Chapter 5

Natural Products as Cytotoxic Agents in Chemotherapy against Cancer

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Additional information is available at the end of the chapter

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Abstract

Nature continues to produce a great wealth of natural molecules endowed with cytotoxic activity toward a large panel of tumor cells. Some of these molecules are used in chemotherapy, and others have shown great anti-tumor and anti-metastatic potential in preclinical trials. This review discusses some examples of these molecules that have been studied in our laboratory and others. We report a differential cytotoxic activity of some monoterpenes (carvacrol, tymol, carveol, carvone, and isopulegol) against a panel of tumor cell lines. The carvacrol was the most cytotoxic molecule both *in vitro* and *in vivo* as demonstrated by preclinical studies using the DBA2/P815 mice model. On the other hand, polyphenols were also studied with respect to their cytotoxic effects. Interestingly, these compounds showed a prominent cytotoxic activity toward a panel of cancer cells with differential molecular mechanisms. In addition, we report a very strong antitumor efficacy of artemisinin, a sesquiterpen lactone from *Artemisia annua*, together with an antimetastatic potential as demonstrated by preclinical experiments. Furthermore, some of the molecular mechanisms involved in these effects are described.

Keywords: natural products, monoterpenes, polyphenols, artemisinin, cytotoxicity

1. Introduction

Natural drugs have formed the basis of traditional medicine systems that have been used for centuries by different cultures [1]. An immense number of these natural sources and their isolated components have demonstrated beneficial therapeutic effects, such as anticancer, antioxidant, immunomodulatory, antimicrobial, and anti-inflammatory properties [2, 3].

Studies reported that plant-derived drugs represent about 25% of the American prescription drug market [4]. Also, natural products play an important role in the health care of 20% of the world's people who mainly reside in developed countries and 119 chemicals compounds, derived from 90 plant species, can be considered as important drugs in many countries [5]. Based on a recent review, from 79 Food and Drug Administration anticancer and antiviral approved drugs from 1983 to 2002, 9 of them were isolated directly from plants and 21 among them were natural-products-based drug. Furthermore, between 39 conventional anticancer molecules, 13 of them were derived on a pharmacophore obtained from natural drugs [5, 6]. Actually, nature continues to be an attractive source of new molecules discovery due to important chemical diversity of the thousands of plant, animal, marine organisms, and micro-organism species. Today, about 60% of drugs are of natural origin [7] (**Tables 1–3**).

Several molecules used as conventional chemotherapy are of natural origin. Some of these molecules and their use are described in **Tables 2** and **3**.

Drug	Utilization	Mechanism of action	Source
Aspirine	Analgesic, anti-inflammatory, anti-pyrtic	Inhibition of cyclo-oxygenase	Plant
Atropine	Pupil dilatator	Anti-cholinergic on muscarinic receptors	Plant
Cafeine	Stimulating	Antagonist of adenosine receptors	Plant
Codeine	Analgesic, anti-tussive	Antagonist of opoide receptors	Plant
Digoxine	Cardiotonic	Inhibition of membrane pump N^+/K^+ ATPase	Plant
Eugenol	Touth pain	Reduction of sensorial nerve excitability	Plant
Morphine	Analgesic	Antagonist of opoide receptors	Plant
Pilocarpine	Glaucoma	Antagonist of muscarinic receptors	Plant
Quinine	Prophylaxis of malaria	Inhibition of protein synthesis	Plant
Taxol	Anticancer	Antimitotic	Plant
Penicilline	Antibiotic	Inhibition of cell membrane	Micro-organism
Tatracycline	Antibiotic	Inhibition of protein synthesis	Micro-organism
Cyclosporine A	Immunosuppressor	Inhibition of lymphocytes T proliferation	Micro-organism
Aurantosides	Antifungal	Inhibition of tubulin polymerization	Marine organism
Spongistatine 1	Antifungal	Inhibition of tubulin polymerization	Marine organism
Manoalide	Analgesic, anti-inflammatory	Inhibition of phospholipase A2	Marine organism

Table 1. Some natural drugs derived from plants, micro-organisms, or marine organisms [8].

Drug	Utilization
Actinomycine	Germinale cells tumor, sarcoma
Bléomycine	Cervix cancer, Germinale cells tumor, and neck
Daunomycine	Leukemia
Doxorubicine	Lymphoma, breast, lung and ovarian cancer, sarcoma
Epirubicine	Breast cancer
Idarubicine	Leukemia and breast cancer
Mitomycine C	Colorectal, gastric, anal, and lung cancer
Streptozocine	Gastric and endocrine tumors

Table 2. Some anticancer drugs derived from micro-organisms [8].

Drug	Utilization	Mechanism of action
Citarabine	Leukemia, lymphoma	Inhibition of DNA synthesis
Bryostatine 1	Experimental phase	Activation of protein kinase C
Dolastatine 10	Experimental phase	Inhibition of microtubules and pro-apoptotic effect
Ecteinascidine 743	Experimental phase	Alkylation of DNA
Aplidine	Experimental phase	Inhibition cell cycle progression
Halicondrine B	Experimental phase	Interaction with tubuline
Discodermolide	Experimental phase	Stabilization of tubuline
Cryptophycine	Experimental phase	Hyperphosphorylation of Bcl-2

Table 3. Some anticancer drugs derived from marine organisms [8].

2. Phytotherapy and cancer

2.1. Generalities

There is a numerous plants involved in the prevention and/or treatment of cancer. As for other diseases, many anticancer drugs are derived from plants (**Table 4**). Studies reported that more than 200 drugs are of herbal origin. The vinca-alcaloids and the taxans are the main groups, which occupy an important place in anticancer chemotherapy.

