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Antitumor, anti-inflammatory, and antioxidant activities of anthocyanins from food and isolated sources: Methodological insights and interactions with the intestinal microbiota

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ABSTRACT

Anthocyanins are secondary metabolites found in plants, commonly present in fruits such as strawberries, grapes, and blueberries. They act as antioxidants, protecting plant cells from oxidative damage, and are known for their potential role in chronic disease prevention. In studies, anthocyanins have been investigated both in food matrices and in isolated forms. They neutralize free radicals, which can damage cells, and reduce inflammation by downregulating pro-inflammatory cytokines involved in chronic diseases. Additionally, they have shown potential in modulating tumor cell proliferation, making them valuable for human health. This review explores the antioxidant, anti-inflammatory, and antitumor activities of anthocyanins, highlighting the methods used to

Abbreviations: ROS, Reactive oxygen species; ACS, Apoptosis-associated speck-like protein containing a CARD; SCFASs, Short-chain fatty acids; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-8, Interleukin-8; IL-10, Interleukin-10; IL-18, Interleukin-18; TNF-α, Tumor necrosis factor-alpha; iNOS, Inducible nitric oxide synthase; LPS, Lipopolysaccharide; GLP-1, Glucagon-like peptide-1; PYY, Peptide YY; HDACs, Histone deacetylases; GPR43/GPR109A, G-protein-coupled receptors 43/109 A; C3G, Cyanidin-3-O-glucoside; DPPH, 1,1-Diphenyl-2-picrylhydrazyl; ABTS, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, Ferric reducing antioxidant power; IC_{50}, Inhibitory concentration 50 %; MAPK, Mitogen-activated protein kinase; MAP, Mitogen-activated protein; SOD, Superoxide dismutase; GPX, Glutathione peroxidase; CRP, C-reactive protein; hs-CRP, High-sensitivity C-reactive protein; PBMCs, Peripheral blood mononuclear cells; PI3K, Phosphoinositide 3-kinase; AKT, Protein kinase B; mTOR, Mechanistic target of rapamycin; STAT3, Signal transducer and activator of transcription 3; JAK, Janus kinase; AMPK, Adenosine monophosphate-activated protein kinase; FAK, Focal adhesion kinase; LOX, Lipoxygenase; COX-2, Cyclooxygenase-2; MMP-2, Matrix metalloproteinase-2; MMP-9, Matrix metalloproteinase-9; uPA, Urokinase plasminogen activator; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular cell adhesion molecule-1; NO, Nitric oxide; PGE2, Prostaglandin E2; ORAC, Oxygen radical absorbance capacity; 8-isoPGF2α, 8-Isoprostane prostaglandin F2α; 8-OHdG, 8-Hydroxy-2′-deoxyguanosine; CLS, Crown-like structures; CXCL2, C-X-C motif chemokine ligand 2; MIP-2, Macrophage inflammatory protein-2; IP-10, Interferon gamma-induced protein 10; I-TAC, Interferon-inducible T-cell alpha chemoattractant; GRO-α, Growth-regulated oncogene alpha; IκB, Inhibitor of kappa B; JNK1/2, c-Jun N-terminal kinases 1 and 2; ERK1/2, Extracellular signal-regulated kinases 1 and 2; RONS, Reactive oxygen and nitrogen species; GLUT2, Glucose transporter 2; SGLT1, Sodium-glucose transporter 1; NRF2, Nuclear factor erythroid 2–related factor 2; NLRP3, NOD-like receptor family, pyrin domain containing 3; MCP-1, Monocyte chemoattractant protein-1; C3GdM, Cyanidin-3-glucoside-dimalonylated; NCDs, Noncommunicable diseases; BMI, Body mass index; NAFLD, Non-alcoholic fatty liver disease; HIF-1α, Hypoxia-inducible factor 1 alpha; PARP, Poly(ADP-ribose) polymerase; TNBC, Triple-negative breast cancer; ELISA, Enzyme-linked immunosorbent assay; TPTZ, 2,4,6-tripyridyl-*s*-triazine; PAL, Phenylalanine ammonia lyase; CHS, Chalcone synthase; ANS, Anthocyanidin synthase; CHI, Chalcone isomerase; DFR, Dihydroflavonol 4-reductase; HAT, Hydrogen atom transfer; SRB, Sulforhodamine B; qPCR, Quantitative polymerase chain reaction; UAE, Ultrasound-assisted extraction; MAE, Microwave-assisted extraction.

