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Review Article

Calendula officinalis - An Important Medicinal Plant with Potential Biological Properties

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Calendula officinalis L. (Marigold) is globally known for its medicinal importance containing various phyto-chemicals including carbohydrates, amino acids, lipids, fatty acids, carotenoids, terpenoids, flavonoids, quinones, coumarins and other constituents, showing some important biological activities like wound healing, immuno-stimulant, spasmogenic and spasmolytic, hepatoprotective, genotoxic and antigenotoxic, anti-amylase, anti-inflammatory, anti-oedematous, anti-bacterial and anti-fungal, antioxidant, antidiabetic, anti-HIV and anti-cancerous, nephron-protective, prevention of oropharyngeal mucositis, hypoglycemic and gastroprotective activities with no toxic effect .In this review, a detailed account of different phytochemicals and their medicinal properties of *C. officinalis* have been addressed.

Keywords: *Calendula officinalis*; Asteraceae; Marigold; Phytochemicals

Introduction

In India, over 6,000 plants are used in herbal, folk and traditional medicine. Approximately, amongst1500 identified medicinal plants 500 are commonly in use (Chidambaram *et al*., 2014). *Calendula officinalis* L. (pot marigold) is one of the commonly used medicinal plantsin India, China, Europe and US (Muley *et al*., 2009). *Calendula* was known as "gold's" in old English was associated with Virgin Mary and Queen Mary, hence the name marigold (Grieve 1931; Kemper 1999; Mills 1991). The name of this plant comes from a Latin word 'Calend' meaning the first day of each month, because of the long flowering period of plant. As flowers move in the direction of the sun's radiation, it has become an astronomical sun sign "Leo" (Dinda and Craker, 1998). *Calendula* is an annual herb growing about 80 cm tall, having corymbosely branched stem; a long tap root with numerous secondary roots; hispid, acute, oblanceolate, alternate and sessile leaves; flower head inflorescence (surrounded by two rows of hairy bracts). The plant has yellow to orange ûowers with female ray ûorets and hermaphrodite, tridentate, tubular, disc florets; and curved, sickle-shaped and ringed achenes (Bisset, 1994) (Fig. 1).

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 high content 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 *et al*., 2003) and are used as antiseptic, stimulant, diaphoretic, anti-spasmodic and anti-pyretic agents (Kirtikar and Basu, 1993; Weiner, 1990). The flower extracts of the plant have anti-viral effects on HIV (Kalvatchev *et al*., 1997). *In-vitro*, *Calendula officinalis* (CO) plant

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Fig. 1: A. *Calendula officinalis* (young leaflet stage) B. *Calendula officinalis* (young flower bud) C. *Calendula officinalis* (full flowering stage)

extracts show anti-cancerous activity on various tumor cell lines derived from leukemias, fibrosacomas, melanomas, breast, cervix, prostate, pancreas and lung (Medina *et al*., 2006). It has also been internally used for the treatment of 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.

Important Phyto-chemicals

Various phyto-chemical studies have revealed the presence of different chemical compounds including carbohydrates, amino acids, lipids, carotenoids, terpenoids, flavonoids, volatile oil, quinines, coumarins and other constituents (Tables 1 and 2).

Carbohydrates

The water soluble polysaccharides of *C. officinalis* inflorescence contain 9.25 % moisture, 25.77 % acidic sugar, 29.25 % ash, 31.25 % reducing sugars and 84.58 % pectic substances and various mono-saccharides including glucose, arabinose, rhamnose, xylose, galactose and galacturonic acid (Lim, 2013). The ethanolic extract of *C. officinalis* inflorescence was reported to contain monosacccharides along with polysaccharides, PS-I,-II, -III with (1→3)-β-D-galactam backbone and a side chain at C-6 consisting of α-L-rhamnan-(1→3)-araban and α-araban-(1→3)-araban form (Varlijen, 1989; Wanger *et al*., 1985).

Amino Acids

The *C. officinalis* flower extract showed the presence of 15 free amino acids including proline, phenylalanine, histidine, lysine, leucine, serine, alanine, valine, arginine, tyrosine, aspargine, threonine, glutamate, methionine and aspartate and amino acid content, being highest in the flower (4.5%) (Abajova, 1994).

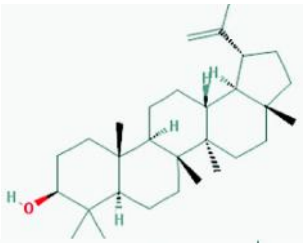
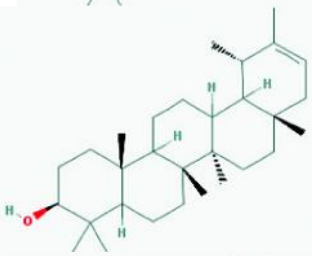
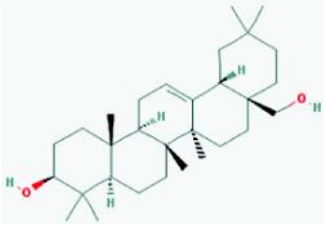
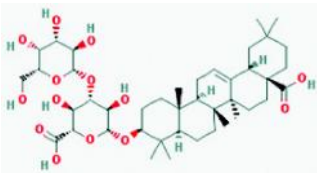
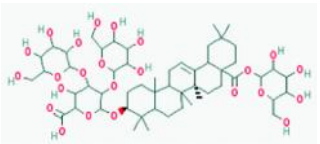
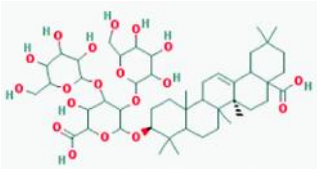
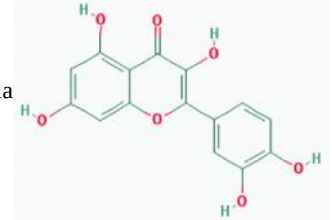
Lipids and Fatty Acids

