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A REVIEW ON PHYTOCHEMICAL PROFILE, ISOLATION, CHARACTERIZATION AND ANTIOXIDANT POTENTIAL OF CALENDULA OFFICINALIS L. SEEDS

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ABSTRACT

Calendula officinalis, commonly known as pot marigold, is the member of the Asteraceae family and plays an important role in addressing various health issues. It has been traditionally used to alleviate inflammation, treat ulcers, and resolve skin and digestive troubles, as well as to ease nervousness and insomnia, among other conditions. The plant contains the variety of compounds, such as lipids, terpenoids, carotenoids, coumarins, and quinones. Many studies have confirmed its medicinal benefits, highlighting its anti-inflammatory, healing, and regenerative properties, along with antimicrobial, anticancer, antigenotoxic, and genotoxic effects. Additionally, it has hepatoprotective, neuroprotective, and cardioprotective qualities, as well as gastroprotective, antioxidant, spasmogenic, and spasmolytic activities. Calendula also shows antiprotozoal, anthelmintic, acaricidal, antiviral, anti-aging, photoprotective, and immunostimulant effects.Clinical trials have highlighted its efficacy for conditions such as ulcers, radiodermatitis, burn wounds, oropharyngeal mucositis, plague, and gingivitis, with no reported toxicity. Due to these properties, calendula is incorporated into various medicines and finds a place in the food industry as its flowers are edible, suitable for fresh salads or dried for dyeing purposes.This study aims to provide a comprehensive overview of pot marigold, coverning its distribution,botanical characyeristics, cultivation methods, traditional applications, phytochemicals, phytochemistry, and pharmacological actions.

KEYWORDS: *Calendula officinalis,* Antioxidants, Phytochemicals, Flavonoids, Phenolic compounds, Odidative stress.

INTRODUCTION

Calendula belongs to the genus *Calendula* within the family Asteraceae,and it is believed to have originated in the southwestern Mediterranean region. Among the species in this genus, *Calendula officinalis* Linn. (CO) is one of the most significant plants used in traditional medicine. Because of its rich content of bioactive secondary metabolites, *C. officinalis* has found widespread use not only in traditional healthcare systems but also in the food and pharmaceutical industries. The genus *Calendula* comprises approximately 25 species, of which *C. officinalis*, *C. arvensis*, *C. tripterocarpa*, *C. stellata*, and *C. suffruticosa* are the most commonly known.

The term “calendula” is derived from the Latin word *calends*, which refers to the first day of the month, which reflects the plant’s prolonged flowering period. The plant is also often called the “herb of the sun” because its flowers open in the morning and close in the evening.

Historically, *C. officinalis* has been widely used for treatment of minor skin ailments, wounds, cuts, and burns. The flowers are a primary source of carotenoids, which are extracted to make commonly used medicinal products such as pot marigold tincture and carophyllenic ointment. Moreover, *C. officinalis* is a key component of the proprietary homeopathic formulation Traumeel, which is used to relieve pain and inflammation associated with acute musculoskeletal injuries.

Traditional medical systems, including Ayurveda and Unani medicine, have extensively documented the therapeutic properties of *C. officinalis*, reporting its antipyretic, anti-inflammatory, antiepileptic, and antibacterial activities, primarily attributed to its leaves and flowers. Botanically, *C. officinalis* is an annual or biennial herbaceous plant, attaining a height of 30–60 cm. It is characterized by sessile, hispid, acute, oblanceolate leaves.

The leaves are hairy, alternate, petiolate, and oblong to spatulate in shape, with entire or sparsely toothed edges. The Upper leaves are lanceolate and pointed, while the lower leaves are elliptic with rounded tips, with leaf bladesrange in length from 2 to 4 inches.

The tribe Calenduleae includes over 110 species across eight genera, with most of them predominantly found in South Africa. As an ornamental plant, *C. officinalis* is widely

cultivated and distributed across Macaronesia, Eastern and Mediterranean regions, Western Asia, Europe, the United States, Cyprus, Turkey, Iran, and several Middle Eastern countries.

Fig. 1: Flower of Calendula Officinalis.

Table 1: Common Name of CO in Various Languages.

Sr. No.	Languages	Common Name
01	English	Pot marigold, Scoth Marigold, Calendula, Marybud, Gold-bloom, Holligold, English Marigold
02	Hindi	Genda
03	Portugese	Wonders, boninas
04	Spanish	Maravilla, calendula
05	German	Ringelblume
06	French	Calendula fleur

Description of plant:

Calendula officinalis is a short-lived, aromatic, herbaceous perennial plant with loosely arranged or erect, sparsely branched stems, that can grow up to about 80 cm in height. The leaves are oblong to lanceolate in shape, measuring 5–17 cm in length, pubescent on both upper and lower surfaces, and have entire to slightly serrated edges.

