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Diverse Belongings of *Calendula officinalis*: An Overview

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ABSTRACT
Herbal drugs therapy is regarded as an important therapy for the treatment of a wide range of diseases. However, enormous improvements have been observed in modern systems of medicine, but Indian herbal plants provide a rich source for health care to prevent different pathological states. *Calendula officinalis* is an aromatic, erect, annual herb that is indigenous to Europe, cultivated commonly in North America, Balkans, Eastern Europe, Germany and India. Ample studies have reported about the anti-inflammatory, antioxidant, antitumour, antigenotoxic, chemoprotective and hepatoprotective potential associated with the drug. The review article describes about the various phytochemical constituents associated with the plant extract. Moreover, numerous pleiotropic effects exhibited by the plant have been clearly discussed.

Keywords: Herbal, *Calendula officinalis,* Pleiotropic.

INTRODUCTION

India has a very long history of using herbal drugs as the main course therapy for treating a number of diseases. [1-2] The herbal drugs have made their importance felt in the last few decades whose prevalence is continuously increasing in both developing and developed countries due to their natural origin and lesser side effects. [3] *Calendula officinalis*, commonly known as marigold related to family *Compositae*, is an aromatic, erect, annual herb that grows up to 60 cm in height with angular and glandular stems; leaves 2.5-7.5 cm long; lower spathulate, entire, upper lanceolate with cordateamplexicaul base; flower-heads terminal, heterogamous, light yellow to deep orange; ray florets fertile; achenes 1.0-1.5 cm long, boat-shaped, faintly ribbed; indigenous to central, eastern and southern Europe, cultivated commonly in North America, Balkans, Eastern Europe, Germany and India. [4-6] *Calendula officinalis* has a long history of usage by the folk systems because of its rich medicinal values that have been reported to possess potent anti-inflammatory, antitumour, antioxidant, antibacterial, anti-HIV, anti-ulcer, antigenotoxic, chemoprotective and antiseptic properties. [7-9] Moreover, a large number of phytochemicals have been found in various parts of the plants that include calenduline and oleanolic acid glycosides, sterol glycosides, alpha-and beta-amyrin, taraxasterol, lupeol, brein, faradiol, arnidiol, erythrodiol, calenduladiol, coflodiol and manilladiol. [10-12]

The present review article discusses about the various phytochemicals present in the plant. Moreover, various pharmacological properties exhibited by the plant have been demarcated

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PHYTOCHEMISTRY IN SUPPORT OF HERB

Phytochemistry has been considered as the heart of herbal therapy and the phytochemical research plays an important role in the development of herbal medicines. A number of studies have well reported about the phytochemicals released with the plant.

Piccaglia *et al.* invesitigated about the agronomic parameters, flavonoid and carotenoid contents of an Italian *Calendula officinalis* which were evaluated over a two-year trial performing two annual cuts during the flowering period. The number of flower heads per plant and the yield of heads and petals were found to be higher in the second cut, but the pigment content greatly differed in the second year. Glycosides of quercetin and isorhamnetin were the predominant components of the flavonoids, while beta-carotene and lutein were the most abundant carotenoids. [13]

Szakiel and Janiszowska evaluated specificity of the tonoplast transport of oleanolic acid monoglycosides in the vacuoles from *Calendula officinalis* leaves. These two glycosides were isolated from leaf protoplasts of the plant with the use of chemically synthesized analogues. The results indicate that the proper structure of both parts of oleanolic acid monoglycoside, i.e. aglycon and the sugar moiety, are required for binding to a specific tonoplast carrier. [14]

Marukami *et al.* investigated the structures of new ionone and sesquiterpene glycosides from Egyptian *Calendula officinalis*. Two new ionone glucosides (officinosides A and B) and two sesquiterpene oligoglycosides (officinosides C and D) were isolated from the flowers of Egyptian *Calendula officinalis*, the structures of which were elucidated on the basis of chemical and physicochemical evidences. [11]

Bako *et al.* evaluated high performance liquid chromatography (HPLC) study on the carotenoid composition of calendula products such as steams, leaves, petals and pollens. In the petals and pollens, the main carotenoids were found namely flavoxanthin and auroxanthin; while the stem and leaves mostly contained lutein and beta-carotene. Five different herbal tea and two tinctures made from the flowers of *Calendula officinalis* were also investigated and the carotenoid composition of the industrial products was compared to the starting material. [15]

