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Review Article

Calendula (*Calendula Officinalis*) Marigold as Medicinal Plant

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Abstract

Calendula (*Calendula officinalis*) has healing and antiseptic properties with actions that are sudorific and analgesic, affect the bile duct, are anti-inflammatory, antiviral and anti-emetic, and tone the skin with vasodilatation. A tincture of 5% calendula positively influences the generation of new cells involved in wound healing, the fibroblasts, and provides more satisfactory healing than other treatments applied to wounds. Calendula officinalis plant extracts show anti-cancerous activity on various tumor cell lines derived from leukemias, fibrosa comas, melanomas, breast, cervix, prostate, pancreas and lung. It has also been internally used for the treatment of gastritis, colitis and bleeding of duodenal ulcers.

The aqueous/ethanolic extract of Calendula flowers caused relaxation of spontaneous contraction and K+ induced contraction of muscles. When the extract was further fractionated with dichloromethane, it inhibited spontaneous contraction. Calcium channel blockade (CCB) was responsible for spasmolytic activity. N-type calcium channel blockade prevents sudden cardiac death. N-type calcium channel blockade (NCC) blockade only or along with L-type calcium channel blockade (LCC) blockade can be beneficial in patients with hypertension, cardiovascular and other metabolic diseases. Hepatoprotective Activity Calendula flower hydro-alcoholic extract caused 28.5% reduction in hepatocytolysis of CCl4 –intoxicated rat liver due to reduction in glutamo-pyruvate-transaminase and glutamo-oxalate-transaminase. Calendula flower hot water extract showed anti-hepatoma activity (25-26% inhibition) against five human liver cancer cells: Hep3B, SK-HEP-1, HepG2/C3A, PLC/ PRF/5 and HA22T/VGH. Moreover, CCl4 intoxicated rats pre-treated with Calendula floral extract afford a protection against CCl4 induced toxicity and showed an improvement in liver function due to significant anti-oxidant activity and free radical scavenging activity of bioactive metabolites including flavonoids and terpenoids present in Calendula. These bioactive metabolites have potent activities for scavenging the hydroxyl radicals (OH) and superoxide radicals (O.2) resulted from CCl4 metabolites. The aqueous-ethanolic extract of Calendula flowers exhibit a genotoxic effect at high concentration and anti-genotoxic effect at low concentration. Calendula glycosides show anti-inflammatory activities against mouse ear edema and calenduloside F 6'-O-n-butyl ester show potent cytotoxicity against melanoma, leukemia and colon cancer. C. officinalis inflorescence extract shows anti-inflammatory activity against the dextran and carrageenan-induced acute paw edema in mice. Abscesses, acne, amenorrhea, analgesia, anemia, antibacterial, antifungal, anti-inflammatory, antioxidant, anti-viral, anxiety, appetite stimulant, atherosclerosis, athlete's foot, bacterial infections, benign prostatic hypertrophy, bladder irritation, blood purification, blood clots, bowel irritation, bruises, burns, cardiac disease, cholera, circulation, colitis, conjunctivitis, constipation, cosmetic, cough, cramps, diaper rash, dizziness, diuresis, dystrophic nervous disturbances, eczema, edema, epididymitis, epistaxis, eye inflammation, fatigue, fever, frostbite, gastrointestinal tract disorders, gastritis, gingivitis, gout, headache, heart disease, hemorrhoids, herpes simplex, herpes keratitis, HIV, indigestion, immunostimulant, influenza, insomnia, jaundice, liver cancer, liver-gallbladder function stimulator, menstrual period abnormalities, metabolic disorders, mouth and throat infections, muscular atrophy, nausea, nosebleed, pain, peptic ulcer disease, periodontal prophylaxis proctitis, prostatitis, purging agent, skin cancer, sore throat, spasms, spleen disorders, stomach ulcers, stones, syphilis, thrombophlebitis, tinnitus, toothache, tuberculosis, ulcerative colitis, urinary retention, uterine tonic, varicose ulcers, warts, yeast infections. Calendula officinalis extract was found to scavenge superoxide radicals generated by photo reduction of riboflavin and hydroxyl radicals generated by Fenton reaction and inhibited in vitro lipid peroxidation.

Keywords: calcium channel blockade; hepatocytolysis; prostatic hypertrophy and thrombophlebitis

