potent cytotoxic activity *in-vitro* (37). Administrations of floral extract of *C. officinalis* to tumor bearing C57BL/6 mice reduce the lung tumor nodules by 74% with 43.3% increase in life span. Elevated levels if hydroxyproline, sialic acid and γ-glutamyl transpeptidase in the metastatic controls were found to reduce in floral extract treated mice. The study revealed anti-metastasis action due to down regulation of pro-inflammatory cytokines and inhibition of the expression of matrix metalloproteases 2 and 9, lysyl oxidase and prolyl hydroxylase by the ingredients present in the floral extract of *C. officinalis* (83). Further it was also found that the extract administration inhibit the cyclooxygenase-2 enzyme, which plays regulatory role in inflammation (83). Dietary lutein present in *C. officinalis* extract has been found to suppress mammary tumor growth, increase tumor latency and enhanced lymphocyte proliferation in mouse models (123). The carotenoids present in calendula flowers like lycopene has been reported to consistently reduce transcript levels of pro-inflammatory cytokines (87). β-carotene, lycopene and lutein were reported to inhibit colonic aberrant crypt foci formation in rats (124). The β-carotenes present in the plant extract have been reported to have anti-metastatic potential against B16F-10 melanoma cells (125).

First active component in calendula is calenduloside F6-0-n-butyl ester, active against leukemia (MOLT-4, RPMI 8226), colon cancer (HCC-2998) and melanoma (LOXIMVI, SK-MEL-5, UACC-62) cell lines with GI_{50} values of 0.77-0.99 µM except for leukemia (CCRF-CEM,GI_{50}), renal (AK-1, UO-31) and breast cancer (NCI/ADR-RES) cell lines. The second component is calenduloside G6'-O-methyl ester, which is active against all the cancer cell lines mentioned for the first compound with a GI_{50} of 20µM except for ovarian cancer (IGROVI) and renal cancer (VO-31) cell lines (37). The plant extract showed potent inhibition of tumor cell proliferation when assayed against a wide variety of human and murine tumor cell lines. The cells got arrested in G_0/G_1 phase and as caspase-3-induced apoptosis and aqueous extract showed less potential than the ethanolic extract of *C. officinalis* (119). When the same extract was assayed against human peripheral blood lymphocyte (PBLs) and human natural killer cells (NKCs), it showed induction of proliferation and activation of B and T lymphocytes, NK cells and CD^{4+} cells *in-vitro*. In addition, the extract also showed anti-tumor activity *in-vivo* in nude mice. The anticancer activity of calendula extract may be due to quenching of singlet oxygen and radicals by carotenoids content of plant extract, which play important role in cancer etiology (120, 121).

Hepatoprotective effect

Study have shown the protective effect of the plant extract against aflatoxins induced oxidative stress and hepatotoxicity in rats liver and results suggested that

calendula extracts had anti-genotoxic effects due to higher content of total phenolic compounds (126). Experimental studies suggested that aqueous and ethanolic floral extract of *C. officinalis* have protective effect against CCl_4 induced (83, 127), acetaminophen induced hepatotoxicity (128) and aflatoxin induced hepatotoxicity (129) in wistar rats. Similarly, hydroalcoholic extract of the flowers results in reduced hepato-cytolysis and reduced hepatic biomarkers *in-vitro* and *in-vivo* models (130). Verma et al., (128) studied the attenuating potential of floral extract of *C. officinalis* on acetaminophen induced hepatotoxicity in Wistar rats and the treatment with ethanolic extract restored hepatic blood markers, increased the level of total thiols, reduced glutathione, total antioxidant status and antioxidant enzymes (CAT, SOD, GPx, GST) and reduced the levels of malondialdehyde and total oxidant status of blood and hepatocytes. These observations are in corroboration with the histological alterations in hepatic tissue in rats (128). Restoration of cellular antioxidant levels viz. increased levels of reduced glutathione, total thiols and enzymatic components of antioxidant system may be the prime mechanisms of polyphenolic compounds present in the calendula to protect the cells from chemical induced cellular damage (122, 128, 130). Calendula extract succeeded to improve the biochemical parameters, inflammatory cytokines, decreased the oxidative stress, and improved the histological pictures in the liver of rats fed aflatoxin-contaminated diet in a dose-dependent manner. It can be concluded that calendula extract has hepatoprotective potential against aflatoxin induced hepatotoxicity due to its antioxidant properties and radicals scavenging activity (129). The partial hepatoprotective activity of calendula in aflatoxin induced hepatotoxicity may be its modulatory effect on cytochrome P_{450} enzymes which may interfere on aflatoxin active metabolite production responsible for the cellular damage (83).

