

Review

An Association Map on the Effect of Flavonoids on the Signaling Pathways in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is the third most common type of cancer in the world, causing thousands of deaths annually. Although chemotherapy is known to be an effective treatment to combat colon cancer, it produces severe side effects. Natural products, on the other hand, appear to generate fewer side effects than do chemotherapeutic drugs. Flavonoids are polyphenolic compounds found in various fruits and vegetables known to possess antioxidant activities, and the literature shows that several of these flavonoids have anti-CRC properties. Flavonoids are classified into five main subclasses: flavonols, flavanones, flavones, flavan-3-ols, and flavanonols. Of these subclasses, the flavanonols have a minimum effect against CRC, whereas the flavones play an important role. The main targets for the inhibitory effect of flavonoids on CRC signaling pathways are caspase; nuclear factor kappa B; mitogen-activated protein kinase/p38; matrix metalloproteinase (MMP)-2, MMP-7, and MMP-9; p53; β -catenin; cyclin-dependent kinase (CDK)2 and CDK4; and cyclins A, B, D, and E. In this review article, we summarize the *in vitro* and *in vivo* studies that have been performed since 2000 on the anti-CRC properties of flavonoids. We also describe the signaling pathways affected by flavonoids that have been found to be involved in CRC. Some flavonoids have the potential to be an effective alternative to chemotherapeutic drugs in the treatment of colon cancer; well-controlled clinical studies should, however, be conducted to support this proposal.

Key words: Colon cancer, Anti-colorectal cancer, Flavonoids, Signaling pathways.

Introduction

Colon cancer is the third most common type of cancer in the world, with nearly 1.36 million new cases diagnosed in 2012 in both genders [1]. Although cancer of the colon and rectum are more common in developed countries than in developing countries, the mortality rate in developing countries is higher. In 2012, approximately 694,000 deaths were reported from 1.4 million diagnosed cases worldwide [1]. Technological developments in the last few decades have resulted in a sedentary lifestyle, leading to changes in nutrition and exercise [2], which may have contributed to growing cancer rates, including those of colon cancer. The incidence of colon cancer is predicted to increase by 90% by 2030 [3].

Colorectal cancer (CRC) originates from the

epithelial cells lining the colon or rectum in the gastrointestinal tract. Like cells in other types of cancer, colon cancer cells have six common hallmarks: autonomous growth, unresponsiveness to growth-inhibitory signals, evasion of apoptotic signals, unlimited replicative potential, ability to induce angiogenesis needed for expansion and survival of tumors, and migration to other parts of the body.

Although great advancements have been made in the treatment of colon cancer and the control of its progression, there remains much room for improvement. A number of undesired side effects sometimes occur during chemotherapy. Although a myriad of available natural products have shown

promising anti-cancer properties *in vitro* and *in vivo*, only a few plant products are being used for therapeutic purposes in CRC [4, 5].

Flavonoids are considered to be the main group of polyphenol compounds for combating colon cancer, more than 5000 of which have been detected for this purpose. Flavonoids comprise five main subclasses: flavonols, flavanones, flavones, flavan-3-ols, and flavanonols [6]. The results of clinical studies investigating the relationship between flavonoid consumption and cancer prevention or development conflict for most types of cancers. Research has shown, however, a relationship between consumption of flavonoids and reduction of CRC risk [6].

In recent years, some studies have been performed to investigate the role of flavonoids in signaling pathways in the treatment of colon cancer. Hence, the objectives of this review are to summarize the findings from articles that have been published since 2000 on the mechanisms of effect of flavonoids on CRC and to describe the different cell signaling mechanisms of cancerous cells affected by flavonoids *in vitro* and *in vivo*.

CRC signaling pathways

The six hallmarks of CRC described earlier appear in several signaling pathways. The most important signaling pathways, however, are mitogen-activated protein kinase (MAPK)/p38, PI3K/Akt, Wnt, and the apoptosis cascades. Flavonoids have been found to affect one or more of these pathways, resulting in the inhibition of CRC.

Flavonoids

We retrieved all data reported in studies published since 2000 on the role of the main flavonoids (23 flavonoids belonging to five subclasses) in the signaling pathways in CRC. As reported in the literature, flavonoids inhibit cell signaling pathways in various CRC cell lines. Some of these flavonoids also demonstrated significant effects *in vivo*. The chemical structures of these flavonoids are described in Figure 1, and a summary of the data is presented in Table 1 and Figure 2.

Flavonols

Flavonols, a group of compounds belonging to the flavonoids that have a 3-hydroxyflavone skeleton, are found in many fruits and vegetables [7]. Some of the compounds in this category, such as quercetin, are known to have anticancer properties [8].

Quercetin

Quercetin is one of the best known flavonoids. It

has been found in onions, tea, apples, cranberries, and beans [9]. As reported in the literature, quercetin has been tested on a wide range of colon cancer cell lines, such as HT-29 (p53 mutant type) [10], HCT-116 (p53 wild type) [11], and SW480 (p53 mutant type) [12]. Quercetin demonstrates antiproliferative activity against CRC by inducing cell cycle arrest in the G2/M phase, cytotoxicity activity, cell differentiation, apoptosis, and attenuation of cell migration [13]. Quercetin reduces cyclin A levels and induces the expression of Cdc-2 and p21 [14]. The chemopreventive activity of quercetin was investigated *in vivo* on 1, 2-dimethylhydrazine-induced colonic tumorigenesis in rats [15]. According to Park et al. [16], quercetin inhibited the activity of β -catenin/Tcf in SW480 colon cancer cells. Data confirmed that inhibition of SW480 cells via quercetin is related to the degradation of β -catenin or downstream compounds and consequently a reduction in the binding of Tcf complexes to DNA. Thus, quercetin leads to the inhibition of the β -catenin/Tcf signaling pathway.

