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Breast Cancer and Flavonoids as Treatment Strategy

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Abstract

Breast cancer is the most prevalent cancer type among women. Despite recent progress in early detection and therapeutic strategies, the rate of mortality is increasing. Anti-estrogens or aromatase inhibitors are preferred to treat the women diagnosed with estrogen-receptor (ER) positive tumors. However, breast tumors usually show intra-tumoral heterogeneity with ER-positive and -negative cells. The advanced breast cancer cells lose the estrogen responsiveness and become aggressive by developing new strategies for rapid proliferation such as mutations in cell cycle machinery. New promising drugs are still being investigated against these types of tumors especially to overcome acquired resistance against chemotherapeutic drugs; however, a successful treatment for metastatic tumors is still unclear. Flavonoids, with various pharmacological activities, are plant or fungus secondary metabolites present in human diet. In plants, beside their role in pigmentation, they may also act as messengers, regulators and cell cycle inhibitors. Therefore, they are being tested in ovarian, cervical as well as breast cancer. Due to the positive correlation between flavonoids-rich diet and lower risk of cancer, flavonoids are referred as chemopreventive agents. The current chapter emphasizes the therapeutic potential of flavonoids and their synthetic analogues as anti-cancer agents in breast cancer providing new insights into the molecular mechanisms.

Keywords: breast cancer, chemoprevention, flavonoids

1. Introduction

The use of natural and dietary agents for cancer chemoprevention and therapy received attention for their health benefits. As consumption of fruits or vegetables has been associated

with a reduced risk of human cancers especially breast cancer [1], dietary flavonoids, found particularly in these alimentary groups with more than 5000 polyphenolic compounds, have been identified as potential cancer-preventive components [2, 3]. Polyphenols can be divided into ten different classes based on their chemical structure [4]. Flavonoids, phenolic acids, stilbenes, and lignans are the most abundant polyphenols in plants. Polyphenols, mainly flavonoids, possess a number of functions including pollination, pollen tube growth, resorption of minerals, and tolerance to abiotic stress [5]. Flavonoids represented greater attention with the decreased incidence of cancer and cardiovascular diseases in Mediterranean population, which was associated with vegetables, fruits, and red wine consumption. Therefore, they have been under investigation for their therapeutic significance in the protection of human health for decades. Flavonoids are one of the common components in the human diet and generally are present as O-glycosides with sugars bound at C3 position [6].

Breast cancer is the leading cause of cancer death among women worldwide. Despite the presence of new promising advances in therapeutics, the breast cancer mortality rate is still increasing. Recent reports suggest that breast cancer prognosis is lower in countries consuming a healthy, plant-based diet [7]. The possible cause to this scenario has been suggested as flavonoids in fruits and vegetables. Epidemiologic investigations showed that flavonoids exhibit important effects on cancer chemoprevention and chemotherapy. They have been shown to interact with different genes and enzymes including those playing role in antiproliferation, cell cycle arrest, apoptosis, angiogenesis, and multidrug resistance. Therefore, this chapter focuses on the chemopreventive and chemotherapeutical roles of flavonoids in the treatment of breast cancer [6].

2. Structure, classification and metabolism in humans

The chemical structure of flavonoids is based on a C₁₅ skeleton with a chromane ring bearing a second aromatic ring B in position 2, 3, or 4 (**Figure 1**).

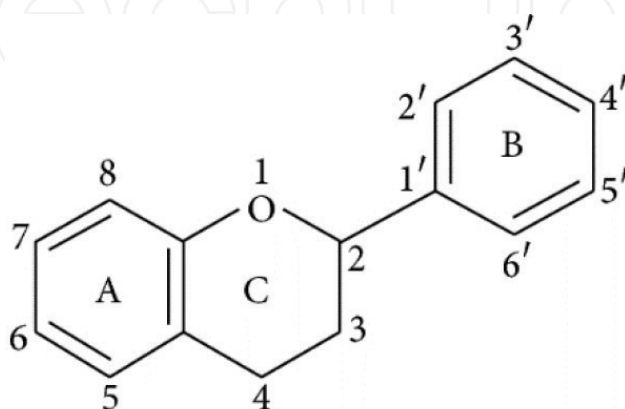


Figure 1. Basic flavonoid structure [8].

Flavonoids are subdivided into different groups based on the nature of C3 element: flavones, flavonols, flavanones, flavanols, anthocyanins, and isoflavones (**Figure 2**).

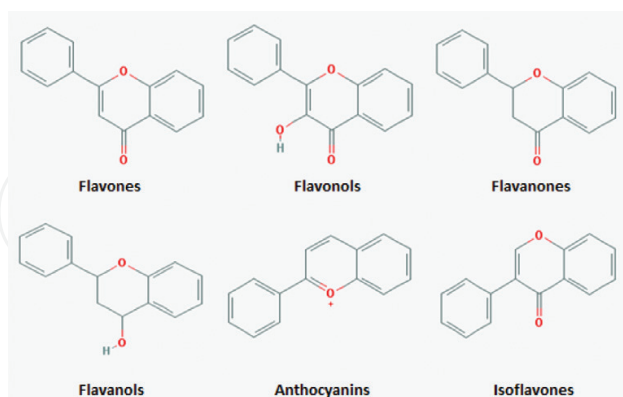


Figure 2. Subclasses of flavonoids (PubChem).