2.2. Examples of natural products with important cytotoxic activity

2.2.1. Cytotoxic activity of some natural monoterpenes

The chemical composition of plant-extracts is known for being very rich and diversified. Thus, a single extract may contain more than hundreds of interactive biomolecules [9]. Therefore, finding and discovering those responsible for the biological Activity become essential. Many monoterpenes, such as eugenol, have been described in the literature to have

Drugs	Utilization
Vincristine	Leukemia, lymphoma, breast cancer, and lung cancer
Vinblastine	Lymphoma, kidney cancer, germinal cells cancer, and breast cancer
Paclitaxel	Breast cancer, ovarian, lung, and d'ovaire, de poumon, bladder, and neck cancer
Docetaxel	Breast and lung cancer
Topotecan	Ovarian and lung cancer
Irinotecan	Colorectal and lung cancer

Table 4. Anticancer drugs derived from plants [8].

a wide range of important biological activities [10]; it possesses *in vitro* and *in vivo* antiviral activity against human herpesvirus [11]. Carvone promoted protection of 75–87.5% against convulsions at 300–400 mg/kg [12]. Isopulegol and carvone showed significant bactericidal and fungicidal activities [13]. Also, the combination of these molecules between them or with conventional molecules could have a synergistic effect [14, 15]. Furthermore, carvacrol, extract of thyme essential oil, is one of natural products with important biological activities. It has been reported to have an important antitumor effect [9, 16]. Here, we present a summary of our findings [17] on the cytotoxic activity as well as their molecular mechanisms of six natural monoterpenes compounds (carvacrol, thymol, carveol, carvone, eugenol, and isopulegol).

2.2.1.1. In vitro cytotoxic effect of the products against a panel of target cells

The antitumor activity of the products was evaluated against the following five tumor cell lines: P-815, K-562, CEM, MCF-7, and MCF-7 resistant to gemcetabine (MCF-7-gem). The results are summarized in **Figure 1**, which shows that the cytotoxic effect depends on the nature of the products as well as on the target cell lines. In general, the effect of the products is dose-dependent. Moreover, the cytotoxic activity of carvacrol, thymol, carveol, carvone, eugenol, and isopulegol is more important against P-815 and CEM tumor cell lines compared to the other tested cell lines. The carvacrol is the most cytotoxic compared to other compounds. Against P-815, K-562 and CEM cancer cell lines, eugenol, carveol, and carvone exhibit also a strong cytotoxic activity. The IC_{50} values are ranging from 0.09 to 0.24 μM (**Table 5**). Nevertheless, those compounds showed a less effect toward MCF-7 and very lowest one against MCF-7-gem cancer cell lines as demonstrated by the IC_{50} values ranging from 0.26 to 0.87 μM. Comparing the activity of thymol and isopulegol on the tumor cell lines studied, it shows that P-815 is the most sensitive with an IC_{50} = 0.15 and 0.09 μM, respectively. Importantly, acquired resistance to gemcetabine by MCF-7 cell line was linked with a development of resistance to thymol, carveol, carvone, and eugenol but not to isopulegol or carvacrol (**Table 5**).

2.2.1.2. Synergy

Our results demonstrate that the combination of natural monoterpene with MTX or Cis showed a synergistic effect at used concentrations (IC_{20}) of each tested molecules (monoterpenes, cisplatine, and methotrexate). The interactions between these molecules exhibit a cell lysis ranging between 53 and 62%. Furthermore, a slight cytotoxicity was shown after the combinations between monoterpene-cisplatin and monoterpene-methotrexate (**Table 6**).