Kaempferol

Kaempferol is present in various plants, including tea, strawberries, cranberries, grapefruit, apples, peas, brassicas (broccoli, kale, brussels sprouts, cabbage), chives, spinach, endive, leek, and tomatoes [17, 18]. Kaempferol induces apoptosis in HCT116 CRC cell lines by increasing the expression of p53 upregulated modulator of apoptosis (PUMA). In the DNA damage response, p53 increases the transcription of PUMA and consequently upregulates the translation of PUMA in the cytoplasm. PUMA protein is associated with antiapoptotic factors such as Bcl-x, and the PUMA/Bcl-x complex can cause the release of apoptotic factors such as Bax. The liberated apoptotic factors then activate mitochondria permeability and cytochrome C release. Kaempferol interferes with the promotion of PUMA, which leads to stimulation of apoptotic cascades [19]. The apoptosis property of kaempferol has also been demonstrated in the HT-29 cell line. HT-29 cells were arrested in G2/M in the presence of kaempferol. Moreover, kaempferol reduced the expression of cyclin-dependent kinase (CDK)2, CDK4, cyclin D1, cyclin E, and cyclin A [20]. The Akt/extracellular signal-regulated kinase (ERK) pathway was blocked in HT-29 cells in the presence of kaempferol. Insulin-like growth factor (IGF)-1 was also attenuated [21]. From the work by Lee *et al.*, the apoptosis features of kaempferol against HT-29 cells might be related to *i*) cleavage of caspase-3, -7, and -9; *ii*) increased mitochondrial membrane permeability; *iii*) increased cytochrome C release; *iv*) attenuation of

Bcl-xL, phosphorylation, and reduction of Akt activity; *v*) augmentation of Bad; and *vi*) activation of the Fas ligand and caspase-8 [22]. Moreover, kaempferol suppressed DLD-1 colorectal cells by inhibiting cyclooxygenase (COX)-2 promoter activity [23].

Kaempferol can also be used as a chemopreventive agent against CRC. Nirmala and Ramanathan showed that kaempferol had a chemopreventive effect on tumors in Wistar male rats that were induced by 1,2-dimethylhydrazine [24].

Myricetin

Myricetin is found in walnuts, grapes, and berries, as well as in fermented grapes such as red wine [25]. The anti-colon cancer property of myricetin has been investigated in HCT-15 and HCT-116 cell lines. The study authors claimed that myricetin increased the level of Bax and promoted release of apoptosis inducing factor (cytochrome C) from the mitochondria [26, 27].