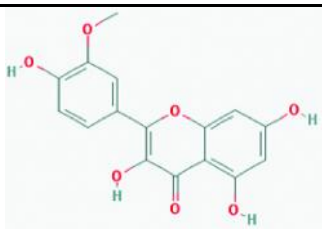
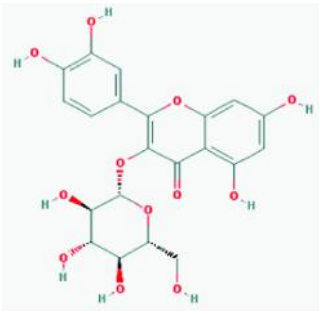
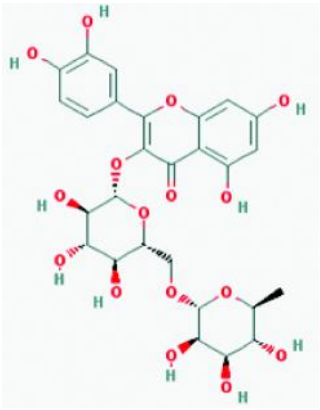
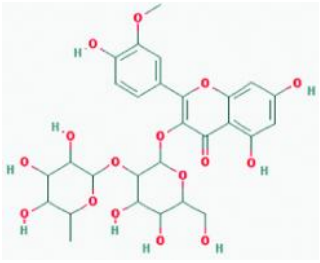
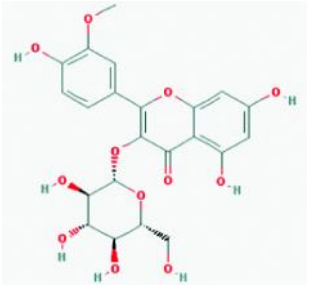
The fatty acids present in the *C. officinalis* flowers

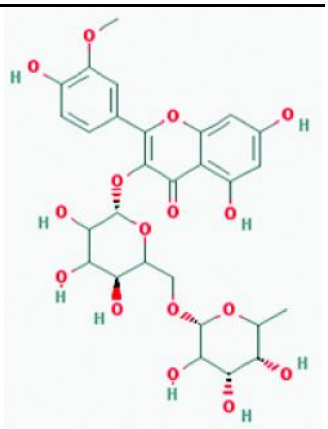
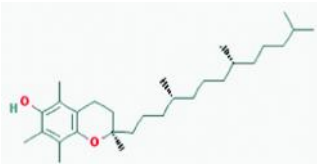
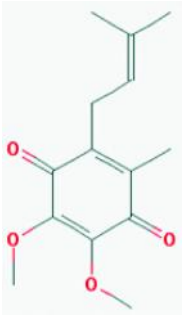
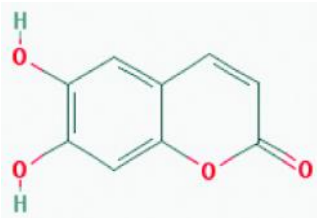
Table 1: Active components present in different plant parts of *C. officinalis*

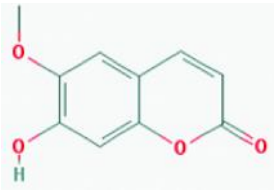
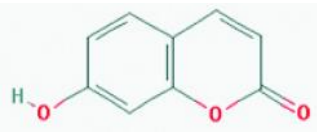
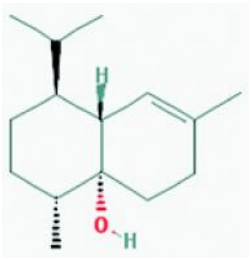
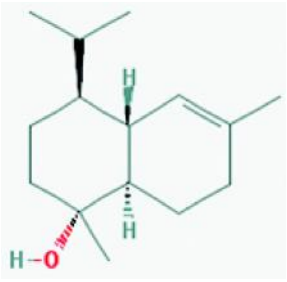
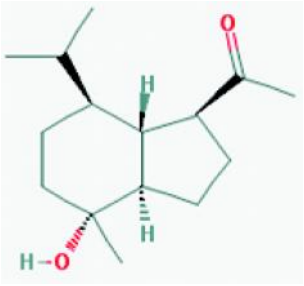
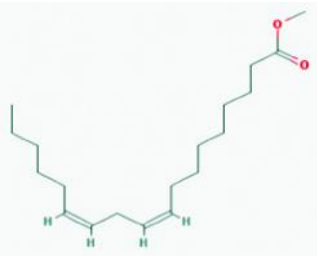
Plant part	Active components	Constituents	Reference
Flower	Terpenoids	Lupeol, Ψ-taraxasterol	(Zittwel-Eglseer *et al*., 1997; Wilkomirski, 1985)
		Erythrodiol	(Wojciechowski *et al*., 1972)
		Calenduloside	(Vecherko *et al*., 1975)
		Calendulaglycoside A, Calendulaglycoside B	(Ukiya *et al*., 2006)
		Cornulacic acid acetate	(Naved *et al*., 2005)
Flower	Flavonoids	Quercetin, Isorhamnetin	(Kurkin and Sharova, 2007)
		Isoquercitrin, rutin, calendoflavoside	(Ukiya *et al*., 2006)
		Isorhamnetin-3-*O*-β-D glycoside, narcissin	(Vidal-Ollivier *et al*., 1989)
Leaves	Quinones	Phylloquinone, α-tocopherol, ubiquinone, plastoquinone	(Janiszowka *et al*., 1976)
Flower	Coumarin	esculetin,scopoletin,umbelliferone	(Kerkach *et al*., 1986)
Flower	Volatile oil	Cubenol, α-cadinol, oplopanonec methyllinoleate	(Nicoletta *et al*., 2003)
		Sabinene, limonene, α-pinene, *p*-cymene, nonanal, carvacrol, geraniol, nerolidol, *t*-muurolol and palustron	(Khalid and J. A. Teixeira da Silva, 2012)
Root	Terpenoid	Calenduloside B	Iatsyno *et al*., 1978

