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Cytotoxic Effect and Mechanisms from Some Plant-Derived Compounds in Breast Cancer

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Abstract

Breast cancer (BrC) is a major health problem in women all around the world. A growing knowledge about these alterations and their associated molecular signaling pathways offers opportunities for therapeutic strategies; chemotherapy is one of the most utilized treatments; however, because of the adverse side effects and multidrug resistance that patients may present, there has been great advancement in search of new alternatives as the use of plant-derived natural compounds. This review describes information on the progress and development of cytotoxic compounds against BrC belonging to the families of flavonoids, terpenes, and alkaloids that through in vitro and in vivo studies have demonstrated to induce cellular death mainly through apoptosis, activating the intrinsic pathway. The in vitro IC_{50} and the in vivo EC_{50} dose-response relationship can vary depending on various factors, including the choice of cell line and/or the model used. Also, the association of some of these compounds with nanoparticles or paclitaxel with antibodies has clearly shown a potential improvement in its effect. The clinical studies that are being conducted with some of them show promising results; however, it is necessary to continue with the effort to develop new and more effective drugs against different types of BrC.

Keywords: breast cancer, cytotoxic agents, natural products, in vitro model, in vivo model, flavonoids, terpenoid, alkaloids, nanoparticles

1. Breast cancer (BrC)

Cancer is the uncontrolled and unregulated growth of cells that generally invade and destroy normal cells [1]. Breast cancer [BrC] is the deadliest malignancy in women worldwide and also an important public health problem being the second leading cause of cancer in women accounting for more than 626,679 deaths only in 2018 [2]. In many cases this disease has become fatal because of its multifactorial origin and also because it has a great number of exogenous and endogenous factors that can stimulate different pathways [3]. In early BrC, gene expression profiles are determined for hormone receptor (HR)-positive disease and human epidermal growth factor type 2 receptor (HER2) status which define if a patient is likely to receive systemic therapy and are therefore used to guide their treatment [4]. Approximately

60–70% of primary BrC cases express positive estrogen receptors (ER+) or positive progesterone receptors (PR+) or both and are hormone responsive. However, about 15–20% of BrC cases are in the category of triple-negative phenotype owing to their lack of ER, PR, and amplified HER2. Commonly, ER+ hormone-dependent BrCs have a better prognosis and are often responsive to antihormone therapy [4].

Because of these reasons, the survival rates for BrC have decreased significantly; however, there have been great advancements in new alternative therapies which not only are safer but are also more effective and inexpensive and have minimal side effects [5]. Therapeutic drugs derived from natural compounds have become of great interest since more than 75% of anticancer drugs were designed and developed from plant-derived natural ingredients which have been proven to have anticancer properties with novel mechanisms [6]. In the last 50 years, nearly 200 new chemical compounds have been approved to fight cancer, of which around 50% are molecules of unmodified natural products and their semisynthetic or synthetic derivatives that are safe and profitable [7, 8]. Small organic molecules such as terpenes, flavonoids, alkaloids, lignans, saponins, vitamins, minerals, glycosides, oils, and other secondary metabolites play a significant role in either the inhibition of proliferation, induction of apoptosis, or other mechanisms that may be altered [9, 10]. The structural diversity of natural products and their wide application in therapeutics have always been recognized by pharmaceutical industries [1].

This chapter summarizes the small novel organic molecules obtained from plants and their derivatives, some of which are on the market and others are found in preclinical studies with encouraging results. We also describe some interesting biotechnological associations between some compounds and nanoparticles or other molecules as antibodies that show a novel potential in the treatment of BrC.

2. In vitro models for studying plant-derived compounds in breast cancer

The evaluation of the therapeutic potential of novel plant-derived compounds or secondary metabolites, either pure compounds or the mixture of active constituents, can serve as chemotherapeutic agents in BrC. The biological models used for the development of new drugs include in vitro models using BrC cell lines and are divided into estrogen receptor positive (ER+, T47, MCF-7) and ER negative (ER-, MDA-MB-231, MDA-MB-453, SKBR3). MDA-MB-231 or triple-negative breast cancer cell (TNBC) line, estrogen negative (ER-) and progesterone negative (PR-), and HER- are known to be models for metastasis, which are more aggressive, containing a high potential to metastasize, and are unresponsive to antiestrogens [5, 8]. TNBC line is used to investigate the mechanism underlying migration and invasion. It is important to find cancer therapeutic compounds which possess multi-targeted and multifunctional potential with anti-metastasis activity [8, 11]. MCF-7 is the most used cell line with a great number of publications due to the presence of ER+ [9].

In vitro experiments have also been used in different studies, in order to characterize and identify compounds derived from extracts, essential oils, and other extractions. Trypan blue dye, MTT, sulforhodamine B, and lactic dehydrogenase assays constitute some of the most utilized assays used to evaluate the cytotoxic effect of essential oils and/or pure compounds in different cell lines of BrC. In order to characterize morphological changes, biochemical and molecular levels of cell death, proteins that are modified (expression and activation), gene regulation, migration, invasion, and cell division, among other changes, experiments such as staining with hematoxylin and eosin, Western blot, TUNEL, annexin V, qRT-PCR, scratch assay, and cell cycle assays are performed [12].

3. In vivo models for studying plant-derived compounds in breast cancer

Wide varieties of animal model systems are now available to investigate plant-derived compounds in different stages, such as cancer initiation, promotion progression, invasion, and metastasis. These models are also used to comprehend therapeutic response, which represents an essential step between in vitro systems and clinical studies [8, 13]. The in vivo models are also used to investigate the capability of plant formulation to induce an anti-BrC effect where it is sought to optimize dose, bioavailability, administration routes, and selective delivery and reduce toxic effects, among others [8, 14]. The two animal species that will be mentioned in this review are those involving mice and rats [14]; however, BrC mouse models are used in a variety of preclinical studies [13].