Introduction

Calendula (*Calendula officinalis* L.) is in the order Asterales and is a member of the family Asteraceae; the genus name Calendula is derived from the Latin word Calendae, which means 'first day of each month'. The genus has 29 species (Missouri Botanical Garden (Mobot, 2013), including *Calendula officinalis* L., which is herbaceous and widely cultivated in several countries for ornamental, medicinal and cosmetic purposes. In Brazil, the plant is popular and is known as marigold and wonder-of-gardens. The origin of calendula is in the region of the Mediterranean Sea (Lorenzi & Matos, 2008; Silva Junior, 2006), and the plant is currently cosmopolitan and cultivated in many parts of the world, including Brazil (Bertoni *et al*., 2006). Calendula is used for cosmetic purposes in moisturizing creams, for both pre- and post-sun exposure, because of the amount of saponins contained in the plant and because the gums and mucilages have large wetting abilities. Moreover, calendula has healing and antiseptic properties with actions that are sudorific and analgesic, affect the bile duct, are anti-inflammatory, antiviral and anti-emetic, and tone the skin with vasodilatation (Martins *et al*., 2004). According Pagnano et al. (2008), a tincture of 5% calendula positively influences the generation of new cells involved in wound healing, the fibroblasts, and provides more satisfactory healing than other treatments applied to experimental wounds in rabbits. The classic knowledge of social groups and communities on the use of plants is essential to discover active compounds and molecules that have some healing action. Data from the World Health Organization (WHO, 2003) show that approximately 80% of the world population uses some type of herb in search of relief from some type of unpleasant symptom. Of this total, at least 30% was of medical value (Martins *et al*., 2004). According to the WHO report released in the early 1990's, 65–80% of the population in developing countries depended on medicinal plants as the only form of access to basic health care (Martins et al., 2004). To target new pharmaceutical products against diseases that plague the population of the world, traditional knowledge about biodiversity is an important resource (World Health Organization (WHO, 2003). In the production of quality medicinal plants, in addition to growing naturally without pesticides, the first aspect for consideration is undoubtedly planting and harvesting at the correct times.

Discussion

In medicinal species, the variability is high in the production of substances with therapeutic activity. The ideal time to harvest varies according to the plant organ, the stage of development, the time of year and the time of day (Martins *et al*., 2004) and should be performed when the quality of plants is the highest. The ideal moment to harvest depends on three interrelated elements: the stage of development with the highest biomass, the stage of development with the highest biosynthesis of bioactive compounds, and finally, the seasonal variation in the content of active ingredients through the developmental stages of the plant (Corrêa Júnior & Scheffer, 2009). Moreira et al. (2005) found variable plant heights from 28.82 to 39.24 cm in plants treated with five levels of nitrogen and phosphorus. Moreira et al. (2005) found that the mass of dried flower heads of calendula varied according to the dose of P, and the maximum (4.70 g pot^{-1}) was obtained using 271.7 mg of P pot^{-1}. Because the mass of the dried flower heads also varied as a function of N application rates, the maximum (0.52 g pot^{-1}) was obtained with a dose of 199.2mg N pot^{-1}.

The aim of drying is to prevent spoilage by reducing the water content of the raw material, which has a negative effect on the action of enzymes; dehydration (Martins et al., 2004) can be performed naturally or artificially. Leite et al. (2005), in cultures with various doses of organic fertilizer, found that flavonoid production ranged from 0.52 to 0.60% flavonoids per t ha^{-1}; however, the authors emphasized that this ratio could increase with attack by aphids and other arthropods. Araújo et al. (2009) used mulch associated with increasing levels of organic fertilizer and obtained a value of 0.70% flavonoids with 59 t $ha^{-1}$of organic compost. Using chemical fertilization associated with mulch, Borella et al. (2011) observed a maximum yield of 0.79% of the total flavonoid content, and using organic fertilization associated with mulch, observed a yield of d 0.70%. The plant species has been reported to contain a variety of phyto-chemicals, including carbohydrates, phenolic compounds, lipids, steroids, tocopherols, terpenoids, quinones and carotenoids (Kishimoto *et al*., 2005; Re *et al*., 2009;Shahrbabaki *et al*., 2013; Wojciak-Kosior *et al*., 2003) with different health benefits (Miliauskas *et al*., 2004; Muley *et al*., 2009; Vodnar, 2012). The major active constituents of plant include triterpendiol esters, saponins, and flavonoids including rutin and hyperoside. The orange flower contains a highcontent of carotenoids including auroxanthin and flavoxanthin (Braun and Cohen, 2005; Neukiron *et al*., 2004; Roopashree *et al*., 2008). The pot marigold extracts possess a wide range of pharmacological effects (Pintea *etal*., 2003) and are used as antiseptic, stimulant, diaphoretic, anti-spasmodic and anti-pyretic agents. In-vitro, *Calendula officinalis* (CO) plant extracts show anti-cancerous activity on various tumorcell lines derived from leukemias, fibrosacomas, melanomas, breast, cervix, prostate, pancreas and lung (Medina *et al*., 2006). It has also been internally used for the treatmentof gastritis, colitis and bleeding of duodenal ulcers (Bone *et al*., 2003). Due to significant biological activity of C. officinalis and its constituents it is imperative that the plant be given attention and developed as a medicine. Nineteen fatty acids were identified in the eleven genotypes of Calendula seed oils with calendic acid and linoleic acid being the predominant fatty acids (51.47%-57.63% and 28.5-31.9%), followed by oleic acid (4.44-6.25%) and palmitic acid (3.86-4.55%) (Dulf *et al*., 2013). The fatty acids present in trace amounts include lauric, stearic, myristic, palmitoleic,á-linolenic, elaidic, gondoic,behenic, linoelaidicic, pentadecanoic, cis-7-hexadecenoic and margaric acids, and a very lowamount of hydroxy-fatty acid, namely 9-hydroxy-trans-10, cis-12-octadecadienic acid (9-HODE) (Dulf *et al*., 2013).