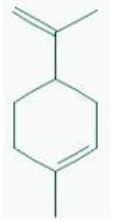
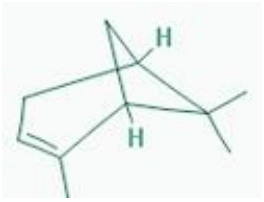
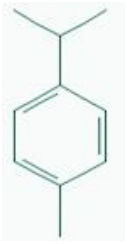
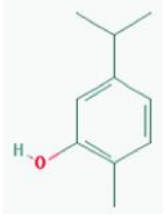
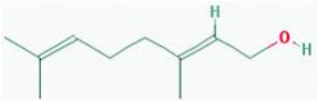
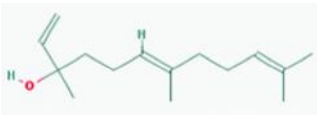
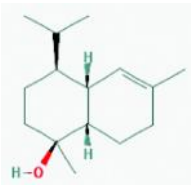
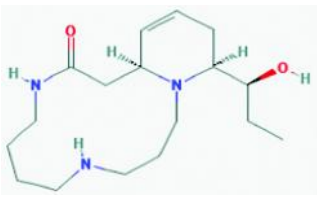
Table 2: Chemical structure, physical, chemical and pharmacological properties of various components present in C. officinalis

Compound	Physical and chemical properties				Pharmacological properties	Chemical Structure
	Molecular weight	Molecular formula	Topological polar surface area	Com-plexity		
Lupeol	426.7174 g/mol	$C_{30}H_{50}O$	20.2 A^2	766	Anti-Inflammatory activity	
Ψ-taraxasterol	426.7174 g/mol	$C_{30}H_{50}O$	20.2 A^2	779	Antitubercular activity	
Erythrodiol	442.7168 g/mol	$C_{30}H_{50}O$	240.5 A^2	810	Anti-viral and Anti-Inflammatory activity	
Calenduloside	794.96504 g/mol	$C_{42}H_{66}O_{14}$	233 A^2	1560	Cytotoxicity against , melanoma leukemia and colon cancer	
Calendulagly-coside A	1119.24624 g/mol	$C_{54}H_{86}O_{24}$	391 A^2	2200	Anti-Inflammatory activity	
Calendulagly-coside B	957.10564 g/mol	$C_{48}H_{76}O_{19}$	312 A^2	1880	Anti-Inflammatory activity	
Quercetin	302.2357 g/mol	$C_{15}H_{10}O_{71}$	27 A^2	488	Anti-proliferative, anti-inflammatory and anti-allergy activity	

Isorhamnetin	316.26228 g/mol	$C_{16}H_{12}O_{71}$	16 A^2	503	Anti-plasmodial activity against chloroquine-sensitive Plasmodium falciparum NF54	
Isoquercitrin	464.3763 g/mol	$C_{21}H_{20}O_{12}$	207 A^2	758	Hepatoprotective activity, Antiviral activity	
Rutin	610.5175 g/mol	$C_{27}H_{30}O_{16}$	266 A^2	1020	Antioxidant and anti-inflammatory activity	
Calendoflavoside	624.54408 g/mol	$C_{28}H_{32}O_{16}$	255 A^2	1040	Antioxidant activity	
Isorhamnetin-3-O-β-D	478.40288 g/mol	$C_{22}H_{22}O_{12}$	196 A^2	773	Vasorelaxation activity glycoside	

Narcissin	624.54408 g/mol	$C_{28}H_{32}O_{16}$	255 A^2	1040	Cytotoxic activity against the human chronic myelogenous leukemia	
Phylloquinone	450.69574 g/mol	$C_{31}H_{46}O_{23}$	4.1 A^2	696	For control of massive hemorrhage and in other coagulation disorders	
α-tocopherol	430.7061 g/mol	$C_{29}H_{50}O_{22}$	9.5 A^2	503	Anticoagulant, neuroprotective, antiviral, immunomodulatory activities	
Ubiquinone	250.29032 g/mol	$C_{14}H_{18}O_{45}$	2.6 A^2	474	Termiticidal activity against Coptotermes formosanus	
Plastoquinone	749.2011 g/mol	$C_{53}H_{80}O_{23}$	4.1 A^2	1590	Antimicrobial and antiparasitic activity	
Eesculetin	178.14154 g/mol	$C_9H_6O_{46}$	6.8 A^2	248	Antioxidant activity	

Scopoletin	192.16812 g/mol	$C_{10}H_8O_{45}$	5.8 A^2	261	Cytotoxicity against human HT1080 cells and human LoVo cells	
Umbelliferone	162.14214 g/mol	$C_9H_6O_{34}$	6.5 A^2	222	Antimicrobial activity	
Cubenol	222.36634 g/mol	$C_{15}H_{26}O_2$	0.2 A^2	292	Anti-inflammatory activity	
α-cadinol	222.36634 g/mol	$C_{15}H_{26}O_2$	0.2 A^2	292	Antiviral activity against SARS coronavirus	
Oplopanonec	238.36574 g/mol	$C_{15}H_{26}O_3$	37.3 A^2	310	Anti-inflammatory	
Methyllinoleate	294.47206 g/mol	$C_{19}H_{34}O_2$	26.3 A^2	279	Cytotoxicity against isogenic chicken DT40 cell lines	
Sabinene	136.23404 g/mol	$C_{10}H_{16}O$	0 A^2	179	Anticancerous activity	