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assess these properties. Moreover, it discusses their interaction with the gut microbiota, which enhances anthocyanin biological effect through the production of short-chain fatty acids and phenolic metabolites derived from microbial metabolism. The review also addresses the challenges and future perspectives for the industrial-scale application of anthocyanins.

Email:

1. Introduction

Anthocyanins are bioactive compounds that belong to the flavonoid group. There are over 700 types of anthocyanins in nature (Ayvaz et al., 2022; Wallace & Giusti, 2015). Anthocyanins play multifaceted roles essential for plant metabolism and physiology. As potent antioxidants, they neutralize reactive oxygen species (ROS) produced in response to environmental stresses, such as ultraviolet radiation, protecting plant cells from oxidative damage (Hoch et al., 2003; de Leonardis et al., 2015; Zarrouk et al., 2016; Zhou et al., 2020). In addition, by absorbing excess light, anthocyanins contribute to photoprotection, preventing the photoinhibition of chloroplasts and ensuring the efficiency of photosynthesis under intense sunlight (Del Valle et al., 2020; Ko et al., 2020; Mannino et al., 2021; Zhao et al., 2022).

These compounds also defend against herbivores and inhibit pathogens, thereby enhancing plant resistance to biological threats. Ecologically, the vibrant coloration provided by anthocyanins is vital for attracting pollinators and seed dispersers, promoting plant reproduction and dispersion. Therefore, the functions of anthocyanins are interconnected supporting the adaptation and survival of plants in diverse environments (Mannino et al., 2021; Zhao et al., 2022).

Fortunately, the benefits of anthocyanins extend beyond the plant kingdom. Studies have demonstrated that these compounds can promote human health primarily due to antioxidants (Alam et al., 2021), anti-inflammatory, and antitumor effects (AlMadalli et al., 2024). These effects could support the treatment of noncommunicable diseases (NCDs) (*e.g.*, cardiovascular diseases, cancer, diabetes), which were reported by the World Health Organization, 2020 as the seven leading causes of death worldwide (Pan American Health Organization, 2020a). This alarming statistic underscores the importance of seeking natural and effective alternatives to prevent and combat these diseases (Fraga et al., 2019).

In this context, this review aims to summarize the main aspects of anthocyanins, addressing their role in modulation and their impact on the gut microbiota, followed by an exploration of their antioxidant, anti-inflammatory, and antitumor activities. This paper also describes the main methodologies used to evaluate the biological effects (the potential to exert physiological effects in the organism) of anthocyanins and discusses the limitations and future perspectives of their use in industrial settings.

2. Source, biosynthesis and chemical structure of anthocyanins

Anthocyanins are present, for example, in strawberries, cranberries, blackberries, blueberries, pomegranates, red radish, grapes, açaí, purple and black cereals, such as purple corn, wheat and rice and some forages such as sorghum (*Sorghum bicolor*), and elephant grass (*Cenchrus purpureus*) (Zhou et al., 2019). They are responsible for the red, purple, orange, blue, and violet colors and can be present in various parts of plants, such as fruits, leaves, stems, and roots, with variable contents depending on the source (de Pascual-Teresa & Sanchez-Ballesta, 2008; Salehi et al., 2020; Husain et al., 2022).

The biosynthesis of anthocyanins in plants, initiated by the conversion of phenylalanine into p-coumaric acid by the enzyme phenylalanine ammonia lyase (PAL), involves a series of enzymatic reactions, including the action of chalcone synthase (CHS), chalcone isomerase (CHI), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS), culminating in the production of these crucial molecules (Ayvaz et al., 2022; Passeri, Koes, & Quattrocchio, 2016; Sui et al., 2018; Zhao & Tao, 2015).

Its structure consists of a flavylium cation core, comprising an aromatic ring with several hydroxyl and methoxyl groups attached, typically at positions C3, C4, C5, C6, and C7. This flavylium cation (AH^+) imparts these pigments their characteristic color, which can range from red to blue-violet depending on the matrix pH (Ayvaz et al., 2022). These compounds are water-soluble and can be classified according to their unique and characteristic chemical structure based on the number of hydroxyls, methylation level, and number of sugar molecules attached to the phenolic group (Chen et al., 2023; Enaru et al., 2021; Merecz-Sadowska et al., 2023).