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 (Pintea *et al*., 2008) due to the presence of a specific conjugase in calendula seeds which converts linoleic acid into calendic acid (Cahoon *etal*., 2006). Some of the carotenoids which were recently identified include -(5Z, 9Z)-lycopene, (5Z)-g-lycopene, (5Z, 9Z, 5'Z, 9'Z)-lycopeneand (5Z, 9Z)-rubixanthin. The carotenoid identified as a synthesized compound is (5Z, 9Z, 5'Z)-lycopene (2) (Kishimoto *et al*., 2005). The total carotenoid present in the petals and pollens is 7.71% and 1.61%, respectively. Lutein, neoxanthin, 9Z-neoxanthin, Z-isomers of lutein, anther axanthin and violaxanthin, are the main carotenoids detected in the leaf and stem (Bako *etal*., 2002). The total carotenoid present in the leaf and stem is 0.85% and 0.18%, respectively (Bako et al., 2002). The carotenoid composition of herbal tea (Calendula flos) is less because of drying of plant material and only anther axanthin and 9Z-antheraxanthin in smaller amounts were detected (Bako *et al*., 2002). The ratio of Z-isomers of lutein and carotene increased with drying of plant material (Bako *et al*., 2002). HPLC analysis of carotenoids in four varieties of Calendula ('Bonbon Abricot', 'Double Esterel Jaune', 'Radio Extra Selected' and 'Double Esterel Orange') showed that all varieties contain common pigments and the difference is only in ratioof individual pigments (Pintea et al., 2003). Orange varieties are rich in carotenoids and contain both hydrocarbons and oxygenated derivatives (Pintea *et al*., 2003). Moreover, orange color intensity of Calendula is determined by the amount of carotene, â-carotene, lycopene and rubixanthinas these pigments are responsible for the orange or even red color of vegetaltissues (Pintea *et al*., 2003). Cornulacic acid acetate (new oleanane triterpeneester) was reported from Calendula flowers (Naved *et al*., 2005).The C. officinalis inflorescence contains various flavonoids including isorhamnetin, quercetin (Kurkin and Sharova, 2007), isorhamnetin-3-O-D-glycoside, isoquercetin, calendoflavoside, narcissi, isoquercitrin, rutin, quercetin-3-O- rutinoside, quercetin-3-O-glucoside, isorhamnetin-3- O-rutinoside, isorhamnetin-3-O-2G-rhamnosyl rutinoside, neohesperidoside, isorhamnetin-3-O-neohesperidoside, calendoflavobioside (Ukiya et al., 2006). The flavonoid content depends on the plant variety, time and place of cultivation, and there appears a relationship between floret color and total flavonoid content of C. officinalis (Raal and Kirsipuu, 2011).The flowers contain minimum VO at pre-flowering stage and maximum at full flowering stage (Okoh *et al*., 2007). Coumarin is the parent molecule of warfarin (a clinically useful anticoagulant), which acts as a vitamin K antagonist (Asif, 2015).

The decline in lipid peroxidation may be due to the antioxidant activity of Calendula (Chandran and Kutton, 2008). Daily use of Calendula gel (2%) causes significant healing of wounds due to its antioxidant and antimicrobial activities (Leach, 2008). Calendula may facilitate the wound healing by increasing wound angiogenesis, epithelialization, and nucleoprotein, glycoprotein and collagen metabolism leading to improvement in local circulation and granulation tissue formation (Leach, 2008). However, this evidence is weak and requires further investigation. The topical application of C. officinalis cream leads to the healing of Achilles tendon by increasing the collagen and non-collagen protein concentration as well as by organizing the collagen proteins (Aro *et al*., 2015). Immuno-stimulant activity was also observed in shrimp (Fenneropenaeus chinensis) against Vibrio harveyi,when injected with SFPSE i.e., a Sargassum fusiforme polysaccharide extract (Huang *et al*., 2006). Moreover, the polysaccharides from Salicornia herbaceaeshow immuno-modulatory activity and are efficiently used against various types of cancers (Im *et al*., 2006).

