Chapter 5

Anthocyanins-Smart Molecules for Cancer Prevention

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Additional information is available at the end of the chapter

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Abstract

Anthocyanins are one of the most widespread natural pigments in the plant kingdom. Being surrounded by so many fruits and vegetables rich in anthocyanins, it is recommended to consume a relatively large amount of them. A daily intake of anthocyanins has a certain demonstrated benefits: lowers the risk of cardiovascular disease, diabetes, arthritis, and cancer due, at least in part, to their antioxidant and anti-inflammatory activities. Lately, great attention is paid to their anticancer properties due to the need for user-friendly approaches to improve the treatment. So far, cancer had been nominated to be the second in top 10 diseases of the twenty-first century. Those colorful pigments have the ability to modulate the activity of multiple targets involved in carcinogenesis through direct interaction or modulation of gene expression and can also inhibit the growth of cancer cells. However, the main concern related to the use of anthocyanins as anticancer agents is their poor bioavailability, more specific poor absorption, and biodistribution. In this chapter, the anticancer activities of anthocyanins or anthocyanin-rich extracts *in vitro* or *in vivo* were reviewed.

Keywords: anthocyanins, berries, cancer

1. Introduction

Anthocyanins are cell vacuole components, abundant flavonoid constituents, which are responsible for the varied colors (red, purple, and blue) of flowers, vegetables, or fruits. Apart from fruits and flowers, anthocyanins also are also accumulated in vegetative tissues where they are considered to confer protection against various biotic and abiotic stresses [1–4]. They are the largest and the most important group of water-soluble plant pigments. Berries, grapes, apples, purple cabbage, black soybean, and black rice are some examples of

rich anthocyanins fruits and vegetables. In their natural environments, plants are vulnerable because of multiple attacks by many different species of herbivores and also pathogens [5]. A vast spectrum of secondary metabolites have been demonstrated to act against their predators [6]. Among them are the phenolics, a large group of structurally diverse compounds, as well as certain flavonoids such as the anthocyanins. There are several ways anthocyanins assist plants in their defense against other organisms, such as chemical repellents and visual signals [7]. Along with other flavonoids, certain anthocyanins have demonstrable antiviral, antibacterial, and fungicidal activities. Also, it is generally accepted that the colors of flowers and fruits enhance reproductive success by facilitating communication between plants, their pollinators, and seed-dispersers [8]. Another positive propriety of anthocyanins is that they have demonstrated to exhibit antioxidant potential *in vitro* and *in vivo*. The antioxidant potential of anthocyanins have been demonstrated *in vitro* using several cell culture lines including ovarian, colon, endothelial liver, breast, leukemic cells, and keratinocytes [9–17]. Applied *in vitro* as treatment, anthocyanins have exhibited multiple antiproliferative and anticarcinogenic effects [18, 19]. The antioxidant activity of anthocyanins is a great property and was demonstrated that their chemical structure seems to be responsible for that [20–23].

2. Chemical structure of anthocyanins

Anthocyanins occur naturally in fruits and vegetables as glycosides, having one or more sugar attached to an aglycone nucleus (anthocyanidin). Their aglycones share a C6-C3-C6 carbon skeleton, characterized by the presence of two benzyl rings (A and B) and a heterocyclic ring (C) [24].

