Calendula officinalis: Potential Roles in Cancer Treatment and Palliative Care

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Abstract

A continuous challenge in cancer management is to improve treatment efficacy and to diminish its side effects. Consequently, new conventional and unconventional drugs and bioactive compounds from plants are constantly developed, characterized, and used for in vitro and in vivo models. This review focuses on the antitumor properties of *Calendula officinalis*, its biological and molecular effects in tumor cells and animal models, as well as its role in cancer palliative care. A systematic review of studies describing the cytotoxic role of *C officinalis* and its therapeutic role on cancer cells were carried out using the PubMed database. Albeit *C officinalis* extracts have cytotoxic activity toward different cancer cell lines, a high grade of variation between studies was observed, depending on plant organ subjected to extraction, extraction method, and the cancer cell lines used for each study. Nevertheless, its cytotoxic activity is related to a few bioactive compounds, presenting multiple roles in both activation of proapoptotic proteins and decreasing the expression of the proteins that inhibit cell death. Moreover, due to its anti-genotoxic/protective as well as antitumor and antimetastatic effects proven in animal models, *C officinalis* could have important future implications in developing novel cancer treatment strategies, while until now it has been used especially for diminishing the side effects of radiotherapy.

Keywords

Calendula, cancer, cytotoxicity, genoprotective, antimetastatic, palliative care

Introduction

Cancer ranks second among all causes of mortality worldwide, with 8.2 million cancer-related deaths in 2012. Regarding its incidence, 12 million new cases were recorded in 2012, while this number is expected to rise by about 70% over the next 2 decades.[1] Depending on cancer stage, its phenotype, and localization, the ongoing treatment options include surgery, radiotherapy, chemotherapy, immunotherapy, and, when suited, hormonal therapies. If the surgery and radiotherapy are successfully used to cure solid tumors in early stages, chemotherapy represents the most important choice of cancer armamentarium for advanced cancers as well as hematological malignancies. However, these treatment options are not always efficient and have significant limitations, probably due to the high heterogeneity of tumors and the fact that the majority of cancers are identified in advanced stages. To increase the treatment efficacy and to reduce the tumor drug resistance, a combinatory regimen is currently applied.

Even if chemotherapy represents one of the most important approaches to kill cancer cells, it produces many side effects by damaging healthy tissues due to its action against all cells entering division. Therefore, both short-term and long-term cumulative cytotoxicity and several adverse effects including fatigue, hair loss, anemia, nausea and vomiting, diarrhea, constipation, mood changes, kidney problems, and dry skin are associated with adjuvant chemotherapy.[2]

Another major problem in cancer treatment is that tumor cells become resistant to radiochemotherapy, thus limiting the response to therapy.[3] Tumor cells might become resistant either by inheriting the resistance traits from a cancerous cell subpopulation, an event known as baseline

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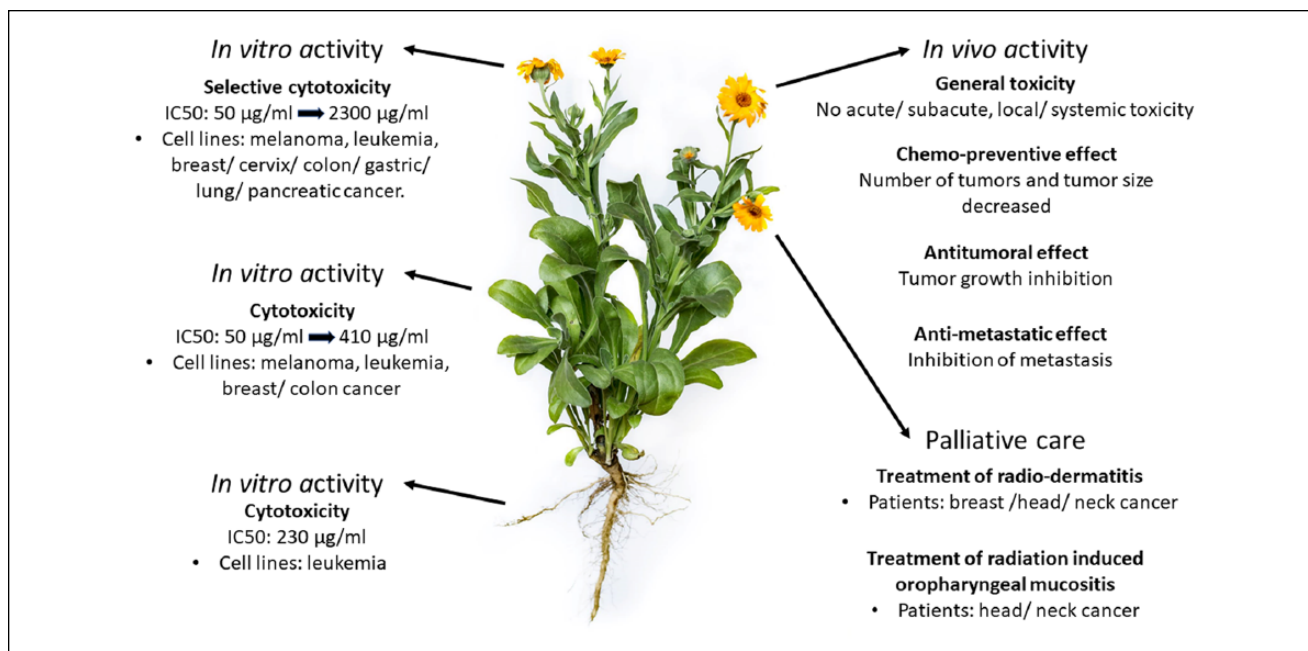
Figure 1. Overview of *Calendula officinalis* anticancer activity. Flowers/flos extracts: in vitro activity, in vivo activity, and in palliative care; leaves/herba extracts: in vitro activity; roots extract: in vitro activity.