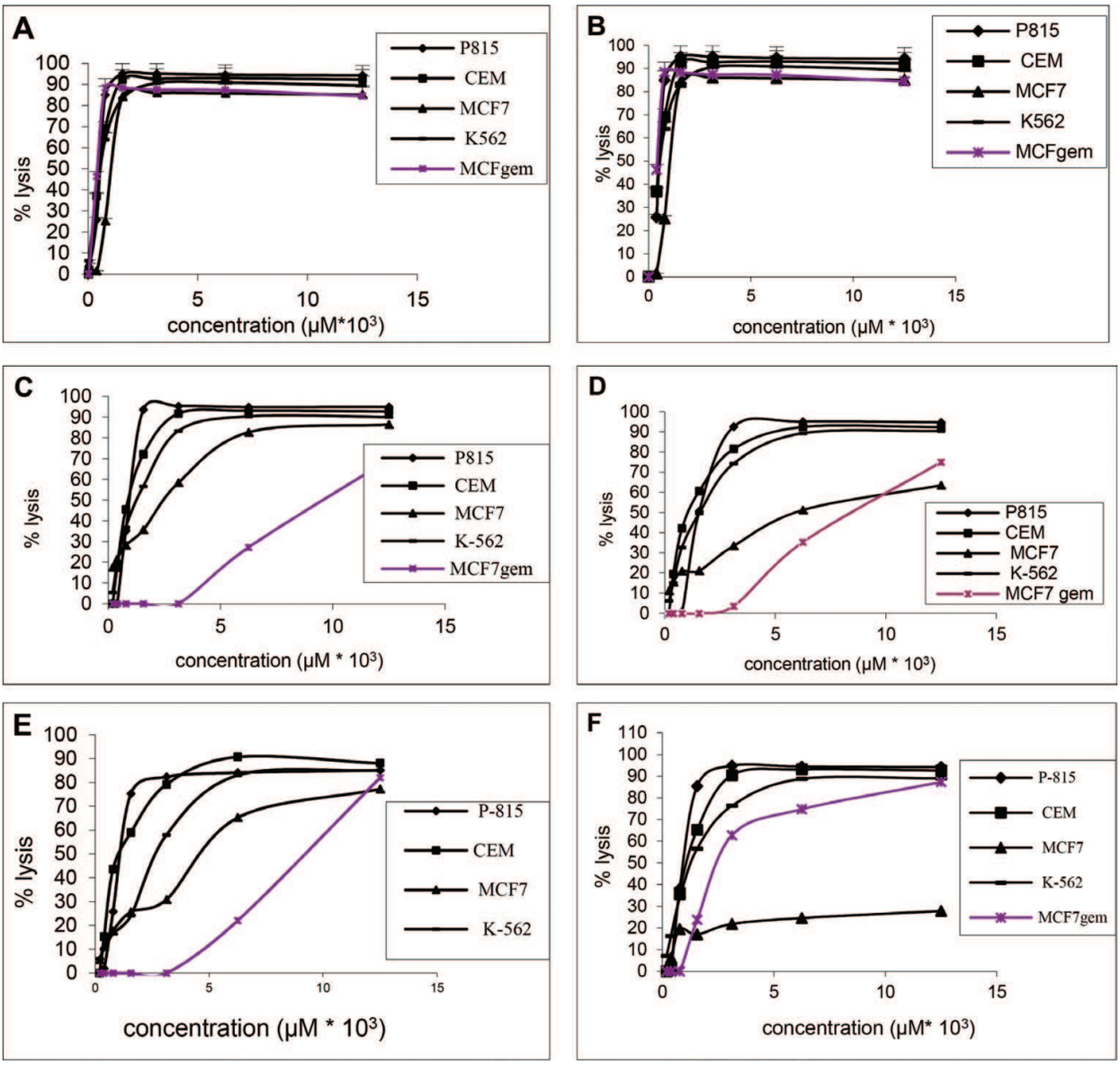

Figure 1. Cytotoxic effect of carvacrol (A), thymol (B), carveol (C), carvone (D), eugenol (E) and isopulegol (F) against different tumor cell lines: P815 (♦), CEM (■), K562 (_), MCF-7 (▲) and MCF-7 gem (*).

Product	P815	CEM	K-562	MCF-7	MCF-7/gem
Carvacrol	0.067	0.042	0.067	0.125	0.067
Thymol	0.15	0.31	0.44	0.48	—
Carveol	0.11	0.11	0.13	0.26	0.45
Carvone	0.16	0.11	0.17	0.63	0.91
Eugenol	0.10	0.09	0.24	0.41	0.87
Isopulegol	0.09	0.11	0.13	—	0.25

Table 5. IC_{50} (μM) of the tested monoterpenes against different target cell lines.

Combination	Fa	CI
C-MTX	54.9	0.17
C-Cis	56.6	0.01
T-MTX	61	0.14
T-Cis	57.6	0.01
Cl-MTX	53.3	0.17
Cl-Cis	57.9	0.01
Cn-MTX	51.2	0.17
Cn-Cis	58.5	0.01
E-MTX	58.6	0.15
E-Cis	55.9	0.01
I-MTX	58.5	0.15
I-Cis	62.3	0.01

Table 6. Affected fraction (Fa) and combination index (CI) of molecule combinations.

2.2.1.3. Effect of carvacrol, thymol, carveol, carvone, eugenol, and isopulego on the cell cycle progression

At the molecular level, carveol- and carvacrol treatment-induced cell cycle arrest in S phase. Nevertheless, thymol and isopulegol stopped it in G0/G1 phase. Regarding the eugenol and carvone, they have no effect cell cycle progression (**Figure 2**).

2.2.1.4. In vivo antitumor effect of carvacrol

Our experimental model was based on the use of the P-815 tumor-bearing DBA-2 mice to investigate the cell-killing induced by carvacrol. Experiments were carried out by oral administration (gavage) of carvacrol dissolved in vegetal oil to 6- to 8-week-old DbA-2/6 mice (6 mice for each group) (Orleans, France) weighting 18–22 g for 7 days. The tumor volume was measured for up to 30 days. The tumor volume at day n, (Tvn) was calculated using the formula: $Tv = (l \times W^2)/2$, where l equals the length of the tumor and W the width, as described by Yoshikawa [18]. Interestingly, during the first 18 days, there was no statistical difference ($p < 0.94$) in the volumes of the tumors in all the groups of mice, including the control group (0.4–0.5 ± 0.1 cm^3). Nevertheless, after 18 days, the tumor volume was reduced for the treated groups; this decrease occurred more rapidly in the group "C" who received 100 mg/kg/day than the group "B" treated with 50 mg/kg/day ($p < 0.05$ at day 21th). Compared to untreated group, the tumor volume increased quickly reaching 1.5 cm^3 at 23rd day. Furthermore and importantly, the tumor volume reduction was accompanied by a notable increase of mice survival (**Figure 3**). The antitumor activity of carvacrol has not been has not been

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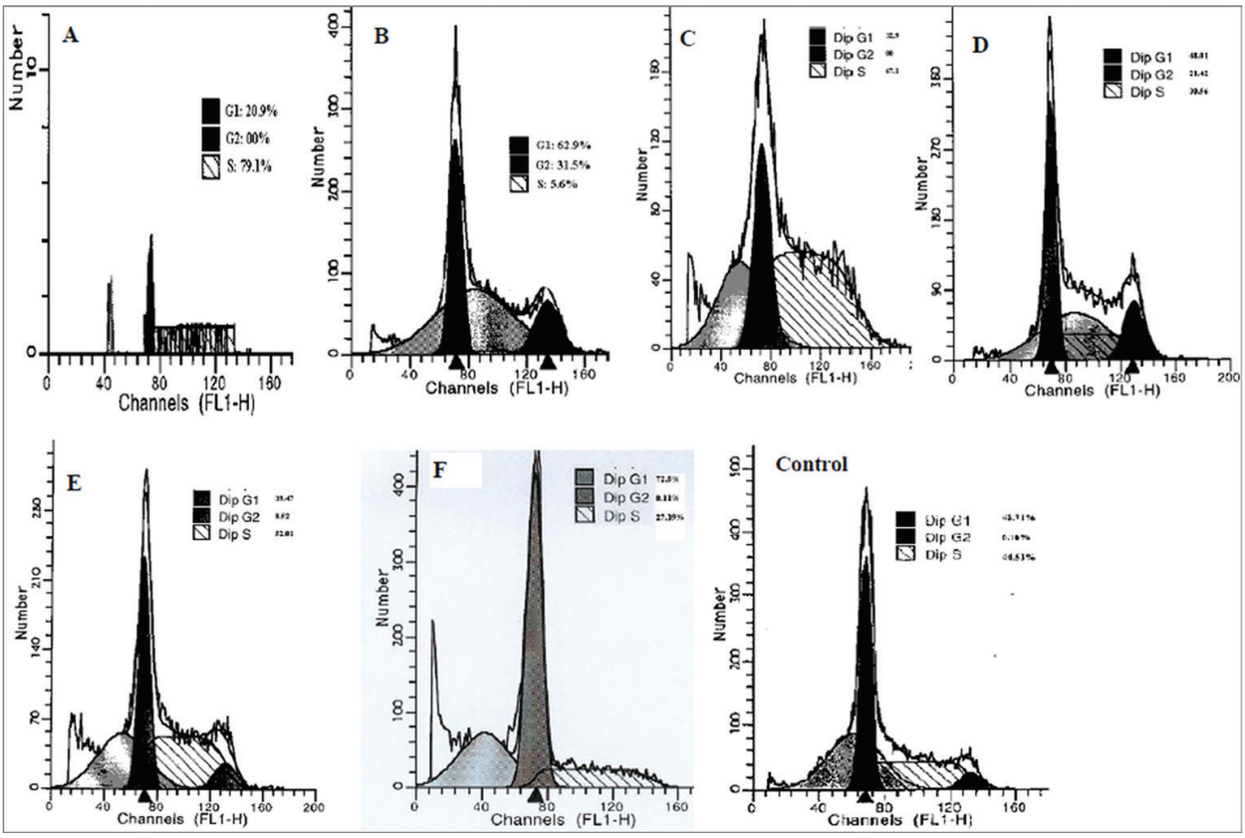

