

Pharmacological Activities of *Calendula Officinalis*

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Abstract: *Calendula officinalis*, belonging to the family of Asteraceae, commonly known as English marigold or Pot Marigold is an aromatic herb which is used in Traditional system of medicine in Europe, China and India amongst several places in the world. It is also known as "African marigold" and has been a subject of several chemical and pharmacological studies. It is used in especially for wound healing, anti-HIV, anti-inflammatory, hepatoprotective, spasmolytic and spasmogenic, antispasmodic, analgesic, and anti-diabetic. In this review, I have explored the organoleptic, pharmacological activities of *Calendula officinalis* in order to existing information on this plant as well as highlight its multi activity properties as a medicinal agent.

Keywords: *Calendula officinalis*, Organoleptic Properties, Asteraceae, Phytochemical constituents, Pharmacological activities.

1. Introduction

Calendula officinalis Linn. is used medicinally in Europe, China, US and India. It belongs to the family, Asteraceae, and is commonly known as Zergul (Hindi), African marigold, *Calendula*, Common Marigold, Garden Marigold, Marigold, Pot Marigold (English), Butterblume (German), Chin Chan Ts'ao (Chinese), Galbinele (Romanian) and Ringblomma (Swedish)^[1,2]. *Calendula officinalis* Linn has been widely used in homeopathic medicine for the treatment of many diseases. It has been reported to possess many pharmacological activities, which include antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral. It also possess cytotoxic as well as tumor reducing potential. It is used as analgesic, anthelmintic, anti-bacterial, anti-emetic, anti-fungal, anti-inflammatory, anti-pyretic, antiseptic, anti-spasmodic, anti-viral, astringent, bitter, candidicide, cardiogenic, carminative, cholagogue, dermagenic, diaphoretic, diuretic, hemostatic, immunostimulant, lymphatic, uterotonic, and as vasodilator. Generally in cases of external it is used for treating skin inflammations, open wounds and laceration wounds with bleeding. It is also used for treating minor diseases like razor burns and wind burns. Internally it is used for mucous membrane inflammations, peptic and duodenal ulcers, spasms of the GI tract, duodenal and intestinal mucosa, dysmenorrhea (painful menstruation) especially in nervous or anemic women, splenic and hepatic inflammations. It is also used as a mouthwash after tooth extractions^[3].

Taxonomic classification of Calendula officinalis^[4,5,6,7]

Kingdom - Plantae
Subkingdom - Tracheobionta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Asteridae
Order - Asterales
Family - Asteraceae
Tribe - Calenduleae
Genus - *Calendula*
Species - *C. Officinalis*

Habitat

The plant is native to Central and Southern Europe, Western Asia and the US^[8].

Organoleptic Properties.

Calendula officinalis has faint, pleasantly aromatic odour and bitter taste.^[9]

2. Pharmacological Activities

Antimicrobial Activity

The essential oil of the flowers inhibits the growth in vitro of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*^[10]. A flavonoid fraction isolated from the flowers inhibited the growth in vitro of *S. aureus*, *Sarcina lutea*, *E. coli*, *Klebsiella pneumoniae* and *Candida monosa*^[11]. However, chloroform, ethanol, methanol or water extracts of the flowers did not inhibit bacterial growth in vitro^[12,13,14]. Acetone, ethanol or water extracts inhibited the growth in vitro of the fungus *Neurospora crassa*^[15]. Extracts of the flowers inhibited the growth in vitro of *Trichomonas vaginalis*^[16]. Oxygenated terpenes appear to be responsible for the antimicrobial activity^[17]. Extracts of the flowers inhibits the growth in vitro of *Trichomonas vaginalis*. Oxygenated terpenes appear to be responsible for the antimicrobial activity.