Flavonoids participate light-dependent phase of photosynthesis [9], and they catalyze electron transport. They have been shown to be synthesized from phenylalanine and tyrosine, the aromatic amino acids, with acetates [10]. First, aromatic amino acids are converted to cinnamic acid and parahydroxycinnamic acid, respectively, by phenylalanine and tyrosine ammonia lyase enzymes [11]. Then, parahydroxycinnamic acid accumulates with acetate units to give rise to cinnamoyl, which is the derivative of caffeic acid and chlorogenic acid. Cinnamoyl, then, is converted to ortho-hydroxyacetophenone with a benzaldehyde derivative generating flavonones. If ortho-hydroxyacetophenone condenses with a benzoic acid derivative, flavones are formed. Anthocyanins are naturally occurring glycosides of flavylium (2-phenyl-1-benzopyrylium) ions substituted by hydroxyl and methoxyl groups. Biotransformation of flavonoids occurs in the gut and various secondary metabolites are produced as well such as phenolic acids, lignins, lignans, and stilbenes [11].

Flavonoids, mainly flavanols and quercetin glucosides, are absorbed from the small intestine, while quercetin, quercetin galactoside, and many others are not [12]. Those absorbed by the intestine have been shown to be transported through membrane and use both ATP-dependent pumps and ATP-independent transporters [13]. Following absorption, they are metabolized *via* microbial catabolism and conjugated in the liver and enterocytes [14]. Depending on the subclass, only 5–10% of the amount consumed was shown to be absorbed in the intestine; the rest excreted through the colon where they are further metabolized. The absorbed part in the duodenum is found as methylated, sulfate-conjugated, glucuronide-conjugated, or glycine-conjugated forms [15]. Firstly, in 1992, Hertog et al. measured the content of flavonoids in different fruits, vegetables, and wine. Their findings indicated that mostly quercetin, kaempferol, myricetin, apigenin, and luteolin are found as flavonoid subclasses in the diet. They also suggested that the mean daily intake of flavonoids was higher than the antioxidants β -carotene, vitamin E, and vitamin C [16]. However, the measurement of daily flavonoid intake is difficult to estimate since a standardized method is lacking [17]. Flavonoids, except catechins, exist in nature as glycosides. Following flavonoid intake, the glucosides are

cleaved and glucuronated. Glucuronides can be metabolized, released, or stored as aglycones by glucuronidases in a tissue-specific manner [18]. Although the glycosylation of flavonoids has been suggested important for their absorption, the non-glycosylated form of catechin intake has been shown relatively efficient [19]. Flavonoids, especially flavanols, flavonols, and anthocyanins, are relatively abundant in human diet and are shown to play role in cancer, cardiovascular, and neurodegenerative disease disorder prevention [20].

3. Flavonoid-rich food and medicinal plants

The plant extracts have been used as folk remedies against various health problems, including metabolic diseases, cancer, and neurodegenerative disorders. According to in vitro and in vivo studies, a number of plant species have antiproliferative and antitumoral role in breast cancer pathogenesis. In addition, plants which have higher amount of flavonoids are accepted as chemopreventive agents. According to the United States Department of Agriculture (USDA) database, the six subclasses of flavonoids are listed for 506 food items. According to the database, flavonols (quercetin, kaempferol, myricetin, isorhamnetin), flavones (luteolin, apigenin), flavanones (hesperetin, naringenin, eriodictyol), flavan-3-ols ((+)-catechin, (+)-gallocatechin (GC), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin 3-gallate, (-)-epigallocatechin 3-gallate, theaflavin, theaflavin 3-gallate, theaflavin 3'-gallate, theaflavin 3,3' digallate, thearubigins), anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin), and isoflavones (genistein, daidzein, glycitein) are listed.

Generally, these dietary compounds are known with their antioxidant, anti-inflammatory, and anticarcinogenic effects. According to the Seven Countries Study report, the average consumption of quercetin, kaempferol, myricetin, luteolin, and apigenin in composite food samples have ranged from 6 mg/day in Finland to 64 mg/day in Japan, with intermediate intakes in the United States (13 mg/day), Italy (27 mg/day), and the Netherlands (33 mg/day). In a similar study report, average flavonoid intake in Hungarian population was lower compared to Dutch, Danish, and Finnish citizens. The intake of five flavonoids in 17 different diets was estimated. When diet types were compared to each other according to flavonoid consumption ratio, it was shown that South African diet is the lowest flavonoid consumed diet type as 1–9 mg/day consumption. In contrary, Scandinavian diet in correlation with population-based study outcomes was the higher flavonoid intake diet type (75–81 mg/day).

In addition dietary origin of the flavonoids varied between countries. While tea is the main dietary source of flavanoids in Japan by 95% and the Netherlands by 64%, alcoholic beverages such as famous resveratrol based popularity of red wine and beer in Italy by 46%. The vegetables and fruits are the most common dietary sources of Scandinavian countries such as Finland by 100%. Similar ratio was also observed in the United States by 80%. In Australia, tea is the major source of flavonoid, and flavan-3-ols are 75% of whole intake. Therefore, it is important to evaluate the chemopreventive and chemotherapeutic potential of flavonoids in breast cancer disease.

In this section, it is aimed to discuss potential molecular mechanism of above-listed flavonoids in breast cancer studies.