There are different types of in vivo models of BrC, such as cell line-derived xenografts (MDA-MB-231 line) that are implanted into immunocompromised animals (cell-derived xenografts, CDX). CDX models represent a relatively homogeneous mass of transformed breast epithelial cells, and depending on where the cells are inoculated, they are classified as ectopic CDX (models advanced disease only, subcutaneous injection of human tumor cells), orthotopic CDX (in mammary gland/fat pad), metastatic CDX (following tail vein or intra-cardiac injection in specific sites, i.e., bone or lung), syngeneic (mouse tissue implanted to strain-matched host) or metastasis with syngeneic model (usually fast-growing tumors and microenvironment derive from the same species, i.e., 4T1 cells), and genetically engineered mouse models (GEMMs) to address early events of tumorigenesis [13]. Nonetheless, researchers need to consider the limitations of each model and the mechanism of action of the compound previously investigated in in vitro models.

4. Cytotoxicity of plant-derived compounds

Plants produce bioactive secondary metabolites such as flavonoids [15], terpenoids [1], alkaloids [16], tannins, and others, which have profusely been studied for BrC (1, 8). Here, we describe some terpenoid compounds such as *D*-limonene, camptothecin (CPT), paclitaxel, and ursolic acid (UA) and some flavonoids such as cynaroside, isoflavones as Biochanin A (BA) or ginsenoside R2, naringenin, and other novel cytotoxic compounds from different natural sources as shown in **Figure 1** and **Table 1**.

The anti-BrC activities of plant-derived compounds discussed in this chapter are taken from published articles that demonstrated an anti-BrC activity against specific cancer cell lines (see **Table 2**) and in vivo models (see **Table 3**) or clinical studies with their mechanism of action.

4.1 Terpenoids as cytotoxic compounds

Terpenoids are organic compounds derived from five-carbon units (isoprene) assembled and modified in different ways. The classification of terpenoids is based on the isoprene units which are commonly classified as monoterpenes (C₁₀) and diterpenes (C₂₀), i.e., paclitaxel, and triterpenes as ursolic acid [1]. Interestingly, essential oils are a rich and complex composition of monoterpenes with anti-BrC activity such as *Decatropis bicolor* essential oil (DBEO) [17].

4.1.1 *D*-limonene

D-limonene, (1-methyl-4-(1-methylethenyl)-cyclohexene) a monocyclic monoterpene, with a molecular mass of 136.23 g/mol [18], is found in the peels of citrus

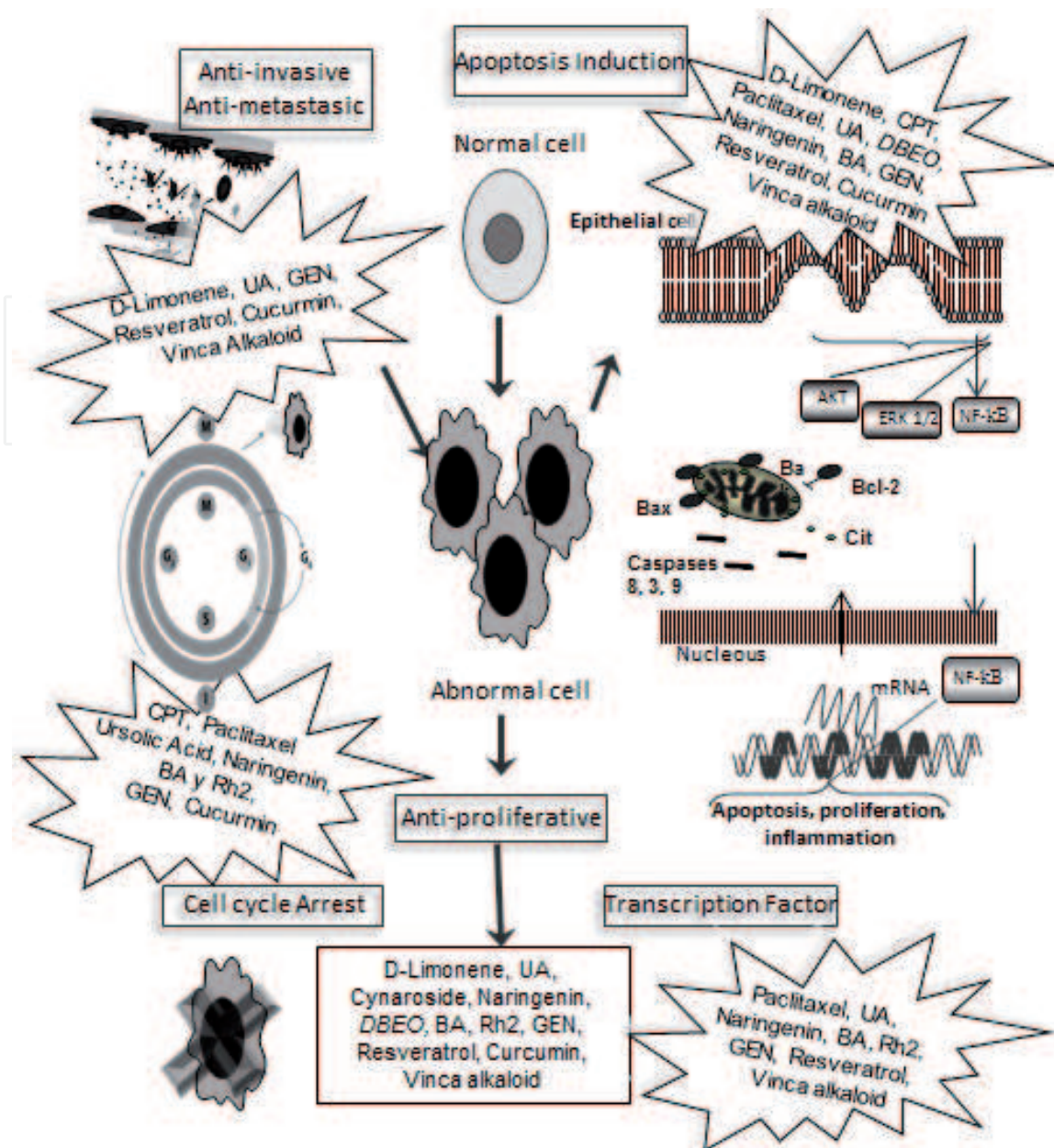


Figure 1.
Molecular targets of plant-derived compounds in BrC.