Figure 2. Effect of the tested products on cell cycle progression. The samples were analyzed using a FACStar plus flow cytometer and the WinMDI software. Results are the mean ± SEM of three tests. (A) Carvacrol, (B) thymol, (C) carveol, (D) carvone, (E) eugenol, and (F) isopulegol.

widely discussed in the literature. At the best of our knowledge, this is the first study that reported the oral administration of carvacrol for successive 7 days significant decrease tumor volume, body weight loss, and delayed mortality (data not shown). These results corroborate those published by Karkabounas who demonstrated that carvacrol exhibited 30% reduction of 3,4-benzopyrene carcinogenic activity *in vivo* [19].

Studies were carried out by gavage of carvacrol dissolved in vegetable oil to mice (6–8 week-old) for 7 days. Group "A" (untreated) treated with 100 μl/day of vegetal oil only. Groups "B" and "C" received 50 and 100 mg/kg/day of carvacrol dissolved in 100 μl of vegetal oil, respectively. Mice were weighted and the tumor volume was calculated by measurement of the width (*W*) and the length (*l*) for three times a week up to day 30. The tumor volume at day *n* (*TVn*) was measured using the following formula: $TV = (l \times W^2)/2$. The experiments are the mean ± SEM of two tests.

2.2.1.5. Discussion

Monoterpenes (carvacrol, thymol, carveol, carvone, eugenol, and isopulegol) have been found to exert antitumor effect. In fact, eugenol was described to exhibit cell death by apoptosis in

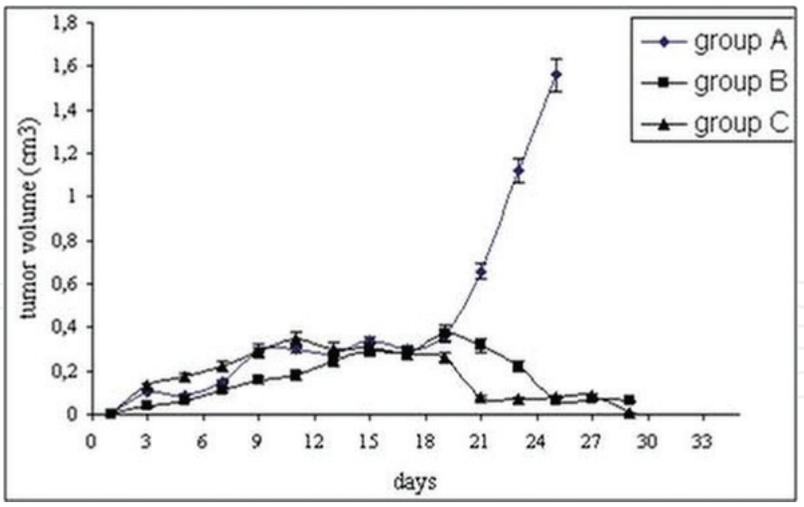

Figure 3. *In vivo* antitumor effect of carvacrol.

mastocyte [20] and melanoma cells [21]. Also, it has been demonstrated not to be mutagenic neither carcinogenic [22]. Carveol has chemopreventive activity against mammary cancer when fed during the initiation phase [23]. Carvone prevents chemically induced lung and for stomach carcinoma development [24]. Carvacrol and thymol significantly reduced the level of DNA damage induced in K-562 cells by the strong oxidant H_2O_2 [25]. Furthermore, carvacrol has an important *in vitro* antitumor effect against tumor cell lines like Hep-2 [26] and A-549 [16, 27]. As shown in **Table 5**, the monoterpenes studied induced a differential cytotoxic activity against a panel of tumor cell lines. P-815 and CEM cell lines are the most sensitive targets to all tested molecules. Although the effects of these products are dose-dependent, the carvacrol is the most cytotoxic molecule as revealed by the IC_{50} values (**Table 5**). Importantly, unlike isopulegol and carvacrol, the acquired resistance to gemcitabine by MCF-7 tumor cell line was associated with a resistance to thymol, carveol, and carvone. Taken together, these results suggest that these compounds could have a similar pathway. The differential sensitivity of the studied monoterpenes toward MCF-7 and MCF-7-gem could be linked to the expression level of ribonucleotide reductase subunit R1 [28]. Furthermore, the cell cycle analysis showed that carveol- and carvacrol treatment-induced cell cycle arrest in S phase when thymol and isopulegol stopped it in G0/G1 phase. Nevertheless, the eugenol and carvone have no effect cell cycle progression. These results suggest that the molecular mechanistic pathway of the cytotoxicity exhibited by those molecules is more complicated and is not related only with the cell cycle. It was reported that monoterpenes decreased expression of cyclin-dependent kinase cdk4, cyclin D1, and cdk2 and increased expression of cyclin E and cdk inhibitor p21 [29]. Furthermore, geraniol, farnesol, and isoprenoids perillyl alcohol exhibited a G0/G1 cell cycle arrest by increasing in the expression level of p27 (Kip1) and the cyclin kinase inhibitor proteins p21 (Cip1) and a decreasing in cyclin B1, cyclin A, and cyclin-dependent kinase (Cdk2) expression [14]. Interestingly, our results demonstrate that the interaction of tested monoterpenes at lowest concentration (IC_{20}) with the conventional anticancer molecules (cisplatine and methotrexate) exhibited a synergistic activity (**Table 6**). Thus, this combination may reduce the toxicity of the conventional chemotherapy drugs by reducing their doses.