Reed *et al.* investigated and identified calendic acid from linoleic acid in whole plant of *Calendula officinalis* by the use of kinetic isotope effect (KIE) measurements. This was accomplished by incubating appropriate mixtures of linoleate and regiospecifically dideuterated isotopomers with a strain of Saccharomyces cerevisiae expressing a functional (1, 4)-desaturase. The data indicates that calendic acid is produced via initial H-atom abstraction at C11 of a linoleoyl substrate and supports the hypothesis that this transformation represents a regiochemical variation of the more common C12-initiated delta-12 desaturation process. [16]

Hamburger *et al.* evaluated preparative purification of the major anti-inflammatory triterpenoid esters from Marigold *(Calendula officinalis*). Faradiol-3-Olaurate, palmitate and myristate, the major anti-inflammatory triterpenoid esters in the flower heads of the medicinal plant *Calendula officinalis* has been identified. During the work-up of the faradiol esters, accompanying minor compounds of the triterpene ester fraction were purified and identified by spectroscopic means as maniladiol-3-O-laurate and myristate. [17]

Neukirch *et al.* carried out simultaneous quantitative determination of eight triterpenoid monoesters from flowers of ten varieties of *Calendula officinalis* and characterisation of a new triterpenoid monoester. Dichloromethane extracts of dried flowers of *Calendula officinalis* contain eight known bioactive triterpendiol monoesters, namely faradiol-3-O-palmitate, faradiol-3-O-myristate, faradiol-3-O-laurate, arnidiol-3-O-palmitate, arnidiol-3-O-myristate, arnidiol-3-O-laurate, calenduladiol-3-O-palmitate and calenduladiol-3-O-myristate. Moreover, the lipophilic extract from the flowers of the plant has been shown to contain low levels of the newly characterized calenduladiol-3-O-laurate. [18]

Kishimoto *et al.* evaluated the analysis of carotenoid composition in petals of *Calendula officinalis*. Nineteen carotenoids were identified in extracts of petals of orange- and yellow-flowered cultivars of calendula. In addition, ten carotenoids were unique to orange-flowered cultivars. The ultraviolet (UV) visible absorption maxima of these ten carotenoids were at longer wavelengths than that of flavoxanthin, the main carotenoid of calendula petals providing the evidevce that these carotenoids are responsible for the orange color of the petals. Six carotenoids had a cis structure at C-5 (C-5') and it is conceivable that these (5Z)-carotenoids are enzymatically isomerised at C-5 in a pathway that diverges from the main carotenoid biosynthesis pathway. Among them, (5Z, 9Z)-lycopene, (5Z, 9Z, 5'Z, 9'Z)-lycopene, (5'Z)-gamma-carotene, (5'Z, 9'Z)-rubixanthin and (5Z, 9Z, 5'Z)-lycopene have been identified. [19]

Matysik *et al.* evaluated the influence of *Calendula officinalis* extracts on cell cultures, and the chromatographic analysis of extracts. Three extracts of *Calendula officinalis* namely heptane, ethyl acetate and methanol were introduced to a human skin fibroblast (HSF) cells culture and a culture of human breast cancer cells (T47D). The ethyl acetate but not the heptane and methanol extracts in concentrations above 25 microg/mL stimulated cell proliferation and cellular metabolism by increase of mitochondrial dehydrogenase activity. However, concentrations exceeding 75 microg/mL have been found to be toxic for cells. The second part of the study concerned elaborating of optimal chromatographic systems for quantitative analysis of these extracts by the use of high performance thin layer chromatography (HPTLC) with densitometry. Oleanolic acid, beta-amyrin, beta-amyrin acetate, rutin, narcissin, 3-glucoside of isorhamnetin, quercetin, isoquercitrin, vanillic acid, caffeic acid, chlorogenic acid, protokatechuic acid, p-coumaric acid and syringic acid were all identified in the plant extract. [20]

PLEIOTROPIC PHARMACOLOGICAL PROPERTIES OF THE HERB

Calendula officinalis has a long history in herbal and folk medicinal systems to possess various beneficial properties referred to as its pleiotropic pharmacological effects. The reports that are available are briefly reviewed as follows.