fruits [18–20]. The main mechanism described by *D*-limonene is the inhibition of the posttranslational isoprenylation of cell growth-regulatory proteins such as Ras, inducing cell death. It has also demonstrated to decrease the viability of cancer cells in a dose-dependent manner by inducing apoptotic cell death. It induced the activation of caspase-3 and caspase-9, PARP cleavage, and Bax protein and cytosolic release of cytochrome c from the mitochondria and through the attenuation of the expression of Bcl-2 protein, suggesting that *D*-limonene induces apoptosis via the mitochondrial pathway and through the suppression of the PI3K/AKT pathway [21, 22]. The interest in this compound as a potential cancer chemotherapeutic agent was stimulated by pronounced chemopreventive and chemotherapeutic efficacy in spontaneous and carcinogen-induced animal tumor models with little toxicity [19, 22]. In the in vivo models, it has been reported to exhibit various effects on several hallmarks of cancer (i.e., proliferation, apoptosis, inflammation) [23, 24]. 7,12-Dimethylbenz(a)anthracene (DMBA) and *N*-methyl-*N*-nitrosourea (MNU) induced mammary carcinogenesis in rats and induced regression of carcinomas [24–26]. Dietary feeding of *D*-limonene also inhibited the development of Ras oncogene in mammary carcinomas in rats [25].

Source	Compound	Study type	Concentration	Ref
Apples, herbs and spices including rosemary	Ursolic acid (UA)	MCF-7, MDA-MB-231	221.39 µg/ml 239.47 µg/ml	[33]
Apiaceae family, <i>Cuminum cyminum</i>	Cynaroside	MCF-7	IC ₅₀ :3.98 µg/ml	[42]
Soybeans, soy foods, legumes	Biochanin (BA)	MCF-7, MDA-MB-231	63.76 µm and 59.76 µm, respectively	[44]
Soybeans, soy foods, legumes	Ginsenoside (Rh2)	MCF-7, MDA-MB-231	57.53 µm and 52.53 µm, respectively	[44]
Soybeans, soy foods, legumes	Ginsenoside + biochanin	MDA-MB-231 MCF-7	27.68, 25.41 µm; 25.2 µm, and 22.75 µm	[44]
Tomatoes (<i>Solanum lycopersicum</i>), citrus fruits and grapes	Naringenin	MDA-MB-231	40 µg/ml	[43]
Soy products	Genistein (GEN)	MCF-7, MDA-MB-231 /ERβ1	100 µm	[48]
Tomatoes (<i>Solanum lycopersicum</i>), other red fruits	Lycopene (LYC)	MDA-MB-231 MCF-7	≥1.25 µm to 5 µl in TNBC. 50 µm	[11, 12]
LYC incorporated biopolymeric nanoparticles with whey protein isolate	Lycopene (LYC-WPI-NPs)	MCF-7	25–50 µm/ml	[49]
A derivative from <i>D</i> -limonene	Perillyl alcohol a hydroxylated product of <i>D</i> -limonene	KPL-1, MCF-7, MKL-F, MDA-MB-231	500 µm	[26]
<i>Taxus brevifolia</i>	Paclitaxel	MCF-7 T47-D, MBA-MB-231, MDA-MB-435	100–500 nm	[28]
<i>Taxus brevifolia</i>	Paclitaxel-loaded nanospheres	MDA-MB-435, ZR.75.1	0.06–0.6 ng/ml	[50]
Grapes, wine, nuts, berries	Resveratrol	MCF-7	10–150 µm	[51]
<i>Camptotheca accuminata</i>	Camptothecin	MDA-MB-231 MCF-7	100 nm IC ₅₀ :0.65 nm	[52, 53]
<i>Curcuma longa</i>	Curcumin	MCF-7 MCF-7 MDA-MB-231	IC ₅₀ : 29 µg/ml IC ₅₀ : 35 µm IC ₅₀ : 30 µm	[54, 55]
Vinca alkaloids	Vincristine	MCF-7, HeLa	IC ₅₀ : 170 and 50 nmol/L	[56]

Table 1.
Cytotoxic effect of plant-derived compounds in in vitro models of BrC.

4.1.2 Paclitaxel

Paclitaxel is a complex diterpene, with a molecular structure of C₄₇H₅₁NO₁₄ and a molecular mass of 853.91 g/mol [18]. This compound induces mitotic arrest and also apoptosis by activating extrinsic or intrinsic pathway. In MCF-7 cells it decreased levels of Bcl-2 protein and increased proapoptotic proteins such as Bax, cytochrome c, caspase-9, and caspase-3 [27, 28]. Likewise, it has also been reported