Anthocyanins are glycosylated forms of anthocyanidins, in which one or more sugar residues are attached to the aromatic nucleus. Anthocyanidins constitute the basic skeleton of these molecules, known as the flavylium ion. Glycosylation, particularly at the C-3 position of the flavylium core, is a common feature of anthocyanins and is associated with enhanced pigment stability. The most frequently involved monosaccharides are glucose, rhamnose, galactose, arabinose, and xylose (Zhang et al., 2014).

Additionally, there are methylated anthocyanins, which contain one or more methoxy groups attached to the aromatic ring, a modification that may also contribute to increased stability. Acylated forms occur when the sugar residues are esterified with aromatic acids (such as p-coumaric, caffeic, ferulic, sinapic, gallic, or p-hydroxybenzoic acid) or with aliphatic acids (such as malonic, acetic, malic, succinic, tartaric, or oxalic acid). Acylation also plays a significant role in the structural stabilization of anthocyanins (Zhang et al., 2014).

This unique molecular configuration and its ability to assume different pH-dependent forms are responsible for the antioxidant properties of anthocyanins and their wide range of vibrant colors (de Pascual-Teresa & Sanchez-Ballesta, 2008; Zhang et al., 2014).

Among the main types of anthocyanins, we can highlight pelargonidin is associated with a bright red hue; cyanidin and its methylated derivative exhibit a red to reddish-purple coloration; while delphinidin and its methylated derivatives, malvidin and petunidin, display blue to violet tones (Fig. 1) (de Pascual-Teresa & Sanchez-Ballesta, 2008; Zhang et al., 2014).

The synthesis of anthocyanins occurs in the vacuoles of plant cells, organelles responsible for storing various substances, including pigments, enzymes, sugars, and minerals (Ayvaz et al., 2022). The biosynthesis of anthocyanins is a complex, multi-step, and highly regulated process involving the activation of specific genes by transcription factor complexes and the action of specialized enzymes in synthesizing these pigments.

3. Bioavailability and intestinal microbiota

Anthocyanins have attracted increasing scientific attention due to their potential health benefits. However, their clinical and nutritional application faces a fundamental challenge related to their low systemic bioavailability. This concept involving both bioaccessibility (the ability to withstand adverse gastrointestinal conditions such as pH variations, digestive enzymes, and diverse fluids) and biological effect (Rein et al., 2013). This process is influenced by several interrelated factors, such as the compound's structure, its interaction with the food matrix and microbiota, and individual physiological or genetic characteristics (Gull et al., 2021; Khoo et al., 2017).