The aqueous/ethanolic plant extract showed spasmogenic activity (Bashir et al., 2006). The aqueous/ethanolic extract of Calendula flowers caused relaxation of spontaneous contraction and K+ induced contraction of muscles. When the extract was further fractionated with dichloromethane, it inhibited spontaneous contraction. Calcium channel blockade (CCB) was responsible for spasmolytic activity (Bashir *et al*., 2006). N-type calcium channel blockade prevents sudden cardiac death (Nattel, 2014). N-type calcium channel blockade (NCC) blockade only or along with L-type calcium channel blockade (LCC) blockade can be beneficial in patients with hypertension, cardiovascular and other metabolic diseases (Kuwahara and Kimura, 2015). Hepatoprotective Activity Calendula flower hydro-alcoholic extract caused 28.5% reduction in hepatocytolysis of CCl_4 –intoxicated rat liver due to reduction in glutamo-pyruvate-transaminase and glutamo-oxalate-transaminase. For instance, histo-enzymological studies showed steatosis reduction bysuccinate dehydrogenase, cytochromoxidase, lactate dehydrogenase and Mg 2+ dependent ATPase (Rasu *et al*., 2005). Calendula flower hot water extract showed anti-hepatoma activity (25-26% inhibition) against five human liver cancer cells: Hep3B, SK-HEP-1, HepG2/C3A, PLC/PRF/5 and HA22T/VGH (Lin *et al*., 2002). Moreover, CCl 4intoxicated rats pre-treated with Calendula floral extract afford a protection against CCl 4 induced toxicity and showed an improvement in liver function due to significant anti-oxidant activity and free radical scavenging activity of bioactive metabolites including flavonoids and terpenoids present in Calendula (Maysa *et al*., 2015). These bioactive metabolites have potent activities for scavenging the hydroxyl radicals (OH) and superoxide radicals (O^{-2}) resultedfrom CCl4metabolites (Maysa *et al*., 2015).

The aqueous-ethanolic extract of Calendula flowers exhibit a genotoxic effect at high concentration and anti-genotoxic effect at low concentration (Perez-Carreon *et al*., 2002). During the evaluation involving the measurement of excretory 24h urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and lymphocyte DNA fragmentation in young pigs, propylene glycol extract of Calendula was also found to have anti-genotoxic effect (Frankic *et al*., 2008). The urinary 8-OHdG acts as a biomarker for carcinogenesis and various degenerative diseases, and has been used as a pivotal marker for measuring the endogenous effect of oxidative damage to DNA and as a factor of initiation and promotion of carcinogenesis (Valavanidis *et al*., 2009). Calendulo glycosides show anti-inflammatory activities against mouse ear edema and calenduloside F 6'-O-n-butyl ester show potent cytotoxicity against melanoma, leukemia and colon cancer (Ukiya *et al*., 2006).C. officinalis inflorescence extract shows anti-inflammatory activity against the dextran and carrageenan-induced acute paw edema in mice (Preethi *et al*., 2009).Abscesses, acne, amenorrhea, analgesia, anemia, antibacterial, antifungal, anti-inflammatory, (Hamburger *et al*., 2003) antioxidant, (Cordova *et al*., 2002; Chaparzadeh *et al*., 2004) anti-viral, anxiety, appetite stimulant, atherosclerosis, athlete's foot, bacterial infections, benign prostatic hypertrophy, bladder irritation, blood purification, blood clots, bowel irritation, bruises, burns, cardiac disease, cholera, circulation, colitis, conjunctivitis, constipation, cosmetic, cough, cramps, diaper rash, dizziness, diuresis, dystrophic nervous disturbances, eczema, edema, epididymitis, epistaxis, eye inflammation, fatigue, fever, frostbite, gastrointestinal tract disorders, gastritis, gingivitis, (Krazhan and Garazha, 2001) gout, headache, heart disease, hemorrhoids, herpes simplex, herpes keratitis, HIV, indigestion, immunostimulant, (Barbour *et al*., 2004) influenza, insomnia, jaundice, liver cancer, liver-gallbladder function stimulator, menstrual period abnormalities, metabolic disorders, mouth and throat infections, muscular atrophy, nausea, nosebleed, pain, peptic ulcer disease, periodontal prophylaxis proctitis, prostatitis, purging agent, skin cancer, sore throat, spasms, spleen disorders, stomach ulcers, stones, syphilis, thrombophlebitis, tinnitus, toothache, tuberculosis, ulcerative colitis, urinary retention, uterine tonic, varicose ulcers, warts, yeast infections (Iauk *et al*., 2003).