Antiviral Activity

A tincture of the flowers suppressed the replication of herpes simplex, influenza A2 and Influenza APR-8 viruses in vitro^[18]. However, an aqueous extract of the flowers was not active¹⁴. A chloroform extract of the flowers inhibited the replication of HIV-1 in acutely infected lymphocytic MOLT-4 cells in vitro (IC₅₀ 0.4 mg/ml)^[19]. A chloroform extract also inhibited HIV-1 reverse transcriptase activity in a dose-dependent manner (ED₅₀ 51.0 mg/ml). A 5% hot aqueous extract of the flowers (2 ml) inhibited the replication of encephalitis virus after intraperitoneal administration to mice^[20].

Anti-inflammatory and Anti-edematous Activity

Topical application of a 70% ethanol extract of the flowers to mice at a dose of 1.2 mg/ear (corresponding to 4.16 mg crude drug) reduced croton oil-induced ear edema by 20%^[21]. External application of a carbon dioxide extract of the flowers (300 mg/cm²) suppressed croton oil-induced ear edema in mice^[22]. The triterpene fraction of an extract of the flowers had marked anti-inflammatory activity in mice (1 mg/ear) against ear edema induced by 12-

Ottridecanoylphorbol-13-acetate^[23]. Faradiol esters isolated from the flowers (240 mg/cm²) inhibited croton oil-induced ear edema in mice^[24]. Intra-gastric administration of an aqueous extract of the flowers (100 mg/kg body weight) inhibited carrageenan-induced footpad edema in rats^[25]. However, an 80% ethanol extract of the flowers was weakly active (11% inhibition) at a concentration of 100 mg/kg body weight administered orally 1 hour prior to induction of edema^[26]. Isorhamnetin glycosides isolated from the flowers inhibited rat lung lipoxigenase in vitro^[27].

Wound-healing and Angiogenic Activity

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 β -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 treated with ethanolic extract 1% revealed an increase in the number of blood vessels compared to solvent control^[28]. 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^[29]. *Calendula* (*Calendula officinalis*) infused oil is considered beneficial for the reversal of numerous skin and tissue conditions. It is used only after the threat of infection has passed. It is not used on deep wounds since it is felt that *calendula* may seal the wound too quickly preventing drainage^[30].

Antioxidant

A 70 % methanol extract of the plant was successively extracted with ether, chloroform, ethyl acetate and n-butanol leaving a residual aqueous extract which was assayed for antioxidant activity by liposomal lipid peroxidation-induced Fe²⁺ and ascorbic acid. The ether, butanol and water extracts, containing flavonoids, showed antioxidant activity^[31]. Propylene glycol extracts of the petals and flower heads, assayed for antioxidant activity by lipid peroxidation, indicate that the extract of the petals was more potent than the flower head extract, based on analysis of plasma and urine malondialdehyde (MDA) and urine isoprostane invent rations (ipf2 α -VI)^[32].

Anti-HIV

A chloroform extract of the flowers inhibited the replication of HIV-1 in acutely infected lymphocytic MOLT-4 cells in vitro (IC₅₀ 0.4 mg/ml). A chloroform extract also inhibited

HIV-1 reverse transcriptase activity in a dose-dependent manner (ED₅₀ 51.0mg/ml)^[33]. Dichloromethane-methanol (1:1) extract of *C. officinalis* flowers exhibited potent anti-HIV activity in in vitro MTT/tetrazolium-based assay. This activity was attributed to inhibition of HIV1-RT at a concentration of 1,000 μ g /mL as well as suppression of the HIV-mediated fusion at 500 μ g/ml^[34].

Immunostimulant

The polysaccharide fraction of *C. Officinalis* extract showed immunostimulant activity, based on in vitro granulocyte test. Polysaccharide III showed the highest phagocytosis (54 – 100 %) at a concentration of 10⁻⁵ - 10⁻⁶ mg/mL, while PS-I and PS-II exhibited 40 – 57 and 20 – 30 % phagocytosis, respectively^[35,36].