Neuroprotective effect

Neurological disorders such as schizophrenia, depression dementia, seizure disorders are the major health problem. Phytochemicals from medicinal plants play a vital role in maintaining the brains chemical balance by influencing the function of receptors for the major neurotransmitters. Numbers of herbal medicines used in ayurvedic practices as well Chinese medicines contain multiple compounds and phytochemicals that may have a neuroprotective effect which may prove beneficial in different neurodegenerative disorders (131). Floral extract of *C. officinalis* produce neuroprotective effect on 3-nitropropeonic acid (3-NP) induced neurotoxicity in rats (132). Administrations of floral extract for seven days significantly attenuated the behavioural alterations, oxidative damage of neuronal cells and striatal neuronal loss in 3-NP-treated rats (132). Similarly *C. officinalis* extract also shows neuroprotective action on monosodium glutamate (MSG) induced neurotoxicity in experimental animals. Protective effect on supplementation caused a significant alteration in animal behavior, oxidative defense (raised levels of lipid peroxidation, nitrite concentration, depletion of antioxidant levels) and hippocampus neuronal histology in MSG-treated animals (132). The plant extract contains mixtures of organic chemicals viz. fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes, etc. Proponents of herbal medicines describe a plant's therapeutic value as coming from the synergistic effects of the various components of the plants, in contrast to the individual chemicals of conventional medicines isolated by pharmacologists, therefore it is believed that traditional medicines are effective, with few or no side-effects (132, 30). Although the brain accounts for less than 2% of the body weight, it consumes about 20% of the oxygen available through respiration. Therefore, because of its high oxygen demand, the brain is the most susceptible organ to oxidative damage (133). The phytochemicals present in the plants (polyphenols, flavonoids, carotenoids, terpenoids, etc) are potent scavenger of free radicals and thus maintains the antioxidant status to combat oxidative stress induced neurological diseases (134, 138). Dietary supplementation of polyphenolic compounds and flavonoids like quercetin and catechin reduced oxidative stress improve memory and learning, by protecting vulnerable neurons, enhancing existing neuronal function or by stimulating neuronal regeneration and protected cultured hippocampal neurons against nitric-oxide-mediated cell death (136).

Reproductive effect

Various studies have been reported the effects of *C. officinalis* extract on the male and female reproductive system in experimental animals. Repeated oral administrations of *C. officinalis* flowers extract caused significant reduction in the weight of testes, epididymis, seminal vesicles and ventral prostate of male rats. Further, sperm motility, sperm density were also reduced significantly, resulting in 80 per cent loss of fertility in experimental animals (137). The weight reduction of the testes and other accessory sex organs might be due to low level of androgens (138, 139). The protein content of the reproductive organs was significantly decreased which may be due to a low level of androgens and decreased level of sialic acid in the testis, epididymis, seminal vesicles and ventral prostate also reflects loss of androgens (131). In another study along with these observation reduction in serum concentration of luteinizing hormone, FSH and testosterone were also observed. It was concluded that aqueous extract *C. officinalis* possess dose related effect on male reproduction and possess contraceptive potential in male rats (141). In a study conducted by Silva et al., (142), to evaluate the effects of administration of a hydroalcoholic extract of *C. officinalis* flowers on the female reproductive function of wistar rats, the result

concluded that extract had no toxic effect on female reproductive parameters and was also non-toxic in the pre-implantation and organogenesis periods of pregnancy. It induced a decrease of the maternal weight gain when administered during the fetal period (142). Thus, in future, more scientific research on *C. officinalis* effects on reproductive system helps in identifying the active principles used for contraceptive of the natural origin.

Cardioprotective effect

The supplementation of *C. officinalis* extracts produces cardioprotective effect against ischemic heart disease. The cardioprotective action may be due to stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocytes apoptosis (143). 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 (143). Contrary to this Perez-Carreon et al., tested the effect of aqueous extract on the heart of male Wistar rats and found it to inhibit heart rate contractility by 100% at a dose of 0.3 mg/l may be due to over-stimulating the myocardium activity (144). This may be possible due to spasmogenic activity of plant extract in a dose dependent manner (107).