3.1. Flavanols

3.1.1. Quercetin

Quercetin is a natural dietary flavonoid which exerts antioxidant, anti-inflammatory and anticancer properties. Quercetin is found in barks of many plants, fruits, and vegetables. It is one of the well-established grape polyphenols like other members, resveratrol, naringenin, and catechin, can exert antitumoral, antioxidant, anti-angiogenic properties and modify selectively estrogen-receptor (ER). According to a recent study, it is found that quercetin at IC₅₀ value (37 μ M) modulated Twist and p38 MAPK signaling, which lead to apoptosis in MCF-7 and MDA-MB-231 breast cancer cells [21]. In addition it is well documented that quercetin exerts its therapeutic effect through modulating different cellular targets. According to the previous study, it was shown that quercetin induced p21 CDK inhibitor with a concomitant decrease of phosphorylation of retinoblastoma (Rb), which inhibits the G1/S cell cycle progression by trapping E2F1. A low dose of quercetin induced mild DNA damage and Chk2 activation, which is the main regulator of p21 expression by quercetin. In addition, quercetin downregulated the cyclin B1 and CDK1, essential components of G2/M cell cycle progression. Inhibition of the recruitment of key transcription factor NF- κ B to cyclin B1 gene promoter by quercetin led to transcriptional inhibition SKBR3, MDA-MB-453, and MDA-MB-231 cells [22, 23].

Similar to previous findings, MCF-7 breast cancer cells were exposed to the increasing concentrations of quercetin; consequently, cell viability ratios were decreased, and apoptosis was triggered. Following exposure of cells to moderate cytotoxic dose of quercetin for 48 h, cells undergo apoptosis due to activation of caspases. In addition, quercetin mediates the disruption of Bcl-2/Bax ratio in MCF-7 cells [24].

3.1.2. Kaempferol

A dietary flavonoid, kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), is found in edible plants such as kale, beans, endive, tea, broccoli, cabbage, tomato, and grapes, is commonly used in traditional medicine (e.g., *Ginkgo biloba*, *Tilia* spp., *Equisetum* spp., *Moringa oleifera*, *Sophora japonica*, and propolis) has been reported as antimicrobial, anticancer, antioxidant, neuroprotective, cardioprotective, and antiallergic activity [25]. Kaempferol leads to anti-angiogenic effect via reducing vascular endothelial growth factor (VEGF) expression in ovarian cancer cells [26]. Moreover, it enhanced the effect of cisplatin in ovarian cancer and induced cell cycle arrest and apoptosis by modulating Bcl-2-Bax expression. In vitro studies about kaempferol on breast cancer cases, time-dependent exposure of cells to flavonoid (50 μ M) induced G2/M arrest *via* inhibiting CDK1; cyclin A, and cyclin B and induced apoptosis by p53 phosphorylation in MDA-MB-453 breast cancer cell [27]. Although kaempferol treatment significantly induced cell viability loss in MCF-7 cells, no significant

effect has been reported in MDA-MB-231 breast cancer cells or HC-11 mammary epithelial cells. Kaempferol-induced ERK activated apoptotic cell death and reactive oxygen species (ROS) generation and N-acetyl cysteine (NAC) co-treatment prevented kaempferol-mediated poly (ADP-ribose) polymerase (PARP) cleavage in MCF-7 breast cancer cells [28]. Kaempferol could be extracted from *Murraya koenigii* leaf, which was reported to induce caspase-3-dependent apoptotic cell death and inhibited endogenous 26S proteasomal enzyme activity in MDA-MB-231 breast cancer cells. Moreover, dose-dependent kaempferol treatment reduced the tumor growth through inhibiting the expression of angiogenic and antiapoptotic genes in breast cancer xenografts [29]. In vivo breast cancer xenograft mice models, kaempferol-mediated anticancer effect reported *via* downregulation of IRS-1, pAkt, pMEK1/2, and ERK gene expression and decreased the tumor growth and volume. In addition, kaempferol treatment prevents breast cancer invasion and metastasis in MDA-MB-231 breast cancer cells due to reduction in MMP-2 and MMP-9 expressions, suppression of transcription factor activator protein-1 (AP-1) and MAPK signaling [30]. Lymphangiogenesis, a new lymphatic vessels formation process, is a major step in spreading of tumor cells. Lymphangiogenesis inhibitors might be focused as an important drug target strategy in breast cancer cells. A VEGFR2/3 kinase inhibitor, kaempferol, inhibited mammalian lymphangiogenesis in metastatic breast cancer xenograft models [31].

3.1.3. Myricetin

The use of plant derivatives, which exert biological functions, has gained importance in recent years. Myricetin (3,5,7,3',4',5'-hexahydroxyflavone cannabiscetin) is a natural flavonol, which has a unique hydrophobic chemical structure found in different varieties of fruits, vegetables, tea, berries, etc. [32]. The dietary intake of myricetin from our foods is about 0.98–1.1 mg per day, which is quite higher than some other flavonols [33]. Recent studies showed that myricetin is an antioxidant and it possesses cytoprotective, anticarcinogenic, antiviral, and antimicrobial effects [34].