Anticancer, lymphocytes and dual

The ethyl acetate soluble fraction of the methanol extract of *C. officinalis* flowers has shown cytotoxic activity in vitro^[37]. Further activity-guided isolation of that fraction showed that the active compounds were: calendulose F6'-O-n-butyl ester, which is active against leukaemia (MOLT-4 and RPMI 8226), colon cancer (HCC-2998) and melanoma (LOXIMVI, SK-MEL-5 and UACC-62)] cell lines with GI₅₀ values of 0.77-0.99 μ mole, except for leukaemia (CCRF-CEM, GI₅₀ = 23.1 μ mole), renal cancer (AK-1, 17.2 μ mole; UO-31, 12.7 μ mole) and breast Muley et al Trop J Pharm Res, October 2009; 8 (5): 461 cancer (NCI/ADR-RES, >50 μ mole)] cell lines; and calendulose G6'-O-methyl ester, which is active against all the cancer cell lines mentioned above with GI₅₀ \leq 20 μ mole except for ovarian cancer (IGROVI, GI₅₀ = 20.1 μ mole) and renal cancer (VO-31, 33.3 μ mole) cell lines^[37]. Aqueous laser-activated *calendula* flower extract (LACE) showed potent in vitro inhibition of tumor cell proliferation when assayed against a wide variety of human and murine tumor cell lines. The inhibition ranged from 70 – 100 % with an C₅₀ concentration of 60 μ g/mL. The mechanisms of the inhibition were identified as cell cycle arrest in G₀/G₁ phase and caspase-3 induced apoptosis. On the other hand, when LACE as assayed against human peripheral blood lymphocyte (PBLs) and human natural killer cell lines (NKL) it showed in vitro induction of proliferation and activation of these cells, mainly B-lymphocytes, CD4+, T lymphocytes and NKT lymphocyte^[38]. Various extracts of the leaf, flower and whole plant have also been found to be cytotoxic to MRC5, HeP2, ascetic cells from Ehrlich carcinoma. The saponin rich fraction of these extracts displayed anti-tumoural activity in vivo in the Ehrlich mouse carcinoma model^[39].

Antifungal and Antibacterial Activities

The methanol extract and 10% decoction of the plant's flowers were assessed for their activity against anaerobic and facultative aerobic periodontal bacteria, namely, *Porphyromonas gingivalis*, *Prevotella* spp., *Furobacterium nucleatum*, *Caphocytophaga gingivalis*, *Veilonella parvula*, *Eikenella corrodens*, *Peptostreptococcus micros* and *Actinomyces odontolyticus*. The results showed marked inhibition against all tested microorganisms with MIC \geq 2048 mg/L^[40]. When the essential oil of the flowers was tested (using disc diffusion technique) against various fungal strains, namely, *Candida albicans* (ATCC64548), *Candida*

dublinsiensis (ATCC777), *Candida parapsilosis* (ATCC22019), *Candida glabrata*(ATCC90030), *Candida krusei* (ATCC6258), and yeast isolated from humans, viz, *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, *Candida guilliermondii*, *Candida krusei* and *Rhodotorella* spp., it showed good potential antifungal activity (at 15 µl/disc)^[41].

Hepatoprotective

The hydro alcohol extract of the flowers, when given to CCl₄-intoxicated liver in albino male Wistar rats at a dose of 10 mL/kg, resulted in a reduction of hepatocytolysis by 28.5 % due to reduction in glutamo-oxalate-transaminase (GOT) and glutamo-pyruvate-transaminase (GPT). However, histoenzymology showed reduction of steatosis of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), cytochromoxidase (Cyox) and Mg²⁺-dependant adenosine triphosphatase (ATPase)^[42]. The hot water extract of *C. officinalis* flowers exhibited anti-hepatoma activity against five human liver cancer cells - HepG2/C3A, SK-HEP-1, HA22T/VGH, Hep3B and PLC/PRF/5 - with an inhibitory effect of 25 – 26 % at a dose of 2000 µg/mL^[43].