Compound	Study type	Dose and activity	Ref
D-Limonene	DMBA and NMU-induced mammary carcinogenesis in rats	10% of limonene diet. Induced complete regression of primary rat mammary tumors and prevented the development of secondary tumors	[24]
Perillyl alcohol a hydroxylated product of D-limonene	Mammary rat tumor induced with KPL-1 cells	75 mg/kg. Suppressed orthotopically transplanted KPL-1 tumor cell growth and regional lymph node metastasis in a nude mouse system	[26]
Paclitaxel-encapsulated liposomes	DMBA mammary tumor	20 mg/kg. In combination with 500 mg/kg of <i>Eruca sativa</i> extract reduced NF-κB, COX-2 and Bcl-2 gene expression	[97]
Paclitaxel-loaded nanospheres	Tumor xenograft model in mice	5–50 mg/kg. Showed an equivalent antitumor efficacy to the clinical formulation and provided a superior safety and improved tolerability to higher paclitaxel doses	[50]
Resveratrol	DMBA induced mammary cancer in rats estrogen-induced breast carcinoma in rats	100 µg/rat. Suppressed COX-2 and matrix metalloprotease-9 expression in the breast tumor. 50 mg. Inhibits breast carcinogenesis via induction of NRF2-mediated protective pathways	[67, 98]
Curcumin	MCF-7 xenografts mouse breast cancer model	100 mg/kg in combination with mitomycin 1–2 mg/kg. The combined treatment inhibited tumor growth, induced G1 arrest, and decreased cyclin D1, cyclin E, cyclin A, CDK2, and CDK4	[99]
Camptothecin	Mouse 4T1 breast tumor model	2 mg/kg in combination with 1.05 mg/kg of doxorubicin. Induced 70% of tumor volume reduction and caspase-3 induction	[100]

Table 2.
Compounds evaluated in in vivo model of BrC.

that the induction of apoptosis was independent of caspases [29]. The combination of paclitaxel with a compound that inhibits the mitotic slippage such as phenylethyl isothiocyanate (PEITC) induced apoptosis in MDA-MB-231 cells which are drug resistance [30]. Also, additional activities of taxol have been described including the effect on cell signaling and gene expression and activation of mitogen-activated protein kinases (MAPKs), Raf-1, and protein tyrosine kinases [29]. Paclitaxel has been approved by the FDA to be used alone, or in combination with other anticancer treatments, to treat BrC and other cancers [31, 32].

4.1.3 Ursolic acid

Ursolic acid (3-β-hydroxy-urs-12-en-28-oic acid) is a pentacyclic triterpenoid natural product and a member of the cyclosqualenoid family, commonly named as UA with a molecular structure of C₃₀H₄₈O₃ and a molecular mass of 456.7 g/mol [18]. UA is derived from diverse plants and fruits, such as rosemary (*Rosmarinus officinalis*), apple (*Malus domestica*), makino, cranberries (*Vaccinium macrocarpon*), pears (*Pyrus pyrifolia*), prunes (*Prunus domestica*), bearberries (*Arctostaphylos alpina*), loquat (*Eriobotrya japonica*), scotch heather (*Calluna vulgaris*), basil (*Ocimum sanctum*), and jamun (*Eugenia jambolana*) [9, 11]. UA posed different activity against BrC via several molecular mechanisms [7, 11, 33–35], and UA


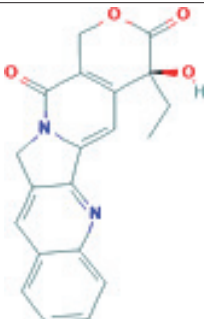
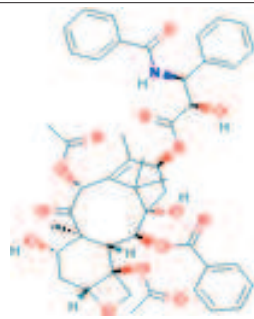
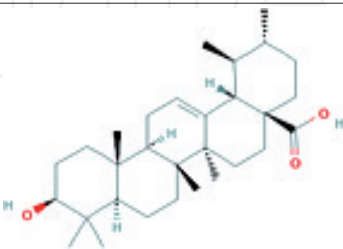

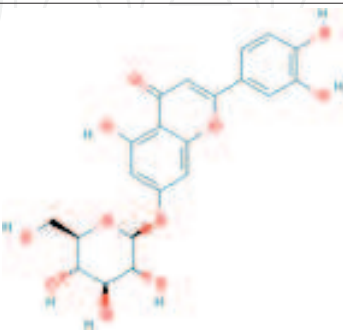
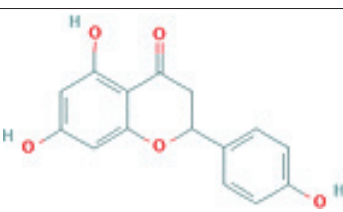
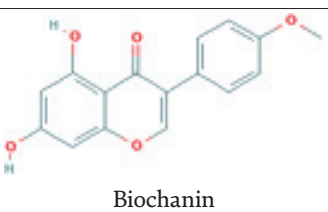
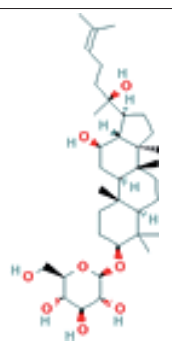
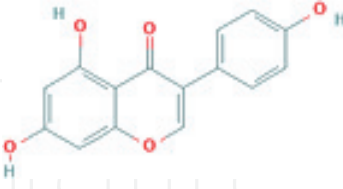
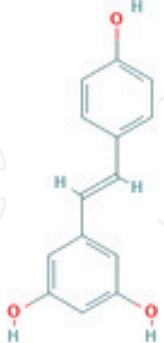
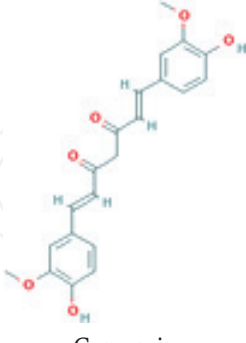
 D-Limonene PubChem CID:22311	 Camptothecin PubChem CID:24360	 Paclitaxel PubChem CID:36314
 Ursolic acid PubChem CID: 64945	 Lycopene PubChem CID: 446925	 Cynaroside PubChem CID: 5280637
 Naringenin PubChem CID: 932	 Biochanin PubChem CID: 5280373	 Ginsenoside Rh2 PubChem CID: 119307
 Genistein PubChem CID: 5280961	 Resveratrol PubChem CID: 445154	 Curcumin PubChem CID:969516

Table 3.
Structures of plant-derived compounds with potential effect in the BrC treatment.

