Chapter 8

Anthocyanins in Berries and Their Potential Use in Human Health

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Abstract

Anthocyanin pigments are responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers, increasing the interest due to their strong antioxidant capacity and their possible use to the benefit of human health. Abundant evidence is available about the preventive and therapeutic roles of anthocyanin in different kinds of chronic diseases. According to the structural differences and anthocyanin content of berries such as blackberry, blueberry, chokeberry, and others, there are different healthy properties in the treatments of circulatory disorders, cancer cell lines, and diabetes as well as antiviral and antimicrobial activities. On the other hand, molecular aspects play an important role in anthocyanin biosynthesis, making it possible to determine how biotic and abiotic factors impact its biosynthesis complex. Thus, the aim of this chapter was to describe the use of anthocyanins from berries for human health and their potential use as a pharmacological bioresource in the prevention of chronic diseases. In addition, an update of the molecular mechanisms involved in anthocyanin biosynthesis will be discussed.

Keywords: anthocyanins, berries, cancer, transcription factors

1. Introduction

The scientific evidence regarding the positive relationship between diet and health has increased consumer demand for more information related to healthy diets, including fruits and vegetables, with functional characteristics that help to delay the aging process and

reduce the risk of several diseases, mainly cardiovascular diseases and cancer [1]. Berries are recognized as an important component of healthy diets due to their bioactive compounds. In this sense, commercial berry species such as blackberry (*Rubus* sp.), bilberry (*Vaccinium myrtillus* L.), blackcurrant (*Ribes rugrum* L.), chokeberry (*Aronia melanocarpa* (Michx.) Elliott.), cranberry (*V. macrocarpon* Ait.), bayberry (*Myrica* sp.), raspberry (*Rubus ideaus* L.), black raspberry (*Rubus occidentalis* L.), strawberry (*Fragaria ananassa* Duch.), highbush blueberry (*V. corymbosum* L.), maqui (*Aristotelia chilensis*), murtilla (*Ugni molinae* Turcz.), and calafate (*Berberis microphylla* G. Forst.) are particularly rich sources of antioxidants, which are usually consumed in fresh and processed products [2–5]. Higher plants, especially berry species, synthesize a diverse group of phenolic compounds such as flavonoids. These plant secondary metabolites have many biological functions, including their key role in plant-microbe interaction, plant-pathogen interaction, pollen-tube growth, UV radiation protection, tissue pigmentation, and others [6, 7]. Flavonoid compounds, which include flavonols, flavones, flavanols, flavanones, isoflavonoids, and anthocyanins, are molecules widely accumulated in vascular plants and to a lesser extent in mosses, being accumulated in all organs and tissues at different stages of development and depending on the environmental conditions [6].

Anthocyanins are natural pigments responsible for the blue, purple, red, and orange colors of many fruits and vegetables [8, 9]. Anthocyanins are a glycoside form of anthocyanidins [9], and the structural differences among them are related to the number of hydroxyl group, position, and kind and/or number of sugars linked to the molecule [10, 11]. These compounds appear to be an interesting natural resource of water-soluble dyes because they are easily incorporated in aqueous media [12]. Another important property of anthocyanins is their remarkable antioxidant activity, playing a vital role in the prevention of neuronal and cardiovascular illnesses, diabetes, cancer, etc. [11, 13]. Many reports have focused on the effect of anthocyanins in cancer prevention [14], human nutrition [15], and their biological activity [10]. Nowadays, there is an increased interest in explaining the role of anthocyanins as a natural antioxidant and their mechanism of action on human health as well as the treatment of chronic diseases and their use as a natural dye, substituting the synthetic dyes, which can be toxic to humans. This review endeavors to describe the use of anthocyanins from berries for human health and their potential use as a pharmacological bioresource in the prevention of chronic diseases. In addition, an update of the molecular mechanisms involved in anthocyanin biosynthesis will be discussed. Finally, recent clinical and preclinical studies about anthocyanin use in the prevention of human diseases are reported.