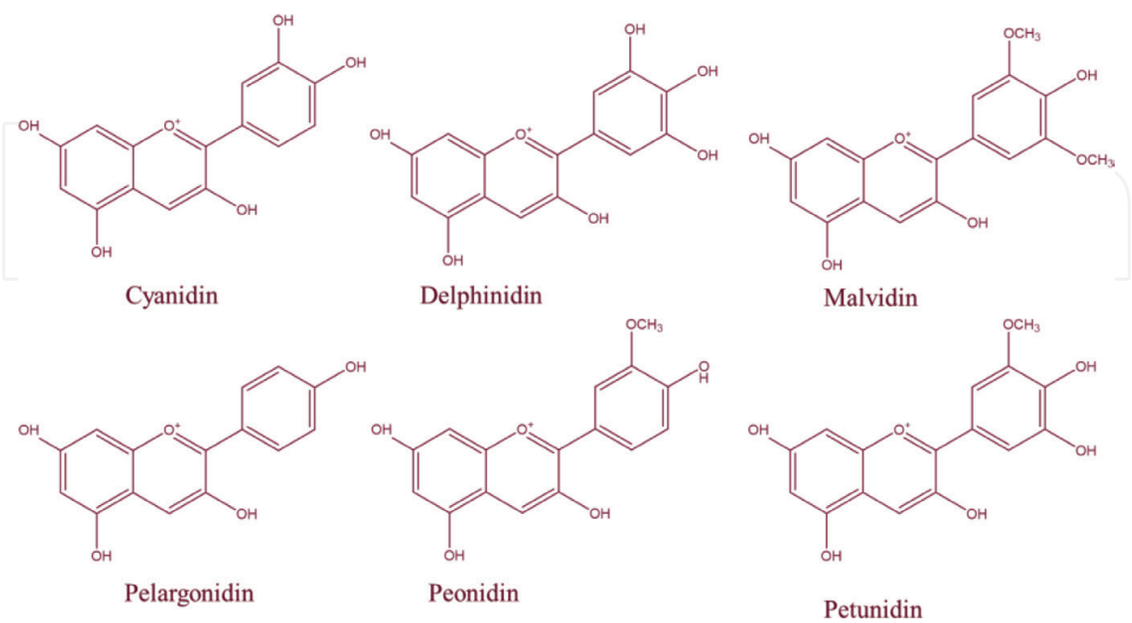

Figure 1. Chemical structure of the six most common anthocyanidins.

According to hydroxylation and methylations on the different positions of the rings, there are close to 25 different aglycones [25]. They exist in natural products, mainly in a form combined with glucose, galactose, and rhamnose, the more common sugar moieties attached to the aglycone but others sugars are also frequently found, and can be divided into at least six common types, such as pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin, according to the different substituent groups on flavylium B-ring (**Figure 1**) [26, 27]. The sugars attached to the aglycone may in turn be further linked to other sugars through glyosidic bonds or acylated with organic aromatic or aliphatic acids [28]. One of the most striking properties linked to their chemical structure is that their color changes depending on the pH. They are natural pH indicators; they appear pink at low pH, purple in neutral conditions, and greenish-yellow in basics but the most stable form dominates at low pH [26].

3. Anthocyanins' potential health benefits

Since we consume a great amount of fruits, the daily intake of anthocyanins is highly variable and dependent on eating habits. Residents of the United States consume about 12.5 mg/day while in Europe, a highest consumption was found in Italy, about 64.9 mg/day [29, 30]. Many studies have suggested that anthocyanins have antioxidant, anti-inflammatory, and anti-carcinogenic properties and lower the risk of cardiovascular disease, diabetes, arthritis, and cancer due, at least in part, to their antioxidant and anti-inflammatory activities (**Figure 2**) [19, 26, 31, 32].