Calendula officinalis (Asteraceae), an important species of the genus Calendula and is well known for its medicinal properties (Mishra *et al*., 2018), was investigated for biological activity based on different extraction protocols. Also known as pot marigold, C. officinalis has been cultivated as a food and medicinal plant since the Middle Ages and the color ant made from this plant was used in the ancient Greece and Rome and by early Indian and Arabic population (Efstratiou *et al*., 2012). In previous studies, the flowers and leaves of C. officinalis have been reported as a source of biologically active compounds. For example, in a study conducted by (Cruceiru et al., 2020) described that the presence of hydroxyl cinnamic acids and flavonols in the flowers and leaves of C. officinalis. Similarly, quercertin and apigenin derivatives were found to be in the hydroalcoholic extract of flower (Escher *et al*., 2019). In addition to phenolics, the leaves and flowers contained saponins, triterpenoids, essential oils, and carotenoids (Chitrakar *et al*., 2019; Hamburger *et al*., 2003; Neukirch *et al*., 2004; Ohmiya, 2011 and Petrovi´c *et al*., 2007). C. officinalis seeds are particularly rich in calendic acid which is used in cosmetic formulations. The flowers are exploited as a commercial source of lutein. The cell rejuvenates; healing, anti-inflammatory, soothing and skin softening properties of C. officinalis flower support its use in skin care products (Mishra *et al*., 2018). The antimicrobial and antifungal properties of C. officinalis flower extract support its application for the treatment of inflammation and skin wounds (Kozlowska *et al*., 2019). The application of C. officinalis cream balm to diabetic patients' feet prevented infection, reduced itching, redness, dryness, pain, and promoted the disappearance of scars (Cioinac, 2016). Favorable proliferation and adhesion to fibroblast cell as well as antibacterial properties was observed while incorporating C. officinalis extract to an electrospun fiber sca old consisting of poly ("-caprolactone), zein, and gum Arabic as compared to plain PCL/zein/GA scaffold, thereby suggesting the possible application of C. officinalis embedded scaffolds biomaterial for skin regeneration (Pedram Rad *et al*., 2019).

The Food and Drug Administration (FDA) has classified C. officinalis as a GRAS (Generally Recognized as Safe) substance (Slavov *et al*., 2020). Edible Calendula flowers possessing nutritional properties and being rich in quinic acid and -tocopherol, were used for the preparation of omelets, salads, and as accompaniment with cheese in Medieval France (Pires *et al*., 2020). The flower extract was used to manage measles, smallpox; the flower juice was used to treat skin diseases, ulcers, costiveness, jaundice, fever, wounds, and to suppress menstruation, promote sweating, and prevent suppuration; infusion of the plant was used to manage anemia and the ointment was used against bruises, sores, cracks, and cuts in the hands (Chitrakar *et al*., 2019). C. officinalis teas were taken against colitis, gastroduodenitis, and duodenal ulcers (Hamzawy *et al*., 2017). Whole or cut, dried, fully opened flowers, which have been detached from the receptacle, of the cultivated, double-flowered varieties of *Calendula officinalis* L. It contains not less than 0.4% of flavonoids, calculated as hyperoside ($C_{21}H_{20}O_{12}$, Mr 464.4) with reference to the dried herbal substance.

Constituents (Willuhn, 2004; Hänsel *et al*., 2007; Muley *et al*., 2009; Ghédira and Goetz, 2016): Triterpene saponins: 2-10% derivatives of the oleanolic acid with glucuronic acid on C3 Triterpene alcohols: free and esterified (with fatty acids) mono-, di- and triols of the ψ–taraxene-, taraxene-, lupine- and ursine-type. Approximately 0.8% Monols (α- and β-amyrin, lupeol, taraxasterol, ψ-taraxasterol), approximately 4% Diols, mostly in form of the mono esters (faradiols and arnidiol esters) Ionon- and sesquiterpene glycosides: isolated from Calendula grown in Egypt (officinosids, Marukami *et al*., 2001). After extraction, the still liquid mixture is filtered; the filtrate congeals as the temperature falls (Kubelka *et al*., 2007). Semi-solid

preparations contain usually 4-20% of the herbal substance (Kubelka *et al*., 2007). The therapeutic use of Calendula flowers and ointments goes back at least to Hildegard von Bingen (Mayer *et al*., 2000).

Dosage frequency accepted for all preparations in the previous version of the monograph: 2-4 times daily. Taking into account the historical references (Fintelmann *et al*., 2002) that indicated "several times daily", the frequency was not changed during the revision.In the standard text book on phytotherapy by Weiss (Fintelmann *et al*., 2002) it is stated that Calendula preparations are superior to Chamomile in the topical treatment of nappy rash.Nicolaus et al.(2017) investigated in vitro the effects of three different extracts from Calendula flowers (n-hexanic, ethanolic, aqueous) on the inflammatory phase of wound healing in human immortalized keratinocytes and human dermal fibroblasts.

A Calendula ointment (containing 5% dry extract; no further detail) enhanced the healing of experimental wounds in buffalo calves (ESCOP, 2003). Shafeie et al. (2015) compared in vivo the effects of different concentrations of *Calendula officinalis* gels (containing a dry ethanolic extract; extraction solvent: ethanol 70% V/V) on cutaneous wound healing in rats. The male rats were randomly divided into three groups (control, placebo, and treatment group). Dina et al. (2016) studied the mechanism of *Calendula officinalis* flowers dry ethanolic extract (CEE; extraction solvent: ethanol 50% V/V) and its active fraction (dry water fraction of ethanolic extract, WCEE) on primary human dermal fibroblasts (HDF). Because of the differences in the structure of the skin between humans and animals these data should be interpreted carefully (Wissinger-Gräfenhahn, 2000). The activity at the higher concentration was comparable to that of indometacin at 120 µg/ear (ESCOP, 2003). Okiya et al. (2006) tested ten oleanane-type triterpene glycosides along with five flavonol glycosides from the flowers of *Calendula officinalis*. Eight triterpenes exhibited a marked anti-inflammatory activity in the TPA-induced inflammation in the mouse ear with ID50 values of 0.05-0.32 mg/ ear. They were almost comparable and in part even more inhibitory than the anti-inflammatory agent indomethacin (ID50 0.3 mg/ear) used as positive control.