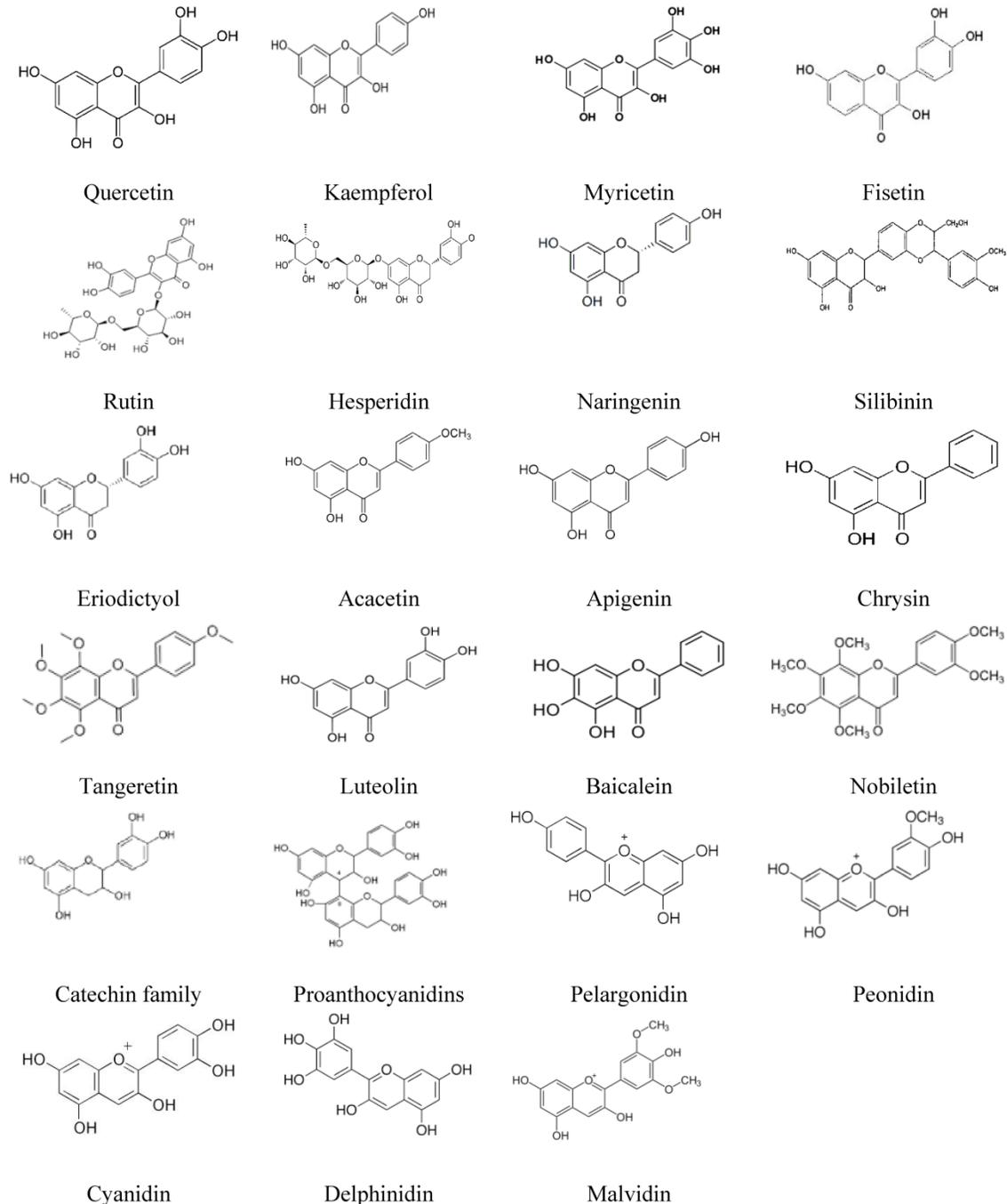


Figure 1: Chemical formulas of main flavonoids.

Fisetin

Fisetin is found in large amounts in strawberries and cucumbers [28]. Studies have demonstrated inhibition of the growth of colon cancer cell lines such as HT-29 and HCT-116 induced by fisetin. Growth inhibition of HT-29 cells via fisetin ensued through two main pathways, namely, the inhibition of cell growth and of DNA synthesis. Fisetin arrested HT-29 cells in the G2/M phase. Downstream experiments demonstrated reduced cyclin-dependent activity (CDK2 and CDK4) and consequently attenuation in cyclin E and D1 and in the strength of p21 expression [29]. Researchers have proposed that fisetin has the potential to reduce the expression level of the COX-2 gene, which could be the cause of growth inhibition [30]. Enhancement of expression of the COX-2 gene could occur through the activation of oncogenic pathways such as the Ras-MAPK pathway. After the expression of the COX-2 gene was increased, cell proliferation was promoted via stimulation of β -catenin/Tcf activity [31].

Although fisetin attenuates the COX-2 and MAPK pathways in HT-29 cells, the growth of HCT-116 cells was inhibited through the apoptosis mechanism. Several factors were involved in this mechanism, such as reduction of antiapoptotic Bcl-xL and Bcl-2 at the protein level and enhancement of proapoptotic Bad and Bim. Moreover, excitation of mitochondrial permeability and consequently activation of the caspase cascade, including caspase-3, -7, -8, and -9, and downstream factors such as cytochrome c are other means of promoting apoptosis. Activation of death receptors (Fas and tumor necrosis factor) by fisetin assisted in promoting apoptosis. In addition, fisetin increased p53 expression [32].

Rutin

Rutin is present in a wide range of fruits, including citrus, berries, peach, apple, and apricot, as well as in some vegetables such as parsley and tomatoes [33]. *In vitro* investigation of the rutin compound showed a slight trend towards inhibition of CRC cells such as HT-29 and CaCo-2 [13]. Rutin has been reported to have a role in causing DNA damage [34]. *In vivo* results on nude mice, which were made cancerous by injecting SW480, indicated that rutin has antiangiogenic activity against CRC [35]. Another study reported that rutin has the potential to reduce the number of aberrant crypt foci (ACF) in azoxymethane (AOM)-induced rats. Rutin also has the potential to induce apoptosis in rats by virtue of it being able to modify the expression level of Bax, Bcl-2, and caspase-9 [36]. The efficiency of rutin had also

been explored in a study on F344 rats, which demonstrated inhibition in the outgrowth of ACF [37].

Flavanones

Flavanones are a group of flavonoids that are present in citrus fruits [38]. Hesperidin, naringenin, silibinin, and eriodictyol are members of this family.

Hesperidin

Hesperidin is a flavanone that is widely found in citrus fruits. It specifically upregulates caspase-3 transcription in SNU-C4 CRC cells [39]. This upregulation in mRNA initiates the apoptotic pathway. Hesperidin has also been reported to decrease the expression of Bcl-2 and to upregulate Bax expression [40].

Naringenin

Naringenin, which can be found in citrus fruits, has shown promising anti-CRC effects on HT-29 cells [41]. These effects were tested by assessing the ability of naringenin to activate a related signaling pathway of the apoptosis cascade in the presence of estrogen receptor β (ER β). One study showed that naringenin regulates ER β activity and increases stimulation of p38/MAPK phosphorylation and caspase-3 activity [42]. *In vivo* studies indicated that naringenin could suppress the early stage of colon cancer by attenuating inducible nitric oxide synthase (iNOS) and COX-2 levels in carcinogen-injected rats [43, 44].