Spasmolytic and Spasmogenic

The aqueous-ethanol extract of *C. officinalis* flowers, when assayed in rabbit jejunum, caused a dose-dependent (0.03 - 3.0 mg/mL) relaxation of spontaneous and K⁺- induced contraction; further fractionation of the extract with dichloromethane showed inhibition of spontaneous contractions in a dose range of 0.01 - 0.3 mg/mL. This is ten times more potent than the parent crude extract, and spasmolytic activity was found to be due to calcium channel blockade (CCB). On the other hand, the aqueous fraction of the parent extract exhibited spasmogenic activity in a dose range of 1 - 10 mg/mL^[44].

Genotoxic and Antigenotoxic Dual Activities

The aqueous (AE), aqueous-ethanol (AEE), ethanol and chloroform extracts of *C. officinalis* flowers were evaluated to determine if they caused induction of unscheduled DNA synthesis (UDS) in rat liver culture and reversal of diethylnitrosamine (DEN)-induced UDS. In the UDS test in liver culture, DEN, at a level of 1.25 µmole, produced a maximum increase of 40% 3H-thymidine (3HdTT) incorporation while AE and AEE extracts showed complete reversal of DEN effect at levels of around 50 ng/mL, and between 0.4 and 16 ng/mL, respectively. In the absence of DEN, these two polar extracts induced UDS at concentrations of 25 and 3.7 - 100 µg /mL for AE and AEE, respectively, in rat liver cell culture. Thus these polar extracts (AE and AEE) at low concentrations (i.e., ng/mL range) showed anti-genotoxic effect while at high concentrations (i.e., µg/mL range) they exhibited genotoxic effect^[45]. The propylene glycol extract of *C. officinalis* also showed anti-genotoxic effect based on an evaluation in young growing pigs which involved the measurement of the excretion of lymphocyte DNA fragmentation and 24 h urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG)^[46].

Inhibition of Heart Rate

The aqueous extract was tested on the heart of male Wistar rats and found to inhibit heart rate contractility by up to 100 % at a dose of 0.3mg/L^[46].

Insecticidal Activity

The acetone: methanol (2:1 v/v) extract of the flowers showed insecticidal activity when it was tested on milkweed bug^[47].

Anti-diabetic and Anti-hyperlipidemia Activities

The anti-diabetic and anti-hyperlipidaemic effect of hydro alcoholic extract of *calendula officinalis* in alloxan induced diabetic rats. Diabetes was inducing by single intraperitoneal injection of alloxan (150 mg/kg) of body weight. The blood glucose and urine sugar were significant elevated in diabetic rats compared to normal rats. Upon oral administration of hydro alcoholic extract of *calendula officinalis* in to diabetic rats at dose 25 and 50 mg/kg body weight significantly lowered the blood glucose and urine sugar as they compared with group of diabetics rats. Hydro alcoholic extract of *calendula officinalis* in to diabetic rats at a dose of 100 mg/kg body weight 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 haemoglobin lever. The extract was similar to that of insulin. Thus, the investigation clearly show that hydro alcoholic extract of *calendula officinalis* has both anti-diabetic and anti-hyperlipidaemic effect^[48]. The structures of the officinosides were elucidated on the basis of chemical and physicochemical evidence. The inhibitory activities of the principle saponins from the flowers of *C. officinalis* on the increase of serum glucose levels in oral glucose-loaded rats, on gastric emptying in carboxymethyl 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^[49].

Anthelmintic Activities

The dried flowers and leaves of *C. officinalis* have anthelmintic activity. 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, *Pheretima posthuma* 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 56.5 min and death of worms at 111.2 minutes. 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^[50].