Limonene	136.23404 g/mol	$C_{10}H_{16}O$	0 A^2	163	Antioxidant activity	
α-pinene	136.23404 g/mol	$C_{10}H_{16}O$	0 A^2	186	Anti-inflammatory, Anticancerous	
p-cymene	134.21816 g/mol	$C_{10}H_{14}O_2$	0 A^2	86.2	Anti-inflammatory activity	
Nonanal	142.23862 g/mol	$C_9H_{18}O$	17.1 A^2	69.1	Anti-micribial activity	
Carvacrol	150.21756 g/mol	$C_{10}H_{14}O$	0.2 A^2	120	Agonist activity at human TRPA1 channel expressed in HEK293 cells	
Geraniol	154.24932 g/mol	$C_{10}H_{18}O$	20.2 A^2	150	Anti-cancerous activity	
Nerolidol	222.36634 g/mol	$C_{15}H_{26}O$	20.2 A^2	269	Anti-inflammatory activity	
t-muurolol	222.36634 g/mol	$C_{15}H_{26}O$	20.2 A^2	292	Antifungal activity	
Palustron	309.44694 g/mol	$C_{17}H_{31}N_3O_2$	64.6 A^2	373	Anti-bacterial activity	

are myristic acid, lauric acid, stearic acid, palmitic acid, oleic acid, linoleic acid and linolenic acid. The lipids present in the seeds of *C. officinalis* are phospholipids, glycolipids and neutral lipids. Seeds also contain 9-hydroxy-18:2(*trans*-9, *cis*-11) acid-dimorphecolic acid and 18:3 conjugated trienic (*trans*-8, *trans*-10, *cis*-12) acid (Vlchenko, 1998; Wilkomirski and Kasprzyk, 1979). The seed oil contains D-(+)-9-hydroxy-10, 12-octadecadienoic acid (oxygenated fatty acid) (Badami and Morris, 1965).

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, β-calendic, arachidic, elaidic, gondoic, behenic, linoelaidicic, pentadecanoic, cis-7-hexadecenoic and margaric acids, and a very low amount of hydroxy-fattyacid, namely 9-hydroxy-trans-10, cis-12-octadecadienic acid (9-HODE) (Dulf *et al*., 2013). Calendic acid is a conjugated trienoic fatty acid containing conjugated trans-Δ^8-, trans-Δ^{10}-, and cis-Δ^{12}-double bonds (Cahoon *et al*., 2001). Conjugated trans-Δ^8- and trans-Δ^{10}-double bonds of calendic acid are formed by the modification of cis-Δ^{12}-double bond of linoleicacid (Crombie *et al*., 1984). CoFADX-1 and CoFADX-2 (cDNAs for two closely related FAD2-like enzymes) are responsible for the formation of conjugated trans-Δ^8-andtrans-Δ^{10}-double bonds of calendic acid (Cahoon *et al*., 2001).

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 *et al*., 2006; Fritsche *et al*., 1999).

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 content and pigment profile. Yellow flower petals of *Calendula* contain 19 carotenoids and orange flower contains10 unique carotenoids. These 10 carotenoids have UV-visible absorption maxima at a wavelength longer than that of flavoxanthin, and provide orange color to the flower petals (Khalid and Teixeira da Silva, 2012). Six carotenoids (5Z) having *cis* form at C-5 may be isomerized at C-5 enzymatically in a pathway deviating from the main pathway of carotenoid biosynthesis. Some of the carotenoids which were recently identified include - (5Z, 9Z)-lycopene, (5Z)-g-lycopene, (5Z, 9Z, 5'Z, 9'Z)-lycopene and (5Z, 9Z)-rubixanthin. The carotenoid identified as a synthesized compound is (5Z, 9Z, 5'Z)-lycopene (2) (Kishimoto *et al*., 2005).

The main carotenoids present in the petals and pollens are flavoxanthin, luteoxanthin, auroxanthin, 9Z-antheraxanthin, neoxanthin, lutein and its Z-isomers, mutatoxanthin, violaxanthin, 9Z-neoxanthin, 9Z-violaxanthin, α- and β-carotene, and α- and β-cryptoxanthin with higher quantity of lycopene in petals (Bako *et al*., 2002). The total carotenoid present in the petals and pollens is 7.71% and 1.61%, repectively. Lutein, β-carotene, neoxanthin, 9Z-neoxanthin, Z-isomers of lutein, antheraxanthin and violaxanthin, are the main carotenoids detected in the leaf and stem (Bako *et al*., 2002). The total carotenoid present in the leaf and stem is 0.85% and 0.18%, respectively (Bako *et al*., 2002; Goodwin, 1954). The carotenoid composition of herbal tea (*Calendula flos*) is less because of drying of plant material and only antheraxanthin and 9Z-antheraxanthin in smaller amounts were detected (Bako *et al*., 2002). The ratio of Z-isomers of lutein and β-carotene increased with drying of plant material (Bako *et al*., 2002). Zeaxanthin or lutein is necessary for photo-protection and also leads to the inhibition of lipid peroxidation (Niyogi *et al*., 1997).

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 ratio of individual pigments (Pintea *et al*., 2003) (Table 3). 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 rubixanthin as these pigments are responsible for the orange or even red color of vegetal tissues (Pintea *et al*., 2003) (Table 4).