These results are supported by previous findings reporting that when combined to isoprenoids perillyl alcohol, farnesol, and geraniol showed an additive antiproliferative activity against the human pancreatic cancer cell line MIA PaCa-2 [14]. Also, Chander et al. reported that in chemotherapy of breast tumors, the combination of limonene, natural monoterpene, and 4-hydroxyandrostenedione, inhibitor of aromatase, was more effective than each drug used alone [30]. Interestingly, in our study, we reported a synergistic effect and not an additive one suggesting that only low doses of each monoterpene combined with tolerable low doses of methotrexate or cisplatine (IC_{20}) showed an important effect (60% lysis).

2.2.2. Polyphenols: a potent cytotoxic molecules

Natural polyphenols have received increasing interest in the human health due to their benefit effects against several diseases attributed particularly to their antioxidant activity [31]. Beside their well-known and effective antioxidant activity [32, 33], several polyphenols shown a high cytotoxic effect against cancer cell lines through targeting cellular and molecular processes involved in cancer progression and metastasis. The antitumor potential of these active ingredients is due to their effect as modulators of oxidative stress [34], apoptosis inducers [35] cell proliferation inhibitor [36], tumor cell cycle blockers [37], and angiogenesis/metastasis suppressors [38]. These bioactive compounds have shown promising antitumor properties in both *in vitro* and *in vivo* interventions [39, 40]. These structural variations may be responsible for their various health benefits, including antioxidant [41], and anti-proliferative mechanisms, as well as regulation of key signaling protein and enzyme functions [42], and as promising immunostimulating effect on normal immune cells [43]. The relationship among natural polyphenols, antitumor activity, and cancer was identified by various studies on the ability of these compounds to act as cancer chemopreventive and/or chemotherapeutic agents [44]. In this purpose, a variety of natural polyphenols have been identified to interfere with carcinogenesis particularly through apoptosis induction and the modulation of oxidative stress [45, 46].

2.2.2.1. Polyphenols and apoptosis induction

Large number of studies has focused on the ability to introduce apoptosis on cancer therapy under cellular control conditions [47, 48]. The intrinsic and extrinsic molecular pathways involved in the regulation of the apoptotic process have recently been evaluated and give promising results. Several proapototic receptors have been selectively developed activating the intrinsic pathway, particularly including the antiapoptotic proteins, the Bcl-2 family proteins, and the p53 signaling pathways [49–51]. In this purpose, polyphenols could inhibit tumor cell proliferation via the programmed cell death (apoptosis) using both intrinsic and extrinsic cell pathways. As reported, polyphenols such as EGCG: (−)-epigallocatechin-3-gallate, resveratrol, naringenin, quercetin, hydroxytyrosol, and curcumin, through different intrinsic signaling pathways from mitochondrial intermembrane space, may inhibit NF-κB-dependent signal related to proliferation and survival [52], cause cell cycle arrest through upregulation of p53 [53], stabilize and activate the tumor suppressor gene p53 [54], and downregulate the expression of Bcl-2, and Bcl-XL anti-death proteins, favoring apoptosis induction via the activation of multiple caspases activity and cytochrome-c (cyt-c) [55, 56]. These polyphenols have

been shown to promote apoptosis in different cancers particularly breast, lung, prostate, leukemia, colon, cervical, or melanoma [57, 58]. In breast cancer cells, naringenin demonstrated anti-estrogenic activity in estrogen-rich status and estrogenic activity in estrogen-deficient status [59]. Additionally, few early studies suggested that gavage of polyphenols in green tea (EGCG), even at low doses, prevented colon carcinogenesis by inhibiting metastasis and angiogenesis through apoptosis induction [60]. Few years ago, our research group has published an article [61] on natural polyphenols extracted from olive mill waste (OMW) and their implication in anticancer activity, where the *in vitro* cytotoxic and apoptotic assays involving several phenolic compounds found in those specific phenolic extracts (particularly including, quercetin, naringenin, apigenin, hydroxytyrosol, oleuropein, and its derivatives) have been discussed. The *in vitro* cytotoxic effect of olive mill waste extracts was evaluated using the MTT assay (methyl tetrazolium test). The IC_{50} values ranged from 4.8 to 7.6 μg/ml (**Table 7**), which demonstrate an effective cytoxicity of these phenolic compounds at low doses. We have demonstrated that the cytotoxic potential of these phenolic extracts was exhibited via apoptosis induction by DNA fragmentation test using agarose gel electrophoresis (**Figure 4A**). DNA isolated from MCF7 tumor cells was treated with OMW extracts at concentrations corresponding to the IC_{50} values and incubated for 24 h. To confirm the cell-death mechanism of these natural extracts, the apoptosis analysis was performed using the Annexin V biotin-streptavidin FITC test. We reported that phenolic extracts induced significantly apoptosis (**Figure 4B**) compared to untreated cells (**Figure 4C**). Interestingly, those polyphenols have not shown any cytotoxic effect against human normal cells (PBMC) (**Figure 5**). Hence, it triggered apoptosis in a dose-dependent manner on a breast cancer cell line (MCF-7) without any effects on normal cells by enhancing the viability with 12–16% in 48 h, compared to methotrexate (conventional cytotoxic drug), which suppressed 20% viability of these cells.