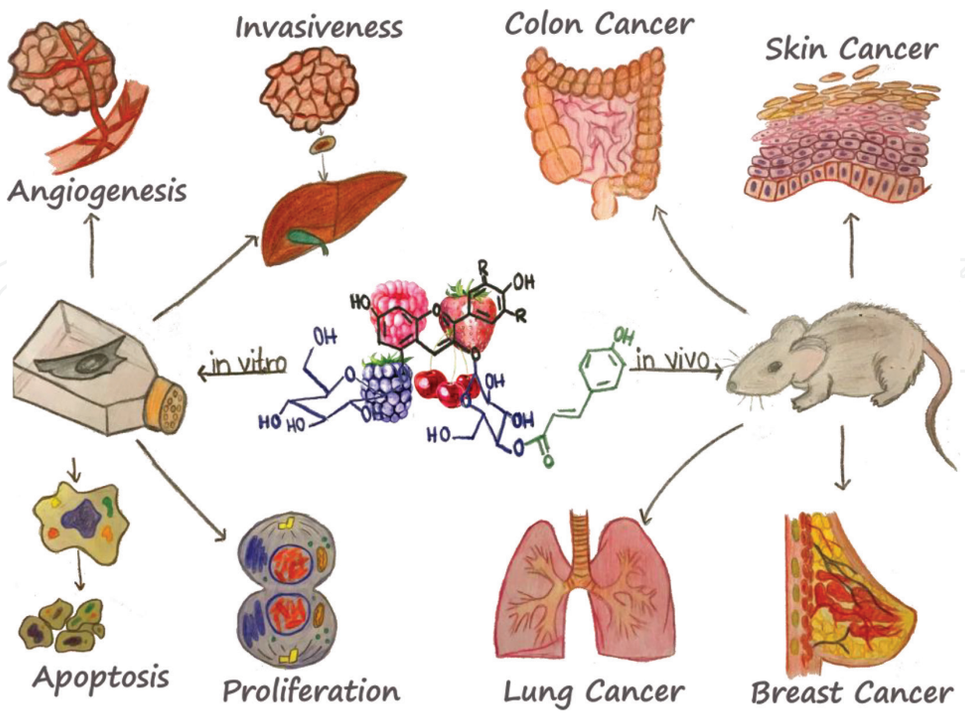

Figure 2. Anticancer properties of anthocyanins.

Reactive oxygen species (ROS) are produced by all aerobic cells and are important to the immune system, cell signaling, and many other normal body functions. They can elicit cellular damage, leading to degenerative diseases such as inflammation, cardiovascular disease, cancer, and aging if ROS are excessively produced [33–35]. As we mention previously, anthocyanins are potent antioxidants and their effectiveness has been tested *in vitro* and *in vivo*. They quench free radicals and terminate the chain reaction that is responsible for the oxidative damage. Because of the pH in the human body, their antioxidant activity at a neutral pH has a particular importance [36]. To assess the *in vivo* antioxidant activity of anthocyanins, an anthocyanin mixture was administered to mice/rats that were subjected to psychological stress [37, 38]. It was noticed that anthocyanins have similar antioxidant potency as vitamin E (α-tocopherol). Dietary anthocyanins have the potential to increase serum antioxidant capacity and thereby protect against LDL oxidation and prevent cardiovascular diseases as demonstrated in a human trial [39]. Deposits oxidized cholesterol into the artery wall can lead to atherosclerosis and eventually cardiovascular diseases [40]. Several studies have shown that anthocyanins have anti-obesity effect on high-fat diets and consequently may contribute to the prevention of type 2 diabetes. One of the studies demonstrates that black soybean anthocyanins were found to effectively reverse the weight gain of high-fat diet group rats [41]. Moreover, a number of different reports indicate that consumption of fruits and vegetables, especially rich in polyphenols, decrease the incidence of type-2 diabetes, a condition associated with insulin resistance [42, 43]. Nevertheless, evidence in the use of anthocyanins to improve night vision was also revealed in other scientific articles [44, 45].

4. Anticancer properties of anthocyanins

The uncontrolled growth of cells which can invade and spread to distant sites of the body is a global health problem, called cancer, with high mortality. Prevention and routine monitoring are critical to early and accurate diagnosis. Most therapeutic options do not offer cure but rather a deceleration of cancer progression. They not only aim at life extension and the improvement of patients' life quality but also often they have multiple side effects. In recent years, fruit and vegetables, including soft fruits such as berries, may represent a valid alternative than drugs with undesirable side and adverse effects, because of their chemopreventive or chemotherapeutic properties against certain diseases, such as cancer. Recent studies on the cancer preventative activities of the anthocyanins include results from *in vitro* cell culture and *in vivo* animal model tumor systems, as well as data from human epidemiological studies. Cancer cells differ from normal cells by a number of characteristics, thus being different in morphology and function. Anthocyanins can attack cancer cells due to these differences and cause a number of effects. A significant characteristic of cancer cells is their uncontrolled cell cycle, which leads to continuous division and proliferation. Pure anthocyanins and anthocyanin-rich extracts have demonstrated to inhibit cell proliferation by the ability of anthocyanins to block various stages of the cell cycle [46, 47]. Moreover, they can selectively inhibit the proliferation of cancer cells, but have little influence on the proliferation of normal cells [48, 49]. Anthocyanins have demonstrated to induce the apoptosis of cancer cells through the internal mitochondrial pathway and the external death receptor pathway. Usually, apoptosis,