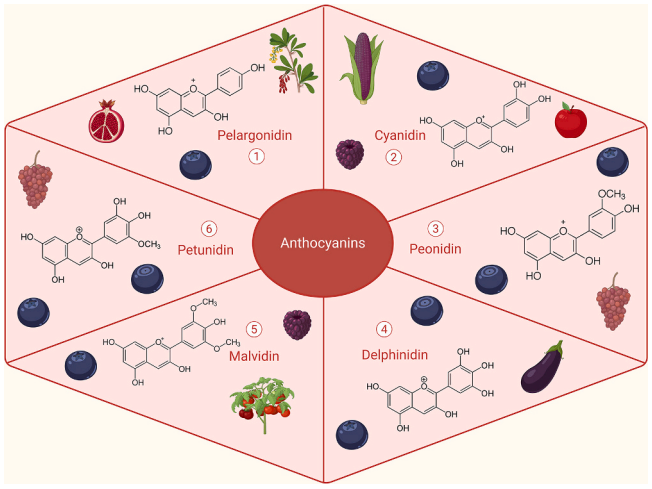

Fig. 1. Main classes of anthocyanins and their respective food sources.
Fig. 1. The image presents the six major anthocyanins that make up anthocyanins, highlighting their chemical structures and the foods in which they are naturally found.These include: (1) pelargonidin, presented in pomegranate, berry plant and raspberries; (2) cyanidin, detected in apples, blueberries and purple corn; (3) peonidin, found in grapes and blackberries; (4) delphinidin, found in blueberries and eggplant; (5) malvidin, found in grapes, tomato, blackberries and blueberries; (6) petunidin, presented in blueberries and dark grapes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Notably, the digestive stability of anthocyanins can be influenced by their glycosylation and acylation patterns. While disaccharide-glycosylated anthocyanins may exhibit resistance to digestive processes under specific conditions, their stability is not universally higher than that of monosaccharide-glycosylated forms or aglycones. Acylation and methylation generally enhance anthocyanin stability, protecting the pigments against digestive degradation, pH fluctuations, and thermal stress, likely through intramolecular copigmentation. However, acylation decreases anthocyanin polarity, hindering their interaction with membrane transporters such as bilitranslocase (Yang et al., 2018; Zhao et al., 2017). These structural characteristics profoundly influence absorption patterns along the digestive tract, which vary not only between different molecules but also across experimental models.

Human studies have provided valuable insights into the metabolic fate of anthocyanins, indicating that a substantial fraction of the ingested dose—sometimes exceeding 80 %—may reach the colon, primarily in a non-absorbed form (Kahle et al., 2006). The extent of this passage, however, depends on structural characteristics such as the type of conjugated sugar and the degree of methoxylation of the molecule (Kahle et al., 2006). In ileostomized patients, the recovery of intact anthocyanins after blueberry consumption ranged from 28 % to 85 % within 2 h, with higher values observed for arabinose-conjugated derivatives compared with glycosylated forms (Kahle et al., 2006). In a study employing a ^{13}C isotopic tracer, the fecal recovery of cyanidin-3-O-glucoside (C3G) was approximately 32.1 ± 6.1 % (Czank et al., 2013). Similar results were observed following raspberry intake, with recoveries ranging from 40 to 75 % within 48 h (González-Barrio et al.,