Table 3: Total carotenoid content in Calendula officinalis L.-varieties (Pintea *et al*., 2003)

Variety	Colour	Carotenoid amount (mg/100g fresh flowers)
Bonbon Abricot	Yellow-orange	48.2
Double Esterel Jaune	Lemon yellow	97.0
Radio Extra Selected	Orange	111.8
Double Esterel Orange	Dark orange	276.0

calendulaglycoside B 6'-*O*-*n*-butyl ester, calendula-glycoside C 6'-*O*-*n*-butyl ester, calendula-glycoside C 6'-*O*-*n*-methyl ester, calendulo-side F 6'-*O*-*n*-butyl ester, calenduloside G 6'-*O*-*n*-methyl ester (Ukiya *et al*., 2006), faradiol-3-*O*-myristate, faradiol-3-*O*-palmitate, faradiol-3-*O*-laurate (Eitterl-Eglseer *et al*., 2001), glucuronides (mainly present in green parts and flowers) and glucosides of oleanolic acid (mainly present in growing and senescing plants) (Ruszkowski *et al*., 2003; Wojciechowski *et al*., 1971).

Table 4: Carotenoid composition of inflorescences of Calendula officinalis L.- varieties (Pintea *et al*., 2003)

Pigment	No. of pigment on HPLC chromatogram	Double esterel orange %	Radio extra selected %	Bonbon abricot %	Double esterel jaune %
Neoxanthin	1	0.92	1.71	2.84	1.74
Luteoxanthin+Auro	8	8.9	11.3	15.43	18.97
Antheraxanthin	9	2.09	4.31	4.56	6.83
Flavoxanthin	10	14.1	17.4	35.42	42.05
Mutatoxanthin	11	0.38	-	2.17	-
Lactucaxanthin	12	4.49	8.02	-	11.31
Lutein	3	9.18	11.38	8.27	12.29
Zeaxanthin	4	0.11	0.23	-	0.15
Rubixanthin	13,14	14.36	7.27	4.58	-
Lycopene	15	14.03	5	0.57	-
?-carotene	16	12.15	6.15	5.11	-
a-carotene	17	0.98	1.15	1.89	0.2
ß-carotene	7	16.68	17.51	10.31	2.37

Terpenoids

Calendula contains various terpenoids including stigmasterols, sitosterols (Alder and Kasprzyk, 1975), lupeol, Ø-taraxasterol, 3-monoesters of taraxasterol (Wilkomirski, 1985; Zittwel-Eglseer *et al*., 1997), ursadiol (Sliwowski *et al*., 1973), diesters of diols (Wilkomirski and Kasprzyk, 1979), brein, erythrodiol (Wojciechowski *et al*., 1972; Kasprzyk and Wilkomirski, 1973), calenduladiol-3-*O*-myristate, aranidiol-3-*O*-myristate, calenduladiol-3-*O*-palmitate, aranidiol-3-*O*-laurate, aranidiol-3-*O*-palmitate (Neukiron *et al*., 2004; Ukiya *et al*., 2006), calenduloside AH (Vecherko *et al*., 1969, 1971, 1974, 1975), calendulaglycoside A, calendulaglycoside B, calendulaglycoside C, calendulaglycoside A 6'-*O*-*n*-butyl ester, calendulaglycoside A 6'-*O*-*n*-methyl ester,

The major triterpenoid esters present in the flower head of *Calendula* are palmitate, myristate and faradiol 3-*O*-laurate (Hamburge *et al*., 2003). Triterpene alcohols and triterpene saponins are found in ligulate flowers (Hansel *et al*., 1992; Issac 1992). Cornulacic acid acetate (new oleanane triterpene ester) was reported from *Calendula* flowers (Naved *et al*., 2005).

Flavonoids

The *C. officinalis* inflorescence contains various flavonoids including isorhamnetin, quercetin (Kurkin and Sharova 2007), isorhamnetin-3-*O*-β-D-glycoside, isoquercetin, calendoflavoside, narcissi (Vidal-Ollivier *et al*., 1989), 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).

Volatile Oil and Its Constituents

The flower heads of *C. officinalis* have been reported to contain volatile oil (VO) consisting of cubenol, α-cadinol, ϒ-cadinene, δ-cadinene, oplopanonec, methyllinoleate, methyltetradecanoate, methylhexanoate, methyloctadecanoate and methyl-9, 12, 15-octadecatrienoate (Nicoletta *et al*., 2003). The flowers contain minimum VO at pre-flowering stage and maximum at full flowering stage (Okoh *et al*., 2007). The VO contain a maximum amount of α-cadinol, α-cadinene, limonene, t-muurolol and 1,8 cineol with a minimum amount of p-cymene during the post-flowering stage (Okoh *et al*., 2007). According to Gazim *et al*. (2007), the main constituents of VO are epi-α-muurolol, α-cadinol, δ-cadinene, sesquiterpenols and sesquiterpene hydrocarbons. The VO contains various monoterpenes and sesquiterpenes: sabinene, limonene, α-pinene, β-pinene, α-thujene, p-cymene, ϒ-terpenene, tras-β-ocimene, δ-3-carene, 1,8-cineol, nonanal, carvacrol, geraniol, terpene-4-ol, α-terpeneol, 3-cyclohexene-1-ol, α-cadinol, bornyl acetate, calarene, aromadendrene, germacrene-D, endobourbonene muurolene, α-bourbonene, α-copaene, α-cubebene, α-gurjunene, α-humulene, α-phellandrene, α-cadinene, α-cadinol, β-cubebene, β-caryophyllene, β-saliene, nerolidol, t-muurolol and palustron (Khalid and Teixeira da Silva, 2012).