the programed cell death, in tumor cells is not present; therefore, dead cells cannot be eliminated normally. Cancer cells have deregulated several genes to avoid the apoptosis, such as p53, and these cells have high resistance to death compared with normal cells. In the intrinsic pathway, cytochrome *c* release and modulation of caspase-dependent anti- and proapoptotic proteins appear as an increase in mitochondrial membrane potential, because of anthocyanin treatment on cancer cells. In the extrinsic pathway, the expression of FAS and FASL is modulated by anthocyanins resulting apoptosis in cancer cells [50–52]. Lately, anthocyanins have been shown to suppress angiogenesis through several mechanisms such as: inhibition of H_2O_2 and tumor necrosis factor alpha (TNF-α)-induced VEGF expression in epidermal keratinocytes and by reducing VEGF and VEGF receptor expression in endothelial cells [53]. Angiogenesis is the physiological process of forming new blood vessels from the existing vascular network for the growth and metastasis of malignant tumors. The process of angiogenesis is controlled by multiple cytokines, of which the most important factor is vascular endothelial growth factor (VEGF); therefore, inhibiting the receptor of angiogenesis vascular endothelial growth factor receptor (VEGFR) could inhibit the metastasis of tumors effectively [18]. Anthocyanins were found to inhibit cancer cell invasion by reducing the expression of matrix metalloproteinase (MMP) and urokinase plasminogen activator (u-PA), both of which degrade extracellular matrix as part of the invasive process and, by stimulating the expression of inhibitors, both of which counteract the action of MMP and uPA [54]. There are two main aspects of cancer cells that threaten patient's health and life: invasion and metastasis. Successful tumor cell extravasation is successful by facilitating degradation of the extracellular matrix barriers. The balance of activated proteases and their naturally occurring inhibitors determine the degradation of the basement membrane [55].

5. *In vivo* studies

In carcinogen-treated animals and also animals with a hereditary predisposition to cancer, anthocyanins have been shown to inhibit the development of cancer. Moreover, they have been proven effective in: esophageal cancer, colon cancer, skin cancer, and lung cancer. After treatment, administration in different forms, such as anthocyanin-rich tart cherry extract, black raspberry powder, lyophilized black raspberries or ethanol: H_2O extract from berries, certain effects, were observed. All diets were equally effective in preventing the development of tumors, reducing tumor numbers by 42–47%, suggesting that anthocyanins in the fruits are important for their chemopreventive activity. A small summary of several types of cancer will be discussed further.

5.1. Colon cancer

Colon cancer is one of the most prevalent diseases across the world. In the United States, colon cancer is the second most prevalent cause of death from cancer in men and women after lung cancer, with approximately 50,310 causes of death [56]. In Europe, colorectal cancer is the second most common cancer, with 50,000 new cases diagnosed in 2012 [57]. The development of colon cancer is associated with high alcohol consumption, high-fat diet poor in fiber, red

meat, obesity, smoking, lack of physical exercise, diabetes, inflammatory bowel disease, and some genetic and epigenetic alterations as: microsatellite instability, chromosomal instability, mutation of p53 gene is one of the familiar genetic changes in the development of colon cancer, and several others [58]. A very recent study published in 2017, used a mouse model, which treated them comparatively with azoxymethan (AOM)/dextran sodium sulfate (DSS) and anthocyanin-rich extract from bilberries for colon cancer development [59]. The anthocyanin extract administered to mice resulted in less inflammation of the colon and a reduced number of tumors than the control group. The formation and the growth of colorectal cancer in AOM/DSS-treated Balb/c mice were prevented by anthocyanins. Another *in vivo* study investigated the chemopreventive activity of commercially available anthocyanin-rich extracts of bilberry, chokeberry, and grape prepared for the food industry [60] Colon cancer male rats treated with a colon carcinogen, azoxymethane, had multiple biomarkers investigated such as: the number and multiplicity of colonic aberrant crypt foci, colonic cell proliferation, urinary levels of oxidative DNA damage, and expression of cyclooxygenase (COX) genes. Compared to the control group, rats fed with different extracts showed several changes. In rats fed with bilberry, chokeberry, and grape extracts, the number of large aberrant crypt foci was reduced. The bilberry and chokeberry diet decreased the colonic cellular proliferation, and the grape and bilberry diets had lower COX-2 mRNA expression of gene. These results clearly support the chemopreventive activity of tested extracts.