2. Anthocyanin and phenolic compounds in berries

Phenolic acid, organic acids, tannins, anthocyanins, and flavonoids are phenolic bioactive compounds with a high concentration in the berry fruits [16]. The chemical structure of phenolic compounds is characterized by one or more aromatic rings with hydroxyl groups. According to their structural characteristics, phenolic compounds are classified into five

major groups: phenolic acids, stilbenes, flavonoids (flavonols or catechins, flavonols, flavones, flavonones, isoflavonoids, anthocyanins), tannins, and lignans [13]. The concentration of phenolic compounds in berry fruits is altered by many factors, such as genotype, species, agronomic management, climatic factors, ripening stage, harvesting time, and postharvest management [17, 18]. Given the plant phenol attributes of berry species, attention has largely focused on anthocyanin and flavonol antioxidant action on human health. In this way, substantial epidemiological and experimental research suggests that intakes of recognized nutritional antioxidants such as vitamin E and carotenoids can decrease the oxidative damage of proteins, lipids, and DNA *in vivo* and may reduce the incidence of developing many chronic diseases in humans [19]. The *in vitro* antioxidant effectiveness of anthocyanins and other polyphenols is due to its donation of free hydrogen atom from an aromatic hydroxyl group of the antioxidant molecules, acting as radical scavenger [20].

It has been reported that the antioxidant capacity of flavonoids is stronger than vitamins C and E [21, 22], and under *in vitro* conditions, flavonoids can prevent injury in different ways, acting as a suppressor of reactive oxygen formation, scavenging free radicals by hydrogen atom donation [22, 23], activating antioxidant enzymes [23, 24], chelating metal, reducing α-tocopheryl radicals, inhibiting oxidases, oxidative stress mitigation by nitric oxide, increasing uric acid levels, and increasing antioxidant properties of low-molecular antioxidants [22]. Anthocyanin concentration in blackberry is much higher than in raspberry and strawberry and similar to red currant blueberry, depending on the cultivar (see **Table 1**).

Anthocyanin concentration widely differs significantly among plant species, even among species of the same genus. In **Table 1**, anthocyanin and total phenolic compounds of different species and cultivars and their analysis are detailed. In blackberry, anthocyanin content is generally similar in all species, but phenolic content shows strong differences (**Table 1**). Anthocyanin content in *Rubus insularis* F. Aresch. represents 36% of the phenolic compounds, whereas in *R. fructicosus* cultivar Hull Thornless, it only represents 6.4% of the total phenolic compounds (**Table 1**). Raspberry (*R. innominatus* S. Moore) showed higher anthocyanin level, representing 41.2% of the total phenolic compounds. *R. ideaus* show high phenolic compounds; however, their high content does not necessarily represent a high anthocyanin content. *R. ideaus* Heritage cultivar has showed the highest anthocyanin percent with respect to the total phenolic compounds, representing 3.8% (**Table 1**). Additionally, blueberry cultivars showed low differences between anthocyanin and phenolic compounds, but they showed greater health benefits than other berries due to their particularly high proportion of anthocyanins. In some cases, high anthocyanin content in blueberries is related to high antioxidant capacity, but the anthocyanin contents and composition are different in each species and cultivar (**Table 1**). More specifically, the *V. corymbosum* cultivar (Duke) contains 63% anthocyanin with respect to the total phenolic compounds, followed by the cultivars CVAC5.001 and Brigitta, with 46 and 41%, respectively, and finally by Bluecrop with 27%. It is therefore necessary to evaluate the correlation between anthocyanin content and total phenolic compounds, because the ratio can exist between the two parameters, but it is not necessary to estimate in all species or among cultivars of the same genus (**Table 1**) [25–29].