Cardiovascular Activity

Calendula could be cardioprotective against ischemic heart disease. Two groups of hearts were used: the treated rat hearts were perfused with *calendula* solution at 50 mM in KHB buffer (in mM: sodium chloride 118, potassium chloride 4.7, calcium chloride 1.7, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulfate 1.2, and glucose 10) for 15 min prior to subjecting the heart to ischemia, while the control group was perfused with the buffer only. *Calendula* achieved cardioprotection by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocyte apoptosis. Cardioprotection appears to be

achieved by changing ischemia reperfusion-mediated death signal into a survival signal by modulating antioxidant and anti-inflammatory pathways as evidenced by the activation of Akt and Bcl2 and depression of TNF α . The results further strengthen the concept of using natural products in degeneration diseases like ischemic heart disease^[51].

3. Conclusions

In this review, we have explored the organoleptic, in-vitro and in-vivo pharmacological activities of *Calendula officinalis* Linn. (Asteraceae), a medicinal plant found in central and southern Europe, western Asia and united states, amongst others. It exhibits several pharmacological activities such as anti-HIV, cytotoxic, anti-inflammatory, hepatoprotective and spasmolytic amongst others. It is potentially an important medicinal plant for mankind.

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References

- [1] Lt. Colonel Kirtikar KR, Major Basu BD. Indian Medicinal Plants. Vol II, Deharadun, India, International Book Distributor, 1993, pp 1413-1414.
- [2] The Wealth of India, Raw Materials, A Dictionary of Indian Raw Material & Industrial Products. Vol 3, New Delhi, Publications & Information Directorate CSIR, 1992, pp 55-58.
- [3] G.S.Chakraborty. International journal of research in Ayurveda and pharmacy. 2010, 1(1), 131-134.
- [4] WHO monographs on selected medicinal plants, Flos Calendulae, 37-44.
- [5] Master data/ monograph – calendula officinalis (pot marigold), 2007; 1-5.
- [6] *Calendula officinalis*. From Wikipedia, the Dictionary of encyclopedia. (Cited 2009).
- [7] Leila Maria Leal Parente; Maria Auxiliadora Andrade; Luiz Augusto Batista Brito; Veridiana Maria de Souza Brianezi Dignan; Marina Pacheco Miguel; Ruy de Souza Lino-Júnior; Leonice Faustino Manrique Tresvenzol; José Realino de Paula; Neusa Margarida Paul. Acta Cirurgica Brasileira., 2011, 26 (1), 19-26.
- [8] Editorial Boards. PDR for Herbal Medicines. 2nd edn, Montvale, Thomson-Medical Economics, 2003; pp 497-500.
- [9] Mukesh Kr. Singh*, Pankaj Sahu, K. Nagori, D. Dewangan, T. Kumar. A. Alexander, H. Badwaik and D.K Tripathi, Organoleptic properties in-vitro and in-vivo pharmacological activities of *Calendula officinalis* Linn: An over review; J. Chem. Pharm. Res., 2011, 3(4):655-663
- [10] Janssen AM et al. Screening for antimicrobial activity of some essential oils by the agar overlay technique. Pharmaceutisch Weekblad, 1986, 8:289-292.