is relatively nontoxic to normal cells [11]. Administration of UA demonstrates inhibitory efficacy against cell proliferation rate and induces apoptosis via both the mitochondrial death pathway (cleavage of caspase-9, caspase-3, and PARP, Bax upregulation and Bcl-2 downregulation, release of cytochrome c to the cytosol, decreased mitochondrial membrane potential) and extrinsic death receptor-dependent pathway (Fas receptor) in MDA-MB-231 cells [9, 11]. The treatment

of BrC cells with UA induced changes in glycolytic pathway leading to cytotoxic autophagy, also at low doses (5–20 μm) caused a G0/G1 cell cycle arrest, increased p21 levels, oxidative stress, and DNA damage [36]. UA has been demonstrated to exhibit strong anti-BrC potential by inducing cell cycle arrest and inhibition of proliferation, angiogenesis, and metastasis in both in vitro and in vivo models [7, 11, 33–35]. Finally, UA inhibited BrC growth by inducing cell death via the inhibition of inflammatory responses through the NF- κ B, PI3K/AKT signaling pathways [9, 33]. Therefore, UA could be used as a potential anti-BrC strategy in clinical studies.

4.1.4 Lycopene alone

Lycopene (LYC) (trans-lycopene) is a terpene assembled from eight isoprene units and is a rich antioxidant compound, a major carotenoid present in tomatoes (*Solanum lycopersicum*), apricots, red oranges, pink grapefruit, watermelon, rose hips, guava, vegetables, and photosynthetic algae [37]. LYC has a molecular structure of $\text{C}_{40}\text{H}_{56}$ and a molecular mass of 536.888 g/mol [18]. LYC is a compound that has displayed antiproliferative, anti-migration, anti-invasive, anti-metastatic, and antioxidant characteristics in numerous in vitro and in vivo studies in BrC [9, 37–39]. Also, LYC induces apoptosis and activates caspase-9 enzyme in human BrC cells [9]. Finally, LYC has inhibited the multiplication of cancer cells by arresting cell cycle at different phases (G1, S, and M phases) and by sustained activation of the ERK $^{1/2}$ with suppression of cyclin D1 and upregulation of p21 [39]. Other mechanism of action of LYC is through the inhibition of I κ B α phosphorylation and decrease in the expression of NF- κ B [40].

4.1.5 Decatropis bicolor essential oil: a mixture of monoterpenes

Essential oils are a complex mixture of secondary metabolites such as monoterpenes that are responsible for their biological activity that includes anti-BrC effect. However, some studies suggested a synergistic activity of the compounds [17]. For example, DBEO was studied on MDA-MB-231 cells.

It had a cytotoxic effect with an IC $_{50}$ of 53.81 $\mu\text{g}/\text{ml}$. It induced DNA fragmentation and apoptosis via intrinsic pathways due to the activation of Bax, caspase-9, and caspases-3, suggesting a synergistic activity of compounds present in the essential oil, such as 1,5-cyclooctadiene, 3-(methyl-2)propenyl, β -terpineol, 1-(3-methylcyclopent-2-enyl)-cyclohexene, D-limonene, pinene, and linalool [41].

4.2 Flavonoids as cytotoxic compounds

Flavonoids which are polyphenolic substances found in different plant-derived food are divided into flavones (cynaroside), flavonols, flavanones (naringenin), flavanols, isoflavones (genistein (GEN), biochanin A), anthocyanidins, and nonflavonoids [10]. Flavonoids have been reported to have an effect on BrC through numerous mechanisms such as antioxidant, anti-inflammatory, antiproliferative, cytotoxic, anti-angiogenic, and anti-metastatic effects in numerous in vitro and in vivo experiments in estrogen-dependent or estrogen-independent BrC [15, 37] (Tables 1–3).

4.2.1 Cynaroside

Cynaroside (luteolin-7-O-glucoside) is a glycosyloxyflavone or a glycoside form. It derives from luteolin. Cynaroside has a molecular structure of $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ and a molecular mass of 448.38 g/mol [18]. Cynaroside is a constituent of the leaves of

Capsicum annuum (red pepper) and seeds and fruits of *Cuminum cyminum*, an old famous medicinal and culinary plant from the Apiaceae family. Currently, Goodarzi and collaborators demonstrated that luteolin-7-*O*-glucoside plays a significant role in cytotoxic effect of *C. cyminum* against MCF-7 cell line (IC₅₀ of 3.98 µg/ml) and can be introduced as a candidate for chemopreventive and chemotherapeutic drugs [42]. More in vitro or in vivo studies are necessary to elucidate its mechanism of action.

4.2.2 Naringenin

Naringenin (4',5,7-trihydroxyflavanone) is a flavanone and member of 4'-hydroxyflavanones; it has a molecular structure of C₁₅H₁₂O₅ and a molecular mass of 272.256 g/mol [18]. This bioflavonoid is a constituent of tomatoes, citrus fruits, and grapes. Naringenin is a phytoestrogen which is also an important anti-BrC, reported to be involved in decreasing the number of ER-α-positive cells by modulating p38 MAPK signaling pathway [9]. Recently, investigators reported that naringenin has antiproliferative effects by arresting the cell cycle at the G2 phase and caused an inhibitory effect on MDA-MB-231 cells via induction of apoptosis and inhibition of caspase-3 and caspase-9 activities [37, 43].

4.2.3 Biochanin and ginsenoside Rh2 as pure compounds or mixture

Biochanin or 4'-methylgenistein (B5,7-dihydroxy-4'-methoxyisoflavone) is an *O*-methylated isoflavone that is isolated from red clover *Trifolium pratense* and the root of *Astragalus membranaceus*, a traditional Chinese herbal medicine. BA has a molecular structure of C₁₆H₁₂O₅ and a molecular mass of 284.267 g/mol [18, 44]. The phytoestrogen BA caused antiproliferative activity by stabilizing and activating p53 through the upregulated expression of phospho-p53, phospho-p38, and p-ASK1 and downregulated expression of TRAF2 in MDA-MB-231 and MCF-7 cells [44] and apoptosis through upregulated expression of mRNA levels of ER-α, Bcl-2, and miR-375 in ER+ BrC cells, that is to say T47D and MCF-7 [37, 44, 45].