Scientific name	Common name	Cultivar	Anthocyanins*	Phenolics**	References
Rubus cyri Juz.	Blackberry	Native	143	545	[25]
Rubus georgicus Focke	Blackberry	Native	89	561	[25]
Rubus insularis F. Aresch.	Blackberry	Native	170	472	[25]
Rubus ursinus (Douglas ex Hook.)	Blackberry	Native	211	629	[25]
Rubus fructicosus L.	Blackberry	Chactaw	125	1703	[26]
Rubus fructicosus	Blackberry	T. evergreen	146	2061	[26]
Rubus fructicosus	Blackberry	Hull Thornless	152	2349	[26]
Rubus idaeus L.	Raspberry	Native	65	517	[27]
Rubus innominatus S. Moore	Raspberry	Native	52	126	[25]
Rubus niveus Thunb.	Raspberry	Native	230	402	[25]
Rubus ideaus	Raspberry	Heritage	49	1280	[26]
Rubus ideaus	Raspberry	Autumm Bliss	39	2494	[26]
Rubus ideaus	Raspberry	Fallgold	3	1459	[26]
Rubus ideaus	Raspberry	Meeker	42	2116	[26]
Ribes sativum	Red currants	London Market	7.8	1115	[26]
Ribes sativum (Lam.) Mert. & Kock	Red currants	Rovada	7.5	1193	[26]
Ribes sativum	Red currants	White Versailes	1.4	657	[26]
Ribes nigrum L.	Red currants	Alagan	169	694	[25]
Ribes nigrum	Red currants	Ben Lomond	261	933	[25]
Ribes nigrum	Red currants	Ojebyn	165	830	[25]
Ribes nigrum	Red currants	Consort	411	1342	[25]
Vaccinium corymbosum L.	Blueberry	Bluecrop	84	304	[25]
Vaccinium corymbosum	Blueberry	Briggita	103	246	[25]
Vaccinium corymbosum	Blueberry	Duke	173	274	[25]
Vaccinium corymbosum	Blueberry	CVAC5.001	430	868	[25]
Vaccinium corymbosum	Blueberry	Native	62-235	181–473	[28]
Vaccinium corymbosum	Blueberry	Bluegold	206	432	[29]
Vaccinium corymbosum	Blueberry	Briggita	190	468	[29]
Vaccinium corymbosum	Blueberry	Legacy	226	570	[29]
Vaccinium angustifolium Ait.	Blueberry	Native	208	692	[25]
Vaccinium myrtillus L.	Bilberry	Native	300	525	[28]

*mg cyanidin 3-glucoside eq./100 g fresh weight.
**mg gallic acid eq./100 g fresh weight.

Table 1. Total anthocyanin and phenolic content of berry fruits.

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Berry species with higher anthocyanin content are interesting for use in breeding programs for increasing their content in fruits, enhancing their antioxidant capacity, and obtaining fruit products with health properties. In addition, the understanding of the molecular network of genes involved in anthocyanin biosynthesis and how biotic and abiotic factors could affect their concentration and gene regulation are a key to use it in genetic engineering and agronomic management.

3. Molecular regulation of anthocyanin biosynthesis

Six structural genes are common in the anthocyanin pathway in all angiosperms, which are divided into two main groups. The first group is the upstream genes or early biosynthesis genes, for example, chalcone synthase (CHS), chalcone flavanone isomerase (CHI), and flavanone 3-hydroxylase (F3H), coding for enzymes that produce precursors for one or more important non-anthocyanin flavonoids. The second group is the downstream genes or late biosynthesis genes, for example, anthocyanidin synthase (ANS), dihydroflavonol-4-reductase (DFR), and UDP-glucose flavonoid 3-oxy-glucosyltransferase (UF3GT), coding for enzymes specific to anthocyanin synthesis [30–32]. In the anthocyanin pathway, L-phenylalanine is converted to naringenin by phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate CoA ligase (4CL), chalcone synthase (CHS), and chalcone isomerase (CHI). Then, the next pathway is catalyzed by the formation of complex aglycone and anthocyanin composition by flavanone 3-hydroxylase (F3H), flavonoid 3'-hydroxylase (F3'H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), UDP-glucoside flavonoid glucosyltransferase (UFGT), and methyl transferase (MT) [33]. It has been described that the transcription of early and late biosynthesis genes to produce anthocyanins appears to be regulated by R2R3-MYB and basic helix-loop-helix (bHLH, also known as MYC) called transcription factors in collaboration with tryptophan-aspartic acid repeat (WDR) or WD40 proteins [32, 34–37].