Various parts of plant such as leaves, flowers have been reported to possess therapeutic activity (Muley *et al*., 2009). Advanced analytical techniques have been used to isolate novel chemical constituents such as isorhamnetin, rutin, quercetin glucoside, which are biologically active as well as used in food and cosmetic industry (Albulescu *et al*., 2004). Traditionally, *Calendula officinalis* was used as anti-inflammatory, diaphoretic, analgesic,antiseptic and in jaundice treatment (Chakraborthy, 2010). The pleiotropic properties of this meritocratic plant include anti-ulcer, anti-HIV, immune- stimulant, wound healing (Arora *et al*., 2013). It is also used for treating minor diseases like razor burns and wind burns. It is also used as a mouthwash after tooth extractions (Mukesh *et al*., 2011). The plant is nativeto Central and Southern Europe, Western Asia and the US (PDR for Herbal Medicines, 2003). Various flavonoids have been isolated from the ethanol extract of the inflorescence of C. officinalis. They include quercetin, isorhamnetin, (Kurkin and Sharova, 2007) isoquercetin, isorhamnetin-3-O- D-glycoside, narcissin, calendoflaside, calendoflavoside, calendoflavobioside, rutin, isoquercetin neohesperidoside, isorhamnetin-3-Oneohesperidoside, isorhamnetin-3-O-2G- rhamnosyl rutinoside, isorhamnetin-3-Orutinoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside (Ukiya *et al*., 2006).

C. officinalis flowers contain maximum volatile oil at full flowering stage (0.97 %) and minimum during the pre-flowering stage (0.13%). The composition also showed different patterns at different phases of vegetative cycles. Various monoterpenes and sesquiterpenes have been reported in the volatile oil: α-thujene, α-pienene, sabinene, β-pienene, limonene, 1, 8-cineol, p-cymene, trans-β-ocimene, ϒ-terpenene, δ-3-carene, nonanal, terpene-4-ol, 3-cylohexene-1-ol, α-phellandrene, α-terpeneol, geraniol, carvacrol, bornyl acetate, sabinyl acetate, α-cubebene, α-copaene, α-bourbonene, cubebene, α-gurjunene, aromadendrene, β-aryophyllene, α-ylangene, α-humulene, epibicyclosequiphellandrene, germacrene D, allo aromadendrene, β-saliene, calarene, muurolene, δ-cadinene, cadina 1,4-diene, α-cadinene, nerolidol, palustron, endobourbonene, oplopenone, α-cadinol, Tmuurolol. The essential oil was found to berich in α-cadinene, α-cadinol, t-muurolol, limonene, and 1,8-cineol with p-cymene at lower levels at the post-flowering periods (Okoh *et al*., 2007).

Total carotenoids (mg/g dry weight) for the leaves are 0.85% and for stems 0.18 % (Bako *et al*., 2002). Nineteen carotenoids were identified in extracts of petals of orange and yellow flowered cultivars of calendula. In addition, ten carotenoids were unique to orange-flowered cultivars. The ultraviolet (UV) visible absorption maxima of these ten carotenoids were at longer wavelengths than that of flavoxanthin, the main carotenoid of calendula petals providing the evidence that these carotenoids are responsible for the orange color of the petals. Six carotenoids had a cis structure at C-5(C-5') and it is conceivable that these (5Z)-carotenoids are enzymatically isomerised at C-5 in a pathway that diverges from the main carotenoid biosynthesis pathway. Among them, (5Z, 9Z)-lycopene, (5Z, 9Z, 5'Z, 9'Z)-lycopene, (5'Z)-gamma-carotene, (5'Z, 9'Z) - rubixanthin and (5Z, 9Z, 5'Z)-lycopene have been identified (Kishimoto *et al*., 2005). These two glycosides were isolated from leaf protoplasts of the plant with the use of chemically synthesized analogues. Structures of new ionone and sesquiterpene glycosides were investigated from Egyptian *Calendula officinalis*. Two new ionone glucosides (officinosides A and B) and two sesquiterpene oligoglycosides (officinosides C and D) were isolated from the flowers of Egyptian *Calendula officinalis*, the structures of which were elucidated on the basis of chemical and physicochemical evidences (Lin *et al*., 2002).