Quinones and Coumarins

Various quinines have been reported from *C. officinalis*. They include phylloquinone in leaves, α-tocopherol, phylloquinone and ubiquinone in mitochondria, and α-tocopherol, phylloquinone and plastoquinone in chloroplast (Janiszowka 1976). The flower contains coumarins, esculetin, scopoletin and umbelliferone (Kerkach *et al*., 1986). Coumarin is the parent molecule of warfarin (a clinically useful anticoagulant), which acts as a vitamin K antagonist (Asif 2015). Coumarins act as phytoalexins as they are produced by plantsfordefense against various pathogens (Berenbaum *et al*., 1991; Weinmann 1997). Coumarins are leached from the roots of some plants (wild *Avena*) into the soil, to provide defense against various micro-organisms (Asif 2015). Coumarins possess a variety of biological activities including estrogenic, anticoagulant, dermal photo-sensitising, vasodilator, anti-microbial, anti-helminthic, molluscacidal, hypnotic, sedative and analgesic activity (O'kennedy and Thornes, 1997).

Other Constituents

Other constituents of *Calendula* include calendulin (Fliesonner 1985), loliolide (calendin) (Willuhn and Westhaus 1987), *n*-paraffins (Komeo and Hayashi 1971) and some bitter constituent.

Medicinal Properties

The use of plants for treating diseases is as old as the human species. Various pharmacological studies have reported that *C. officinalis* has a broad range of biological activities and some of these can be used for further future development.

Wound Healing Property

While studying the healing activity of *Calendula* flower extract against thermal burns in rats, it was found that the extract showed significant healing activity by increasing hexosamine and collagenhydroxyproline content with a significant decrease in the level of tissue damage marker enzymes (aspartate transaminase and alkaline phosphatase) and acute phase proteins (orosomycid and heptaglobin). 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 collagenmetabolism leading to improvement in local circulation and granulation tissue formation (Leach, 2008). The *Calendula* treatment was more effective than other medicines and also reduces discomfort during dressing changes. The use of 10% *Calendula* solution supplemented with 2% *Calendula* gel for cleaning skin lesions, burn, and venous ulcers reduces the time of healing and also

results in a greater number of healed wounds, compared to using of *Calendula* solution alone (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

C. officinalis polysaccharide (PS) fraction exhibits immuno-stimulant activity. PS-I and PS-II showed 40-57% and 20-30% of phagocytosis, respectively, while PS-III exhibited the highest (54-100%) phagocytosis (Varlijen, 1989; Wagner *et al*., 1985). 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, thepolysaccharides from *Salicornia herbaceae* show immuno-modulatory activity and are efficiently used against various types of cancers (Im *et al*., 2006).

Spasmogenic and Spasmolytic Activity

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 ($OH^{\cdot}$) and superoxide radicals ($O^{\cdot}_2$) resulted from CCl_4 metabolites (Maysa *et al*., 2015).

Genotoxic and Antigen-toxic Activity

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).

Anti-inflammatory and Anti-oedematousacitivity

Calenduloside B (trioside of oleinic acid) of *Calendula* roots show sedative and anti-phlogistic activity (Iatsyno *et al*., 1978). Calenduloglycosides 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 showsanti-inflammatory activity against the dextran and carrageenan-induced acute paw edema in mice (Preethi *et al*., 2009). Also a significant increase in the level of pro-inflammatorycytokines like IL-1β, IL-6, and TNF-α in the sera of LPS (lipopolysaccharide)

induced animals has been observed (Preethi *et al*., 2009). The cytokines-like IL-1, IL-6 and TNF-α act as stimulants in the liver to produce C-reactive protein (CRP) which is increased several folds during acute inflammation. Studies suggest that increased levels of pro-inflammatory cytokines IL-6 and tumor necrosis factor-alpha (TNF-α) play important role not only in the development of kidney ailments (Stenvikel *et al*., 2005) but also ovarian epithelial cancer (Mark M. Moradi, *et al*., 2006). *Calendula* flower methanolic extract inhibits 12-O-tetradecanoyl phorbol-13-acetate (TPA) induced inflammation in mice attributed to presence of oleanane-triterpene glycoside in *Calendula* flower (Ukiya *et al*., 2006). After elucidating the structure of faradiol-3-myristic acid ester, faradiol-3-palmitic acid ester and taraxasterol, it was found that these three esters/compounds have anti-oedematous activities as shown by inhibition of Croton oil-induced oedema of the mouse ear (Della, 1990; Della, 1994).

Anti-bacterial and Antifungal Activity

Calendula has various anti-bacterial and anti-fungal activities (Rossiter *et al*., 2006; Tonks *et al*., 2007) and has been used for the treatment of abrasions, burns, ulcers, skin inflammations, eczema and wounds (Schulz *et al*., 2004). CO flower extract has an anti-bacterial activity against various bacteria. *In-vitro*, the essential oil of flowers inhibited the growth of gram-positive bacteria including *Staphylococcus aureus* and *Bacillus subtilis*, and gram-negative bacteria including *Pseudomonas aeruginosa* and *Escherichia coli,* showing maximum inhibition for *Pseudomonas aeruginosa*. Moreover, the reproductive parts of CO show less anti-bacterial activity than the petals (Hamad *et al*., 2011)**.** The flower decoction and methanolic extract showed anti-bacterial activity against various facultative aerobic and obligate anaerobic periodontal bacteria including *Furobacteriumnucleatum*, *Porphyromonosgingi - valis*, *Caphocytophagagingivalis*, *Prevotella* spp., *Veilonellaparvula*, *Peptostreptococcus micros*, *Eikenellacorrodens* and *Actinomycesodontolyticus* (Iauk *et al*., 2003). The flower volatile oil showed anti-fungal activity against various fungal strains: *Candida dubliniensis* (ATCC777), *Candida krusei* (ATCC6258), *Candida glabrata* (ATCC90030), *Candida albicans* (ATCC64548), *Candida parapsilosis* (ATCC22019) and against yeast isolated from humans: *Candida krusei*, *Candida dubliniensis*, *Candida guilliermondii*, *Candida glabrata*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicals,*and *Rhodotorella* spp. (Gazim *et al*., 2008) (Table 5). *Streptococcus aureus* was more susceptible to the aqueous extracts than ethanolic, methanolic and petroleum ether extracts of *Calendula* flower, suggesting the better anti-bacterial activity of aqueous extracts (Roopashree *et al*., 2008). *Calendula* leaf, stem, root and flower extracts show anti-microbial activity against various