Hypoglycemic and hypolipidemic effect

Experimental studies suggested floral extract of *C. officinalis* have hypoglycemic and hypolipidemic effect on rats (145, 146). Oral administration of hydroalcoholic extract of *C. officinalis* to diabetic rats at a dose of 100mg/kg body weight resulted in a significant reduction in blood glucose, urine sugar and serum lipids in alloxan induced diabetic rats. It worked similar to insulin thus confirming its antidiabetic as well as hypolipidemic effect (145). Similarly, repeated administration of ethanolic floral extract (300mg/kg) have more effective than aqueous extract of *C. officinalis* in restoring the mean blood glucose, per cent glycosylated hemoglobin, total cholesterol, triglycerides, low density lipoprotein, total thiols, malondialdehyde levels, antioxidant enzymes and renal parameters in streptozotocin induced diabetic rats (146).

Renal effect

Experimental studies suggested that floral extract of *C. officinalis* have nephroprotective potential against cisplatin induced nephrotoxicity in animals (83, 135). Verma *et al*., showed that treatment with ethanolic floral extract of *C. officinalis* protect cisplatin induced nephrotoxicity by restoring antioxidant system of the renal tissue. These observations were supported by restoration of plasma renal biomarkers and histopathological findings in renal tissue (135). The molecular mechanism of nephroprotective action is not well established but supposed to be due to its antioxidant potential and free radicals scavenging potential of carotenoids (litein, zeaxathin and lycopene) present in the extract.

Antibacterial effect

The areal part of the calendula is used in various preparations due to antibacterial potential of phytochemical ingredients present in the extract. Iauk *et al*., studied the methanolic extract and 10% decoction of the flowers of *C. officinalis* showed marked inhibition against anaerobic and facultative anaerobic periodontal bacteria (*Porphyromonos gingivalis, Prevotella spp., Furobacterium nucleatum, Caphocytophaga gingivalis, Veilonella parvula,* and *Actinomyces odontolyticus*) (147). Aqueous floral extract of *C. officinalis* exhibited better antibacterial activity especially against *Streptococcus aureus* than the petroleum ether, methanolic and ethanolic extracts (148). Flowers, leaves, stems and roots extract of *C. officinalis* made in n-butanol, ethanol and distilled water were tested for their antimicrobial properties and all the three solvents were effective against pathogenic microbes (149).

In another study using disc diffusion and MIC level, *C. officinalis* have been susceptible to 100% *Streptococcus, Enterococcus*, Methacillin resistant *Streptococcus aureus* (MRSA) and 50% *Staphylococcus*. The antibacterial activities of free oleanic acid and its glucosides and glucoronides isolated from *C. officinalis* were investigated. Oleanic acid inhibited bacterial growth and survival influenced cell morphology and enhanced the autolysis of gram positive bacteria suggesting that bacterial envelopes are the target of its activity (150). Moreover, Ghaima *et al*., studied the antibacterial and anti-biofilm activities of water extract of *C. officinalis* flowers against some of the entero-pathogenic bacteria was studied and the result showed a good antibacterial activity against all pathogenic isolates of bacteria. The reported activities for *C. officinalis* flowers extract allow their listing as potential anti-biofilm, antibacterial and antioxidant natural agents (151). Chloroform, ethanol and water leaf extracts of *C. officinalis* exhibits *in-vivo* antibacterial activity against gram positive and gram negative bacteria. Experimental studies results suggest that oleanic acid and its glycosides from *C. officinalis* can be considered as potential therapeutic agents against bacterial infections (150).

Antifungal effect

Floral extract of calendula have also tested against different pathogenic fungal strains isolated from humans and the extract showed significant antifungal properties (151). The *in-vitro* antifungal activity of floral extract of

C. officinalis showed effective against *Aspergillus niger, Rhizopus japonicum, Candida albicans* and *Rhodotorula glutinis* (152). Similarly, steam distillate volatile oils from flower of *C. officinalis* have potential antifungal activity against various strains of *Candida* spp. comparable to Nystatin as standard antifungal drug (151). The studies also proven that leaf extract of *C. officinalis* can also be used as antifungal agent (151, 153).