resistance and recorded in about 50% of cancer patients or by acquiring resistance over time, as a cellular response to specific drug exposure.[4]

If chemotherapy is considered a nontarget therapy, inducing many side effects, immunotherapy, including monoclonal antibodies, adoptive cell transfer, cytokines, and cancer vaccines, represents a reliable targeted therapy. However, it is limited to some specific tumor phenotypes. Furthermore, due to the high tumor heterogeneity and the high mutation rate, cancerous cell may acquire resistance and thus cause patient relapse.[5]

In this context, new conventional and unconventional anticancer drugs and compounds are constantly developed in an effort to improve cancer therapy and to diminish treatment side effects. In this regard, one of the most important research fields aiming to identify new compounds useful for cancer treatment is that related to plants.

Plants have been proved to be a relevant source of bioactive phytochemicals with cytotoxic and antitumor activity, increasing efforts being made to identify novel plant compounds as possible effective drugs in cancer treatment. Bioactive agents originating from plants like *Catharanthus roseus*, *Podophyllum* species, *Taxus brevifolia*, *Camptotheca acuminata*, *Cephalotaxus* species, or *Curcuma longa* have been long known to possess anticancer properties, with some of them being used in the treatment of different malignancies in clinical setting.[6,7] Paclitaxel, a drug widely used nowadays in the treatment of different types of cancer and part of the World Health Organization's List of Essential Medicines, represents a good example of such a compound.[8] It was originally isolated from the bark of the Pacific yew, *Taxus brevifolia*,[9] and based on its success, it has begun to be semisynthetically made under the name of Taxol/Onxol. Besides their potential of becoming part of the standard treatment in cancer, plant compounds or whole extracts are used in patient palliative care and in complementary and alternative medicine (CAM). CAM are used by around 49% of all cancer patients after the year 2000,[10] herbal medicine being one of the most used types of CAM, even though many such therapies have not demonstrated their effectiveness yet.[11,12]

Calendula officinalis L (*Asteraceae*), the pot marigold, shows promising results regarding its potential use in cancer management. In this article, we review the latest updates on *C officinalis* anticancer activity, both in vitro and in vivo and its phytochemical constituents that present cytotoxic activity, thereby its potential usage in cancer treatment and CAM. Furthermore, we emphasize its putative role in patient's palliative care, based on relevant recent research. An overview of *C officinalis* anticancer activity is presented in Figure 1.

Calendula officinalis: Overview

Calendula officinalis has long been used in traditional medicine, and since 2008, it is recognized as an herbal medicinal product by the European Medicines Agency. Both flowers and leaves of *Calendula* are used nowadays in folk
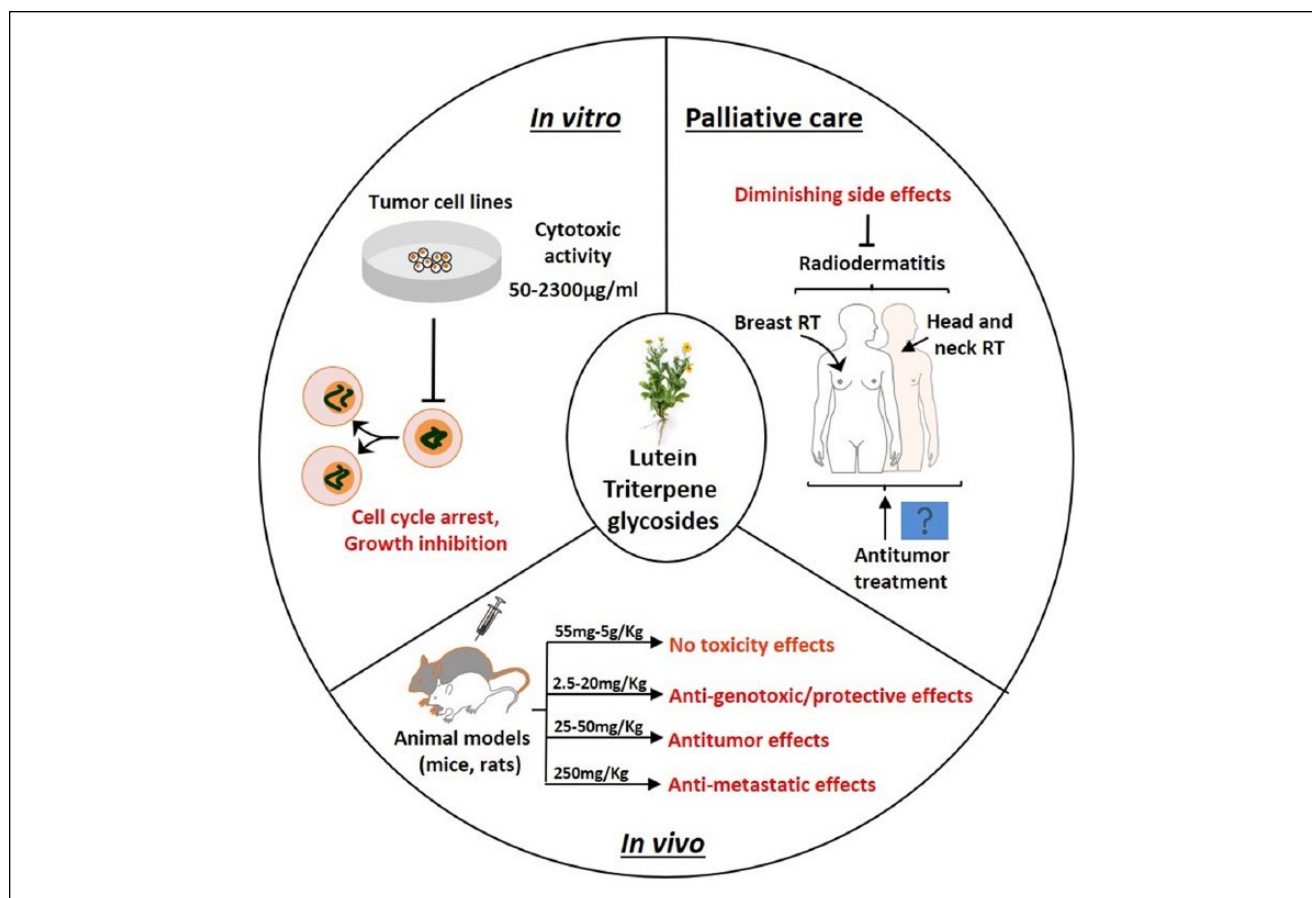
Figure 2. The main antitumor properties of *Calendula officinalis* on in vitro and in vivo models, as well as its role for palliative care in human cancers.