The inflorescences are capitula borne terminally, exhibiting yellow to orange coloration, and are surrounded by two series of pubescent involucral bracts. Each capitulum measures approximately 4–7 cm in diameter. In wild populations, the central region consists of tubular, bisexual disc florets, which are surrounded by a single whorl of peripheral ray florets. The disc florets typically display a more intense orange-yellow pigmentation compared to tridentate, pistillate ray florets. Under favorable environmental conditions, flowering may occur throughout the year. The fruit is a curved, spiny achene.

Flower **Seeds** **Leaves** **Roots**

TAXONOMICAL PROFILE:

Kingdom: Plantae Subkindom: Tracheobionta Division: Magnoliophyta Class: Magnoliopsida Subclass: Asteridae

Order: Asterales Family: Asteraceae Tribe: Calendulea Genus: Calendula Species: Officinalis

CHEMICAL CONSTITUENTS:

The diverse pharmacological activities of *Calendula officinalis* are attributed to its complex chemical composition. Phytochemical investigations have revealed that the plant contains various classes of bioactive compounds, including terpenoids, quinones, coumarins, flavonoids, essential oils, carotenoids, amino acids, triterpenoid esters, and saponins. Among these constituents, flavonoids such as isorhamnetin, rutin, quercetin, and their glycosides are prominent and are widely used in the food and cosmetic industries.

Extracts obtained from the leaves of *Calendula officinalis* have been reported to contain fatty acids, triterpenes, sterols, and chloroform-soluble compounds, while aqueous extracts mainly consist of tannins, phenolic compounds, and saponins. Additional flavonoids, including quercetin, isorhamnetin, and isoquercetin, have also been isolated from the plant. The inflorescences of *Calendula officinalis* are particularly rich in carotenoids, which are responsible for the characteristic yellow to orange pigmentation of the flowers. The major bioactive constituents identified in the plant, especially in the flowers, include:

- **Triterpenoids:** These are considered the primary active constituents of Calendula, particularly oleanolic acid glycosides and faradiol esters. These include saponins (e.g., calendulosides A-H), which contribute to the plant's anti-inflammatory and wound-healing properties, and triterpene alcohols (e.g., faradiol, arnidiol), known for their anti-inflammatory and antitumor activities.

- **Flavonoids:** These phenolic compounds (e.g., quercetin, rutin, isorhamnetin) contribute to the potent antioxidant and anti-inflammatory effects of Calendula officinalis. They also exhibit antimicrobial and anticancer properties.

- **Carotenoids:** The bright orange and yellow colors of *Calendula officinalis* flowers are due to the presence of carotenoids (e.g., beta carotene, lycopene, lutein), which act as antioxidants and contribute to skin protection.

- **Volatile Oils:** The essential oil of *Calendula officinalis* contains a variety of monoterpenes and sesquiterpenes, including α-cadinol, T-cadinol, and β-caryophyllene, which contribute to the plant's antimicrobial, anti-inflammatory, and wound-healing properties.

- **Polysaccharides:** Calendula contains polysaccharides, which have been shown to stimulate immune responses. These complex carbohydrates contribute to the plant's immune-modulating and wound-healing effects.

- **Coumarins:** Coumarin derivatives such as umbelliferone are present in Calendula and contribute to its anti-inflammatory and anticoagulant properties. The specific composition of *Calendula officinalis* can vary depending on factors such as geographical location, cultivation methods, and extraction techniques.

Extraction and isolation of *Calendula officinalis L. seeds*

Extraction plays a vital role in phytochemical studies as it enables the separation of bioactive constituents from plant materials. The effectiveness of the extraction process depends on several factors, such as the type of plant material, the polarity of solvent used, extraction technique, and the chemical characteristics of the compounds of interest.

Seeds of contain valuable phytochemicals, particularly fixed oils, fatty acids, and other secondary metabolites. Different solvent systems have been reported for the extraction of seeds constituents based on their chemical properties. Non- polar solvents are generally used for the recovery of lipid components and fatty acids, whereas polar solvents may assist in extracting phenolic compounds and other polar constituents.

Conventional extraction methods such as soxhlet exraction, maceration, and solvent extraction are commonly applied for the investigation of plant drived compounds. These approaches allow the recovery of phytochemicals that can be further analysed for their biological activities. After extraction, the compounds can be separated and purified using techniques such as thin-layer chromatography (TLC), column chromatography,and high- performance liquid chromatography (HPLC).