Taken together, these data showed the differential and selective cytotoxic effect of natural polyphenols. In this sense, Miccadei et al. [43] have shown that polyphenolic extracts from the edible part of artichoke (*Cynara scolymus* L) may selectively inhibit the growth of human hepatoma cells with little or no toxicity against normal hepatocytes cells based on their differential redox status. Interestingly, the authors have shown that Artichoke extracts exhibit a pro-oxidant activity in breast cancer cells and an antioxidant effect on normal hepatocytes. Moreover, some flavanols may have a significant effect on cytokine release from both unstimulated and lipopolysaccharides-stimulated PBMCs [62]. Oral administration of naringenin suppressed breast cancer metastases after surgery by modulating the host immunity [63].

2.2.2.2. Role of polyphenols in therapy-induced senescence

Cellular senescence is a physiological process of irreversible cell-cycle arrest that contributes to various physiological and pathological processes of aging. It is an alternative and a novel

Samples	S1	S2	S3	S4	S5
IC_{50} (μg/ml)	6.95 ± 0.15	5.3 ± 0.1	4.75 ± 0.05	7.75 ± 0.15	5.3 ± 0.2

Table 7. IC_{50} values of the cytotoxicity of OMW polyphenolic extracts against MCF-7 breast cancer cell line.

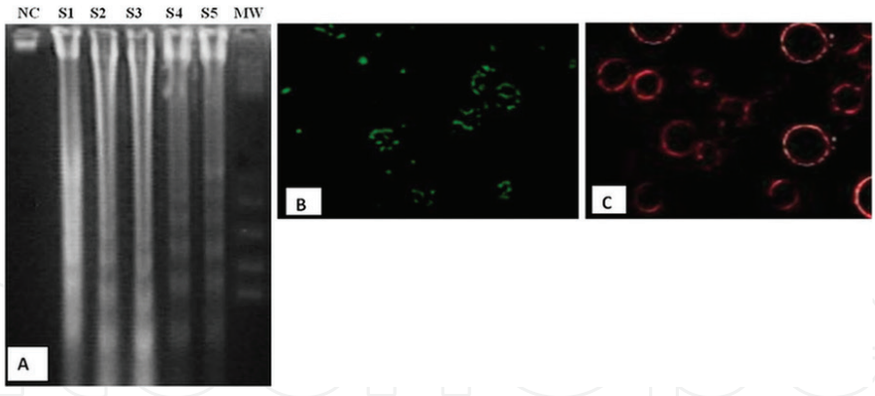

Figure 4. Apoptosis-induction analysis in MCF-7 cell line. (A) Phenolic extracts induced DNA fragmentation was detected by agarose gel electrophoresis of DNA isolated from MCF-7 tumor cells. Cells were incubated for 24 h with OCE (corresponding to IC_{50} concentrations). S1 to S5: Olive mill waste extracts samples. Positive control (WM): DNA weight marker. DNA of untreated cells was used as negative control (NC). (B) Annexin V biotin-streptavidin FITC test. MCF-7 tumor cells (2×10^6 cells) were treated with 25 μg/ml of OCE and incubated for 24 h. The assay is based on the ability of Annexin V (green fluorescence) to bind to the phosphatidylserine exposed on the surface of cells undergoing apoptosis. Cells cultured in a medium without serum were used as a positive control (C).

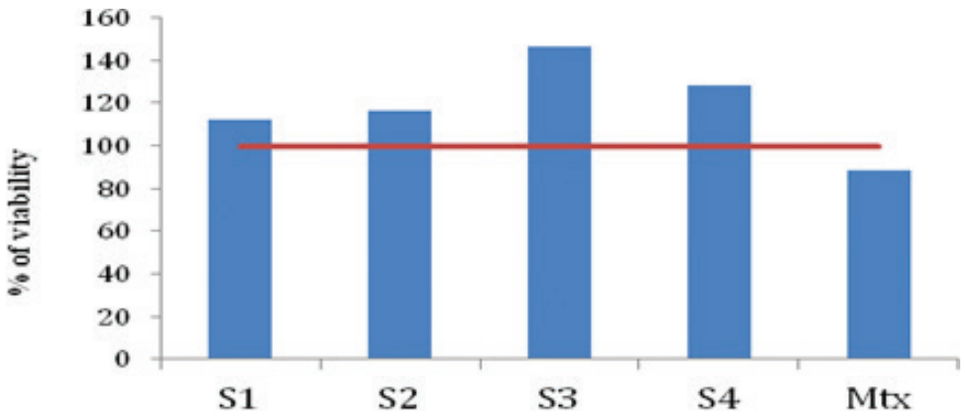

Figure 5. Cytotoxic effect of OMW polyphenolic extracts on normal human peripheral blood mono mononuclear cells (PBMC) from normal donors.