medicine as anti-inflammatory and antispasmodic medicine, in the treatment of poorly healing wounds, minor burns, bruises, and rashes, and also in discomfort alleviation caused by stomach ulcers or inflammation of the oral and pharyngeal mucosa.[13]

Topical *C officinalis* formulations are generally considered safe to use. According to the Safety Assessment of *C officinalis*–derived Cosmetic Ingredients implemented by the Cosmetic Ingredient Review Expert Panel, different *Calendula* extracts and oils are not significantly toxic at conventional concentrations but might be mild ocular irritants.[14] For the majority of patients, *Calendula* products are not allergenic, but rare cases of sensitization are reported in the literature.[15]

Several pharmacological activities were reported for different fractions of *C officinalis* extracts, among which the most important are the following: anti-inflammatory,[16] anti-edematous,[17] and antioxidant activity[18]; antibacterial[19] and antifungal activity[20]; anti-HIV[21] and antiviral activity[22]; wound healing[23] and immunostimulant activity.[24] The biological activity of each extract is due to its constituents, mostly plant secondary metabolites. The major classes of

compounds found in *C officinalis* are terpenoids, flavonoids, phenolic acids, carotenoids, coumarins, quinones, volatile oils, amino acids, and lipids.[25]

With the expansion of CAM based on herbs as cancer treatment,[10] the interest in the putative anticancer efficacy of *C officinalis* extracts and compounds has increased. Its cytotoxic effect on tumor cell lines and its anticancer activity in vivo were first described more than 25 years ago,[26] but valuable insight have been gained since. The main antitumor properties of *C officinalis* on tumor cells and animal models, as well as its role for palliative care in human cancers, are presented in Figure 2.

Calendula officinalis: Cytotoxic Constituents

In the past decade, 3 different bioactive compounds isolated from *C officinalis* extracts were identified as possessing significant cytotoxicity toward cancer cell lines in vitro.

Lutein, isolated through silica gel column fraction chromatography from a methanol extract of *C officinalis* aerial parts and detected with nuclear magnetic resonance, was

Table 1. The Solvents Used for *Calendula officinalis* Flowers/Flos Extracts; the IC_{50} Doses and the Selective Coefficients in the Antitumor Action for the Extracts on Different Cancer Cell Lines; Other Cytotoxic Effects (Viability, Growth Inhibition) at Specific Doses and Time Intervals[a].

Nr Crt	Solvent	Tested Cell Line	IC_{50} at 24 Hours	Coefficient of Selectivity	Other Cytotoxic Effects		Reference
1	Methanol	T47D	~50 µg/mL	No selectivity	Dose at 24 hours	Viability	27
					75 µg/mL	14.2%	
2	Heptane		~50 µg/mL	1.2		18%	
3	Ethyl acetate		~125 µg/mL	1.8		58.4%	
4	Boiled distilled water[30]	HeLa	750 µg/mL	4.16	NS	NS	28
		Fem-X	360 µg/mL	8.67			
		MDA-MB-361	2250 µg/mL	1.38			
		LS174	2300 µg/mL	1.36			
		K562	870 µg/mL	3.59			
5	LACE	B16	NS	NS	Dose at 72 hours	Growth inhibition	29
					250 µg/mL	76%	
		B9				72%	
		ANDO-2				100%	
		MDA-MB-231				100%	
		AGS				100%	
		DU-141				72%	
		A-549				90%	
		IMIN PC-1				100%	
		DLD 1				100%	
		HeLa				83%	
		U937				21%	
		Jurkat T				100%	

Abbreviations: IC_{50}, half maximal inhibitory concentration; NS, not specified.
[a]The selectivity coefficient was calculated as the ratio between the IC_{50} doses obtained for healthy cell lines (human skin fibroblasts by Matysik et al[29]; peripheral blood mononuclear cells by Mati et al[30]) and the IC_{50} doses for the tumor cell lines.

found to possess selective cytotoxic activity toward breast cancer cell lines.[27] The ¼ IC_{50} value was 100 µg/mL for MDA-MB-231 cell line and >100 µg/mL for MCF7 cell line, suggesting that lutein has a greater cytotoxic activity toward triple-negative breast cancer rather than luminal breast cancer. Furthermore, significantly lower doses of lutein were cytotoxic toward breast cancer cell lines, when compared with its activity against the healthy breast cell line MCF10A. The expression levels of several proapoptotic proteins like p53, bax, and caspase-3 were increased in lutein-treated cancer cells (except caspase-3 in MCF7, where the protein was absent), while the expression of Bcl-2, a protein that inhibits programmed cell death, decreased after treatment.