Also, BA stopped cell growth by blocking the activity of aromatase enzyme which is encoded by the gene CYP19 [5]. On the other hand, ginsenoside Rh2 (protopanaxadiol-type) is the major type of saponin ginsenoside that is separated from *Panax ginseng* and other species. Rh2 has a molecular structure of C₃₆H₆₂O₈ and a molecular mass of 622.884 g/mol [18, 44]. Rh2 exhibits antitumor activity in ER+ (MCF-7) and ER- (MDA-MB-231). However, Ren and collaborators determined that BA plus Rh2 synergistically enhanced the antiproliferative effect in both BrC cells, with decreased EC₅₀ values of both the compounds and a mechanism through the stabilization and activation of p53, p38, and ASK1 proteins (Table 1) [44].

4.2.4 Genistein

Genistein (4',5,7-trihydroxyisoflavone) is an isoflavonoid derived from soy products [46]. It has a molecular structure of C₁₅H₁₀O₅ and a molecular mass of 270.24 g/mol [18]. This agent has an antineoplastic effect in BrC [47]. GEN inhibits the growth of MDA-MB-231 cells by altering the phosphorylation of proteins included in cell cycle regulation and DNA damage response predominantly, and GEN induced apoptosis via the upregulation of Bax and p21WAF1 proteins in MDA-MB-231 cells and downregulating the expression of caspase-3 [5, 47]. In a recent study, GEN increases cell cycle arrest in G2/M phase in MDA-MB-231/ERβ1 cells, even though there is a high dose of GEN-arrested cells in G0/G1, just like in the MCF-7 cells. Thus, the combinatorial effect of GEN and overexpressed ERβ1 resulted in an active blockade of cell cycle progression and a dramatic inhibition

of proliferation in vitro in MCF7 and MDA-MB-231 cells [48]. GEN could be a potential therapeutic agent for ER β 1-positive cancer, which merits further clinical research in the future.

4.2.5 Resveratrol

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is a natural nonflavonoid polyphenol with a molecular formula of C₁₄H₁₂O₃ [18]. These compounds are isolated from more than 72 species of plants including peanuts, grapes, mulberries, bilberries, and blueberries [9].

Numerous in vitro studies have shown that resveratrol has multiple anticancer effects, which protect the cells against both tumor initiation and cancer progression pathway [9, 57]. The activity of this compound was described in hormone-dependent or non-hormone-dependent BrC cells, in which it was found to induce apoptosis by intrinsic pathway through the upregulation of Bax, Bak, caspase-3, p53, and Akt pathway in different breast cancer cell lines and downregulated Bcl-2 and NF-kB and VEGF [51, 58–62]. Also, it can induce the extrinsic pathway through the expression of CD95 receptor [63]. A cell surface resveratrol receptor on the extracellular domain of heterodimeric α V β 3-integrin in MCF-7 human BrC cells induces extracellular-regulated kinases 1 and 2 (ERK1/2) and serine-15-p53-dependent phosphorylation leading to a p53-dependent apoptosis [57]. In several in vivo models, resveratrol supplementation was shown to decrease the incidence of mammary tumor formation, tumor volume, metastasis, and induced apoptosis [64–66]. The effect of resveratrol demonstrated lower tumor growth, decreased angiogenesis, and increased apoptotic index in ER α – and ER β + [62]. Also, the following can suppress mammary carcinogenesis in rats induced by DMBA: dietary administration of resveratrol (10 ppm), downregulation of NF-kB, cyclooxygenase-2 and matrix metalloprotease-9 expression in the breast tumor, and decreased tumor incidence [67, 68].

The effect of resveratrol in cancer patients has been investigated in a few clinical trials. The first clinical trial dealing with resveratrol and cancer was performed by Nguyen and collaborators in 2009, through the administration of 0.07 mg/day of resveratrol which resulted in the reduction of Wnt target gene expression, indicating that it may play a beneficial role in the prevention of cancer. These clinical trials have demonstrated resveratrol to be a promising therapeutic and chemopreventive agent [64, 69, 70].

4.2.6 Curcumin

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is an orange-yellow component of turmeric or curry powder; it is a polyphenol natural product isolated from the rhizome of *Curcuma longa* [9, 71]. Curcumin has anti-proliferative and proapoptotic effects against a variety of cancer cells in vitro. The anticancer effects observed by activating intrinsic apoptotic pathway by interacting with reactive oxygen species can release cytochrome C; upregulate caspase-9, caspase-3, Bax, and Bad; downregulate Bcl-2 antiapoptotic proteins; and induce DNA fragmentation in different BrC cell lines [72–75]. Also, it was found that curcumin inhibits the expression of Ki-67, proliferating cell nuclear antigen (PCNA), p53, and VEGF in BrC cells [54, 76]. Curcumin prevents carcinogen-induced cancers in rodents [77]. Banerjee et al. [79] reported that curcumin-induced G2/M arrest and apoptosis inhibited cell proliferation in MCF-7 cells, leading to an accumulation in the G1 phase, and suppressed the expression of zeste homolog 2 (EZH2) gene via MAPK pathway [78, 79].