3.1. MYB transcription factor

The MYB transcription factors involved in the flavonoid pathway have been identified and described for several kinds of model plants, crops, and ornamental plants. The first identified and reported MYB transcription factor in plants was in *Zea mays*, which included C1 (Colorless 1) and PL1 (Purple Leaf 1) [38]. The MYB transcription factors are composed of the so-called N-terminal MYB domain, consisting of one to three imperfect repeats of almost 52 amino acids (R1, R2, and R3), beginning with R2R3, the most abundant subfamily in plants [39]. The MYB domain is involved in DNA binding and dimerization. The C-terminal region is responsible for establishing protein-protein and regulates activation or repression of gene expression [34, 40, 41]. The MYB genes are exclusive to eukaryotic organisms [42]. In animals, these genes are associated with cell proliferation and differentiation [43, 44], whereas in plants, MYB is associated with responses to different biotic and abiotic stressors (drought, cold, pathogen disease resistance), plant development (trichome formation, seed development), stomatal movement, and many other functions [34, 40, 45, 46]. Anthocyanin biosynthesis mediated by MYB transcription factors has been reported in *Arabidopsis thaliana*

(L.) Heynh. [41, 47–49], strawberry (*F. ananassa*) [50], Chilean strawberry (*Fragaria chiloensis* (L.) Duch.) [51], apple (*Malus domestica* Borkh.) [52–54], and tomato (*Solanum lycopersicum* L.) [55]. Grape (*Vitis vinifera* L.) is the main plant species studied in this way due to its agricultural and commercial importance worldwide. Thus, many MYB transcription factors have been reported in this species by different researchers. *VvMYBPA1* and *VvMYBPA2* are involved in proanthocyanidin synthesis [46, 56], while *VvMYBF1* regulates flavonol synthesis [57]. In addition, *MYBA1* and *MYBA2* genes control the last biosynthetic step of anthocyanin synthesis [58, 59]. It is reported that a glycosylation reaction mediated by the UDP-glucose flavonoid-3-O-glucosyltransferase (UFGT) enzyme produces anthocyanins in grapes [31, 39]. It is important to highlight that MYB transcription factor is conserved between different species and is one of the most important primary proteins involved in structural and biological functions. Furthermore, MYB transcription factor regulates the flavonoid pathway apparently in two ways: (a) due to variations in the C-terminal region of the protein or (b) modulating the interaction with DNA, bHLH, and WD40 protein [34, 60].

3.2. Basic helix-loop-helix (bHLH)

After MYB, bHLH proteins, also known as MYC, are the second most important family of transcription factors involved in anthocyanin biosynthesis [34, 61]. The bHLH protein domain is constituted of about 60 amino acids and is characterized by the presence of 19 conserved amino acids, five in the basic region, five in the first helix, one in the loop, and eight in the final second helix [61]. The basic region of bHLH has basic residues (5.8 on average) essential for DNA binding. In Arabidopsis, 20% of bHLH transcription factors do not have this domain and can act as a repressor because forming heterodimers are unable to bind to DNA [61]. Two cis-element boxes have been reported to bind with bHLH proteins, the E-box (5'-CANNTG-3'), and G-box (5'-CACGTG-3') elements. The G-box is the most commonly recognized sequence representing 81% of the proteins predicted to bind DNA [61, 62]. In the basic region, two amino acids conferred the property on binding DNA in Arabidopsis plants. The Glu13 and Arg16 are the E-box recognition motif [63]. Glu13 has contact with CA bases of E-box and Arg16, apparently helping Glu13 to bind and stabilize. In G-box, specific stabilization is mediated by His/Lys9, Glu13, and Arg17. The Arg17 interacts with inner G base, and His/Lys9 interacts with the last G of the G-box [61, 62]. The alpha-helix function is involved in homo- and hetero-dimerization and is formed by hydrophobic residues of isoleucine, leucine, and valine [34, 61]. Arabidopsis has been demonstrated that this residue is conserved in all bHLH proteins, indicating the importance of the basic region of the bHLH transcription factor in DNA binding [61, 63]. The second helix is involved in DNA binding through direct contact with the E-box. Finally, the loop is responsible for the three-dimensional arrangement of alpha-helices, and residues from the first helix loop junction are involved in association with bHLH proteins [34, 61, 63, 64]. Basic helix-loop-helix transcription factors in plants are involved in processes such as flower development [65, 66], hormonal response [67, 68], metal homeostasis [69], and others. Regarding bHLH and their relation to flavonoid synthesis, the first bHLH involved in this pathway was detected in maize in 1989 [70]. In this context, in *Z. mays* (ZmB, ZmR, and ZmLc), bHLH is involved in the regulation of the anthocyanin pathway [70–72], and