Two triterpene glycosides, calenduloside F 6'-*O-n*-butyl ester and calenduloside G 6'-*O*-methyl ester, were identified by nuclear magnetic resonance analysis in the *n*-BuOH-soluble fraction of the methanolic extract obtained from *C officinalis* dried flowers. Both compounds were tested in vitro for their cytotoxic activity in 60 cell lines derived from leukemia, non–small cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer. At 48 hours after treatment, the IC_{50} values for the calenduloside F 6'-*O-n*-butyl ester compound were <10 µM for a wide majority of the tested cell lines, while for the calenduloside G 6'-*O*-methyl ester compound, they were <20 µM, with the exception of 2 cell lines.[28]

Calendula officinalis: In Vitro Cytotoxic Activity of Extracts

Several recent reports have demonstrated that *C officinalis* extracts have cytotoxic activity toward different cancer cell lines, but the half maximal inhibitory concentration (IC_{50}) varies widely among the studies, depending on the extraction solvent and method, the plant organs subjected for extraction, and the cancer cell line used for each study.

C officinalis flowers/flos extracts were investigated extensively in the past decade, a wide variety of solvents being used for extraction and numerous cell lines being tested (Table 1). Matysik et al[29] tested the methanol, ethyl acetate, and heptane extracts of *C officinalis* flos against T47D human breast carcinoma cell line and normal human

skin fibroblasts (HSF). After a 24-hour stimulation at a concentration of 75 μg/mL, the ethyl acetate extract had the lowest cytotoxicity against both cell lines, the percentage of viable cells being 70.7% for HSF and 58.4% for T47D. Furthermore, it was observed that higher cell densities were better adapted to higher doses, the authors suggesting that the compounds found in the ethyl acetate fraction might be involved in the wound healing activity. Both methanol and heptane extracts were highly toxic, no HSF cells, and only around 15% of the T47D cells being viable after 24-hour treatment with each extract at a concentration of 75 μg/mL. The cytotoxic activity of the heptane extract was assigned to different triterpenes like β-amyrin, while phenolic acids and flavonoid glycosides were considered responsible for the in vitro effect of the methanol fraction.[29]

Matić et al[30] investigated the selective cytotoxicity of *C officinalis* flower extract against 5 different cancer cell lines (HeLa, Fem-X, MDA-MB-361, LS174, and K562) in comparison with its effect on healthy immunocompetent peripheral blood mononuclear cells (PBMCs). The extraction was carried through infusion in boiled distilled water. The IC_{50} value after a 24-hour treatment for the cancer cell lines varied between 360 μg/mL for the FemX human melanoma cell line and 2300 μg/mL for LS174 human colon carcinoma. Interestingly, the lowest cytotoxicity was found against PBMCs, with an IC_{50} value of 3120 μg/mL. In this context, the pot marigold extract proved to be selective against cancer cell lines when compared with PBMCs, the selectivity coefficient (ratio between IC_{50} for PBMCs–IC_{50} for tumor cells) in the antitumor action being 4.16 for HeLa cells, 8.67 for FemX cells, and 3.59 for K562 cells.

In the attempt to increase the performance of *C officinalis* flower extracts, Jimenez-Medina et al[31] investigated the cytotoxic effects of a novel aqueous laser-activated extract of *C officinalis* flowers (LACE). The extract was obtained by subjecting the flowers to laser therapy at a wavelength of 650 nm for 15 minutes and further on by suspension of the treated plant material in water. The flower suspension was maintained at 4°C for 7 to 15 days, and during this period several additional laser treatments were applied. The in vitro effects of the LACE extract were determined on 12 different cancer cell lines and also on human peripheral blood lymphocytes (PBLs). At a concentration of 250 μμg/mL, the treatment with LACE determined a growth inhibition (GI) of 70% to 100% for all tested cell lines, except the leukemia cell line U937. LACE was found producing significantly higher inhibition of tumor cell proliferation when compared with the regular aqueous extract, yielding similar results as those obtained for the chemotherapeutic drug paclitaxel. After a 96- to 144-hour treatment with 250-μg/mL LACE, cells belonging to several cancer cell lines accumulated in the G0/G1 phase, suggesting a cell cycle arrest in this phase. Furthermore, several proteins like cyclin E and D1, involved in the progression through the G1 phase and entry in the S phase, had decreased expression levels after treatment with LACE, in the same cell lines. Treatment with LACE also induced apoptosis, in some cell lines the caspase-3 protein being activated by the extract. In contrast with the cytotoxic activity of LACE on cancer cell lines, the extract proved to significantly increase the proliferation of PBL cells, with a 3- to 5-fold increase in comparison to the control. Similar results were also obtained with the regular aqueous *Calendula* extract. The PBL subpopulations that proliferated after LACE treatment were mainly B lymphocytes, CD4+ T lymphocytes, and natural killer T lymphocytes.

Although most of the studies were focused on *C officinalis* flower/flos extracts, 2 recent studies have presented data on the effects of other parts of this plant. dos Santos et al[32] studied the cytotoxic effect of leaves extracted in methanol for 51 species from Brazil, and *C officinalis* was proved to be one of the most active plants against tumor cells. The IC_{50} values measured at 72 hours after treatment on 4 different cancer cell lines (HL-60, HCT-8, B16, and MCF7) varied between 50.5 and 83.9 μg/mL, with the highest cytotoxicity against murine skin melanoma (B16 cell line) and the lowest against breast cancer (MCF7 cell line). When the concentration was increased to125 μg/mL, the extract caused a cell GI (%) of more than 90% against all tested cell lines.