Silibinin

Silibinin is a flavanone that has been isolated from milk thistle [45]. The anticolorrectal effect of this compound was extensively studied in various colon cancer cell lines such as HT-29, HCT-116, SW480, and LoVo. Silibinin has been shown to have the potential to inhibit HT-29 cells through different pathways [46]. In the presence of silibinin, cell cycle arrest occurred in G2/M. In mechanistic studies related to cell cycle progression, silibinin was associated with decreasing levels of CDK2, CDK4, cyclin E, and cyclin D1 and with the upregulation of p27 and p21 [47]. Although the antiproliferation and apoptotic effects of silibinin on HT-29 cells likely depend on attenuation of ERK and Akt, suppression of iNOS, COX, hypoxia-inducible factor-1 alpha (HIF-1 α), and vascular endothelial growth factor (VEGF) also leads to an antiangiogenesis effect [48].

The inhibitory effect of silibinin on SW480 was reported to occur through four mechanisms: *i*) cell death and apoptosis via upregulation of caspase-3, -8, and -9 [49]; *ii*) the Wnt- β -catenin pathway, whereby silibinin decreases β -catenin and Gsk- β levels; *iii*) a

decrease in angiogenesis regulators such as VEGF and iNOS; and *iv*) targeting of signaling molecules involved in proliferation and survival such as cyclin D and c-Myc [50]. Although silibinin has the potential to inhibit SW480 through apoptosis, growth inhibition of HCT-116 cells in the presence of this compound was independent of apoptosis and more related to suppression of p27, p21, cyclin B1, cyclin D1, and CDK2 [46]. Another study conducted in 2012 demonstrated that silibinin also had inhibitory effect on LoVo colon cancer cells and that this effect occurred through the suppression of matrix metalloproteinase (MMP)-2 and AP-1 binding activity [51].

In vivo studies of Wistar rats and A/J mice induced by AOM also confirmed the anti-colorectal activity of silibinin. These extensive studies were performed to the level of gene expression and indicated that silibinin might be used as a chemopreventive compound in the battle against CRC in Wistar rats through two mechanisms: *i*) suppression of inflammatory receptors such as interleukin-1 (IL-1) and *ii*) induction of apoptosis via the downregulation of Bcl-2 and the upregulation of Bax [52]. The chemopreventive mechanism of silibinin was also under investigation in A/J mice. Silibinin targeted β -catenin and IGF-1 in the Wnt and PI3K/Akt pathways, respectively [53].

Eriodictyol

Eriodictyol inhibited the proliferation of DLD-1 colorectal cell lines at a concentration of 22 μ g/ml. Eriodictyol is potent in preventing the growth of colon cancer by suppressing the transcription of the COX-2 gene [54]. No other data or research supports the use of this compound as an anti-colon cancer agent.

Flavones

The main sources of flavones are cereals and herbs [55]. The most important members of this family are acacetin, apigenin, chrysin, tangeretin, luteolin, baicalein, and nobiletin. In recent years, scientific and public interest in flavones has grown.

Acacetin

The main source of acacetin is *Robinia pseudoacacia*. SW480 cells that were exposed to acacetin showed reduced cell viability and cell arrest in G2/M. Investigators claimed that the functional group on the 4' position of acacetin and its analogs confers an effect on cell arrest [56]; the exact mechanism by which acacetin acts against colon cancer is, however, still unclear.

Apigenin

Apigenin is found in vegetables such as chamomile, celery, and parsley [57]. It has shown an inhibitory effect on some colon cancer cell lines such as SW480, HCT-116, CaCo-2, and HT-29. Further investigations showed that colon cancer cells were arrested in G2/M [56, 58]. The inhibitory effect of apigenin against HCT-116 cells occurred through the downregulation of cyclin B1, Cdc-2, and Cdc-25 and the upregulation of p53 and p21 [59]. Apigenin also induced ERK and p38 in the MAPK cascade [60] and decreased the expression of mammalian target of rapamycin (mTOR) and cyclin D1 in the p53 mutant HT-29 human colon adenocarcinoma cell line [61]. Moreover, apigenin inhibits SW480 cells by increasing the expression of caspase-3 and Bax and downregulating Bcl-2 [62].

In summary, it appears that *in vivo* findings were in accordance with *in vitro* outcomes. Turner *et al.* reported that apigenin has the potential to inhibit CRC induced by AOM in Sprague-Dawley (SD) rats via apoptosis [63].

Chrysin

Chrysin has been found in *Passiflora caerulea*, *Pleurotus ostreatus*, and *Oroxylum indicum* [64]. Exposure of SW480 CRC cells to chrysin inhibited cell growth, and colorectal cells were arrested in G2/M in the presence of chrysin [56]. The inhibitory effect of chrysin on cancer cells might cause inhibition of COX-2 expression via inhibition of nuclear factor IL-6 (NF-IL-6) [65]. Chrysin not only induces DNA fragmentation, but it may also promote apoptosis in CaCo-2 cells [66]. *In vivo* studies reported the chemopreventive effect of chrysin in male rats with colonic cancer induced by AOM [67]. Another *in vivo* study on preneoplastic colorectal lesions induced by 1, 2-dimethylhydrazine in female Wistar rats confirmed the efficiency of chrysin against CRC [68].

Tangeretin

The main source of tangeretin is citrus peel [69] and thus tangeretin is categorized as a citrus flavonoid. A paper published in 2007 reported that tangeretin suppressed proliferation by cell cycle arrest in G1 without apoptosis in the human colon cancer cell line HT-29 [70]. Multidrug-resistant (LoVo/Dx) human colon adenocarcinoma cells that were exposed to tangeretin were inhibited; the investigators concluded that the activity of tangeretin probably enhanced apoptosis activity through the induction of caspase-3 in this cell line [71]. Tangeretin suppresses the proliferation of COLO-205 by blocking cell cycle progression in G1; reducing the expression of cyclin

A, D, and E; reducing the effect of CDK2 and CDK4; and stimulating the activity of p21, p27, and p53 [72].