ZmIn1 is involved in the repression of flavonoid gene expression in maize aleurone [73]. In *A. thaliana*, it has been reported that *AtTT8* gene encodes a bHLH transcription factor involved in the control of proanthocyanidins and anthocyanins in seeds and seedlings [74]. Quatroccio et al. [75, 76]. reported PhAN1, PhJAF13 hBLH transcription factor from *Petunia hibrida* as being involved in the control of the anthocyanin pathway in flowers. For *Vitis vinifera,* VvMYCA1 (also known as bHLH) was reported as involved in promotion of anthocyanin accumulation in grape cells [37].

3.3. WDR proteins

Tryptophan-aspartic acid repeat protein (WDR) or WD40 proteins are characterized by around 44–66 amino acids, delimited by the GH dipeptide on the N-terminal size (11–24 residues from the N-terminus) and the WD dipeptide on the C-terminus [34, 77]. In Arabidopsis, WDR protein contains four (or more) tandem repeats composed of around 40 amino acids [78]. In contrast to the majority of proteins, WDR is not involved in catalytic activities such as DNA binding or gene expression regulation, mostly acting as a platform due to its capacity to interact with more than one protein at the same time [34, 78]. The work of WDR involves eukaryotic cellular process such as cell division, vesicle formation, signal transduction, RNA processing, and transcription regulation [78]. On the other hand, MYB and bHLH transcription factors have few WDR proteins involved in the flavonoid pathway, as shown in *Z. mays* (ZmPAC1), where it regulates the anthocyanin pathway in seed aleurone [79]. In Arabidopsis (AtTTG1), WDR proteins control trichomes, root hair, and seed mucilage production [80]. In petunia, AN11 regulates anthocyanin production as well as the pH of the flower vacuole [81], whereas in grape, *V. vinifera* WDR1 contributes to anthocyanin accumulation [37]. Although WDR proteins are not directly involved in the flavonoid pathway, particularly in anthocyanin synthesis, it is important to note that these proteins are highly conserved among species [34]. Nevertheless, few WDR proteins have been reported in plants, and it must be highlighted that WDR is involved in several metabolic and physiological processes [79, 80, 82]. To clarify the characteristics of WDR proteins and the complex formed with MYB and bHLH, which is involved in anthocyanin biosynthesis, species such as petunia and Arabidopsis have been used [34, 35].

3.4. MYB-bHLH-WDR (MBW complex)

MBW complex has been reported in Arabidopsis, petunia, and some varieties of grape [35, 82]. The most important function of these transcription factors is involved in the process related to DNA binding, activation of gene expression involved in the flavonoid pathway, and stabilization of the three-dimensional configuration of the complex [34]. Basic helix-loop-helix-WDR interaction is needed to WDR protein translocation into the nucleus, and this was demonstrated in onion cells using green protein fluorescent (GPF), which when expressed alone is localized in the cytosol, whereas its co-expression with PFWD and MYC-RP enables the transport and localization in the nucleus [35]. The AN11 from petunia showed the same results, being detected in the cytosol [81]. *V. vinifera* subjected to high salt concentrations showed a cultivar-dependent response for anthocyanin accumulation, which was correlated with the expression of MYBA1-2, MYCA1 and WDR1 genes [37].