Diabetes was induced to the rat by single intraperitoneal injection of alloxan (150mg/kg) of body weight. The blood glucose level and urine sugar level were significantly elevated in diabetic rats compared to normal rats. Upon oral administration of hydro alcoholic extract of *Calendula officinalis* in diabetic rats at dose 25 and 50mg/kg body weight significantly lowered the blood glucose and urine sugar as they compared with group of diabetic's rats. Hydro alcoholic extract of *Calendula officinalis* in diabetic rats at a dose of 100mg/kg body weight was found to be highly significant as it restored all the parameters to the normal levels of blood glucose, urine sugar and serum lipid in alloxan diabetic rats. The extract increases the total hemoglobin level. The extract was similar to that of insulin. Thus, the investigation clearly shows that hydro alcoholic extract of *Calendula officinalis* has both antidiabetic and antihyperlipidemic effects (Chakraborthy *et al*., 2011). The structures of the officinosides were elucidated on the basis of chemical and physicochemical evidence. The inhibitory activities of the principal saponins from the flowers of C. officinalis was noted on the increase of serum glucose levels in oral glucose-loaded rats, on gastric emptying in carboxy methyl cellulose sodium salt test meal-loaded mice, and on ethanol or indomethacin induced gastric mucosal lesions in rats and also discussed the structure requirements for these activities (Marukami *et al*., 2001).

Calendula officinalis could be cardio-protective against ischemic heart disease .Two groups of hearts were used: the treated rat hearts were perfused with *Calendula officinalis* solution at 50mM in KHB buffer (in mM) sodium chloride 118mM, potassium chloride 4.7mM, calcium chloride 1.7mM, sodium bicarbonate 25mM, potassium biphosphate 0.36mM, magnesium sulfate 1.2mM, and glucose 10mM) for 15min prior to subjecting the heart to ischemia, while the control group was perfused with the buffer only. Calendula achieved cardio protection by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocytes apoptosis. Cardio protection appears to be achieved by changing ischemia reperfusion-mediated death signal into a survival signal by modulating antioxidant and anti-inflammatory pathways as evidenced by the activation of Akt and Bcl2 and depression of TNFα. The results further strengthen the concept of using natural products in degeneration diseases like ischemic heart disease (Ray *et al*., 2010).

The 80% methanolic extract of *Calendula officinalis* leaves was investigated against acetaminophen-induced hepatic damage in 30 male albino rats. Acetaminophen produces 100% mortality at dose of 1gm/kg in mice; whretreatment of mice with *Calendula officinalis* (1.0gm/kg) reduced the death to 30%. Pretreatment of mice with leaves extract (500mg/kg orally, four doses at 12 hours interval) prevented ($p<0.05$) the acetaminophen (640mg/kg induced rise in serum transaminases (SGOT, SGPT), serum

bilirubin and serum alkaline phosphatase. Post treatment with three successive doses of leaves extract (500mg/kg. 6 hourly)) restricted the hepatic damage induced by acetaminophen ($p<0.05$) (Ali and Khan, 2006). An extract of *Calendula officinalis* Linn., was evaluated for its antioxidant potential by oral administration of Calendula alcoholic extract inhibit superoxide generation in macrophages in female swiss albino mice by 12.6% and 38.7% at doses of 100 and 250 mg/kg body wt. Oral administration of *Calendula officinalis* to mice for 1 month significantly increased catalase activity. The extract produced significant increase in glutathione levels in blood and liver. Glutathione reductase was found to be increased, whereas glutathione peroxidase was found to be decreased after administration of Calendula extract (Preethi *et al*., 2006).

The aqueous extract of dried flowers and leaves of C. officinalis were prepared by decoction method. The assay was performed on Indian adult earth worm, due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. *Calendula officinalis* flowers and leaf extracts were also shown to have anthelmintic activity the crude extracts of C. officinalis flowers and leaf extracts demonstrated paralysis at (Parente *et al*., 2011).5min and death of worms at 111.2minutes. The plants contain saponins and have also shown anthelmintic potential which are in accordance with previous reports which reveals that saponins are known to have anthelmintic activity (Jain *et al*., 2010). Topical application of a 70% ethanol extract of the flowers to mice at a dose of 1.2mg/ear (corresponding to 4.16mg crude drug) reduced croton oil-induced ear oedema by 20%. External application of a carbon dioxide extract of the flowers (300mg/cm2) suppressed croton oil induced ear oedema in mice (Preethi *et al*., 2009).