Table 5: Anti-fungal activities of the essential oil of flowers of *Calendula officinalis* (Gazim *et al*., 2008)

Microorga-	Origin* nisms	Mean zone of inhibition a (mm)	
		Calendulaoil 5 µl/disc	Nystatin20 µg/disc
C. albicans	ATCC 64548	16	12
C. albicans	orotracheal tube	11	13
C. albicans	OC–HIV	26	11
C. albicans	VVC	18	12
C. albicans	VVC	15	12
C. albicans	VVC	15	12
C. albicans	Urine	27	11
C. dubliniensis	ATCC 777	24	11
C. parapsilosis	ATCC 22019	20	12
C. parapsilosis	Onychomycosis	14	13
C. parapsilosis	Paronychia	30	11
C. parapsilosis	Blood	30	11
C. glabrata	ATCC 90030	15	12
C. glabrata	Hands colonization	23	11
C. glabrata	Hands colonization	28	11
C. tropicalis	Urine	11	13
C. tropicalis	Granulomatous lesion	15	12
C. tropicalis	Urine	21	12
C. tropicalis	Urine	22	11
C. guilliermondii	Hands colonization	25	11
C. guilliermondii	Hands colonization	24	11
C. krusei	ATCC 6258	15	12
Rhodotorulla sp	Hands colonization	30	11

Except ATCC microorganisms all of others are human clinical isolates OC–HIV: oral candidiasis; VVC: vulvo vaginal candidiasis. Mean of inhibition zone by oil of flowers of CO: Good activity (11-18 mm); high activity (20-27 mm); highest activity (28-30 mm)

human pathogenic microbes: *Candida albicans, Candida parapsilosis, Pseudomonas aeruginosa, Escherichia coli, Cogulase* (+) *Staphylococcus* sp., *Enterococcus* sp. and *Cogulase* (-) *Staphylococcus* sp.

Glycosides of oleanolic acid and saponins of *C. officinalis* showed anti-parasitic activity against *Heligmosomoides polygyrus* (Al-Snafi, 2016). The ethanolic and methanolic extract of leaves of *C. officinalis* also showed anti-parasitic activity against *Pheretimaposthuma* (Dorwal, 2012). α-pinene in *C. officinalis* showed anti-Listeria activity against L. monocytogenes (Viuda-Martos *et al*., 2010). Nerolidol of *C. officinalis* showed anti-malarial activity by inhibiting the parasite to synthesize co-enzyme Q in all intraerythrocytic stages (Boyom *et al*., 2003).

Anti-oxidant Activity

C. officinalis contain various phyto-chemical constituents: alkaloids, carotenoids, flavonoids like quercetin, lupeol, protocatechuic acid, isorhamnetin, etc. and triterpenoids (Matysik *et al*., 2005). Most of these phyto-chemicals have free radical scavenging activity and also enhance healing of wounds by artificial cross linkage (Kuppast and Nayak 2006). *C. officinalis* being rich in flavonoids, carotenoids, saccharides, organic acids, lipids and saponosoides show a very effective anti-oxidant activity. Both flavonoids and carotenoids inhibit the production of various reactive oxygen species and free radicals, which can otherwise cause chronic inflammatory and autoimmune diseases in humans, like pulmonary hypertension syndrome (ascites) of broilers (Iqbal *et al*., 2002). Flavonoids and carotenoids inhibit oxidation because of their capacity to inhibit oxidases, activate anti-oxidant enzymes, chelate metal catalysts, transfer free radical electrons, and reduce alpha-tocopherol radicals (Middleton *et al*., 2000; Nijveldt *et al*., 2001). Bio-flavonoids can reduce the oxidative stress and improve the performance of various farm animals (Abd El-Gawad *et al*., 2001; Hager-Theodorides *et al*., 2014). Flavonoids and carotenoids may effect by their interactions with specific proteins that are important to various intracellular signaling cascades. Particularly, flavonoids may selectively act with various components of protein kinase signaling cascades like protein kinase C, asphosphoinositide 3-kinase, Akt/protein kinase B, etc. (Hou and Kumamoto, 2010). Moreover, *C. officinalis* extract shows activity against reactive oxygen species (ROS) and reactive nitrogen species (RNS) with an effective activity even at low concentration (Braga *et al*., 2009). *In vitro*, *C. officinalis* butanolic fraction possesses a high anti-oxidant and free radical scavenging activity (Cordova *et al*., 2002). Butanolic fraction (BF) decreased the concentration of hydroxyl radicals ($OH^{\cdot}$) and superoxide radicals (O^{2-}). BF also showed 100% inhibition of lipid peroxidation in rat liver microsomes induced by Fe^{2+}/ascorbate.

Sabir *et al*. (2015) also reported anti-oxidant activity of *C. officinalis* with flower extract showing higher anti-oxidant activity than leaf extract. *C. officinalis* flower extract having anti-oxidant activity protects the human skin cells against oxidative damage which otherwise can lead to ageing or skin cancer (Alnuqaydan *et al*., 2015).

Anti-HIV and Anti-cancerous Activity

In vitro flower tincture showed antiviral activity by suppressing the replication of influenza APR-8, influenza A2 and herpes simplex virus (Silva *et al*., 2007). *In-vitro* dichloromethane-methanolic extract of *Calendula* flowers showed an effective anti-HIV activity through the inhibition of HIV1-RT and suppression of HIV-mediated fusion (Kalvatchev *et al*., 1997).