3.1.4. Isorhamnetin

Isorhamnetin is one of the important flavanols found in *G. biloba* leaf extracts. Isorhamnetin is also found in parsley, and thereby it is a common dietary flavonoid as the metabolite of quercetin. Generally, it is well known as antagonist of peroxisome proliferator-activated receptor γ (PPAR γ), which inhibits adipocyte differentiation induced by the PPAR γ agonist rosiglitazone [35]. Isorhamnetin is a naturally occurring compound in fruits and vegetables; recent study showed that isorhamnetin could significantly inhibit the invasion of MDA-MB-231 cells by downregulating matrix metalloproteinases (MMP-2 and MMP-9) through inhibiting p38 MAPK and STAT3 [36]. Similar results were also obtained in another study, which showed that isorhamnetin inhibited cell proliferation and led to apoptosis. In addition, isorhamnetin was found effective on Akt/mTOR/MAPKs signaling axis. It was established that isorhamnetin-induced autophagy can be reversed by the co-treatment of 3-methyl-adenine in lung cancer cells. The results indicated that isorhamnetin exerts antitumor effect in breast cancer through targeting multiple molecular targets [37].

3.1.5. Silymarin

In recent years, chemopreventive potential of fruits, vegetables, and medicinal herbs such as tea due to ingredients rich in phytochemicals that act as an antioxidant become an important agent. One of the polyphenolic flavonoids silymarin that is isolated from milk thistle (*Silybum marianum* (L.) Gaertn) has been shown for its antioxidant action against liver toxicity [38]. Recently, studies reported the anticarcinogenic effect of silymarin in several mouse skin tumorigenic samples and cervical, prostate, liver, and breast cancers. The molecular machinery of silymarin was more frequently shown in the treatment of human umbilical vein endothelial cells (HUVECs), and downregulation of survivin, Akt, and nuclear factor (NF)- κ B was observed [39]. Beside cell growth and proliferation inhibition, silymarin was demonstrated as an inhibitor of MMPs and *in vitro* angiogenesis. Silymarin induced liver cancer prevention due to membrane stabilizing antioxidant effect through acting on tumor necrosis factor alpha (TNF- α) expression in hepatocellular carcinoma cells [40]. Moreover, VEGF secretion suppression instead of normal epithelial cells, in prostate and breast cancer epithelial cells, has also been reported in Ref. [41]. In MCF-7 breast cancer cells, silymarin treatment inhibited cell growth *via* upregulating ERB. However, ER alpha (ER α) downregulation is reported to be an important key player in drug-induced autophagy and apoptosis in MCF-7 breast cancer cells *via* Akt/mTOR/ERK signaling pathway [42].

3.2. Flavanes

3.2.1. Luteolin

Luteolin (3',4',5,7-tetrahydroxyflavone) which belongs to flavonoids is a heat-stable and non-toxic compound. It is found in vegetables and fruits such as celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, apple skins, and mignonette and chrysanthemum flowers. As well as other flavanoids, cardiovascular protection, immune system stimulation, antioxidant, anti-inflammatory, and anticarcinogenesis capacities of luteolin have been shown in previous studies [43]. Luteolin exerts its molecular effect *via* inducing different signaling routes in dose-dependent manner. According to previous studies, it is well documented that lutein is both pro- and antioxidant compound. The pro-oxidant activity of flavonoids may be related to their ability to undergo autoxidation catalyzed by transition metals to produce superoxide anions [44]. Due to structural differences including bioactive phenolic ring, prooxidant status of luteolin may increase the cytotoxicity in cells. Luteolin is important in ER-expressing cells. It was shown that luteolin at low concentrations is an antiestrogenic agent and reduces cell proliferation. In addition, luteolin may inhibit aromatase whose function is to catalyze the production of estrogens [44]. A recent study indicated that luteolin downregulates ER and thus caused the degeneration of ER protein. Because the etiology of breast cancer is strongly correlated with nuclear hormone receptor activity, the consumption of luteolin in diet may reduce risk through regulation of estrogen-induced cellular effects. *In vitro* and *in vivo* studies showed that luteolin prevented estrogen-induced cell proliferation. It was shown that luteolin impaired estrogen signaling pathway (ESP) in MCF-7 cells by microarray analysis [45]. Luteolin altered cell cycle regulation signaling targets, including CCNA2, PLK1, PCNA, and CDKN1A. This result was considered as the final consequence of ESP modulation, which

suppresses cell proliferation in breast cancer cells. A previous study showed that luteolin (5 μ M) could be utilized as a chemosensitizing mechanism to target the expression level of cyclin E2 and to overcome tamoxifen resistance in breast cancer patients. In vivo studies also showed that luteolin may exert its effect *via* modulating miRNA expressions such as miR-34a and miR-181a which bidirectionally reduced notch and suppressed invasion mechanism [46].

3.2.2. Apigenin

Apigenin is known as the phytoestrogen, used in postmenopausal symptom treatment, and presented in various plant species. Although it is a nontoxic and non-mutagenic plant derivative, it exerts antitumoral activity in different types of cancers and induces oxidative stress in breast cancer cells [47]. However, there are contradictory reports showing that apigenin might stimulate cell proliferation in ER α -positive MCF-7 and T47D cells, but not effective in ER α -negative MDA-MB-435 breast cancer cells [48]. The molecular mechanism of apigenin-induced apoptotic cell death was caspase-dependent, mitochondria [49] and NF- κ B, STAT signaling-mediated [50]. Moreover, apigenin inhibited cell growth, metastasis, and invasion in breast cancer cells *via* acting on PI3K/Akt signaling and beta 4 integrin in MDA-MB-231 breast cancer cells [51].