Wegiera et al[33] conducted an experiment in which the cytotoxic effects of *C officinalis* roots, herba, and flowers were compared. The plant material was subjected to extraction in methanol, and the effect was quantified against J-45.01 cell line (human acute T leukemia). Surprisingly, the highest cytotoxicity (IC_{50}) was found for the roots extract (230 μg/mL), followed by the flower (330 μg/mL) and herba (410 μg/mL) extracts. After a 24-hour treatment at a concentration close to the IC_{50} value, 35% of the cells were found in an early apoptotic stage and approximatively 15% in a late apoptotic/necrotic stage for the roots and herba extracts. On the other hand, using the same experimental design, 35% of the cells that were stimulated with the flower extract were found in a late apoptotic stage and 15% in an early stage. An increase in both early and late apoptotic cells was reported in a time-dependent manner, with a 10-fold increase in the late apoptotic cells fraction after a 48-hour stimulation with the flowers extract.

Calendula officinalis: In Vivo Toxicity, Chemoprotective Activity, and Anticancer Activity

The in vivo activity of *C officinalis* flower extract was investigated recently regarding toxicity, anti-genotoxic/protective and antitumor activity, as well as anti-metastatic effects, in different animal models (Table 2).

Silva et al[34] investigated the toxicity of the *C officinalis* flowers hydro-alcohol extract, in Wistar rats and in Swiss

Table 2. The In Vivo Activity of *Calendula officinalis* Flowers Extracts; Doses of Administration, the Cellular Effects, and the Mechanisms/Genes Modulated by These Extracts on Specific Animal Models.

Nr Crt	Solvent	In Vivo Activity	Animal Models	Doses (BW)/Treatment Time	Physiological Effects	Mechanisms/Genes Involved/ Activation	Reference
1	Hydro-alcohol	Toxicity	Swiss albino mice	≤5 g/kg	No acute toxicity		32
			Wistar rats	≤5 g/kg	No acute toxicity		
				≤1 g/kg/30 days	No subacute toxicity; no alteration in hematological profile	Renal and hepatic overload: ALT ↑; BUN ↑	
2	LACE	Toxicity	Balb/c, C57BL/6, and CBA mice	Balb/c 55 mg/kg/30 days	No local or systemic toxicity	LD_{50} = 550 mg/kg (day 15)	29
			Wistar rats	Balb/c 550 mg/kg/30 days	No local or systemic toxicity	LD_{50} = 2750 mg/kg (day 15)	
		Antitumoral effect	Nude mice bearing ANDO-2 cell line	50 mg/kg, orally/12 weeks	60% tumor growth inhibition	Survival rate: 75% (day 135)	
				25 mg/kg, intraperitoneally/9 weeks	60% tumor growth inhibition	Survival rate: 60% (day 135)	
3	Hydro-alcohol	Chemopreventive effect	Fischer 344 rats	2.5 mg/kg/7 days before carcinogenesis initiation	Number of AHF: 49% decrease; area of AHF: 55% decrease	GGT ↓	33
		Genotoxic effect	Fischer 344 rats	20 mg/kg/7 days before carcinogenesis initiation	Number of AHF: 42% increase; area of AHF: 46% increase	GGT ↑	
4	Methanol	Chemopreventive effect	Swiss albino mice	10 mg/kg/32 weeks (16 during promotion and 16 after carcinogenesis)	Number of tumors: 15% decrease; tumor size: decreased	Cell proliferation ↓: PCNA ↓, p38 MAPK ↓; apoptosis ↑: p53 ↑; inflammation ↓: NFκB↓, COX-2 ↓; immune surveillance ↑: LC ↑	34
5	Ethanol	Antimetastatic effect	C57BL/6 mice bearing B16F-10 cell line	250 mg/kg/10 days	Life span: 43.3% increase; 74% inhibiton of lung tumor nodes formation	Sia ↓; GGT↓; Lox ↓; P4H ↓; MMP-2 ↓; MMP-9 ↓; TIMP-1 ↑; TIMP-2 ↑;	35

Abbreviations: BW, body weight; ALT, alanine aminotransferase; BUN, blood urea nitrogen; LD_{50}, lethal dose 50%; AHF, altered hepatocyte foci; GGT, γ-glutamyl transpeptidase; PCNA, proliferating cell nuclear antigen; p38 MAPK, p38 mitogen-activated protein kinase; p53, p53 tumor suppressor protein; NFκB, nuclear factor NFκB; COX-2, cyclooxygenase-2; LC, Langerhans cells; Sia, sialic acid; Lox, lysyl oxidase; P4H, prolyl hydroxylase; MMP-2, matrix metalloproteinases-2; MMP-9, matrix metalloproteinases-9; TIMP-1, tissue inhibitor of metalloproteinase-1; TIMP-2, tissue inhibitor of metalloproteinase-2.

albino mice strain. No acute toxicity was recorded after oral administration of the extract in doses up to 5 g/kg body weight in both animal models. No subacute toxicity was observed in Wistar rats after treatment with doses up to 1 g/kg body weight. Several hematologic (erythrocyte count, mean corpuscular volume, different leucocytes count, etc) and biochemical (glucose, triglycerides, proteins, cholesterol quantities, etc) parameters were determined in blood or serum and proved to remain within the reference range after treatment. However, both blood urea nitrogen and alanine aminotransferase were found increased in blood in a dose-dependent manner, suggesting the possibility of renal and hepatic overload.