4.3 Alkaloids as cytotoxic compounds

Alkaloids are a highly diverse group of compounds containing an organic nitrogen atom and a ring structure. Additionally, in most alkaloids the nitrogen atom is located inside the heterocyclic ring structure, which gives them a great biological diversity [16]. The structural diversity of this family is due to the wide number of amino acids used as building blocks [80]. Indeed, the peptide ring that they contain has one or more of its hydrogen atoms replaced with various alkyl radicals, most of which contain oxygen [81, 82]. Consequently, alkaloids can interact with a wide spectrum of molecules. They have a wide distribution in the plant kingdom and are a chemically heterogeneous group of ~17,000 molecules which have displayed pronounced biological and pharmacological activities. Furthermore, several alkaloids exhibit significant biological activities, with their unlimited supply of variable structures as well as their relatively low toxicity and well-documented stability; therefore, alkaloids are being used for their anticancer activity against various cancers [83].

4.3.1 *Camptothecin*

Camptothecin is a monoterpene indole alkaloid that consists of five rings [18], commonly named as CPT with a molecular structure of $C_{20}H_{16}N_2C_4$ and a molecular mass of 348.35 g/mol [18]. The antitumor activity of this compound is mainly due to its interaction with topoisomerase I (Top1), an enzyme involved in the regulation of DNA topology during replication, recombination, and transcription. It induces cell death by stabilizing a covalent complex between DNA topoisomerase I and the nicked DNA, leading to a DNA lesion [84–87].

CPT antitumoral activity has been reported in different cancer cell lines. Low doses of this compound lead to cell cycle arrest in the G2/M phase and inhibit DNA synthesis but at higher doses cause cell cycle arrest in S phase [52]. Also, the expression of some genes as c-Myc, Bax, BFL1, Bak, pRb2, c-Jun, and Jun-B was upregulated, and Cdk4, cyclin B1, Wee1, CRAF1, and DP1 were downregulated. Among these derivatives, camptothecin-20(s)-O-(2-pyrazolyl-1)acetic ester exhibited antitumor activity which demonstrated cytotoxicity, DNA fragmentation, and apoptosis toward MCF-7 cell line [53]. Also, in different studies in vivo, where it was delivered using an intralipid formulation through intramuscular (IM) route, CPT showed nearly 100% growth inhibition and regression in the colon, lung, breast, stomach, and ovary and malignant melanoma xenografts [88, 89].

4.3.2 *Vinca alkaloids: vinblastine, vincristine, and vinorelbine*

There are some vinca alkaloids in clinical use such as vinblastine, vinorelbine, and vincristine. Many alkaloids have poisonous characteristics but also have physiological effects that make them useful as medications. The oldest group of the plant alkaloids used to treat cancer is the vinca alkaloids. They have a dimeric chemical structure composed of two basic multi-ringed units, an indole nucleus (catharanthine) and a dihydroindole nucleus (vindoline), joined together with other complex systems. Structurally, vincristine and vinblastine are identical except for a single substitution on the vindoline nucleus, where vincristine and vinblastine possess formyl and methyl groups, respectively [90, 91] (**Table 2**). The main mechanisms of vinca alkaloid cytotoxicity is due to their interactions with tubulin and disruption of microtubule function, particularly of microtubules comprising the mitotic spindle apparatus, directly causing metaphase arrest. The disturbing effects occur at drug concentrations below those that decrease microtubule mass [91, 92]. Also, disorganization of the microtubule structure provokes the induction of tumor suppressor gene

p53 and activation/inactivation of several protein kinases involved in key signaling pathways, including p21, WAF1/CIP1, Ras/Raf, and PKC/PKA, the apoptosis inhibitor Bcl2 and induction of Bax triggering the process of apoptosis in the cell [93]. These alkaloids demonstrated significant antitumor activity in patients with BrC. Also, xenograft mice models were used to evaluate low doses of vinblastine, which resulted in significant but transient xenograft regression, diminishing tumor vascularity, and direct inhibition of angiogenesis. Also, a combination therapy resulted in full and sustained regressions of large established tumors, without an ensuing increase in host toxicity or any signs of acquired drug resistance during treatment [94].

The risk of side effects and multidrug resistance limited the development of vinca alkaloids for clinical applications. To solve these problems, researchers have developed numerous strategies, such as using liposome-entrapped drugs, chemically modified drugs, and polymeric packaging drugs, to reduce the toxicity and enhance the therapeutic efficiency of vinca alkaloids. Many liposome products are still being tested in clinical trials. Another strategy for reducing chemotherapeutic toxicity involves using chemically modified drugs [95, 96].

5. Biotechnological and clinical advances of plant-derived compounds in breast cancer

Nanotechnology has been found to potentially improve current methods for disease, diagnosis, disease-state imaging, and treatment in BrC.

Targeted nanoparticle drug delivery is intended to reduce the side effects of anti-cancer drugs with both decreasing consumption and treatment expenses, which are the major hurdles in conventional cancer treatment. These small entities can be used in combination with a variety of plant-derived compounds in BrC with a variety of formulations being developed, making them a desirable choice of drug formulation [8].

5.1 Use of phytochemical compounds coupled to nanoparticles

5.1.1 Lycopene with nanoparticles

Recently, Jain and collaborators designed and synthesized LYC incorporated with biopolymeric nanoparticles with whey protein isolate nanoparticles (WPI NPs) with encouraging results in the compatibility of LYC-WPI-NPs over plain LYC as an optimum delivery system in vitro because encapsulation process did not affect its anticancer activity even in in vivo tumor model of DMBA where ~57% of LYC group of animals developed tumor compared with ~29% of LYC-WPI-NPs. The new formulation (LYC-WPI-NPs) posed higher cytotoxicity and cellular uptake efficacy as compared to the plain LYC in MCF-7 cells. Antitumoral effect of LYC-WPI-NPs and survival data indicate that proposed formulation strategy is a novel approach for the synchronized delivery of bioactive compound, leading to increased bioavailability, therapeutic efficacy, and safety profiling because of improvement in animal survival (100%), in contrast to animals free of LYC (66.67%) and negative control group (16.67%). This will certainly open new avenues to explore cancer treatment [49].