The fractions obtained from the extract of *Calendula officinalis* seeds can be analyzed using advanced analytical methods, including gas chromatography-mass spectrometry (GC-MS), liquid chromatography- mass spectrometry (LC-MS), and spectroscopic methods such as infrared (IR) and nuclear magnetic resonance composition and structural identification of seeds- derived compounds.

These methods help determine the composition and structure of compounds derived from the seeds. Despite numerous studies on different parts of *Calendula officinalis,* seeds –based investigation remain comparatively limited. Further research studies focusing on improving extraction methods, compound isolation, and detailed characterization of seeds constituents may help explore their potential antioxidant and pharmaceuitcals properties.

PHARMACOLOGICAL ACTIVITIES OF *Calendula officinalis*:

Calendula officinalis, commonly known as pot marigold, exhibits a wide range of therapeutic activities, making it a significant plant in traditional and modern medicine. Modern scientific research pharmacological has investigated the properties of *Calendula officinalis*, providing evidence to support its traditional uses and uncovering new potential applications. The following sections outline the key activities reported for *Calendula officinalis*.

Anti-inflammatory

Its superior anti-inflammatory properties, *Calendula officinalis* is presently the subject of research. The plant contains a variety of secondary metabolites that are linked to its anti-inflammatory properties, including alkaloids, tannins, flavonoids, essential oils, sterols, saponins, carotenoids, triterpene alcohols, mucilage, polysaccharides, and resin. Mostly used as a component of tinctures, ointments, and infusions, *Calendula officinalis* is used as a wound healing remedy for blisters, scars, mucous membranes, skin inflammations, tissue repair, and allergic responses. Calendula extract cream has shown promise in treating burn oedema, according to research. Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans are all inhibited in vitro by the essential oil extracted from the flower petals. *Calendula officinalis* exhibits potent anti-inflammatory activity, primarily attributed to its triterpenoids, flavonoids, and carotenoids. These compounds inhibit the production of pro-inflammatory cytokines (e.g., TNF-α, IL-1β) and enzymes (e.g., COX-2) involved in the inflammatory response. In vitro studies have shown that *Calendula officinalis* extracts can effectively reduce inflammation in various cell types, including macrophages and keratinocytes.

Antioxidant activity

Flavonoids and other plant polyphenols are some of the most important natural substances with potent antioxidant qualities. The presence of hydroxyl groups in flavonoids is what causes them to scavenge or chelate radicals. Antioxidants can be found naturally in the leaves and petals of the *Calendula officinalis* plant. *Calendula officinalis* extract has been reported to scavenge superoxide and hydroxyl radicals due to the photo reduction of riboflavin.

Flavonoids, carotenoids, and phenolic acids are among the several antioxidants found in *Calendula officinalis*. These substances help the plant's anti-inflammatory, wound-healing, and anti-aging properties by scavenging free radicals and shielding cells from oxidative damage. The antioxidant activity of C. officinalis flower extracts is being studied both in vitro and in vivo. According to reports, the extracts reduce lipid peroxidation in vitro by scavenging nitric oxide, superoxide, hydroxyl, ABTS, and DPPH radicals. An oral extract raised catalase and intracellular glutathione levels and prevented superoxide production in macrophages in vivo in mice, indicating strong antioxidant action.

Cytotoxic and anti-tumor activity

One of *Calendula officinalis* isolated active ingredients, saponin, has been demonstrated to have antimutagenic properties. With the growth of complementary and alternative medicine based on herbs as a cancer treatment, interest in the alleged anti-tumor potential of *Calendula officinalis* extracts and components has increased. Found that calenduloside F 6′-O-n-butyl ester and calenduloside G 6′-O-methyl ester, two triterpene glycosides extracted from marigold flowers, had strong cytotoxic action against human colon cancer, leukaemia, and melanoma cancer cells in vitro. It has been demonstrated that the triterpenoids and flavonoids in the plant prevent the growth and multiplication of a number of cancer cell lines, such as leukaemia cells, breast cancer, and colon cancer. Furthermore, Calendula officinalis has been shown to induce apoptosis (programmed cell death) in cancer cells and inhibit angiogenesis, thereby suppressing tumor growth and metastasis.

Wound-healing activity

Calendula officinalis flower extract has wound and burn healing properties when applied topically and over time. Increases in collagen hydroxyproline and hexosamine indicate that the patient's or animal's wounds are getting better. [30] *Calendula officinalis* may significantly boost collagen metabolism and wound angiogenesis, leading to emollient properties and softer scars. *Calendula officinalis* flower extract provides therapeutic benefits

for burns and wounds when applied topically and taken orally. An increase in hexosamine and collagen hydroxyproline indicates that the patient or animal is healing their wounds. *Calendula officinalis* has the ability to prevent macrophage activation and accelerate the migration and growth of fibroblasts and keratinocytes, which are involved in wound healing. This was achieved by lowering oxidative stress at the wound site and inhibiting the release of proinflammatory cytokines.