5.2. Breast cancer

Breast cancer is the second most common cause of cancer-associated mortalities in women. The American Cancer Society estimated that 60,290 new cases of breast carcinoma *in situ* were expected to be diagnosed among women in the United States during 2015 [61]. Understanding the biology of the human epidermal growth factor receptor 2 (HER2) helps with the classification, prognosis, and treatment of breast cancer because of the overexpression of HER2 identified in 15–20% cases. HER2 is involved in proliferation, angiogenesis, invasion, and metastasis [62]. A group of scientists have used injection of cyaniding-3-glucoside and peonidin-3-glucoside to evaluate the effect on the tumors of the rats used in the experiments [63]. Compared with the control group, the tumors treated with cyanidin-3-glucoside and peonidin-3-glucoside expressed lower levels of HER2 as well as Ki67, a proliferation marker, demonstrated with histopathological studies. Also, the treated tumors expressed higher levels of caspase 3, showing the apoptotic effect of the treatment. A recent published study [64] evaluated the cytotoxicity of an anthocyanin-rich extract from black rice (AEBR) on breast cancer cells *in vitro* and *in vivo*. This study demonstrated that black rice extract has promising roles against breast cancer. The oral administration of anthocyanin-rich extract from black rice (100 mg/kg/day) on nude mice bearing MDAMB-453 cell xenografts, significantly suppressed tumor growth and angiogenesis, as well as antagonized VEGF activity.

5.3. Lung cancer

Lung cancer emerged as the most common cancer worldwide, with 1.8 million new cases in 2012 [57]. The treatment and prevention for lung cancer remains scarce, comparing too

many other types of cancer (e.g., breast and prostate). Also, there are no standard practices for the prevention of lung cancer recurrence and metastasis, so there is a great need for some unconventional, user-friendly approaches to improve the treatment and prevent or delay the recurrent lung disease. A recent study published in 2016, investigated the tumor inhibitory activity of diet supplemented with blackberry, alone and in combination with black raspberry, against lung tumor xenograft using nude mice [65]. Their findings indicated that the mixture of blackberry and black raspberry resulted in higher inhibition of tumor growth vs. blackberry alone. Also, the combination between delphinidin (bioactive in blackberries) and punicalagin (bioactive in black raspberry, which gets converted to ellagic acid *in vivo*) determined a higher tumor growth inhibition than delphinidin alone. In another study, two bioactive compounds, peonidin 3-glucoside and cyanidin 3-glucoside, were isolated and identified the from *Oryza sativa* L. Moreover, those compounds were used to treat various cancer cells. They have demonstrated the inhibition on the growth of Lewis lung carcinoma cells *in vivo* [66].