Luteolin

Luteolin is mainly found in vegetables rather than fruits [73], including celery, carrots, parsley, and mint. The anti-colon cancer effect of luteolin has been investigated *in vitro* in several colon cancer cell lines such as HCT-15, HT-29, CaCo-2, COLO-320 DM, and SW480. The studies together indicated that luteolin has the potential to control CRC through three main pathways: *i*) the apoptosis pathway by activating caspase-3, -7, and -9 in HT-29 cells [74]; *ii*) the PI3K/Akt and ERK pathways by inhibiting the IGF receptor (IGF-R) in HT-29 cells [75]; and *iii*) the Wnt/ β -catenin/GSK-3 β signaling pathway by modulating GSK-3 β in HCT-15 cells and inhibiting β -catenin in COLO-320 DM cells [76, 77]. Through these main pathways, luteolin was able to activate DNA damage in CaCo-2 cells [78]. Furthermore, cell cycle arrest of HT-29 cells was induced by modulation and inhibition of CDK2 and cyclin D [75, 76]. Luteolin also inhibited SW480 and CaCo-2 cells by inducing cell cycle arrest in G2/M [79].

The outcome data of luteolin against CRC was evaluated in an *in vivo* study by using BALB/c mice induced by AOM. Findings from this animal experiment demonstrated that luteolin inhibited metastatic colon cancer by reduction of MMP-2 and MMP-9 [80]. Luteolin also inhibited iNOS and COX-2 [81, 82]. In addition, it activated lysosomal enzymes, inhibited caspase-3, and modulated Bcl-2 and Bax in the apoptosis pathway [83]. Inhibition of tumor growth occurred through the Wnt/ β -catenin pathway in the presence of luteolin. Moreover, the incidences of mucin-depleted foci and ACF were decreased by luteolin in BALB/c mice [84].

Baicalein

The inhibitory effect of baicalein, which is present in Indian trumpet flower and Chinese skullcap, or *Scutellaria baicalensis*, on human colon cancer was studied *in vitro* and *in vivo* [85]. Research indicated that baicalein had a significant inhibitory effect on HCT-116 cells. The mechanisms of effect of baicalein occur through three pathways: *i*) the extrinsic pathway of apoptosis, *ii*) by decreasing the incidence of inflammation, and *iii*) by impairment of tumor formation through inactivation of the PI3K/Akt pathway. Baicalein increased the expression of caspase-3 and -8, which are involved in apoptosis. The expression of NF- κ B was inhibited, resulting in inhibition of iNOS, MMP-9, and MMP-2 genes, all of which are involved in inflammation [86, 87]. The effect of baicalein on HT-29 cells was also

investigated. The data indicated that baicalein had the ability to increase cell arrest in the G1 phase. Baicalein attenuated the expression of Bcl-2, whereas the expression of Bax was augmented. Moreover, induction of apoptosis was achieved by inactivation of the PI3K/Akt pathway [88]. Further studies on cancerous Institute for Cancer Research (ICR) mice induced by AOM supported the preventive effect of baicalein [86].

Nobiletin

The anti-CRC ability of nobiletin has been detected in both *in vitro* and *in vivo* studies. Nobiletin was able to suppress the proliferation of HT-29 cells through cell cycle arrest in G1 without inducing apoptosis [70]. One study indicated that this citrus flavonoid prevented cancer metastasis through reduced expression of MMP-7 [89]. The anti-cancer efficiency of nobiletin was tested at the *in vivo* level in several models, such as F344 rats, SD rats, C57BL/KsJ-db/db mice, and ICR mice. Nobiletin was introduced into cancer models and showed a chemopreventive effect as a result of reduced expression of prostaglandin E2 [89-92].

Flavan-3-ols (flavanols)

Flavan-3-ols are a class of flavonoids found in human foods and beverages [93]. There has been great interest in flavanols in nutrition and medicine because they have antioxidant properties. The best known member of the flavan-3-ol family is the catechin group, which is abundantly present in tea [94, 95].

Catechins

Besides being the main component of tea, catechins can also be found in grapes, apple, peas, wine, and cocoa. Catechins contain a benzopyran skeleton with an additional phenyl group at the second position. The catechin family includes the following: (+)-gallocatechin (GC), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), and (-)-epicatechin 3-gallate (ECG) [96].

The antiproliferative properties of these compounds were investigated in CaCo-2, HT-29 (p53 mutant type), HCT-116 (p53 wild type), and SW480 (p53 mutant type) cell lines [97-100]. When these compounds were compared, EGCG had the most potent anti-colorectal inhibitory potential. Although EGCG had a significant inhibitory effect on HCT-116 and SW-480 cells, even stronger inhibition was observed against HCT-116 cells. Therefore, p53 might play an important antiproliferative role against colon cancer. Cell arrest occurred in the G1 and G2/M phases. Apoptosis was one of the main effects in the

inhibition of HCT-116 cells. Moreover, when the different members of this family were compared, GC, EGC, and EGCG were shown to have better efficiency than EC and ECG, indicating that esterification of gallic acid with catechin could enhance the anti-cancer property of the compound [101].