Angiogenic activity of *Calendula officinalis* L. (Asteraceae) ethanolic extract and dichloromethane and hexanic fractions were evaluated by using Models 36 rats and 90 embryonated eggs to evaluate healing and angiogenic activities of extracts and fractions of the plant, through the induction of skin wounds and the chorioallantoic membrane, respectively. The effect of vascular proliferation was also tested from the study to verify the intensity of expression of vascular endothelial growth factor (VEGF) in cutaneous wounds in rats. In morphometric evaluation increase of the vascular area and of percentage of red-marked areas was observed in CAM treated as positive control 1% (17 ß-estradiol), ethanolic extract 1%, dichloromethane fraction 1% and hexanic fraction 1%, compared to solvent control (ethanol 70%). Digital planimetry by point counting performed on mice derm trated with ethanolic extract 1% revealed an increase in the number of blood vessels compared to solvent control (Parente *et al*., 2011). They reported a statistically significant difference in reduction of total wound area compared with the control ($p<0.05$), showing an overall decrease of 41.71% in the experimental group compared with 14.52% in the control group. They conclude that application of Calendula extract significantly increases epithelization in chronic venous ulcerations. Marigold therapy offers a non-invasive and gentle treatment for difficult to treat plantar verruca, painful hyperkeratotic lesions, and inflamed bursa secondary to hallux abducto valgus (Robert *et al*., 2008). The results obtained indicated that none of the extracts had a direct mitogenic effect on human lymphocytes or thymocytes (stimulation index, $SI<0.07$). Among the plants studied, C. officinalis showed a complete inhibitory effect on the proliferation of lymphocytes in the presence of PHA (SI range 0.01-0.49) (Amirghofran *et al*., 2000).

Pre-incubation of hepatocytes with either *Calendula officinalis* or morus alba extracts ameliorated the hepatotoxicity and oxidative stress induced by CCl4, as indicated by significant improvement in cell viability and enzymes leakages (ALT, AST and LDH) and also, significant improvement of GSH content and significant decrease in TBARS formation as compared to CCl4treated cells. The dose response effect of different concentrations of *Calendula officinalis* and morus alba extracts (1, 10, 100 and 1000µg/ml) on CCl4induced decrease in the viability% of isolated rat hepatocytes. The results revealed that CCl4 (5mM) induced significant decrease in the viability% of isolated rat hepatocytes after 30 min of incubation period. This decrease in the viability was a time dependant compared to a control group (Hussein *et al*., 2010). Ethanolic and aqueous extracts of *Calendula officinalis* inhibit the growth of the bacteria used in the study, at concentrations ranging from 125µg/ml to 64mg/ml. Methanolic extract inhibited the growth of both S. aureus and E. coli at 64mg/ml. Aqueous extract of *Calendula officinalis* exhibited highest antibacterial activity against all the bacteria tested. S. aureus was found to be more susceptible as compared to other bacteria (Roopashree *et al*., 2008).

The microorganisms used for antibacterial and antifungal were Bacillus subtulis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Candida albicans and Aspergillus niger. Gentamicin 5µg/ml was used as standard. The extracts showed antimicrobial activity were subjected to minimum inhibitory concentration assay by two-fold dilutions method. Petroleum ether, chloroform, ethanol and water extract exhibited in-vitro antibacterial activity (Dahake *et al*., 2009). Antimicrobial activity of ethanolic, methanolic, acetone and chloroform extract of C. officinalis was studied against the gram-positive besterial strains were Escherichia coli, Staphylococcus anrens, and the gram-negative strains were Salmonella typhae and Vibrio cholera. The fungal strain used was Candida albicans. Ethanolic extract gave activity against E. coli, Vibrio choleraand Candida albicans. Methanolic extract gave only against Candida albicans. Chloroform gave antimicrobial activity against all microbes while acetone gave only against E. coli (Safdar *et al*., 2010). The flavonoid extracts (0.05-50µg/ml) did not cause significant effect on the proliferation of two cell lines. It may be related to the attached sugar molecule at the position 3 of flavones that can reduce its ability to bind aromatase and other enzymes (Ostad *et al*., 2005).

The hydro alcoholic extract of *Calendula officinalis* can suppress the activities of 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX^{-2}) (key enzymes) in the formation of pro inflammatory eicosanoids from arachidonic acid (Herold *et al*., 2003). The occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% v 63%; $p<.001$) with the use of calendula than with trolamine. Moreover, patients receiving calendula had less frequent interruption of radiotherapy and significantly reducedradiation-induced pain. Calendula is highly effective for the prevention of acute dermatitis of grade 2 or higher and should be proposed for patients undergoing postoperative irradiation for breast cancer (Pommier *et al*.,2004).

An alcoholic extract of *Calendula officinalis* Linn (Composite) was evaluated for its antioxidant potential in vitro. *Calendula officinalis* extract was found to scavenge superoxide radicals generated by photo reduction of riboflavin and hydroxyl radicals generated by Fenton reaction and inhibited in vitro lipid peroxidation. Concentrations needed for 50% inhibition (IC50) were 500, 480, and 2000 mg/ml, respectively. Extract scavenged ABTS radicals and DPPH radicals and IC50 were 6.5 and 100 mg/ml, respectively (Preethiy *et al*., 2006). For *Calendula officinalis* extract of 7.5mg/ml concentration, the LPO decreased slowly with dose from 68% to 40% at 20kGy. The LPO for 3.75mg/ml concentration decreased suddenly from 56% to 28% at 1kGy dose. Then, it decreased slowly with dose (Minea *et al*., 2004).

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