5.4. Skin cancer

Malignant melanoma of skin accounted for 232,000 new cases, and the regions affected are largely those with white populations [57]. Melanoma skin cancer originates in melanocytes, specialized pigment-producing cells found in both the basal layer of the epidermis. Solar UVB radiation has been implicated as the main cause for skin cancer [67]. Early diagnosis is the key for curing this potentially deadly disease. Also prevention is playing a crucial role in spotting melanomas at earlier and more curable stages [68]. Biochemotherapy, the coadministration of traditional chemotherapeutic drugs and biological agents, show a higher response rate for patients than classical treatments that are based only on chemotherapy alone [69–72]. Most anticancer treatments are derived from natural resources such as marine, microbial, and botanical sources [72]. Natural supplements, a rich diet in antioxidants used as a complementary medication, become a common field of research in order to develop new products originating from natural sources with antioxidant and chemopreventive properties. The ability of anthocyanins to influence parameters of skin tumor development on mice was demonstrated in various studies. SKH-1 hairless mouse was used in order to investigate the photo-chemopreventive effect of delphinidin on UVB-induced biomarkers of skin cancer development [17]. After the treatment, the results suggest that delphinidin inhibited UVB-mediated oxidative stress and reduced DNA damage, thereby protecting the cells from UVB-induced apoptosis. The antitumor activity of the anthocyanins extract from *Fructus Sorbi aucupariae* on B-16 melanoma in C57BI/6 mice was also demonstrated [73]. The study revealed an increase in the counts of stromal progenitor cells in the tumor node and their accelerated maturation. The potentiation of the antimetastatic activity of the cytostatic was demonstrated as well. The inhibitory effects of mulberry anthocyanins on the metastasis of B16-F1 cells under noncytotoxic concentrations were investigated. The findings of the study have demonstrated that mulberry anthocyanins have strong anticancer effects by inhibiting the metastasis ability of B16-F1 cells. Further investigations revealed that the antimetastatic effect of these compounds was also evident in a C57BL/6 mice model.

5.5. Prostate cancer

Prostate cancer is the most common malignancy in men and affects most men over the age of 50 and also presents one of the main causes of mortality. For the *in vivo* study, athymic nude mice are used. To highlight the effects of anthocyanins on tumor growth *in vivo*, DH145 tumor xenograft have been established in these mice. The group of animals treated with anthocyanins received an oral dose of 8 mg/kg per day. The effects of treatment were analyzed every 4 weeks. In the first 4 weeks after incubation, the difference between the control and the treated group was insignificant. In the second set of analyses (8 weeks), the difference between the groups was very clear, the control group tumors being much bigger. These differences were observed until the end of the experiment, demonstrating the ability of anthocyanins to reduce tumor growth [74]. Another study has found that delphinidin is effective *in vitro* on PC3 cells and has determined whether these results are also visible in *in vivo* models. The delphinidin doses were not toxic to the animals because they did not lose weight and did not affect the amount of food they consumed. After measurements for 12 weeks, the differences between the tumors of the two groups (control and treated) each week were significant, suggesting an antiangiogenic effect on tumor cells. At the end of the experiment, tumors were extirpated and analyzed, where effects similar to *in vitro* studies were observed [75].

5.6. Leukemia

Acute myeloid leukemia is a hematological malignancy that has numerous causes such as chromosomal abnormalities and various gene mutations. Fifty years ago, this type of cancer was incurable, but now around 35–40% of the cases is treatable [85]. Mice Balc/c has been used to identify *in vivo* benefits of mulberry anthocyanins. Leukemia mice treated with anthocyanins had a higher survival rate than the untreated ones, this survival being correlated with the concentration of treatment. All leukemia-induced mice had the spleen and liver measured at autopsy, indicating splenomegaly and hepatomegaly. The size of these organs was significantly reduced for those treated compared to the control group. The organs were evaluated histopathologically as well and again the treated group had less infiltrated tissue with leukemic cells. Taken all this into consideration, we can say that mulberry anthocyanins can improve or eliminate the leukemic mice disorder [86].

6. *In vitro* studies

6.1. Colon cancer

Based on the substitution pattern of anthocyanidins, a recent study reported that growth inhibition of HT29 cells (human colon cancer) was highly affected by delphinidin and malvidin, while pelargonidin exhibited the lowest growth inhibitory potential. Moreover, same study reported that malvidin could inhibit the activity of phosphodiesterase (PDE) and the hydrolysis of cAMP effectively in HT29 cells thereby inhibiting the MAPK signaling pathway [76]. Another research paper [77] investigated anthocyanin-rich extracts from grape (*Vitis vinifera*), bilberry (*Vaccinium myrtillus* L.), and chokeberry (*Aronia melanocarpa* E.) for their potential