Overall, the literature indicates that EGCG can cause apoptosis, cell cycle arrest, and DNA damage [102]. EGCG inhibited the binding of epidermal growth factor (EGF) to EGF receptor (EGFR) in the receptor tyrosine kinase pathway, induced the expression of caspase-3 and -9, and caused mitochondrial damage in HT-29 cell lines [98]. EGCG instigated mitochondrial damage and apoptosis by interference with JNK [103]. EGCG has been shown to cause reduced MMP-7, MMP-9, and IGF-1R levels in HT-29 and CaCo-2 cell lines [104]. The inhibitory effect of EGCG on EGFR in SW480 cell lines occurs through the internalization of EGFR from the endosome. EGFR is therefore not able to return to the cell surface [105]. EGCG can block HCT-116 cells through two main mechanisms *i*) induction of reactive oxygen species (ROS), thereby activating p53, p21, and PUMA, which then leads to DNA damage; and *ii*) stimulation of caspase-3, caspase -9, and cytochrome C release, which leads to cell apoptosis [106, 107].

The antiproliferative activity of EGCG was tested *in vivo* in an animal model. Colitis was induced in mice by injection of dextran sulfate sodium. Results indicated that the formation of ACF was decreased in the presence of EGCG. Further investigations into the molecular mechanism of EGCG revealed that it controlled the formation of ACF by attenuating the expression of PI3K/Akt/nuclear factor kappa B (NFκB) and the activation of the ERK pathway [108].

Proanthocyanidin

Proanthocyanidin is present in tea, cranberries, and grape seeds and has been shown to interrupt the proliferation of colorectal cells such as CaCo-2, HT-29, SW620, SW480, and HCT-116 [109, 110]. It suppresses HT-29 cells by *i*) disrupting the actin cytoskeleton, *ii*) inducing apoptosis by increasing caspase-3, *iii*) damaging DNA by increasing ROS levels, *iv*) arresting the cell cycle in G2, and *v*) inhibiting COX-2 expression [111-114]. The inhibitory effect of proanthocyanidin on HCT-116 cells was reported to be related to DNA damage, inhibition of the COX-2 gene, and promotion of apoptosis [109, 115]. The expression of PI3K was attenuated in the CaCo-2 cell line in the presence of proanthocyanidin [116]. Proanthocyanidin might also increase apoptosis in CaCo-2 cells [116]. The inhibition of SW620 cells (metastatic colon carcinoma) by proanthocyanidin can occur through several mechanisms such as increased

activation of caspase-3, c-jun, NFκB, and p53 [117, 118].

In vitro results of proanthocyanidin activity has been confirmed in an *in vivo* study. AOM was used to induce colon cancer in F344 rats. ACF were shown to decrease in the presence of proanthocyanidin because of the activation of caspase-3 [119].

Flavanonols

Flavanonols are a class of flavonoids that possess a 3-hydroxy-2,3-dihydro-2-phenylchromen-4-one backbone. Flavanonols are present in red wine, as well as in many red, purple, and blue fruits and vegetables.

Pelargonidin

Pelargonidin is found in berries such as raspberries and strawberries. The efficiency of pelargonidin has been investigated against a variety of cell lines such as primary (CaCo-2) and metastatic (LoVo and LoVo/ADR) CRC cell lines and human colorectal adenocarcinoma (HT-29). No significant inhibitory effect has been reported in these cell lines [120, 121]. Mild inhibition of cell proliferation was reported, however, against HCT-116 cells at a concentration of 200 μg/ml [122].

Peonidin

Bilberry, blueberry, cherry, cranberry, and peach are the main sources of peonidin [114], which inhibits the growth of SW480 cells in a dose-dependent manner. Cell cycle arrest of SW480 cells occurred at the G1 phase in the presence of peonidin [123]. No other studies have been found in the literature describing anti-CRC effects of peonidin.

Cyanidin

Cyanidin is present in various fruits such as red apple, blackberry, blueberry, cherry, cranberry, peach, and plum [124]. Retrieved published articles did not show any significant results for cyanidin against primary (CaCo-2) or metastatic (LoVo and LoVo/ADR) colon cancer cell lines [125].

Delphinidin

Delphinidin is one of the main compounds in blueberries, and an anticancer effect of delphinidin on a human colon cancer cell line (colo205) has been reported [126]. The inhibitory effect of delphinidin on LoVo/ADR cell lines was also investigated. The data revealed that delphinidin inhibited metastatic CRC and that this may have been due to cellular ROS accumulation [125]. Further investigation of delphinidin indicated that it inhibited HT-29 human tumor cells through the suppression of EGFR [97]. A

recent study on delphinidin claimed that it had antioxidant activity against human CRC HTC-116 and HT-29 cells and could also induce DNA damage [127]. In addition, delphinidin potently inhibited HTC-116 and HT-29 cell lines through the downregulation of HIF-1 and p27 by affecting the PI3K/Akt/mTOR signaling pathway [128]. HCT-116 cells treated with delphinidin suppressed the NF-kappa B pathway and activated the expression of caspase-3, -8 and -9,

resulting in cell cycle arrest in the G2/M phase, thereby leading to apoptosis [129].

Malvidin

Blueberries are the main source of malvidin [130]. The antiproliferation effect of malvidin has been studied in colon cancer. Malvidin had no significant inhibitory effect on CaCo-2 and HCT-116 cells, nor did it demonstrate any effect on LoVo or LoVo/ADR cells [125, 131].

Table 1: Summary of the main flavonoids and their anti-colorectal cancer properties.