- [11] Tarle D, Dvorzak I. Antimicrobial substances in Flos Calendulae. Farmaceutski Vestnik (Ljubljana), 1989, 40:117-120.
- [12] Rios JL, Recio MC, Villar A. Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. Journal of Ethnopharmacology, 1987, 21:139-152.
- [13] Dornberger K, Lich H. Screening for antimicrobial and presumed cancerostatic plant metabolites. Pharmazie, 1982, 37:215-221.
- [14] Acevedo JG, Lopez JL, Cortes GM. In vitro antimicrobial activity of various plant extracts used by purepecha against some Enterobacteriaceae. International Journal of Pharmacognosy, 1993, 31:61-64.
- [15] Kubas J. Investigations on known or potential antitumoral plants by means of microbiological tests. Part III. Activity of some cultivated plant species in *Neurospora crassa* test. Acta Biologica Cracoviensia Series Botanica, 1972, 15:87-100.
- [16] Racz G et al. Trichomonicidal and anthelmintic activity of Roumanian folkloric plants. Planta Medica, 1980, 39:257A.
- [17] Gracza L. Oxygen-containing terpene derivatives from *Calendula officinalis*. Planta Medica, 1987, 53:227.
- [18] Bogdanova NS et al. Study of antiviral properties of *Calendula officinalis*. Farmakol Toksikol (Moscow), 1970, 33:349.
- [19] May G, Willuhn G. Antiviral activity of aqueous extracts from medicinal plants in tissue cultures. Arzneimittel-Forschung, 1978, 28:1-7.
- [20] Kalvatchev Z et al. Anti-HIV activity of extracts from *Calendula officinalis* flowers. Biomedicine and Pharmacotherapy, 1997, 51:176-180.
- [21] Fokina GI et al. Experimental therapy of tick-borne encephalitis. Soviet Progress in Virology, 1991, 1:27-31.
- [22] Della-Loggia R et al. The role of triterpenoids in the topical anti-inflammatory activity of *Calendula officinalis* flowers. Planta Medica, 1994, 60:516-520.
- [23] Akihisa T et al. Triterpene alcohols from the flowers of *Compositae* and their anti-inflammatory effects. Phytochemistry, 1996, 43:1255-1260.
- [24] Zitterl-Eglseer K et al. Anti-edematous activities of the main triterpendiol esters of marigold (*Calendula officinalis* L.). Journal of Ethnopharmacology, 1997, 57:139-144.
- [25] Peyroux J et al. Anti-edemic and anti-hyperhaemic properties of *Calendula officinalis* L. Plantes médicinales et Phytothérapie, 1981, 15:210-216.
- [26] Mascolo N et al. Biological screening of Italian medicinal plants for anti-inflammatory activity. Phytotherapy Research, 1987, 1:20-31.
- [27] Bezakova L et al. Inhibitory activity of isorhamnetin glycosides from *Calendula officinalis* L. on the activity of lipoxygenase. Pharmazie, 1996, 51:126-127.
- [28] Leila Maria Leal Parente; Maria Auxiliadora Andrade; Luiz Augusto Batista Brito; Veridiana Maria de Souza Brianezi Dignan; Marina Pacheco Miguel; Ruy de Souza Lino-Júnior; Leonice Faustino Manrique Tresvenzol; José Realino de Paula; Neusa Margarida Paul. Acta Cirurgica Brasileira., 2011, 26 (1), 19-26.