In vitro ethyl acetate soluble fraction of *Calendul*a flower extract showed cytotoxic effect due to the presence of two main compounds: calenduloside G'6-*O*-methyl-ester and calenduloside F'6-O-butyl-ester (Ukiya *et al*., 2006). Calenduloside G'6-*O*-methyl-ester showed anti-cancerous activity against melanoma (UAAC-62, SK-MEL-5 and LOXIMVI), leukaemia (RPMI-8226 and MOLT-4) and colon cancer (HCC-2998) cell lines. Calenduloside F'6-O-butyl-ester also showed anti-cancerous activity against these cell lines (Ukiya *et al*., 2006). *In vitro* aqueous laser activated *calendula* extract (LACE) showed 70-100% proliferation inhibition of murine and human tumor cell lines through cell cycle arrest at G0/G1 phase and caspase-3-induced apoptosis (Medina *et al*., 2006). Moreover, *invivo* LACE exhibited anti-tumor activity in nude mice (Medina *et al*., 2006).

Laser Activated *Calendula* Extract (LACE) showed 100% inhibition of growth of cancer cell lines by inducing cell cycle arrest in G0/G1, mediated via down-regulation of CDK1-Cdc2, CDK2, CDK4 and CDK6, and cyclin E y A, D1 and D3 (Medina *et al*., 2006). LACE induces the apoptotic death and increased concentration of LACE increases the rate of apoptosis. LACE treatment causes 100% growth inhibition in Jurkat cells in leukemia cell lines (Medina *et al*., 2006).

Nephroprotective Activity

CO flower extract inhibit the cisplatin (cis-dichloro diamine platinum II/ platinum containing anti-cancerous drug) induced oxidative stress and reduces the kidney damage (Preethi *et al*., 2009). The renal accumulation of platinum leads to nephrotoxicity. The *Calendula* extract reduces the kidney damage due to its anti-oxidant activity. The increased activity of SOD, CAT and increased level of GSH in extract treated group leads to the protection against cisplatin induced renal damage (Preethi *et al*., 2009).

Prevention of Oropharyngealmucositis

Oropharyngealmucositis (OM) is reported as the main side effect of cancer radiotherapy, involving oral mucosa inflammation, atrophy, erythema, swelling and ulceration (Raber-Durlache *et al*., 2010; Sonis, 2004; Trotti *et al*., 2003). The initiation phase of radio-therapy induced injury in OM leads to the production of reactive oxygen species (ROS) by injured cells and clonogenic cell death (Babaee *et al*., 2013). However, the phenolics and the hydroxyl group containing flavonoids present in *Calendula* are the antioxidants with free radical scavenging and chelating activities (Jacobo-Velazquez and Cisneros-Zevallos, 2009; Es-Safi *et al*., 2007; Heijnen *et al*., 2001; Younes and Siegers 1981), and play a very important role in protecting the body from ROS induced oxidative stress. The presence of these phenolics and flavonoids in CO and their high anti-oxidant activity is responsible for its protective effect in radiotherapy-induced OM (Babaee *et al*., 2013). *Calendula* mouthwash decreased OM intensity without any side effect (vomiting and nausea) but could not completely prevent it, which can be further explained by OM patho-biology (Babaee *et al*., 2013).

Hypoglycemic and Gastroprotective Effect

The butanol-soluble fraction and methanolic extract of *C. officinalis* flowers showed hypoglycemic effect in oral glucose-loaded mice due to the presence of saponins [two oleanolic acid 3, 28-bisdesmosides (5,7) and two oleanolic acid 3-monodesmosides (8,10)] and glycosides A, B, C, D and F. The two oleanolic acid 3-monodesmosides (8,10) showed significant hypoglycemic activity, while seldom hypoglycemic activity was observed with the two oleanolic acid 3, 28-bisdesmosides (5,7) (Yoshikawa *et al*., 2001). The significant hypoglycemic activity of extracts and individual compounds of *Calendula* flowers shows that it can be used as a forth coming anti-diabetic drug (Yoshikawa *et al*., 2001).

Saponins (5-8, 10) present in the flowers of *C. officinalis* showed gastro-protective effect against ethanol and indomethacin-induced gastric mucosal lesions in rats. The saponins (5-8, 10) also showed inhibitory activity in the ethanol-induced gastric lesions, while two oleanolic acid 3-monodesmosides (8, 10) and two oleanolic acid 3,28-bisdesmosides (5, 7) showed a protective effect against indomethacin-induced gastric lesions. The oleanolic acid 3-monodesmoside (8) and oleanolic acid 3, 28-bisdesmoside (5, 7) activities were more effective than the activities of two oleanolic acid 3-monodesmosides (6, 10) (Yoshikawa *et al*., 2001).

The ethanolic extract of *Calendula* has been found to possess anti-acid and anti-ulcer activity in rats due to its gastro-protective and anti-secretory effect (Chandra *et al*., 2015). The ethanolic extract of *Calendula* stimulates mucus secretion and glutathione (GSH) level, while suppressing the pepsin level, thus being the mechanism responsible for gastro-protection (Chandra *et al*., 2015).

Toxic Effect

C. officinalis extract has been found to be non-toxic, non-mutagenic and non-genotoxic (Taylor and Francis Health Sciences, 2001) with no reports of toxicity and mortality (Silva *et al*., 2007, Lagarto *et al*., 2011).

Conclusion

As the plant *C. officinalis* - possesses wide variety of phyto-chemicals and pharmacological activities, so it can be considered as an excellent source of new

drugs. Many reports are available on the *Calendula* having highly effective anti-bacterial, anti-fungal, anti-helminthic, anti-molluscal and anti-inflammatory properties with no toxicity. It is a promising plant which needs to be investigated thoroughly and can be exploited for extraction of active ingredients that can be used in the synthesis of different drugs, for the protection against various maladies and management of various diseases.

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