4. Antioxidant capacity of anthocyanins in berries and their use in human health

The radical scavenging activity (RSA) of anthocyanins is largely due to the presence of hydroxyl groups in position 3 of ring C and also in the 3', 4', and 5' positions in ring B of the molecule. In general, RSA of anthocyanidins (aglycons) is superior to their respective anthocyanins (glycosides), and this decreases when the number of sugar increases [16]. Hanachi et al. [83] showed that fruits of *Berberis vulgaris* L. (barberry) have a high antioxidant activity, reducing the viability of cell cultures associated with liver cancer (HepG2). Furthermore, extracts of leaves and twigs of *B. vulgaris* have more antioxidants than fruits. Končić et al. [84] studied the antioxidant activity of extracts of leaves, branches, and roots of two species of *B. vulgaris* and *Berberis croatica* and demonstrated that all these organs exhibited antioxidant activity. In all cases, the activity was positively correlated with the content of phenolic acids and flavonols, and the flavonols played the main role in the total antioxidant activity of the studied species [84]. They also concluded that the antioxidant activities were significantly different (being higher in *B. croatica* than *B. vulgaris*) and among organs (being higher in leaves followed by branches and roots). The result of the anthocyanin concentration in different organs besides the fruits is interesting, because acquisition of anthocyanin in every season of the year has advantages for making new products with health properties. Thus, interesting results such as a new natural resource for promoting these compounds for human health have been reported. Končić et al. [84] suggested that studies into different species are needed to analyze all the organs of the plant, not just the fruits. Shin et al. [85] reported that in human liver cancer HepG2, cell proliferation was inhibited by strawberry extracts. Moreover, Chang et al. [86] reported that *Hibiscus sabdariffa* Linne (roselle) anthocyanin extracts mediated the apoptosis of human promyelocytic leukemia cells via the p38/Fas and Bid pathways. Research examining the use of black currant extract (BCE) with high concentrations of phenolic compounds on antiproliferative activity against gastric cancer SGC-7901 cells showed a positive antiradical activity and anticarcinogenic effects [87]. Moreover, extracts of mulberry showed an inhibition on the growth of human gastric carcinoma cells [88]. In this study, anthocyanins extracted from mulberry had notable promotive effects on the p38/jun/Fas/FasL and p38/p53/Bax signaling pathways, which accounted for its *in vitro* and *in vivo* growth-inhibitory and apoptotic responses in AGS (gastric cancer) cells. The effects of berries on diseases are shown in **Table 2**.

With respect to *in vivo* studies, Wang and Stoner [89] reported the effect of an anthocyanin-rich extract from black raspberries on the development of tumors in rat esophagus by N-nitrosomethylbenzylamine (NMBA), the most potent inducer of tumors in rat esophagus. This extract inhibited cell proliferation, inflammation, and induced apoptosis in the esophageal tissues (**Table 2**). Stoner et al. [90] compared the effect of black raspberry, red raspberry, strawberry, and blueberry anthocyanin and ellagitannins in fruit extract on the prevention of esophageal cancer induced by N-nitrosomethylbenzylamine (NMBA) in rats. Inhibition of NMBA-induced tumorigenesis in the rat esophagus was observed. The authors detected a reduction in cytokine levels in serum, interleukin 5 (IL-5), and GRO/KC, which is the rat homolog for human interleukin-8 (IL-8), and these cytokines

Disease	Scientific name	Common name	Compound	Experimental conditions	Reference
Liver cancer	*Fragaria x ananassa* Duch.	Strawberry	Crude extract	*In vitro*	[85]
Leukemia	*Hibiscus sabdariffa* L.	Rosselle	Anthocyanin rich extract	*In vitro*	[86]
Gastric cancer	*Morus alba* L.	Mulberry	Anthocyanins	*In vitro*	[88]
Gastric cancer	*Ribes nigrum* L.	Black currant	Crude extract	*In vitro*	[87]
Colon caner	*Vaccinium myrtillus* L.	Bilberry	Anthocyanin-rich extract	*In vivo* (rats)	[91]
Colon cancer	*Aronia melanocarpa* E.	Chokeberry	Anthocyanin-rich extract	*In vivo* (rats)	[91]
Colon cancer	*Vitis vinifera* L.	Grape	Anthocyanin-rich extract	*In vivo* (rats)	[91]
Esophagus cancer	*Rubus occidentalis* L.	Black raspberries	Anthocyanin-rich extract	*In vivo* (rats)	[89]
Esophagus cancer	*Rubus occidentalis*	Black raspberries	Anthocyanins and ellagitannins	*In vivo* (rats)	[90]
Esophagus cancer	*Rubus ideaus* L.	Red raspberries	Anthocyanins and ellagitannins	*In vivo* (rats)	[90]
Esophagus cancer	*Fragaria ananassa*	Strawberries	Anthocyanins and ellagitannins	*In vivo* (rats)	[90]
Esophagus cancer	*Vaccinium corymbosum* L.	Blueberries	Anthocyanins and ellagitannins	*In vivo* (rats)	[90]
Hepatic cancer	*Berberis vulgaris* Duch.	Barberries	Crude extract	*In vivo* (rats)	[83]
Liver cancer	*Vaccinium corymbosum*	Blueberries	Anthocyanin extract	*In vitro* (mice)	[94]
Liver cancer	*Berberis vulgaris*	Barberries	Crude extract	*In vitro*	[83]
Oral cancer	*Rubus occidentalis*	Black raspberries	Crude extract	*In vivo* (mice)	[96]
Mammary	*Vitis vinifera*	Grape	Crude extract	*In vivo* (rats)	[95]
Skin cancer	*Punica granatum* L.	Pomegranate	Crude extract	*In vivo* (mice)	[92, 93]