chemopreventive activity against colon cancer. The growth of colon-cancer-derived HT-29 and nontumorigenic colonic NCM460 cell lines exposed to semipurified anthocyanin-rich extracts (AREs) was monitored for up to 72 h. All extracts inhibited the growth of HT-29 cells, chokeberry extract being the most potent inhibitor. Most importantly, the growth of NCM460 cells was not inhibited at lower concentrations of all three extracts, illustrating better inhibition of colon cancer, as compared to nontumorigenic colon cells. Lately, another study [78] investigated and observed the effects of extracts from five cultivars of strawberries on the proliferation of colon cancer cells HT29 and breast cancer cells MCF-7. Using strawberry as a source of anthocyanins, they demonstrated that strawberry extracts decreased the proliferation of two cell lines in a dose-dependent manner.

6.2. Breast cancer

Human epidermal growth factor 2 (HER2) is a member of the epidermal growth factor receptor family which is overexpressed in breast cancer, and to study the *in vitro* effect of anthocyanins, the cell lines in breast cancer are usually HER2 positive; unfortunately, there are many other types of breast cancer that occur due to other causes. Regarding potential chemopreventive effects of anthocyanins, recently, it was demonstrated that black rice anthocyanins reduce the adhesion, migration, and invasion of HER2 MDA-MB-453 cells. The morphology of these cells was significantly altered, moving from a mesenchymal to an epithelial state. The western blot analysis shows an increase of the epithelial marker, E-cadherin, and decreased the expression of the mesenchymal markers, fibronectin and vimentin; this shows the effect that BRAC has on epithelial mesenchymal transition (EMT). EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties which occurs in the initiation of metastasis in cancer progression [4]. An important role in the metastasis of MDA-MB-453 cells is the focal adhesion kinase (FAK)-signaling pathway. FAK promotes the increased expression of transcription factors associated with EMT. The cells used in certain analysis were treated with Y15 (FAK inhibitor) that inhibits the autophosphorylation site of FAK. The study shows that BRAC has a similar effect to Y15, and also BRAC decreases the activation and transduction of FAK signaling [79].

6.3. Lung cancer

Inhibitory effect of anthocyanins on the migration and invasion of lung cancer was also studied. A previous study reported that glycosylated cyanidins isolated from mulberry exerted a dose-dependent inhibitory effect on the migration and invasion of metastatic A549 human lung carcinoma cells. Their results showed that the applied treatments could decrease the expressions of matrix matalloprotinase-2 (MMP-2) and urokinase plasminogen activator (u-PA) in a dose-dependent manner and also enhance the expression of tissue inhibitor of matrix matalloprotinase-2 (TIMP-2) and plasminogen activator inhibitor (PAI). Moreover, Western blot analysis revealed that anthocyanins treatment to A549 cells inhibited the activation of c-Jun (p48) and NF-kB (p65). Further, another study using anthocyanins from fruits of *Vitis coignetiae Pulliat* (AIMs) reported their anticancer effects on lung cancer cells. AIMs inhibited the growth; migration and invasion of A549 cells; and also some proteins involved with cancer effects are inhibited. AIMs suppressed MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B), both involved in the

proteolytic digestion of the ECM (extracellular matrix) and cell migration through the basement membranes to reach the circulatory system. Through the immunoblotting results, a large number of proteins have been demonstrated to be suppressed by AIMs. A couple of these proteins are involved in cancer proliferation (COX-2, cyclin D1), migration and invasion (MMP-2, MMP-9), as mentioned before, anti-apoptosis (XIAP), adhesion, and angiogenesis (VEGF). However, they were not able to identify in which signaling pathway is AIMs mainly involved. This study identifies that AIMs might have anticancer effects on human lung cancer [80].

6.4. Skin cancer

Several studies have demonstrated that flavonoids are one of the candidates for prevention of the adverse effects of UV radiation due to their UV absorbing property, and antioxidant properties. In this context, a published study revealed that grape seed proanthocyanidins (GSP) inhibits cell growth, induces G1-phase arrest, promotes apoptosis in human epidermoid carcinoma A431 cells through alterations in Cdki-Cdk-cyclin cascade, and caspase-3 activation via loss of mitochondrial membrane potential [81]. Many other studies have also proved the antiproliferative and proapoptotic effects of anthocyanins on melanoma or others skin diseases [82–84]. Our latest published revealed that anthocyanins may inhibit melanoma cell proliferation, increase the level of oxidative stress, and diminished mitochondrial membrane potential [84].