Anti-viral effect

The dichloromethane-methanolic (1:1) extract of *C. officinalis* flowers exhibited potent antiviral activity against viral infections. Studies showed anti-HIV activity in an *in-vitro* MTT/ Tetrazolium based assay. The antiviral activity of chloroform extract of calendula was attributed to inhibition of human immunodeficiency virus-1 reverse transcriptase (HIV1-RT) at a concentration of 1000µg/ml as well as suppression of HIV-mediated fusion at 500µg/ml (154). An 85% RT inhibition was achieved after a 30 min treatment of partially purified enzyme in a cell-free system. These results suggested that organic extract of flowers from *C. officinalis* possesses anti-HIV properties of therapeutic interest. 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) (154). Similarly, *in-vitro* flower tincture showed antiviral activity by suppressing the replication of influenza APR-8, influenza A_2 and herpes simplex virus (33).

Antiparasitic potential

Studies on methanolic and ethanolic extract of *C. officinalis* leaves showed significant anthelmintic activity as compared to the standard drug albendazole (155). Similarly, aqueous extract of leaves and flowers of *C. officinalis* have potent vermicide activity against Indian adult earthworm (*Pheretima posthuma*) (156). Glycosides of oleanic acid inhibited larval stage of parasitic worm *Heligmosomoides polygyrus*. These results suggest that oleanic acid and its glycosides from *C. officinalis* can be considered as potential therapeutic agents (150). Alexenizor and Dorn, (157) reported the insecticidal effect of acetone: methanolic (2:1) extract of *C. officinalis* flowers (157). The extract showed potential insecticidal effect against milk weed bug. α-pinene in *C. officinalis* showed activity against *Listeria monocytogenes* (158). Whereas, nerolidol of *C. officinalis* showed anti-malarial activity by inhibiting the parasite to synthesize coenzyme Q in all intra-erythrocytic stages (159). 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 (160). Further methanolic floral extract of *C. officinalis* is effective against extracellular (promastigote) and intracellular (amastigote) forms of *Leishmania major* in controlling cutaneous leishmaniasis (161). Godara *et al.*, reported acaricidal activity of aqueous and ethanolic floral extract of *C. officinalis* against *Rhipicephalus* (Boophilus) *microplus* resistant to pyrethroids (deltamethrin and cypermethrin). The results indicated that the ethanolic extract of *C. officinalis* had better acaricidal properties against resistant larvae and adult *Rhipicephalus* (Boophilus) microplus than the aqueous extract (162). The supplementation of saponins of *C. officinalis* reduces the infectivity of *Heligmosomoides polygyrus* in mice as indicated by reduced cytokine (IL-6, IFN-γ, TNF-α, IL-17) production on re-infection with *H. polygyrus* (163). Further research needs to characterize the fraction or ingredient from the extract of *C. officinalis* for their use dewormer or pest control programmes.

Toxicological effect

Calendula flowers preparations are of moderate acute toxicity. Intravenous and intra-peritoneal median lethal dose (LD_{50}) in mice of aqueous extract is reported to be 300 and 375mg/kg body weight. The hydroalcoholic preparation the LD_{50} is reported to be 45mg/mouse, subcutaneously and for the rats it was reported to be 5260mg/kg body weight, intravenously (164). Hematological parameters were not altered but the biochemical parameters viz. blood urea nitrogen level and aspartate aminotransferase activity were elevated which may be due to renal and liver overload. No toxic symptoms were observed in the hamster orally given 0.15 g/kg/day for a period of 18 months of a floral extract of *C. officinalis* (164). Some clinical studies reported dermal application of floral extract produces allergy in some human cases assessed by the patch testing method (165). Moreover, Bone (166); Braun and Cohen (167) also advised to persons who are allergic to Asteraceae family should use calendula preparations with caution. Additionally, some allergic reactions are also recorded in persons who are in close contact with calendula with low immunity (168). Pregnant women are also advised to take care while handling calendula because it is believed to stimulate labor (169). The hydroalcoholic extract of *C. officinalis* was evaluated for its acute toxicity by the oral route in rats and mice and for subacute effect on hematological, biochemical and morphologic parameters in rats. Acute toxicity did not induce hematological alterations when compared with control group. There was an increase in blood urea nitrogen and ALT levels. Inflammatory sites were found in lung and liver, which were associated with oral gavage and a possible hepatotoxic effect. Overall the extract was non toxic except for some evidenced of renal and liver overload (33). Lagarto et al., studied the acute and subchronic oral toxicities of *C. officinalis* extract in male and female rats. A single dose of 2000 mg/kg dissolved in distilled water was administered by oral gavage for acute toxicity. Subchronic dose of 50, 250 and 1000 mg/kg/day were administered in drinking water. In acute toxicity, there were no mortality and signs of toxicity. In subchronic study, different blood biochemical parameters viz. aspartate and alanine aminotransferase

were significantly affected in male and females after 90 days. Histopathological examination if tissues showed slight abnormalities in hepatic parenchyma that was consistent with biochemical variations observed this indicate the low hepatic toxicity on chronic exposure of calendula extract (170).