Table 2. Anticarcinogenic effects of anthocyanin/anthocyanin-rich extract from different berry species under *in vivo* and *in vitro* conditions in different chronic diseases.

were associated with an increase in serum antioxidant capacity. At molecular level, Stoner et al. [90] also reported that the use of extracts showed a differential expression in 626 and 625 genes per 4807 and 17846 of preneoplastic esophagus and esophageal papilloma genes, respectively. These genes are involved in carbohydrate and lipid metabolism, cell death and proliferation, and inflammation. These results are an important approach to estimate the relation of anthocyanin gene expression and its influence on proteins associated with cell proliferation, apoptosis, angiogenesis, and esophageal carcinogenesis. Lala et al. [91] observed an anticarcinogenic effect of anthocyanins on colon cancer induced by

azoxymethane in a rat model. In that study, anthocyanin-rich extract from bilberry, chokeberry, and grapes significantly reduced azoxymethane-induced aberrant crypt foci and decreased cell proliferation and *COX-2* gene expression [91]. Delphinidin and pomegranate extracts enriched with anthocyanins, and tannins showed an inhibition in skin cancer induced by UV-B or TPA (12-O tetradecanoylphorbol-13acetate) when applied to mouse skin [92, 93]. Here, delphinidin inhibited DNA damage mediated by UV-B radiation, and pomegranate modulated the mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-kB) pathways. Similarly, studies on the protective effect and antioxidant mechanism of anthocyanin extract from blueberries were conducted using a liver injury induced by CC4 in mice—the effect of which increased lipid peroxidation and reduced liver cell viability [94]. The results indicate that anthocyanin extract effectively protected mice from CC4-induced liver injury by attenuation of lipid peroxidation. In mammary adenocarcinoma induced by dimethylbenzaanthracene (DMBA) in rats, the antitumoral effect of grape juice was evaluated by Singletary et al. [95]. They demonstrated that the tumor mass was ultimately reduced by suppressing cell proliferation (**Table 2**). In general, the strong antioxidant capacity of berry species is attributed to their anthocyanin content, suggesting that it might offer potential chemopreventive properties, including the inhibition of gastric, leukemia, liver, and breast cancer cell proliferation, among others; however, the mechanism of action must be evaluated for each disease because apparently their mechanism of effects varies (inhibiting cell proliferation, activating different enzymatic activity, inducing or repressing gene expression, etc.) depending on the extract from each plant species.

5. Conclusions and future challenges

The potential use of anthocyanins from different plant species as natural compounds with a health benefit for humans opens a new trend for the prevention and alternative treatments of chronic diseases. Several reports have demonstrated that anthocyanins from berries could inhibit or decrease the growth of carcinogenic tumors by affecting cell proliferation, increasing or inhibiting enzymatic systems, and increasing expression of genes involved in cell protection. On the other hand, it is important to highlight that synthesis of anthocyanins in different tissues of plants species should be considered. In addition, the discovery and characterization of new regulatory elements of anthocyanin biosynthesis are crucial to understand and manipulate this pathway in breeding programs. Improving knowledge about increasing anthocyanin synthesis in crops of research and commercial interest, together with more animal and human model studies under *in vivo* conditions, is essential to generate better human anticarcinogenic or antichronic disease supplement products with chemopreventive effects from berries.

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