therapeutic strategy to the cytotoxic treatment which leading to cytostasis approach target for aging and aging-related diseases [64]. Although senescence cells have irreversibly lost their capacity for cell division, they are still viable and remain metabolically active [65]. Prosenescence is usually associated with telomere erosion after repeated cell divisions and occurs in response to abnormal oncogenic signaling, oxidative stress, and DNA damage [66]. To this purpose, natural compounds targeting the epigenetic control of senescence are under investigations to develop additional prosenescence cancer therapeutic strategies [67]. Several anticancer polyphenolic compounds from fruit and vegetables have been shown to be potential chemopreventive and anticancer bioactive compounds [68] to induce cellular growth arrest through the induction of a ROS-dependent premature senescence. Among them, 20(S)-ginsenoside Rg3, a compound extracted from ginseng, and bisdemethoxycurcumin, a natural derivative of curcumin, caused senescence-like growth arrest and increased ROS production, respectively, in human glioma

cells [69] (and human breast cancer cell [70]. Therefore, high doses of polyphenolic extracts from artichoke may induce apoptosis and decrease cell proliferation of the human breast cancer cell line, MDA-MB231 via the induction of premature senescence through epigenetic and ROS-mediated mechanisms [71]. Importantly, the authors have shown that Artichoke extracts have a pro-oxidant activity in breast cancer cells [72] and an antioxidant effect on normal hepatocytes [43]. Therefore, it has been hypothesized that Polyphenolic artichoke extracts could selectively inhibit the tumor cells growth with no cytotoxicity on healthy cells related on their differential cellular redox status. Furthermore, treatment with a low dose of resveratrol exhibits its chemopreventive and anticancer activities by induction of premature senescence in lung cancer cells. This event is associated with an increase in ROS generation and DNA double strands break through the up-regulation of NAPDH oxidase-5 expression [73]. The inhibitory effect of resveratrol was verified *in vitro* and *in vivo*, respectively, on gastric cells cancer and nude mice xenograft model. Low doses of resveratrol treatment arrested gastric cancer cells in the G1 phase and led to senescence instead of apoptosis and exerted inhibitory effect on gastric development and significantly decreased the fraction of Ki67-positive cells in the nude mice tumor specimens [74]. Interestingly, Resveratrol and quercetin administers in subapoptotic doses can induce senescence-like growth arrest in glioma tumors treatment [75]. The concept of prosenescence therapy has emerged over the past few years as a novel therapeutic approach to treat cancers, which may be viewed either as an independent anticancer approach or as a combined strategy with conventional chemo/radiotherapy [76]. In a neoadjuvant setting, prosenescence therapy could be also used with traditional treatments in order to reduce tumor mass before surgery; whereas in adjuvant therapy, the engagement of prosenescence could be helpful in reducing the statistical risk of cancer relapse [77]. Although the effective potential of polyphenol in anticancer therapy as well as their other various beneficial effects on human health, the poor bioavailibility of these active ingredients still a pending issue which limits their potential effects and their incorporation on western medicine. Further aimed challenging studies are needed to improve the absorption, distribution, and metabolism in order to develop the *in vivo* use and in clinical interventions.

2.2.3. Artemisinin: a cytotoxic molecule with medical interests

Artemisinin, the active component of Qinghao (Chinese name of *Artemisia annua* L.) was discovered in 1972 by Professor Tu's team [78], a discovery that was recognized by her receipt of the Nobel Prize in medicine in 2015. Artemisinin belongs to the family of sesquiterpene lactone with an endoperoxide bridge found to be important for its activity. The yield of artemisinin that can be extracted from *Artemisia annua* ranges from 0.01 and 0.8% of the dry weight [79]. This amount of extraction represents a serious limitation on the drug commercialization. Consequently, genetic engineering techniques have been used with the aim to improve the production of artemisinin in cell plant cultures and in transgenic plants as well.

2.2.3.1. In vitro cytotoxic properties of artemisinin

A significant cytotoxicity of artemisinin against tumors has been recently documented. It suggests that artemisinin, commonly used against malaria, can be used to prevent and treat cancer [80–83]. It is a relatively safe drug, with known pharmacokinetics and pharmacodynamics studies. In fact, *in vitro* work on the effects of artemisinin, at different concentrations, shows

that artemisinin significantly inhibited growth and colony formation of human hepatocellular carcinoma cells through inducing apoptosis pathway [84]. Artemisinin at 20 μmol/l for 24 or 48 h of exposure inhibits growth and cell viability of human ovarian carcinoma cell lines (OVCAR-432 and SK-OV-3) [85]. Moreover, a recent study from our laboratory demonstrated that artemisinin has a differential effect on cancer cells. In fact, artemisinin induced lysis on the murin mastocytoma cancer cell line (P815) with IC_{50} = 12 μM and on kidney adeno-carcinoma cell line of hamster with IC_{50} = 52μM [86]. Furthermore, artemisinin was described to possess an anticancer effect on breast, lung, prostate, colon, leukemia, and other cancer cell types. Despite its efficacy, artemisinin has pharmacokinetic limitations such as poor bioavailability and low solubility in water or oil [87]. Thus, it was developed with semi-synthetic derivatives drugs to overcome some of these problems. So far, semi-synthetic derivative of artemisinin such as artesunate and dihydroartemisinin have been demonstrated to exert an important *in vitro* anticancer activity against different cancer cell lines. In breast cancer cells (MCF-7), artemisinin is less active, and the activity in these cells can be estrogen receptors-mediated (ERβ and ERα) which are implicated in cell proliferation [88]. In metastatic nasopharyngeal carcinoma cell lines (CNE-2 and CNE-1), the less sensitivity to artemisinin seems to be related to the over expression of polycomb complex protein BMI-1 [89]. Previously, Efferth et al. described a profound cytotoxic activity of artesunate, a semi-synthetic derivative of artemisinin, against 55 cancer cell lines of the U.S. National Cancer Institute with IC_{50} ranged from 246 nM to 100 μmM, by activating the expression of CDC25A and EGFR genes in cancer cells [83]. Another study by Efferth et al. showed that artesunate cytotoxicity on isogenic *Saccharomyces cerevisiae* with defined genetic defects, involved the implication of two putative target genes, BUB3 and CLN2 [82]. Furthermore, it was described that the anticancer activity of artesunate, arteether, and artemether (semi-synthetic derivative of artemisinin) is associated with the basal mRNA expression of 464 genes linked to proliferation of cells [90]. Generally, artemisinin molecules have been described to be more cytotoxic against cancer than normal healthy cells [86, 91], since normal cells contain significantly less free iron than cancer cells. In general, cancer cells, express more cell surface transferrin receptors and uptake significantly more iron than do normal cells [91].