Based on the in vitro success of LACE extract on different cancer cell lines, Jimenez-Medina et al[31] also investigated the in vivo toxicity of LACE extract in Balb/c, C57BL/6, and CBA mice and also in Wistar rats. After oral administration of 11 and 55 mg/kg body weight LACE extract for 30 consecutive days in all tested mice, no toxicity, local or systemic, was observed. Similar results were obtained for a dose of 11, 55, and 550 mg/kg body weight LACE in Wistar rats. Following the toxicity assays, the in vivo antitumor activity was investigated in nude mice bearing ANDO-2 human melanoma cell line. The mice were administered with 50 mg/kg body weight (orally) and 25 mg/kg body weight (intraperitoneal route) of LACE. The extract showed a tumor GI of 60%, similar result being also obtained with the chemotherapeutic drug Taxol. Furthermore, at 135 days after ANDO-2 injection, the survival rate was 75% in LACE-oral group and 60% in LACE-intraperitoneal group, while no mice were still alive in the control group.

Changing the approach, Barajas-Farias et al[35] evaluated the anti-genotoxic effects of *C officinalis* flowers aqueous-ethanol extract in male Fischer 344 rats in which the carcinogenesis was initiated with *N*-nitrosodiethylamine and promoted with 2-acetylaminofluorene. The in vivo activity of the extract was evaluated based on the area and number of altered hepatocyte foci (AHF) or γ-glutamyl transpeptidase–positive foci in the resistant hepatocyte model in rats. In the control group, in which the animals were treated only with the carcinogens, the area and number of AHF were 5.5% and 17.5 AHF/cm^2, respectively. In the treated group, after the administration of the extract in the range of 0.1 to 2.5 mg/kg body weight, both parameters decreased, with a maximum inhibition of 55% in the area and 49% in the number of the AHF, at a dose of 2.5 mg/kg body weight. On the other hand, when the treatment was administered in doses higher than 20 mg/kg body weight, the extract proved to have genotoxic activity, with an increment of 40% and 53% in the area and number of AHF at a dose of 40 mg/kg body weight. Furthermore, at the same dose of 40 mg/kg body weight, the extract could promote the carcinogenesis but not initiate it. Therefore, the *C officinalis* extract has a

dual dose-dependent effect in vivo, being anti-genotoxic (protective) when administered in low doses but becoming genotoxic in high doses.

Ali et al[36] investigated the chemopreventive effects of a methanolic extract obtained from *C officinalis* flowers in a DMBA-initiated, croton oil-promoted skin carcinogenesis model in Swiss albino mice. The extract was administered at a dose of 10 mg/kg body weight every time the mice were treated with croton oil and further on for 16 weeks, after the promoter application ended. The extract reduced the number, incidence, and multiplicity of tumors on the skin, the average number of tumors per tumor-bearing mouse decreasing with approximatively 15% in the extract-treated group. The tumor size was also reduced, and a normal skin stratification was observed in the treated group. The expression of the proliferating cell nuclear antigen (PCNA) and the cell cycle progression marker p38 MAPK were both suppressed by the extract, suggesting a reduction in tumor cell proliferation in the treated group. Furthermore, higher levels of the p53 tumor suppressor protein were found in the tumors of the extract-treated group when compared with the control tumor-bearing mice; thus, the extract might induce apoptosis. Other relevant effects observed in the *C officinalis*–treated group were reduced skin inflammation, which might be a good chemopreventive target, and enhanced immune surveillance, by retention of the Langerhans cells in the epidermis.

Preethi et al[37] investigated the antimetastatic activity of *C officinalis* flowers extract in C57BL/6 mice injected with B16F-10 melanoma cells. The extract, ethanol based, was orally administrated at a dose of 250 mg/kg body weight for 10 consecutive days. The extract increased the life span by 43.3% among treated animals when compared with untreated tumor-bearing mice. Levels of salicylic acid, often found increased in tumors, and γ-glutamyl transpeptidase, a proliferation marker, were found significantly reduced in the serum of treated animals. At 21 days after injection with B16F-10 cells, the treated group showed reduced number of lung tumor nodes, reduction of tumor size, relatively tumor-free alveoli and bronchioles, and alveolar passage similar to the healthy group, suggesting a lung metastasis inhibition by the *C officinalis* extract. Furthermore, several metastasis markers involved in the synthesis or degradation of extracellular matrix components were differently expressed in treated animals when compared with the untreated group, supporting the histopathological results. Hydroxyproline, a marker for lung fibrosis and hexosamine, a substrate for collagen synthesis, were found reduced in the extract-treated group. Lysyl oxidase (Lox), an enzyme inducing cross-linking in the extracellular matrix, and prolyl hydroxylase (P4H), an enzyme implicated in the synthesis of collagen, were both inhibited by the extract. MMP-2 and MMP-9, matrix metalloproteinases responsible for degrading the basal membrane, and thus

promoting invasion, were found downregulated by the extract. Moreover, the expression of the tissue inhibitors of the metalloproteases TIMP-1 and TIMP-2 was activated. Therefore, the authors conclude that *C officinalis* flower extract increases life span but also inhibits lung metastasis in tumor-bearing mice.

Calendula officinalis in Cancer Palliative Care

Albeit *C officinalis* has demonstrated the cytotoxic and antitumor effects in in vitro and in vivo models, its use in human cancer management is generally limited to the treatment of the secondary effects induced by radiochemotherapy. Palliative care, an important part of cancer management nowadays, is focused on improving the quality of life in cancer patients by treating the symptoms and side effects of the disease and its treatment. With this purpose, more and more cancer patients are using CAM, including herbal remedies,[10] even though little supporting scientific data are available at this moment.[12] Several recent clinical trials have suggested that *C officinalis* extracts could be a relevant resource in diminishing the side effects of radiotherapy in breast, head, and neck cancer patients.