3.3. Flavanones

It was shown that flavanone-rich diet mediated 0.1–100 μ M physiologically achievable concentration in the plasma. One of the mostly known flavanones is naringenin, which is especially abundant in the Mediterranean diet, rich for consumption of grapes, tomato, and citrus. Naringenin was shown with anticancerous effect in various cancer cells. According to in vitro studies, it was shown that naringenin modulated NF- κ B to induce apoptosis in the cells. Naringenin was effective in MCF-7 ER α +/ER β + cell line, but not in ER-independent SKBR-3 (ER α -/ER β -) cell line [52–55]. *Thymus vulgaris* ethanol extraction originated in naringenin induced cell cycle arrest at S and G2/M phases, which led to apoptotic induction in HTB26 and HTB132 breast cancer cells [52]. The apoptotic efficiency of naringenin was found due to alteration of different cell cycles and apoptosis-related genes such as cyclin-dependent kinases and Bcl-2 family members. Naringenin may activate T cells to induce antitumoral activity in mice and lead to increased interferon (IFN)- γ and interleukin (IL)-2 expressing T cell population [54, 56]. Naringenin promotes the therapeutic effect of tamoxifen in breast cancer cells [57, 58]. Thus, naringenin might promote the potential therapy outcomes and good prognosis in breast cancer cases.

Similar to naringenin, eriodictyol has promising therapeutic effects in cancer cells. Eriodictyol, a flavanone, activated Nrf2 and induced phase II proteins to exert its antioxidant effects [59, 60]. However, there are less studies to evaluate the molecular mechanism of eriodictyol compared to naringenin.

Hesperetin is also a promising flavanone and induced cell cycle arrest at G1 phase. According to the previous study, hesperetin regulated CDK4 and p21 (Cip1) in MCF-7 cells and led to block of cell cycle. Hesperetin is also known with its apoptotic effect in breast cancer cells

without effecting normal mammary epithelial cells. It was shown that hesperetin induced apoptosis in dose- and time-dependent manner in MCF-7 cells through triggering ROS generation. Pretreatment of NAC prevented hesperetin-induced apoptosis, which is under control of ASK1/JNK pathway. In addition, hesperetin also induced apoptosis in triple-negative breast cancer MDA-MB-231 cells *via* intrinsic apoptotic pathway [53, 61–64]. In the light of previous findings, both naringenin and hesperetin are known as promising therapeutic candidates in breast cancer due to their sensitizing effect of HER2-positive breast cancer cells.

3.4. Flavan-3-ols

The most important member of flavan-3-ols (catechins) is abundantly present in green and black tea, red wine and chocolate. Catechins, which are generally found in green tea, comprise epigallocatechin gallate (EGCG), epicatechin (EC), gallic catechin (GC), epigallocatechin (EGC), catechin gallate (CG), epicatechin gallate (ECG), gallic catechin gallate (GCG), and catechin (C). Although green tea is a favorable catechin source, it is required to design more bioavailable structures to treat various cancer types, including breast cancer. The detailed investigation for EGCG was established in different studies. According to xenograft model studies, EGCG with tamoxifen has potential in ER-negative breast cancer models. The MDA-MB-231-mediated tumor volume was decreased following 25 mg/kg treatment of EGCG and/or EGCG + tamoxifen in athymic nude female mice model [65–68]. The potentiation of green tea catechins is generally acted on mTOR and EGFR pathways. Similar to these findings, studies indicated that EGCG produces anticancer effect by modulating the activity of MAPKs, IGF/IGF-1 receptor, Akt, NF- κ B, and HIF-1 α [69–73]. Although catechins have multiple molecular targets in the cells, it is required to improve their structural properties to achieve powerful treatment results.

3.5. Anthocyanins

Anthocyanins confer the bright red, blue, and purple colors to plants such as berries, grapes, and apples. Anthocyanidins lack the sugar component of the anthocyanin [74]. Six anthocyanidins occurred most commonly in nature are pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. It has been suggested that the consumption of cyanidin lowers the risk of cardiovascular disease, diabetes, and cancer due to the antioxidant and anti-inflammatory activities [75]. The phenolic structure is responsible for the antioxidant activity such as the ability to scavenge superoxide ($O_2^{\cdot-}$), singlet oxygen (O_2), peroxide (ROO^{\cdot}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\cdot}), members of ROS [76], in in vitro cell lines including colon, liver, and breast cancer cells [77].

3.5.1. Cyanidin

The anticancer effect of cyanidin-rich extracts of different plant has been shown in MCF-7 ER α (+), MDA-MB-231 ER α (–), and MDA-MB-453 ER α (–) breast cancer cell lines. Moreover, apoptotic induction in MDA-MB-453 cells through the intrinsic pathway of apoptosis by activating caspase cascade, cleaving poly (ADP-ribose) polymerase (PARP), depolarizing mitochondrial

membrane potential, and releasing cytochrome C has been shown [78]. In addition, in the same study, 100 mg/kg/day oral administration of cyanidin has been shown to reduce tumor growth and angiogenesis by affecting the expression of angiogenic factors MMP-9, MMP-2, and cell/extracellular matrix (ECM) interaction in nude mice bearing MDA-MB-453 cell xenografts [78]. Furthermore, inhibition of proliferation and cell cycle arrest were induced in MCF-7 human breast cancer cells after the treatment of bilberry extract, which contains high amount of cyanidin [79]. The same study also compared the effect of cyanidin with a well-known antioxidant Trolox, a vitamin E analog, and showed that cyaniding induced apoptosis and cell cycle arrest as much as Trolox [80]. In another study, pycnogenol, derived from pine bark, which contains high amounts of procyanidins, has been shown to induce cell death in breast cancer cells (derived from human fibrocystic mammary tissue) but not in normal human mammary MCF-10A cells [81].