Several studies have tried to explain, at molecular level, the mechanism of its anti-cancer action. A study on HL-60 cancer cell line demonstrated that rapid production of reactive oxygen species is associated with cell death by apoptosis after artemisinin treated cells [92]. Other factors such as endoplasmic reticulum stress and calcium metabolism can also be associated with the anticancer activity of artemisinins [93, 94]. Endoplasmic reticulum seems to be a possible site for artemisinin action, in HepG2 cancer cell line a derivative fluorescence accumulates preferentially in the endoplasmic reticulum as described by Crespo et al. [95].

Artemisinin has been described to induce apoptosis effect [86, 96, 97], as well as cell cycle arrest, especially at G0/G1 cell cycle transition phase [89, 98]. Multiple lines of evidence suggest that the apoptotic pathway could be due to intra and/or extra-mitochondrial mode of action, and the involvements of iron/heme as well [81, 99]. Two mechanistic pathways have been frequently described to explain the apoptotic effect of artemisinin, vascular endothelial growth factor decrease [100–102], and nuclear factor-kappa B inhibition [103, 104]. Recently, other processes have also been illustrated in different cancer cell types, by the involvement of NOXA [105], mitogen-activated protein kinase (MAPK) [106], Wnt/β-catenin

[107], surviving [108], COX [109], c-MYC oncoprotein [93, 110], and epidermal growth factor [111]. Furthermore, it was also reported that the sensitivity to artemisinin action was related to the expression level of proapoptotic (Bax) and antiapoptotic (Bcl2) genes [112]. Also, artemisinin role in the inhibition of cancer is postulated to be associated with direct DNA damage [113] or indirectly in tumor cells involving a cascade of signaling pathways in many hallmarks of cancer [114]. Taken together, these results could explain the apoptotic pathway induction by artemisinin on tested cancer cells [101, 102, 115, 116]. However, we have also reported the possibility of the involvement of another cell death process of artemisinin; probably necrosis [86]. Artemsinin-induced necrosis remains not well documented and may be linked with the increasing level of ATP, defective apoptotic pathways, reactive oxygen species-independent mechanism of programmed cell death and cancer cell line type [86]. Furthermore, we have described that artemisinin interacted synergistically and additively with vincristin to reduce cancer cell proliferation [86], suggesting a possible use of artemisinin as an adjuvant to treat cancer.

2.2.3.2. In vivo anti-tumor and antimetastatic effects

Artemisinin treatment in oral route at 80 mg/kg considerably reduced the tumor volume growth of P815/DBA2 mice as described by our team [86]. In HepG2 and Hep3B human hepatoma mouse xenograft, artemisinin administered at 50 or 100 mg/kg/day delayed tumor onset, respectively, by 30 and 39.4% [117]. Also, in another study, artemisinin reduced tumor growth at 50% on day 20, when injected intraperitoneally at a concentration of 2.8 mg/kg/day on mammary gland ductal carcinoma in mice [118]. Inhibition of tumor growth and antiangiogenic effect in MCF-7 mouse xenograft after subcutaneous treatment with artemisinin at dose 100 mg/kg/day for 2 weeks was reported [98]. Interestingly, artemisinin exhibited an anti-metastatic effect [116]. In fact, these authors showed that after orally artemisinin treatment with 50 mg/kg, a reduction of 63.5% of lung metastasis and lymph node metastases decrease in cervical and mediastinal lymph nodes, as well as an inhibition of lymphangiogenesis by 63% of mice. Artemisinin also exhibited inhibitory effects in lung tumor metastasis by 51.8 and 79.6% for 50 and 100 mg/kg/day, respectively. Furthermore, it was described that artesunate given in the drinking water at 167 mg/kg/day suppressed growth of Kaposi's sarcoma-IMM xenograft tumors in nude mice [119]. The antimetastatic effect of artemisinin seems to be associated with the expression of metalloproteinase genes and their effect on $\alpha v\beta 3$ integrins [120]. Moreover, the decrease of MMP2 with an increase of TIMP-2 in HepG2 and SMMC772 cancer cell lines after artemsinin treatment were reported [121]. Interestingly, the antimetastatic effect of artemisinin could be triggered by enhancing Cdc42 and E-cadherin activation [121]. However, in highly metastatic cancer such as nasopharyngeal cancer (CNE-1,CNE-2 cancer cell lines), artemisinin seems to have a low response due to the overexpression of BMI-1 gene that makes these cancer cells more sensitive to artemisinin drug [122]. In highly metastatic MDA-MB-231 breast tumor cells, artesunate induced resistance as described by Beatrice Bachmeier et al. (2011). This resistance was induced by the activation of transcription factors NF-κB and AP-1 [123]. Another study showed suppression of invasive and metastatic non-small cell lung cancer after artesunate treatment by the inhibition of urokinase-type plasminogen activator (u-PA), and matrix metalloproteinases (especially MMP-2 and MMP-7) transcription [10].

3. Conclusion

Nature continues to produce a great wealth of natural molecules endowed with cytotoxic activity towards a large panel of tumor cells. More than 60% of these molecules such as vinblastine, vincristine, etoposide, teniposide, taxol, navelbine, and camptothecin are used in chemotherapy and others have shown great anti-tumor and anti-metastatic potential in preclinical trials [124, 125]. Other natural product (i.e., Romidepsin 14, *Omacetaxine mepesuccinate*) [126] and natural product-derived drugs (i.e., metformin, metformin Polyphenon E, retinoids, soy isoflavones) [127] are in clinical trials. This chapter discusses some examples of these molecules (carvacrol, thymol, carveol, carvone, eugenol, isopulegol, and artemisinin) as well as polyphenols extract that have been studied in our laboratory. Other natural compounds are also under studies and remain promising. It is clear that if we understand the molecular mechanisms of the various interactions between these cytotoxic molecules on the one hand and the tumor cells in their tumoral environments on the other hand, we can develop new therapeutic modalities to overcome the side effects of these molecules and to fight cancer.

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