In a simple-blinded phase III randomized clinical trial including 254 breast cancer patients, the liposoluble fraction of *C officinalis* extracted in petroleum jelly was evaluated about its putative role in prevention of acute radiation-induced dermatitis of grade 2 or higher. The prevention capacity was assessed compared with trolamine, a topical agent often prescribed during radiotherapy as part of the breast cancer palliative care. The incidence of grade 2 or 3 skin acute toxicity in the *Calendula*-treated group was 41%, while 63% of the patients treated with trolamine showed mild to severe dermatitis. Furthermore, in the *Calendula*-treated group, no allergic reactions were observed and the extract proved to be more effective in reducing pain among patients. Therefore, *C officinalis* might be a good nonsteroid agent for the prevention of radiation-induced dermatitis in breast cancer patients.[38] Albeit the effectiveness of *Calendula* was better than that of trolamine, it should be noted that the choice of trolamine as a reference was not based on its effectiveness in the treatment of radiotherapy-induced dermatitis, but on the data of the randomized RTOG (Radiation Therapy Oncology Group) study, which suggest its curative properties.[39] Moreover, in a recent meta-analysis, trolamine was found to be ineffective in the prevention and treatment of radiation dermatitis[40]; thus, its usage as a control in such a clinical trial is questionable.

Another randomized, 2-armed, blinded, phase III trial was conducted on 420 patients to compare the effectiveness of 2 different commercial products: the topical *Calendula* cream (Weleda AG, Sweden) and an aqueous emulsion with strong moisturizing and protective qualities (Essex-Schering-Plough), in prevention of acute radiation skin reaction (ARSR) in breast cancer patients. The incidence of severe ARSR was similar in the 2 treated groups, with 23% of the patients presenting severe skin reactions for the topical *Calendula* cream and 19% for the aqueous cream–treated group. Therefore, the skin care product chosen had little effect on radiation-induced dermatitis.[41] Overall, the patients in both groups reported lower levels of skin toxicity (23% and 19%) when compared with the patients included in the previous clinical trial (41% and 63% for the *Calendula*- and trolamine-treated group, respectively). The authors suggest that the decreased levels of the skin reaction symptoms are due to an improved photon therapy and a fewer smokers included in their study. Moreover, the differences between the study design and the number of patients could stand of the bottom of the differences between the data provided by these studies.

Radiodermatitis is a common side effect of radiotherapy, but it occurs more often in patients with head and neck cancer, due to the area of the treatment field. A randomized double-blind controlled clinical trial was conducted on 51 head and neck cancer patients to evaluate the effectiveness of *C officinalis* in the prevention and treatment of radiodermatitis in comparison with a lotion based on essential fatty acids (EFA), an often recommended palliative therapy in head and neck cancer patients. Each product was applied on the skin, twice a day, during the radiotherapy period. *Calendula* proved to be more effective in preventing the development of radiodermatitis, after 15 treatment sessions, the incidence of grade 1 dermatitis being 40.73% for the EFA group and only 25% in the *Calendula*-treated group. Furthermore, after the last session of radiotherapy, the incidence of grade 2 or 3 radiodermatitis was 21.43% for the *Calendula*-treated group, while in the EFA group, it was almost double (46.16%). Although *Calendula* proved to be a better palliative care option than EFA treatment for the prevention of radiation-induced dermatitis in head and neck cancer patients, it has to be mentioned that only 27 patients were finally included for statistical analysis.[42] Furthermore, even though EFA lotions are an often recommended palliative therapy in some clinics,[42] there are no available studies in the literature that prove their effectiveness in the prevention of radiation-induced skin toxicity.

Another relevant side effect of radiotherapy in head and neck patients is the radiation-induced oropharyngeal mucositis (OM), with more than 80% of the patients reporting an inflammation of the oral mucosa during treatment.[43] In this context, a placebo-controlled clinical trial conducted on 40 head and neck cancer patients proved that *C officinalis* flower extract mouthwash significantly decreased the intensity of radiation-induced OM after 2, 3, and 6 weeks of treatment. The authors suggest that the inhibition of OM occurrence is at least partially caused by the extract antioxidant capacity.[44]

Using different referent agents, such as hyaluronic acid *Aloe vera*, corticosteroid cream, commercial emulsion with strong moisturizing and protective qualities, or placebo, makes the data difficult to compare between studies. Moreover, different kinds of study designs including simple- or double-blind randomized studies may introduce sources of bias in treatment assignments. Generally, the studies have partially described the biases risks and how they could be avoided. In a previous study, it was highlighted that trials with inadequate or unclear randomization design lead to overestimation of the treatment effects up to 40% compared with data from trials with proper randomization.[45]

However, even if data from previous studies are not really comparable, it is already stated that *Calendula* could be considered for human cancer management, especially for the treatment of the secondary effects induced by radiochemotherapy. Further studies based on proper randomization design and using high number of patients will certainly establish the efficiency and usefulness of *Calendula* in cancer management.