Human epidermal growth factor receptor 2 (HER2) is overexpressed in 20% cases of breast cancer. Therefore, HER2-targeted therapies have been evaluated in recent years. In Liu et al.'s [82] study, cyanidin-3-glucoside, extracted from black rice, inhibited phospho-HER2 and phospho-AKT and induced apoptosis both in vitro and in vivo HER2-positive breast cancer cells and tissues. Another study also revealed that anthocyanidin-rich extracts from berries and grapes have been shown to exhibit proapoptotic effects in multiple cell types in vitro [83]. They induce apoptosis through both intrinsic (mitochondrial) and extrinsic (FAS) pathways.

3.5.2. Delphinidin

Delphinidin is a member of anthocyanins mainly found in pomegranate extract and found in many dietary supplements as complementary cancer medicine. A recent study showed that delphinidin treatment inhibited cell proliferation and induced apoptosis in ER-positive, triple-negative, and HER2-overexpressing breast cancer cell lines without any toxic effect in normal breast epithelial cells [84]. In addition, the same study also indicated that MAPK signaling was inhibited in both triple-negative and ER-negative breast cancer cells but not in MCF-10A normal epithelial cells.

Breast cancer cells overexpressing p65, the unit of NF- κ B responsible from cell survival and proliferation, underwent apoptosis following delphinidin treatment. The possible explanation to this process was shown as the inhibition of phosphatidylinositol 3,4,5-trisphosphate (PI3K)-dependent phosphorylation of AKT in vitro and inhibition of the activation of NF- κ B in vivo [85]. The same study also pointed out that miR-27a and miR-155 were able to inhibit PI3K and NF- κ B and responsible from the anti-inflammatory and cytotoxic activity of delphinidin in MDA-MB-231 breast cancer cell line [86]. Delphinidin has been also shown to inhibit hepatocyte growth factor (HGF)-mediated tyrosyl phosphorylation of focal adhesion kinase (FAK), Src, paxillin, Gab-1, and GRB-2, which are inducers of cell proliferation upon phosphorylation by growth factor signaling. Delphinidin, in the same study, was found to repress Ras-ERK MAPKs and PI3K/AKT/mTOR/p70S6K pathways [16]. The compound also has anti-angiogenic and anti-invasive properties by decreasing MMP-9 activity in ER+ MCF-7 cells. Im et al. showed that delphinidin inhibited MMP-9 transcription by blocking NF- κ B through MAPK signaling pathways [87].

3.5.3. Pelargonidin

Pelargonidin, a subclass of anthocyanin with estrogenic activity, was tested in MCF-7 breast cancer cells. The cytotoxic dose (5 µg/ml) of strawberry extract containing pelargonidin-3-O-glucoside caused 50% decrease in cell proliferation [88]. A study performed in breast cancer tissue of rats showed that pelargonidin could inhibit the synthesis of cytochrome c p450 family 1 subfamily A member 1 (CYP1A1) enzyme which converts estradiol into 2-hydroxy-estradiol that can cause DNA damage [89]. The inhibition of the estrogenic activity by 55% was also indicated following pelargonidin containing pomegranate seed oil in ER+ MCF-7 cells. On the other hand, pelargonidin treatment induced apoptosis in both MCF-7 and MDA-MB-231 (ER-). Seventy-five percent inhibition of invasion across a Matrigel was also observed in MCF-7 cells at 10 µg/ml pomegranate seed oil concentration. Studies suggest that further investigations on chemopreventive and therapeutic applications of pelargonidin should be performed against human breast cancer [90].

3.6. Isoflavonoids

3.6.1. Daidzein

Daidzein, is one of the isoflavonoid present in various plants and herbs such as soybeans, tofu, kwao krua (*Pueraria mirifica*), kudzu (*Pueraria lobata*), and also isolated from *Maackia amurensis* cultures [91]. Breast tumor growth inhibition by lower concentration of daidzein (10 µM) treatment in *in vitro* and *in vivo* has been reported. Daidzein prevented T47D breast cancer cell proliferation and acted as antiestrogenic agent [92]. Fifty micrometer daidzein concentration decreased the cell viability in MDA-MB-231 and MCF-7 breast cancer cells by 50 and 42%, respectively, for 48 h. Moreover, daidzein inhibited cell migration and invasion in breast cancer cells by using MicroRaman techniques [93]. As a soybean extract, daidzein stabilized proto-oncogene BRF2 mRNA and decreases BRF2 promoter methylation in ERα and ERβ breast cancer cells and female breast cancer mice models [94]. Antiproliferative effect of daidzein on breast cancer cells was reported by acting on TNF-α expression, down-regulating MMP-9 mRNA expression, and suppressing hedgehog signaling through preventing the Gli1 nuclear translocation. In *in vivo* MCF-7 athymic nude mice breast cancer models, fed daidzein prevented tumor growth through suppressing ATP2A3 and BLNK expression and decreased MYC oncogene expression [95]. However, no significant association between breast cancer risk and plasma equol and/or equol: daidzein concentrations have been reported in the Chinese population [96].