Flavonoid	Colon cancer cell lines	Animal model	Signaling and target pathways	References
Quercetin	HT-29 HCT-116 SW480	Rats	Reduces cyclin A; induces Cdc-2, p21, and Wnt- β -catenin	[9-16]
Kaempferol	HCT-116 HT-29 DLD-1	Wistar male rats	Induces DNA damage response; upregulates p53 and PUMA; activates mitochondria permeability; induces cytochrome C release; reduces expression of CDK2, CDK4, cyclin D1, cyclin E, and cyclin A; attenuates IGF-1; induces cleavage of caspase-3, -7, -8, and -9; attenuates Bcl-xL, phosphorylation, and Akt activity; increases Bad; activates Fas inhibition of COX-2 activity	[17-24]
Myricetin	HCT-15 HCT-116	---	Induces Bax; induces release of apoptosis inducing factor	[25-27]
Fisetin	HCT-116 HT-29	---	Reduces CDK2 and CDK4 and consequently attenuates cyclin E and D1 and strength of p21 expression; reduces expression of COX-2 and MAPK-Ras; reduces Bcl-xL and Bcl-2; enhances Bad and Bim, including caspase-3, -7, -8, and -9 and cytochrome c release; activates FasL and TNF; increases p53	[28-32]
Rutin	HT-29 CaCo-2	Nude mice, F344 rats	Damages DNA; induces apoptosis; changes expression level of Bax, Bcl-2, and caspase-9	[13,33-37]
Hesperidin	SNU-C4	---	Increases caspase-3 and Bax; decreases Bcl-2	[39-40]
Naringenin	HT-29	Rats	Regulates ER- β ; induces MAPK/p38 and caspase-3; attenuates iNOS and COX-2	[41-44]
Silibinin	HT-29 HCT-116 SW480 LoVo	Wistar rats, A/J mice	Decreases CDK2, CDK4, cyclin E, and cyclin D1; upregulates p27 and p21; attenuates ERK and Akt; suppresses iNOS, COX, HIF-1 α , and VEGF; upregulates caspase-3, -8, and -9; decreases β -catenin and Gsk- β levels; decreases c-Myc; suppresses MMP-2 and AP-1; suppresses IL-1; downregulates Bcl-2 and upregulates Bax; targets β -catenin and IGF-1	[45-53]
Eriodictyol	DLD-1	---	Reduces COX-2 level	[54]
Acacetin	SW480	---	Unknown mechanism	[56]
Apigenin	SW480 HCT-116 CaCo-2 HT-29	SD rats	Downregulates cyclin B1, Cdc-2, and Cdc-25; upregulates p53 and p21; induces ERK and p38; decreases mTOR and cyclin D1; increases expression of caspase-3 and Bax; attenuates Bcl-2 expression	[56-63]
Chrysin	Caco-2 SW480	Wistar rats	Inhibits COX-2 and NF-IL-6; induces DNA fragmentation; induces apoptosis	[56,64-68]
Tangeretin	HT-29 LoVo/Dx COLO-205	---	Increases caspase-3 level; reduces cyclin A, D, and E; attenuates CDK2 and CDK4 activity; instigates activity of p21 and p27	[69-72]
Luteolin	HCT-15 HT-29 Caco-2 COLO-320DM SW480	BALB/c mice	Induces caspase-3, -7, and -9; inhibits PI3K/Akt, ERK, IGF, β -catenin, GSK-3 β , MMP-2 and -9, iNOS, COX-2, Bcl-2, Bax, CDK2, and cyclin D; damages DNA	[73-84]
Baicalein	HCT-116 HT-29	ICR mice	Increases caspase-3 and -8; inhibits PI3K/Akt, NFRB, iNOS, and MMP-2 and -9; attenuates Bcl-2; induces Bax	[85-88]
Nobiletin	HT-29	C57BL/KsJ-db /db mice, ICR mice, SD rats, F344 rats	Reduces MMP-7 and PGE2	[70,89-92]
Catechin family	CaCo-2 HT-29 HCT-116 SW480	Mice	Induces DNA damage and mitochondrial damage by interference of JNK; induces caspase-3 and -9; releases cytochrome C; inhibits binding of EGF to EGFR in receptor tyrosine kinase pathway; reduces MMP-7, MMP-9, and IGF-1R; induces ROS; activates p53, p21, and PUMA; attenuates PI3K/Akt/ NFRB; activates ERK	[93-108]
Proanthocyanidin	CaCo-2 HT-29 SW620 SW480 HCT-116	F344 rats	Inhibits COX-2 and PI3K; induces caspase-3; activates c-jun; increases NFRB and p53 activity	[109-119]
Pelargonidin	Caco-2 LoVo LoVo/ADR	---	No significant effect	[120-122]

	HT-29			
	HCT-116			
Peonidin	SW480	---	No significant effect	[114,123]
Cyanidin	CaCo-2	---	No significant effect	[124,125]
	LoVo LoVo/ADR			
Delphinidin	Colo205	---	Induces ROS accumulation; suppresses EGFR; damages DNA; downregulates HIF-1, p27, PI3K/ Akt/ mTOR, and NFkB; induces caspase-3, -8, and -9	[97,125-129]
	LoVo/ ADR			
	HT-29			
	HCT-116			
Malvidin	Caco-2	---	No significant effect	[125-131]
	HCT-116 LoVo			
	LoVo/ADR			