Conclusions, Challenges, and Perspectives

Calendula officinalis extracts and isolated compounds have revealed a reliable potential in cancer management, both in treatment and in palliative care. Several extracts present significant in vitro selective cytotoxicity toward large panels of cancer cell lines when compared with healthy cells. The cytotoxic activity against cancer cell lines reported for different *C officinalis* extracts varies widely among recent studies (IC_{50} values between 50.5 and 2300 μg/mL). Most of the recent research was conducted on flowers/flos extracts, even though Wegiera et al[33] suggested that root extracts might possess higher cytotoxicity toward cancer cell lines. Accordingly, even if no study presented data on a combination of flower and roots extracts, we consider that this approach could represent a challenge that has to be explored in the near future. Methanol is the most used solvent for extraction. Extracts obtained through infusion in distilled water have lower cytotoxic activities, even though Jimenez-Medina et al[31] reported much better results if the aqueous extract is laser-activated. Nevertheless, the methanolic extract of *C officinalis* flowers did not have selective cytotoxicity toward cancer cell lines when compared with HSFs, while the ethyl acetate extract selectively killed tumor cells in the same experimental design.[29] The aqueous extract obtained from flowers also proved to possess selective cytotoxic activity against different cancer cell lines when compared with healthy PBMCs.[30] Furthermore, the laser-activated aqueous extract proved to have immunostimulant activity, by increasing the proliferation of several PBL subpopulations.[31] As a consequence, different extraction procedures

that use distilled water as the extraction solvent might be better options than extraction in methanol, even though the cytotoxic activity against cancer cell lines is lower for the aqueous extracts.

However, all the IC_{50} values reported for the *C officinalis* extracts are rather high, none of them being smaller than 20 μg/mL, the standard threshold concentration used in conventional drug discovery studies, according to the US National Cancer Institute.[46] In this context, the required dose for effective cytotoxicity in vivo, based on the in vitro results, would be most probably higher than the safe dose. Furthermore, achieving in vivo plasma concentration higher than 20 μg/mL for extracts is difficult, leading to limitations in the uptake of the active compounds.[47] Therefore, the chances that singular extracts from *C officinalis* to be used as a treatment option in clinical settings are greatly diminished. However, the required doses for effective cytotoxicity in vivo might be decreased by using synergistic combinations with other plant formulas, a highly recommended approach for drugs isolated from plants.[47] Further in vitro investigations are needed to assess the synergistic effect of *C officinalis* extracts with other known anticancer plant compounds.

C officinalis extracts proved to have no or low general toxicity in animal models, while they possessed chemopreventive, antitumor, and antimetastatic activity in vivo. Research conducted to date has emphasized different *C officinalis* extracts without in vivo toxicity when used for oral administration from 1 to 5 g/kg body weight in mice and rat models, respectively,[34] or up to 55 mg/kg body weight in mice and 550 mg/kg in rats.[31] Nevertheless, the posttreatment possibility of hepatic and renal overload was suggested.[34] Both aqueous-ethanol[35] and methanol[36] extracts of *C officinalis* flowers proved to have chemopreventive/anti-genotoxic activities in 2 different carcinogenesis models, at much lower effective concentrations than the toxicity threshold concentration identified in all other studies.[29,32] However, Barajas-Farias et al[35] suggest that the extract not only becomes genotoxic if administrated in high doses but also could replace the carcinogenesis promoter. The in vivo antitumor cytotoxic activity was proven in nude mice bearing ANDO-2 human melanoma cell line, in which the LACE extract inhibited the tumor growth by 60% and significantly extended the animal's life span.[31] Moreover, another *C officinalis* flowers extract increased the life span by 43% in lab mice injected with B16F-10 melanoma cells and possessed antimetastatic activity in these tumor-bearing mice.

Only 3 compounds were individually identified in *C officinalis* extracts to possess cytotoxic activity against different cancer cell lines. However, there is a high possibility for other constituents, mainly terpenoids and polyphenols, to be further identified as antitumor agents. Lutein was found to have selective cytotoxicity toward breast cancer cell

lines, by inducing apoptosis in these cells.[27] Other 2 triterpene glycosides were identified as highly cytotoxic against a large panel of cancer cell lines in vitro.[28] According to the US National Cancer Institute plant screening program, for a compound to be considered cytotoxic toward cancer cell lines, the IC_{50} values have to be <10 μM.[46] Therefore, at least the calenduloside F 6'-*O-n*-butyl ester compound shows promising results as a potential anticancer drug.

Finally, *C officinalis* extracts might be good alternatives to current palliative treatments, which aim to diminish the side effects of radiotherapy. Two phase III randomized clinical trials, totalizing 674 breast cancer patients, tested the effectiveness of *Calendula* extracts in the prevention of acute radiation-induced dermatitis.[38,41] In the first one, the extract proved to be superior in its activity when compared with trolamine.[38] However, in a recent study, trolamine proved to be ineffective in the prevention of radiotherapy-associated dermatitis.[40] In the second clinical trial, Sharp et al[41] found no differences in the prevention of ARSR when comparing the standard *Calendula* cream and a topical aqueous cream often prescribed during radiation treatment. None of these studies use a vehicle-controlled placebo; thus, the effectiveness of the *Calendula*-based treatments is not actually assessed. Taking into consideration these conflicting data and the absence of the placebo groups from the studies, *Calendula* products might be good alternatives to the topical palliative treatments of radiation-induced dermatitis in breast cancer patients, but further investigations regarding their efficacy are necessary.

Two other clinical trials suggest that different *C officinalis* extracts are effective in the prevention and treatment of radiodermatitis[42] and of radiation-induced OM[44] in head and neck cancer patients. The first extract showed better results in preventing dermatitis than the EFA treatment.[42] However, there are no data available at this moment to prove the effectiveness of EFA lotions in preventing radiation-induced OM. Furthermore, no placebo group was used in this clinical trial; thus, issues regarding the actual effectiveness of the *Calendula* extract are raised again.

In conclusion, *Calendula officinalis* shows promising results regarding its potential usage in cancer management, especially in cancer prevention, treatment, and in palliative care. However, without knowing the bioactive constituents responsible for the in vitro and in vivo selective cytotoxicity as well as for the prevention of radiotherapy-induced side effects, moving forward to relevant preclinical trials is hampered. Therefore, intensive research is essential toward identifying novel *C officinalis* constituents, which might become relevant resources in cancer management.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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