3.6.2. Genistein

Soybean is one of the dietary components, which contains phytoestrogens and genistein acting as a chemopreventive agent against various cancer cells such as prostate and breast cancer. As a predominant isoflavone, genistein inhibits growth and proliferation of ER-positive and ER-negative breast cancer cells by inhibiting receptor-associated tyrosine kinase (RTK) signaling [97]. Genistein inhibited cell proliferation, growth, invasion, and metastasis and acted as anticarcinogenic and anti-angiogenic compound on breast cancer *in vitro* and *in vivo* models

[98]. The molecular mechanism of anticarcinogenic effect of genistein on breast cancer cells is due to DNA topoisomerase, 5-reductase enzyme inhibition, suppressing the NF- κ B, Akt and MAPK signaling pathways [99]. Genistein is one of the flavonoids that has been shown to effect on chronic diseases such as atherosclerosis and hereditary hemorrhagic telangiectasia. The molecular target of genistein was reported to enhance the action of transforming growth factor- β (TGF- β) [77]. Like other plant secondary metabolites (tocopherols, curcumin), flavonoids reported to regulate VEGF in breast tumors in vivo and in vitro studies. The anti-angiogenic, anticarcinogenic effect of genistein target VEGF receptor-2 (VEGFR-2) mediated PI3K/Akt/mTOR signaling pathway [100]. Angiogenesis is the formation of new blood vessels and sprouting of circulation by activation of VEGF family member, VEGF-A, leading to endothelial cell proliferation, migration, and destruction of matrix metalloproteins. Although the anti-angiogenic effect of isoflavonoids has been reported in various studies, the exact molecular inhibition mechanism has not been clarified yet. One of the anti-angiogenic effects of genistein is the inhibition of VEGF and its receptor secretion. Ten to fifty micromolar genistein prevented basal VEGF expression both in breast cancer and human umbilical vein endothelial cells (HUVECs) [101]. Moreover, under hypoxia conditions genistein has been shown to induce both VEGF downregulation and inhibition of hypoxia-inducible factor 1 (HIF-1) activation. The anti-angiogenic effect of genistein has been accelerated with curcumin-combined treatment in HUVEC cells by VEGFR-1 and VEGFR-2 downregulation [102]. According to in vivo experiments such as xenografts, chick chorioallantoic membrane or zebra fish experimental models showed the reduction of microvessel density due to genistein treatment mediated by plasminogen activator inhibitor-1, endostatin, angiostatin, and thrombospondin-1 activation. Pretreatment with genistein leads to the reduction of MMP-2, MMP-3, MMP-13, and MMP-15 mRNA expression and VEGF-mediated plasminogen activator (PA) and PAI1 expression blockage. However, no significant effect has been determined on MMP-2 and MMP-9 activity. Antiproliferative and anti-angiogenic effect of genistein is also shown by inhibition of cadherin, integrin V, connexin 43 mRNA expression, and genistein (40 mmol/L), or daidzein (110 μ m/L) treatment suppresses epidermal growth factor (EGF) and insulin-like growth factor (IGF-I) [103]. NF- κ B signaling pathway plays an important role in not only angiogenesis but also cell growth apoptosis, inflammation, and invasion. Thus, genistein treatment inhibited MMP-9 by NF- κ B nuclear translocation-induced NF- κ B signaling inactivation [104]. In addition, genistein induced cell proliferation suppression by acting on MAPKs such as ERK-1/2, c-Jun N-terminal kinases (JNK), and p38 dephosphorylation. In order to clarify the anti-angiogenic effect of flavonoids, genistein, one of the major catalytic enzymes of prostaglandin production [cyclooxygenase-2 COX-2], associated VEGF production was investigated [105]. COX isoenzyme catalyzes the production of prostaglandins, VEGF production, and angiogenesis induction. In MCF-7 breast cancer cells, genistein alone or combined treatment with capsaicin leads anti-angiogenic and anticarcinogenic effect *via* acting on reduced COX-2 expression. According to in vivo studies, in TPA-treated animals, genistein or daidzein suppresses NF- κ B and COX-2 activity [77]. During cancer progression, various molecules have been involved in various steps such as cell proliferation, differentiation, migration, and extracellular matrix formation. Moreover, some tumor microenvironment modulators such as immunosuppressive and/or angiogenic-inducing factors play essential roles. One of the key targets during these hypoxic breast tumors is galectin-3 that

is involved by overexpression in cancer niche. Soy compound isoflavonoid genistein chemopreventive effect has been reported due to potential action on galectin-3 expression inhibition in breast cancer. The phytoestrogen genistein, which induced G2/M arrest due to galectin-3 downregulation, has been determined in human breast carcinoma cell lines [106]. As shown in **Figure 3**, analysis of transcriptomic profile of mammary epithelial cells of rat females fed a diet containing the soy isoflavone genistein or soy protein isolate showed that soy consumption is associated with reduced breast cancer risk in women. Results provide insight into the molecular basis of the beneficial effect of soy-rich diets.