Della *et al.* evaluated the role of triterpenoids in the topical anti-inflammatory activity of *Calendula officinalis* flowers. The anti-inflammatory activity of different CO2 extracts is proportional to their content of faradiol monoester, which can be taken as a suitable parameter for the quality control of *Calendula* preparations. [21]

Kalvatchev *et al.* evaluated Anti-HIV activity of extracts from *Calendula officinalis* flowers which were examined for their ability to inhibit the human immunodeficiency virus type 1 (HIV-1) replication. Both organic and aqueous extracts were relatively nontoxic to human lymphocytic Molt-4 cells, but only the organic one exhibited potent anti-HIV activity in an in vitro MTT/tetrazolium-based assay. *Calendula officinalis* flowers caused a significant dose-and time-dependent reduction of HIV-1 reverse transcription (RT) activity. 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 *Calendula officinalis* possesses anti-HIV properties of therapeutic interest. [22]

Ramos *et al.* carried out genotoxicity studies of an extract of *Calendula officinalis* that displayed genotoxic properties when assayed for mitotic segregation in the heterozygous diploid D-30 of *Aspergillus nidulans*. The extract of Calendula exhibited dose-dependent toxicity and genotoxicity (both mitotic crossing-over and chromosome malsegregation being observed) to Aspergillus in the range of five plate concentrations from 0.1 to mg of solids/ml assayed providing the evidence of genotoxicity. [23]

Marchenko *et al.* evaluated the activity of cytoplasmic proteinases from rat liver in Heren's carcinoma during tumor growth and treatment with medicinal herbs. It was determined that during tumor development, the enzymatic activity level of both the acid and neutral proteinases increased by two to six folds. The natural preparation of the

herbs *Calendula officinalis* investigated enzymes and coefficients of the liver weights of the sick animals. The chemical medicinal preparation 5, 6-benzcumarine-5-uracil normalized the activity of the neutral cytoplasmatic proteinases and reduced the level of the proteolytic activity of the acid enzymes in comparison with the control group of the animals as well as increased the liver weight coefficients. [24]

Krazhan *et al.* carried out the treatment of chronic catarrhal gingivitis with polysorb immobilized calendula. The use of traditional and modern methods of periodontal diseases treatment in clinics showed that the highest effect of calendula immobilized on the polysorb in the nearest period after its treatment. [25]

Cordova *et al.* evaluated the extract of *Calendula officinalis* against lipid peroxidation of rat liver microsomes by acting as a potent free radical scavenger and an antioxidant. The results obtained from the study suggested that the butanolic fraction of *Calendula officinalis* possessed a significant free radical scavenging and antioxidant activity and that the proposed therapeutic efficacy of this plant could be due, in part, to these properties. [26]

Herold *et al.* evaluated anti-inflammatory activity of the hydroalcoholic plant extracts of *Calendula officinalis* that showed that it suppress the cell-free systems activities of 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2), the key enzymes in the formation of proinflammatory eicosanoids from arachidonic acid (AA). [27]

Lin *et al.* evaluated the in vitro anti-hepatoma activity of *Calendula officinalis* on five human liver-cancer cell lines. The samples were examined by in vitro evaluation for their cytotoxicity. The results showed that the effects of crude drugs on hepatitis B virus genome-containing cell lines were different from those against non hepatitis B virus genome-containing cell lines confirming its anti-hepatoma activity. [28]

Hamburger *et al.* has shown the major anti-inflammatory potential of *Calendula officinalis* triterpenoid esters obtained from the flower heads of the medicinal plant. Gram quantities of the individual compounds were obtained with 96 to 98% purity by a combination of supercritical fluid extraction (SFE), normal-phase and reversed-phase column chromatography, which showed the potent anti-inflammatory potential of the triterpenoid esters of the plant. [17]

Herold *et al.* evaluated the antioxidant properties of hydroalcoholic plant extracts *Calendula officinalis* along with minor anti-inflammatory properties. The hydro-alcoholic extracts of *Calendula officinalis* exhibited potent antioxidant and anti-inflammatory activities as evidenced by the fact that the plant extracts showed strong reactive oxygen species (ROS) scavenging property along with inhibition of pro-inflammatory mediator generation. Thus, these extracts could be a useful tool for obtaining new antioxidant/anti-inflammatory agents. [29]

Iauk *et al.* investigated about the antibacterial activity of medicinal plant extracts against periodontopathic bacteria. *Calendula officinalis* extracts showed lower inhibiting activity (MIC>or=2048 mg/L) against all the tested species that confirmed its antibacterial potential. [30]

Barbour *et al.* evaluated the modulation of immune response of *Calendula officinalis* extract exposed to live viral vaccines. Administration of *Calendula officinalis* extract reduced the immune response to three different viruses in broiler chickens, associated with improvement in body weights. Moreover, there was a reduction in immune response to IB virus at 42 days of age, to ND virus at 29 and 42 days of age that further confirmes the immunomodulatory effect of the plant extract against three different live viruses in broiler. [31]