- [29] A Robert; BS Hadfield; C Tracey; DPM Vlahovic; M. Tariq Khan. *The Foot & Ankle Journal*. 2008, 1(7), 1-8.
- [30] Cheryl Lans; Nancy Turner; Tonya Khan; Gerhard Brauer; Willi Boepple. *Journal of Ethnobiology and Ethnomedicine*. 2007, 3(11), 1-22.
- [31] Popovic M, Kaurinovic B, Mimica-Dukic N, Vojinovic-Miloradov M, Cupic V.. Combined effects of plant extracts and xenobiotics on liposomal lipid peroxidation. Part 1. Marigold Muley et al *Trop J Pharm Res*, October 2009; 8 (5): 465 extract-ciprofloxacin/pyralene. *Oxidation Commum*, 1999; 22: 487-494.
- [32] Frankic T, Salobir K, Salobir J. The comparison of in vivo antigenotoxic antioxidative capacity of two propylene glycol extracts of *Calendula officinalis* (Marigold) and vitamin E in young growing pigs. *J Anim Physiol Anim Nutr*, 2008; 41: 1-7.
- [33] Z Kalvatcev; R. Walder; D. Garzaro. *Biomedicine and Pharmacotherapy*. 1997, 51(4), 176-180.
- [34] Kalvatene Z, Walder R, Gabzaro D. Anti-HIV activity of extracts from *Calendula officinalis* flowers. *Biomed & Pharmacother*, 1997; 51: 176-180.
- [35] Varlijen J. Structural analysis of rhamnoarabinogalactans and arabinogalactans with immunostimulating activity from *Calendula officinalis*. *Phytochemistry*, 1989; 28: 2379-2383.
- [36] Wagner H, Proksch A, Riess-Maurer I, Vollmar A, S. Odenthal, Stuppner H, Jurcic K, Le Turdu M, Fang Jn.. Immunstimulierend wirkende Polysaccharide (Heteroglykane) aus höheren Pflanzen. *Arzneimittel-Forschung*, 1985; 7: 1069-1075.
- [37] Della LR, Della LR, Tubaro A, Sosa S, Becker H, Saar S, Isaac O. The role of triterpenoids in topical anti-inflammatory activity of *Calendula officinalis* flowers. *Planta Med*, 1994; 60: 516-520.
- [38] Medina EJ, Lora AG, Paco L, Algarra I, Collado A, Garrido F. A new extract of the plant *Calendula officinalis* produces a dual in vitro effect: cytotoxic antitumor activity and lymphocyte activation. *BMC Cancer*, 2006; 6: 119-132.
- [39] Boucard-Maitre Y, Boucard-Maitre Y, Algernon O, Raynaud, J. Genotoxic and antitumoral activity of *Calendula officinalis* extracts. *Pharmazie*, 1988; 43: 220-221.
- [40] Iauk L, Lo-Bue AM, Milazzo I, Rapisarda A, Blandino G. Antibacterial Activity of Medicinal Plant Extracts Against Periodontopathic Bacteria. *Phytother Res*, 2003; 17: 599-604.
- [41] Gazim ZC, Rezende CM, Fraga SR, Svidzinski TE, Cortez DG. Antifungal activity of the essential oil from *calendula officinalis* l. (asteraceae) growing in brazil. *Braz. J. Microbiol* 2008; 39: 61-63.
- [42] Rasu MA, Tamas M, Puica C, Roman I, Sabadas M. The hepatoprotective action of ten herbal extracts in CCl4 intoxicated liver. *Phytother Res*, 2005; 19: 744-749.
- [43] Lin LT, Liu LT, Chiang LC, Lin CC. In vitro anti-hepatoma activity of fifteen natural medicines from Canada. *Phytother Res*, 2002; 16: 440-444.
- [44] Bashir S, Janbaz KH, Jabeen Q and Gilani AH. Studies on Spasmogenic and Spasmolytic Activities of *Calendula officinalis* flowers. *Phytother Res*, 2006; 20: 906-910.
- [45] Perez-Carreón JI, Cryz-Jimener G, Licea-Vega JA, Popoca EA, Fazenda SF, Villa-Trevinos. Genotoxic and antigenotoxic properties of *Calendula officinalis* extracts in rat liver culture treated with diethylnitrosamine. *Toxicology in vitro*, 2002; 16: 253-258.
- [46] Perez-Guitierrez S, Vargas-Solis R, Miguel ZS, Perez-G C, Perez-G RM. Inhibitory effect of five plant extracts on heart rates of rats. *Phytother Res*, 1998; 12: S49-50.
- [47] Alexenizor M, Dorn A. Screening of medicinal and ornamental plants for insecticidal and growth regulating activity. *J Pestic. Sci*, 2007; 80: 205-215.
- [48] G.S Chakraborty; R Arora; C Majee; *International research journal of pharmacy*. 2011, 2(1), 61-65.
- [49] Toshiyuki Marukami; Akinobu Kishi; Masayuki Yoshikawa. *Chem. Pharm. Bull*, 2001, 49(8), 974-978
- [50] Umesh kumar jain; Lipi Purwal; Vivek Shrivastav ; Krishna K Makode. *Scholars research library Der pharmacia letter*. 2010, 2(4), 476-481.
- [51] D Ray; S Mukherjee ; M Falchi; A Bertelli; DK Das. *Curr Pharm Biotechnol.*, 2010, 11(8), 849-54.