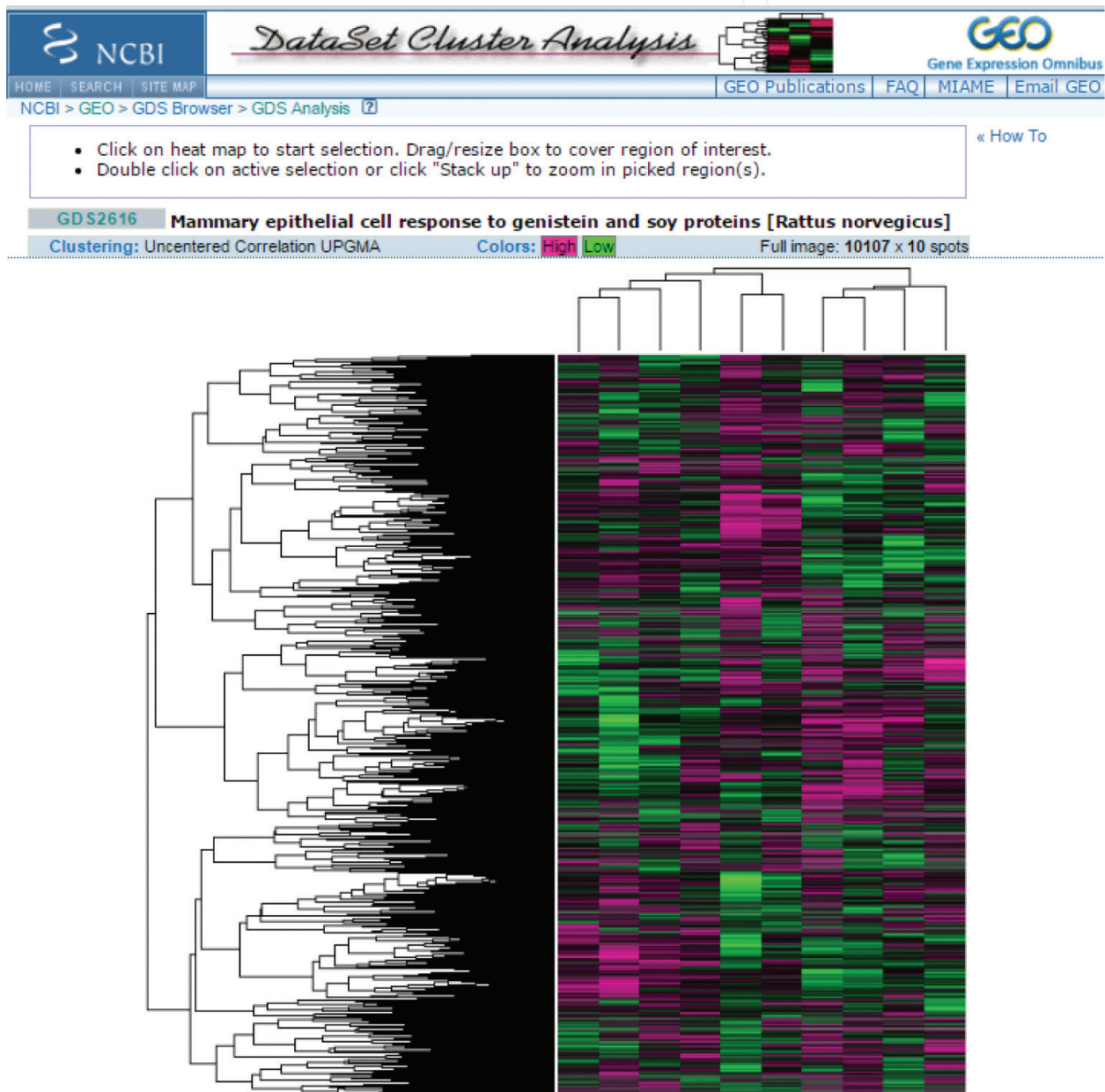


Figure 3. GEO Dataset (GDS2616) demonstration for soy protein genistein protective effect against mammary epithelial cells in *Rattus norvegicus*.

4. Conclusion

In summary, flavonoids can potentially contribute to breast cancer prevention and treatment either by antioxidant or apoptotic activity (**Table 1**). Previous studies highlighted that plant-derived flavonoids are promising when their bioavailability is increased to provide better therapeutic approach in the treatment of disease. However, elucidation of their molecular targets in cell type-specific manner may increase their potential therapeutic effects. Noteworthy that consumption of dietary flavonoids in diet types might be advised to control disease and poor prognosis.

Group of flavonoid	Subgroup	The effect caused by flavonoid treatment in breast cancer models		
		Apoptotic	Anti-angiogenic	Antioxidant
Flavanols	Quercetin	+		+
	Kaempferol	+	+	+
	Myricetin			+
	Isorhamnetin	+	+	
	Silymarin	+	+	
Flavanes	Luteolin		+	+
	Apigenin	+	+	
Flavanones	Naringenin	+		
	Eriodictyol			+
	Hesperetin	+		
Flavan-3-ols	Cyanidin	+	+	+
	Delphinidin	+		
	Pelargonidin	+		
Isoflavonoids	Daidzein	+		
	Genistein	+	+	

Table 1. The summary of the effects of flavonoids in breast cancer.

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