Chaparzadeh *et al.* investigated antioxidative responses of *Calendula officinalis* under salinity conditions. A decrease in total glutathione and an increase in total ascorbate (AsA+DHA), accompanied with enhanced glutathione reductase (GR, EC 1.6.4.2) and ascorbate peroxidase (APX, EC 1.11.1.11) activities, were observed in leaves extract. In addition, salinity induced a decrease in superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POX, EC 1.11.1.7) activities. The decrease in dehydroascorbate reductase (DHAR, EC 1.8.5.1) and monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) activities further suggested the antioxidant potential of the plant. [32]

Pommier *et al.* investigated the comparison of *Calendula officinalis* with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. 254 patients who had been operated on for breast cancer and who were to receive postoperative radiation therapy were randomly allocated for application of either trolamine (128 patients) or calendula (126 patients) on the irradiated fields after each session. 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 confirming its use in acute dermatitis. Additionally, calendula showed high effectiveness for the prevention of acute dermatitis of grade 2 or higher and thus, should be proposed for patients undergoing postoperative irradiation for breast cancer. [33]

Duran *et al.* showed the results from the clinical examination of an ointment with *Calendula officinalis* extract for the treatment of venous leg ulcers. The experiment was carried out in 34 patients with venous leg ulcers and the results obtained suggested the positive effects of the ointment with plant extract on venous ulcer epithelialization. [34]

Ferreyra *et al.* evaluated simultaneous determination of bifonazole and tinctures of C*alendula officinalis* flowers in pharmaceutical creams by a method involving reversed-phase liquid chromatography (RFLC). The method was especially developed for the analysis and quantitative determination of I and II in pure and combined forms in pharmaceutical formulations containing creams without using gradient elution at room temperature. The results obtained from the study suggested that the developed LC method is selective and specific method for the analysis of I and II in pharmaceutical products that can be applied in stability studies. [35]

Fuchs *et al.* evaluated protective effects of different *Calendula officinalis* and rosemary cream preparations against sodium-lauryl-sulfate-induced irritant contact dermatitis. A statistically significant protective effect was observed with the preparations containing *Calendula officinalis* in the cream preparations that confirmed its protective effect against sodium-lauryl-sulfate-induced irritant contact dermatitis. [36]

Rusu *et al.* investigated the hepatoprotective activity of herbal extracts containing *Calendula officinalis* in carbon tetrachloride (CCl_4) intoxicated liver failure in albino wistar rats. Biochemical parameters, including serum transaminase activity (GPT and GOT), histoenzymological measurements

(lactate dehydrogenase, LDH; succinate dehydrogenase, SDH, cytochromoxidase, CyOx; Mg (2+)-dependent adenosine triphosphatase, ATP-ase) and histochemical (Sudan black) and histological examinations (haematoxylin-eosin staining) of the liver were investigated. A number of positive effects such as the reduction of hepatocytolysis and steatosis, and return to normal values of the activity of various enzymes were observed that confirmed the hepatoprotective potential of *Calendula officinalis* extract. [37]

Barajas *et al.* evaluated the dual and opposite effect of *Calendula officinalis* flower extract as a chemoprotector and promoter in rat hepatocarcinogenesis model. It was reported that a protective activity of the plant extract was noted at low doses, while the doses above 10 mg/kg increased altered hepatocyte foci. Such a dual effect is an example of the phenomenon of hormesis. [38]

Jimenez *et al.* evaluated the cytotoxic anti-tumor activity and lymphocyte activation of the whole plant extract of *Calendula officinalis*. 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 was also analyzed. Studies of cell cycle and apoptosis were performed 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-tumoral activity in nude mice against tumor growth of Ando-2 melanoma cells that further confirmed its dual effect. [39]

Calendula officinalis has long been used because of its rich ethanomedicinal importance. Moreover, a number of phytochemicals have been found to be associated with the plant extract that include oleanolic acid glycosides, sterol glycosides, alpha-and beta-amyrin, taraxasterol, lupeol, brein, faradiol, arnidiol, erythrodiol, calenduladiol, coflodiol and manilladiol. In addition, numerous pleiotropic effects have been noted to be exhibited by the plant including anti-inflammatory, antioxidant, anti-tumour, hepatoprotective and immunomodulatory effects. These pleiotropic effects support the fact that the plant may be used for the treatment and prevention of a number of therapeutic conditions. However, future studies are needed to investigate the other hidden effects exhibited by the plant in order to make the plant as a proficient herbal drug therapy.

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