Hepatoprotective activity

Calendula officinalis extracts were shown to possess hepatoprotective properties against the cytotoxicity and oxidative stress caused by carbon tetrachloride. Overall, it raises haemoglobin levels. Likewise, the hydroalcoholic extract of the flowers exhibits reduced hepatocytolysis and liver indicators in both in vitro and in vivo settings. Normal levels of hepatic blood markers were restored after the ethanolic extract treatment. Malondialdehyde and total oxidant status were reduced in both the blood and the hepatocytes, while total thiols and antioxidant enzymes (CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; and GST, glutathione s transferases) were also reduced. The extract from *Calendula officinalis* enhanced the liver'shistological appearance, biochemical markers, and inflammatory cytokines.

Antimicrobial activity

Over the past few decades, it has been noted that the prevalence of harmful bacteria that are resistant to antibiotics has increased, despite the fact that antibiotics have been effectively used for the past 60 years to treat infectious diseases brought on by bacteria and fungi. Broad-spectrum antibacterial action against a variety of bacteria, fungi, and viruses is exhibited by *Calendula officinalis*. By rupturing microbial cell membranes and interfering with microbial metabolism, the plant's essential oil and flavonoid contents help to produce its antimicrobial actions. *Calendula officinalis* is efficient against common infections such as Staphylococcus aureus, Escherichia coli, Candida albicans, and herpes simplex virus (HSV), according to in vitro research. Clinical pathogens such as Aspergillus Niger, Bacillus subtulis, Staphylococcus aureus, Escherichia coli, Candida albicans, and Klebsiella pneumonia have all been studied in relation to the antibacterial qualities of methanol and ethanol extracts derived from calendula petals.

When it came to antibacterial activity against the majority of tested microorganisms, *Calendula officinalis* methanol extract was particularly better than the ethanol extract. Both

methanol and ethanol extracts showed excellent antifungal efficacy against the test fungus strain. *Calendula officinalis* plants have demonstrated the anthelmintic effect of saponins, demonstrating their anthelminthic properties.

Cardiovascular effect

Calendula extract has been found to reduce myocardial infarctions. Cardio protection seems to be achieved by transforming the ischaemia-reperfusion-mediated death signal into a survival signal. Extracts from *Calendula officinalis* have been shown to be beneficial in the treatment of ischaemic heart disease. By modifying antioxidant and anti-inflammatory pathways, a cardio-protective action is achieved by converting the death signal caused by ischaemia-reperfusion into a survival signal, as demonstrated by the decrease in TNF-α and the activation of protein kinase B and Bcl-2.

CLINICAL APPLICATIONS: The diverse pharmacological properties of *Calendula officinalis* have led to its use in a variety of clinical applications. While some applications are supported by strong evidence, others require further investigation.

- **Wound Healing:** Clinical trials have investigated the efficacy of Calendula in promoting wound healing. Studies have shown that Calendula ointments can accelerate the healing of surgical wounds, leg ulcers, and pressure sores. However, the quality of evidence varies, and more large-scale, well-designed trials are needed to confirm these findings.

- **Radiation Dermatitis:** Calendula has shown promise in preventing and treating radiation dermatitis, a common side effect of cancer radiation therapy. Several studies have found that Calendula cream is more effective than conventional treatments, such as trolamine, in reducing the severity of radiation dermatitis.

- **Oral Mucositis:** Calendula mouthwashes have been investigated for the treatment of oral mucositis, an inflammation of the oral mucosa that can occur as a result of chemotherapy or radiation therapy. Some studies have found that Calendula mouthwashes can reduce the severity and duration of oral mucositis.

- **Diaper Rash:** Calendula ointments are commonly used to treat diaper rash in infants. Studies have shown that Calendula is effective in reducing inflammation and promoting the healing of diaper rash.

- **Vaginal Yeast Infections:** Some studies suggest that Calendula creams may be effective in treating vaginal yeast infections. However, more research is needed to confirm these findings. Other Applications: Calendula is also being investigated for its potential use in treating other conditions, such as acne, eczema, hemorrhoids, and varicose veins. However, more research is needed to determine its efficacy in these applications.

Future Perspectives

Future research on L.f. is necessary for the isolation and identification of bioactive compounds, particularly from seeds. The application of advanced analytical techniques, conducting standardization studies, evaluating toxicity, and performing clinical investigations may facilitate the development of its potential pharmaceutical applications.

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