6.5. Prostate cancer

Cyanidin-3-O-β-glucopyranoside (C3G) is well known to be found in a lot of anthocyanin-rich fruits, like berries. To study its effect on cancer, two cells lines were used, LnCap and DU145. These cell lines were chosen because DU145 is a tumor cell line androgen-independent and LnCap is androgen-dependent. Androgen-dependent prostate cancer is characterized by the absence of the androgen receptor due to promoter methylation. In this case, the treatment is based on hormone elimination, yet other approaches are needed if the amount of hormones does not affect the development of cancer [79]. C3G causes a decrease in cell viability in both cell lines, and apoptosis is also induced, DU145 being more responsive in this aspect. The positive effect of treatment is demonstrated by the activation of caspase 3 and a significant increase in expression of p21 protein, evidence that cells undergo apoptosis [80]. Another study focuses on proteins that indicate the presence of apoptosis such as p53 and Bax. P53, or "the guardian of the genome" is a suppressor tumor protein that initiates apoptosis in degraded DNA cells, and Bax is a pro-apoptotic protein of the Bcl-2 family [81].

6.6. Leukemia

An bilberry extract (Antho 50) was used to determine its effect on Jurkat cells. The main interest of this study is the result of Antho 50 on certain proteins, polycomb group (PcG), which are epigenetic regulators. These proteins reduce the expression of suppressor tumor genes, promoting the survival of tumor cells [81]. The aim is to see if the extract is able to inhibit these PcG proteins. The extract was able to downregulate the PcG and related proteins

and induces apoptosis. All these events have an effect on the intracellular ROS formation, causing an increase, resulting in the death of tumor cells [82]. In another study, delphinidin and cyanidin, two major compounds in *Hibiscus sabdariffa*, were investigated. They are able to induce cell cycle arrest in human leukemia cell line HL-60. This effect occurs because of their action on signaling pathways whose role is to induce cell cycle arrest. This indicates promising anticarcinogenic effects [83].

7. Conclusions

Interests in anthocyanins have increased substantially during the past two decades. In this review, we discussed at what level anthocyanins act when talking about anticancer effects. *In vitro*, we saw that anthocyanins affect: the proliferation of the cancer cells, inhibiting of the ability of cancer cells to divide uncontrollably, the induction of apoptosis, the process of angiogenesis where tumors form new blood vessels, and the cancer cells invasion through healthy tissue. Also, a few of the *in vivo* studies demonstrate that dietary anthocyanins inhibit the growth of different types of tumors, angiogenesis, and show apoptotic effect against the cancer cells. It remains to be determined whether the anticancer activity of anthocyanins is due to anthocyanins or their metabolites.

Conflicts of interest

The authors indicate no potential conflicts of interest. "The authors declare no conflict of interest"; "the founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results."

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Abbreviations

ROS	reactive oxygen species
PDE	phosphodiesterase
ARE	anthocyanin-rich extract

LC-MC	chromatography: liquid chromatography-mass spectrometry
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
TNF-α	tumor necrosis factor alpha
MMP	matrix metalloproteinase
u-PA	urokinase plasminogen activator
AOM	azoxymethan
DSS	dextran sodium sulfate
COX	cyclooxygenase
HER2	human epidermal growth factor receptor 2
UVB	ultraviolet B
BRAC	black rice anthocyanins
EMT	epithelial mesenchymal transition
FAK	focal adhesion kinase
Y15	FAK inhibitor
AIMs	anthocyanins from fruits of *Vitis coignetiae Pulliat*
ECM	extracellular matrix
GSP	grape seed proanthocyanidins
C3G	cyaniding-3-O-β-glucopyranoside
Antho 50	bilberry extract
PcG	polycomb group
MAC	mulberry anthocyanins

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