Clinical Studies

C. officinalis flowers are reported to be used in almost 200 cosmetic formulations, over a wide range of products used for treating wounds, ulcers, herpes, scars, skin damage, frost-bite, burn, etc. In herbalism, calendula in suspension or in tincture is used topically for treating acne, reducing inflammation, controlling bleeding, and soothing irritated tissue. Calendula extract heals wounds as well as internal and external ulcers. It is an antiseptic and in addition improves blood flow to the affected area. Different formulations containing calendula (flower, areal part) are used in different conditions of gastric or hepatic disorders. Some of the clinical studies having beneficial potential are evaluated in limited human clinical studies are mentioned in the table 3.

Genotypic development

Calendula genotypes investigated in the VOSFA program showed considerable diversity in flower structure and site stem branching crop height, flowering period and maturity. By applying modern breeding technology, performing crop physiological studies, studying genotype environment interactions and investigating seed cleaning and processing systems capable of meeting the specifications of the oil extraction processes, improved cultivars and production systems will be developed for the main production areas of Europe.Technology to meet the seed cleaning specification of oil extraction production will be developed. In calendula officinalis seed oil content could be improved by reducing the seed hull and increasing kernel mass (177).

Calendula as agronomic raw material for industrial applications (178) project was directed towards development of the best technology to process specialty oil from seeds. Refinement methods were investigated in order to produce different oils meeting the various requirements of the specific applications investigated in this program. Functional ingredients present in flower head extracts may be of interest for application as food ingredient. Further research was conducted and calendula oil was produced and tested in the VOSFA program. Only a limited use in paint formulations was possible due to suboptimal quality of oil. In Calendula as agronomic raw material for industrial applications (CARMINA) these oils were made and tested. Moreover, other industrial research was also conducted related to synthesis and characterization of specialty surfactant for application in lubricants, pharmaceuticals, disinfectants, agrochemical, cleaning products. In CARMINA, the production, processing and testing of calendula oil were integrated through continuous quality monitoring, product evaluation and information feedback up the production chain.

Conclusion

Looking at the present scenario, the ethno-botanical and traditional uses of herbal products has gained much popularity as these products are easily available, cheap, have high efficacy and are safe for human and animals use. Calendula has been reported to be used a medicine for centuries. Flowers of the plant possess greater degree of medicinal importance due to presence of high qualitative as well as quantity of phytochemical ingredients. Numbers of formulations are available and are therapeutically used in various dermatological disorders ranging from skin ulcerations to eczema, gastrointestinal, hepatic renal, cardiac and neurological disorders, etc. The phytochemical constituents are commonly used in formulations used for the wound

Table 3 Clinical studies of *C. officinalis* conducted in human trials for therapeutic applications.

Preparations	Dose	References
Calendula powder	1-2gm in a cup of water orally for the gastrointestinal disorders	[171, 172]
Tincture (1:9 in 20% alcohol)	2-4ml in water three times daily for the gastrointestinal disorders	
Ointment base of *C. officinal (*2 - 5%)	Used to treat athlete's foot, ringworm and *Candida* infection	[113, 117, 118, 173]
Cream with 0.9% of calendula extract	effective long term protection of the skin against ROS caused damage	[174]
C. officinalis containing cream	Sodium-lauryl-sulfate-induced irritant contact dermatitis	[175]
2% Calendula extract mouthwash or as oral gel	Radiation-induced oropharyngeal mucositis	[176]

healing potential along with increased blood supply to affected areas which additionally boost the healing process. Apart from this, very little molecular work has been done on *C. officinalis*. Emphasis needs to be paid on the molecular characterization of the plant as well as using other parts such as roots and analyzing its chemical constituents and properties. The traditional systems have widely proven the significance of *C. officinalis* up to greater extent. Due to rich phytoconstituents these ingredients have wide applicability in food and cosmetic industries besides medical applications. Being useful therapeutic agents in their own right, an understanding of these traditional medicines has provided new plant derived drug leads to modern medicine.

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Conflict of interest statement

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