5.1.2 Paclitaxel with nanoparticles

As a strongly hydrophobic drug, it requires suitable delivery vehicles to effectively distribute into tumor tissues. For efficient distribution of this hydrophobic anticancer drug, paclitaxel is currently formulated and administered to patients via polyethoxylated castor oil (Cremophor EL, CrEL), but it is reported as causing

hypersensitivity reactions and neurotoxicity [101]. To date, paclitaxel albumin-bound nanoparticles (Abraxane®) have been approved by the FDA for the treatment of metastatic BrC and non-small cell lung cancer [8]. Nanoparticle-based delivery systems can take advantage of the enhanced permeability and retention (EPR) effect for passive tumor targeting; therefore, they can improve the therapeutic index and decrease the side effects of paclitaxel. In addition, there are a number of novel paclitaxel nanoparticle formulations in clinical trials [50, 101–104]. Nanoparticle-assisted chemotherapeutic drug delivery has been used because it enhances therapeutic effectiveness. Studies on metastatic BrC demonstrate the inhibition of metastasis by co-delivering chemotherapeutic agent paclitaxel and twist shRNA via complex nanoparticles [105].

5.1.3 Camptothecin with nanoparticles

Research have concentrated on the development of potential delivery system to increase the aqueous solubility, stability, and bioavailability as well as controlled delivery of camptothecin at or around cancer tissues. For that purpose nanoencapsulation of drugs in a biodegradable polymer has been reported to protect the drug in the core of the polymeric shell [106–108]. Camptothecin encapsulated in nanoparticles demonstrated antitumor activity in in vitro and in vivo models; in MCF-7 cells the IC₅₀ was lower (0.23 μ m) than the pure compound (0.57 μ m) [106, 109].

5.1.4 Curcumin with nanoparticles

As other compounds, in order to increase photostability and enhance its anticancer activity against BrC cells, scientists have formulated the transferrin-mediated solid lipid nanoparticle, which enhances the anticancer effect of curcumin in BrC cells in vitro [110]. A polymer-drug conjugate called polycurcumins also has advantages of high drug-loading efficiency, fixed drug-loading contents, stabilized curcumin in their backbones, and tailored water solubility. The polycurcumins are cytotoxic to cancer cells, but a polyacetal-based polycurcumin is highly cytotoxic to MCF-7 cells. The effect of these polymers induced cell cycle arrest and apoptosis partially through the caspase-3-dependent pathway. In vivo, this polymer showed antitumor activity in SKOV-3 intraperitoneal xenograft tumor model [111].

5.2 Novel therapies: antibody-phytopharmaceutical conjugates in breast cancer

In recent years, natural products and their derivatives have been among the major sources of drugs for the treatment of cancer as well as nanoparticles or antibodies. Furthermore, new treatments for different cases of BrC are necessary; this involves linking each cytotoxic drug concerned with a mAb by a linker group to produce a tripartite drug called an “antibody-drug conjugate” (ADC). A means of selective delivery of highly cytotoxic natural products as “prodrugs” to tumor cells has proven necessary in order to reduce off-target effects and increase therapeutic outcomes [8, 112].

5.2.1 Plant natural products as components of development ADCs: nab-paclitaxel or Abraxane®

The plant-derived compounds are secondary metabolites that have a different mechanism of action; although all of them are cytotoxic for BrC cells, new tools are being sought to increase their effectiveness, with less toxic effects. Also, incorporation of paclitaxel in liposomes can facilitate its delivery to cancer cells and eliminate the adverse reactions associated with the Cremophor EL vehicle. The lipid components of

the liposomal formulation were nontoxic, but the intracellular paclitaxel levels were higher when MCF-7 cells were treated with the liposomal paclitaxel formulation; also, liposomal paclitaxel was as effective as conventional paclitaxel in inducing G2/M arrest after 1 day of treatment with 10 mmol/L, increasing the percentage of cells in this population from about 20% in cycling cells to over 60% after 7 days [104].

Abraxane® or nanoparticle albumin-bound paclitaxel (nab-paclitaxel) suspension demonstrated greater efficacy with less toxicity than docetaxel in metastatic BrC. This treatment has been approved to reduce toxicity and increased overall survival rates, compared to the parent compound [8]. Nab-paclitaxel is a neoadjuvant chemotherapy in HER2-negative BrC stages I, II, and III.

The recommended dose of Abraxane® is 260 mg/m² administered intravenously for 30 minutes, every 3 weeks. The results suggest that Abraxane® is effective in patients with highly proliferative cancers (81).

6. Conclusion

In nature, plants contain secondary metabolites, which have been used by humans to treat different diseases, since they have a complex diversity of chemical structures that have been specifically related to have anti-BrC activity in several preclinical studies with more than one mechanism of action; as a result, they can provide greater degree of efficacy. Several natural compounds are highlighted in this chapter, and their mechanism of action, synergistic action, nano-formulations, and future potentials are widely discussed, and due to the promising potential they represent, in fact, some of them are already used as treatment for BrC. However, it is necessary to have a greater diversity of drugs to be able to treat each one of the different tumors of BrC, since each BrC is different and many of these drugs may still induce several side effects and the development of mechanisms of resistance to drugs must be avoided. Subsequent from this review, we can conclude that although there are many compounds that have been characterized mainly in in vitro models and only around 10–20% of these were also evaluated in in vivo models and less than 10% are being evaluated already in clinical phases, it is essential to conduct more research on these compounds to learn their mechanism of action. Also, in their cellular, biochemical, and molecular levels, clinical effects as well as their genetic toxicities should be investigated sufficiently. Compounds that meet the eligibility criteria in these tests should be taken into clinical trial phase, and they may be administered in combination with other compounds or materials that make their pharmacological effect more efficient, make their arrival to the target site more selective, and guarantee their stability, bioavailability, pharmacokinetics, etc. In conclusion, addressing the study of these compounds in clinical phase is a pressing need.

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Conflict of interest

The authors declare that they have no competing interests.

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