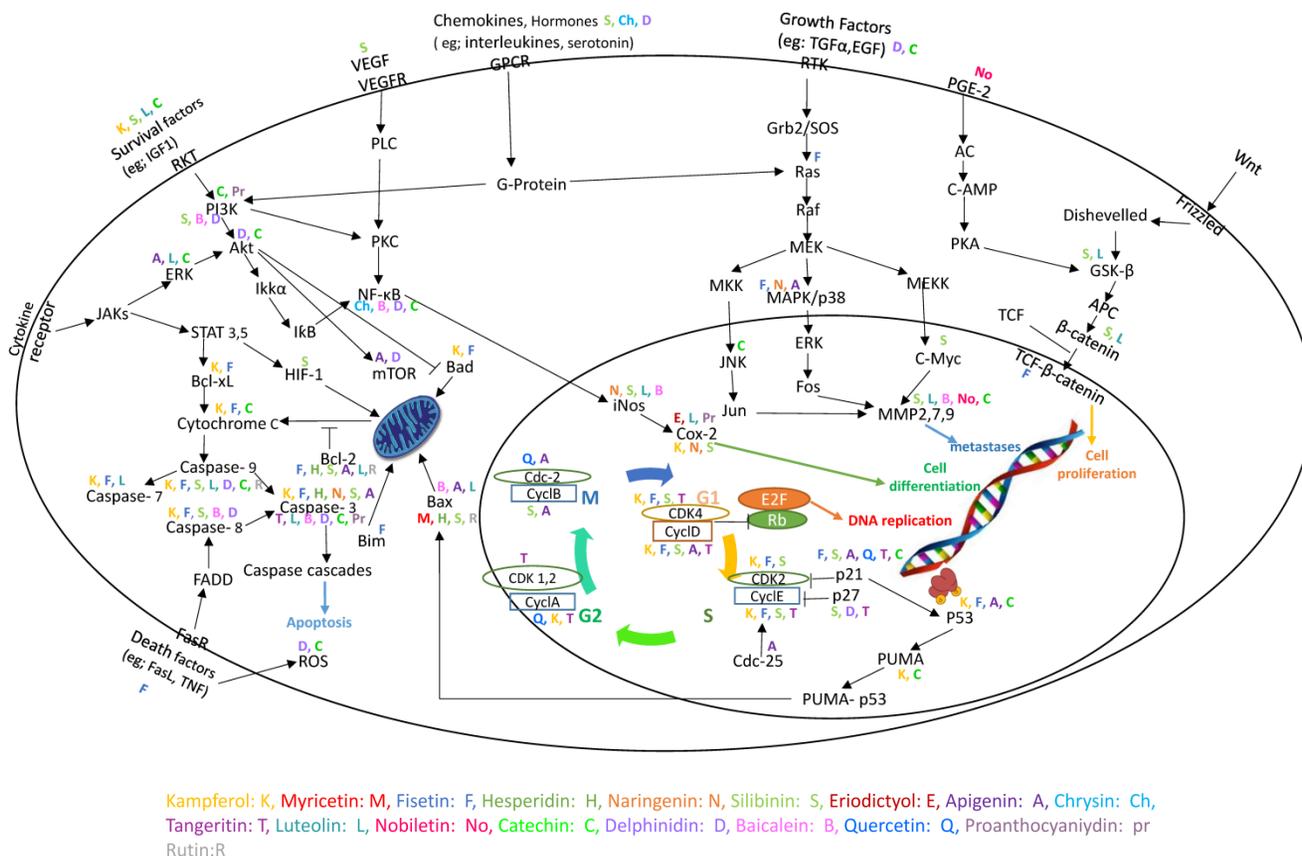


Figure 2: Association map of the role of the main flavonoids in their effect on signaling pathways in colorectal cancer. Different flavonoids are indicated by different colors.

Conclusion

Flavonoids belong to a large family of polyphenols and are well-known for their antioxidant properties. Furthermore, flavonoids have potential effects against several types of cancers such as breast, lung, and prostate. Although the mechanisms by which flavonoids act against colon cancer have been investigated, the exact details of these mechanisms are still unclear. In this review, we classified flavonoids on the basis of their chemical structures and the cell signaling pathways that each compound affects in CRC. We also summarized the *in vitro* and *in vivo*

studies that have been performed for these compounds.

We prepared an association map from the information gathered in this review. Some points can be highlighted on the basis of this map (Figure 2). First, most of the compounds appear to activate the caspase cascades. Second, cyclin D, cyclin E, CDK2, and CDK4 are the major targets for most of the flavonoids that affect the cell cycle. Third, compounds that have effects on Jun and c-Myc prevent cancerous cells from undergoing metastasis. Fourth, Bax and Bcl-2 are the main targets for flavonoids through the induction of apoptosis. Fifth, the majority of

compounds have effects on iNOS, COX-2, and Nf κ B through the PI3K/Akt pathway.

From these findings, we can draw two conclusions. First, flavonoids are good candidates against colon cancer with strong efficacy. Part of this efficacy might be related to the ability of these compounds to block cancer cells through several pathways. Second, there may be an association between the chemical structure of flavonoids and specific signaling pathways. Although flavonoids share the same core ring, the targeted signaling pathway for each compound may be different. In addition, some of these flavonoids possess potent activity against colon cancer; there is a paucity of information, however, regarding the pathways. More clinical and preclinical studies are needed to elucidate the role of flavonoids against colon cancer.

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Conflict of Interest

The authors declare no conflict of interest.

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