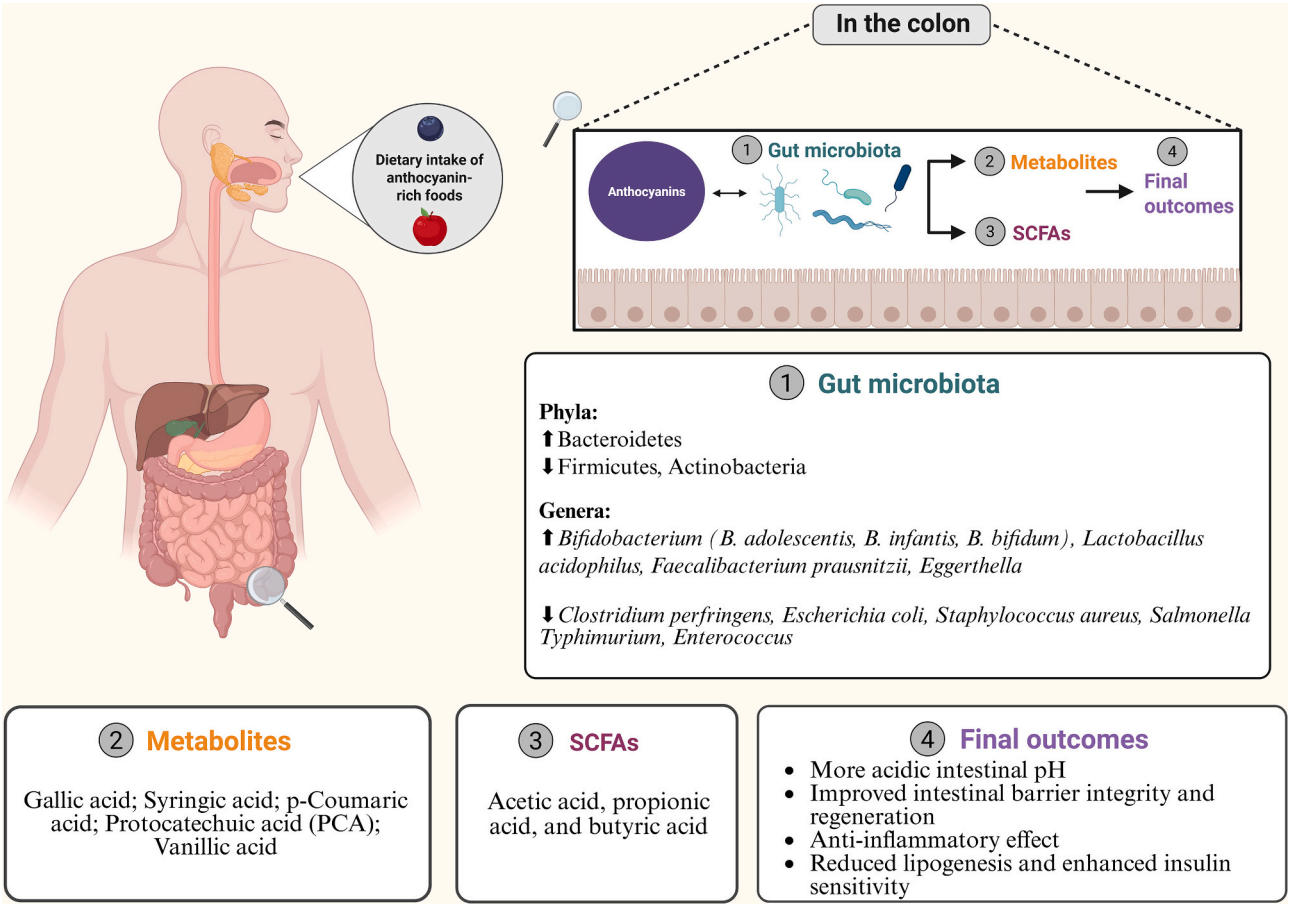

Fig. 2. Impact of anthocyanin intake on gut microbiota and intestinal health.
Fig. 2. Interaction between anthocyanins and the gut microbiota. Anthocyanins of ingested anthocyanins reach the colon, where they are metabolized by commensal microorganisms, resulting in the production of phenolic metabolites and SCFASs. These bioactive compounds positively modulate the intestinal environment by promoting epithelial barrier integrity, reducing inflammatory processes, and improving insulin sensitivity.

2010). In another study, individuals with an intact colon exhibited about 29.6 ± 6.2 % of total anthocyanins in feces after 8 h, along with higher plasma and urinary concentrations compared to ileostomized patients, demonstrating that the colon not only receives a significant proportion of anthocyanins but also plays an active role in their metabolism and absorption (Mueller et al., 2017) (Fig. 2).

Estimates obtained through conventional analytical methods often reflect methodological variability and are influenced by factors such as the specific chemical structure of each anthocyanin and the complexity of the food matrix (Singh et al., 2020).

In the stomach, the highly acidic environment (pH 1.5–2.0) favors the protonation of anthocyanins, stabilizing them in the flavylium cation form (McGhie et al., 2003). In humans, a study evaluating the relative bioavailability of cyanidin-3-O-glucoside (C3G) reported a value of 12.38 ± 1.38 %. This estimate was based on the total elimination of the absorbed 13C-labeled C3G dose. However, considerable interindividual variability was observed in the recovery of the 13C tracer, ranging from 15.1 % to 99.3 % among participants. The authors suggest that this variability may be associated with factors such as gastrointestinal transit time, the composition and catabolic activity of the colonic microbiota, as well as individual capacity for absorption and excretion of anthocyanin-derived catabolites and metabolites (Czank et al., 2013).

In contrast, animal models such as rats exhibit absorption rates (5–10 %), attributed to the expression of bilitranslocase transport proteins in rodents (Passamonti et al., 2002; Passamonti et al., 2003). This transport system demonstrates particular affinity for glycosylated anthocyanins, as evidenced by the detection of malvidin-3-O-glucoside in rat portal plasma just six minutes post-administration (Passamonti et al., 2003), and the identification of cyanidin-3-O-glucoside (C3G) in human plasma 30 min after consumption (Felgines et al., 2007; McGhie & Walton, 2007).

In the small intestine, where neutral pH (6.5–7.5) promotes the conversion of flavylium cations into chalcones, quinoidal bases, and carbinol pseudobases (Wahyuningsih et al., 2017), absorption reaches more significant levels (5–10 % in human clinical trials) (Gonçalves et al., 2021), while animal studies (rats and pigs) indicate higher rates of 10–15 % (Ferrars et al., 2014). These interspecies discrepancies arise from physiological and methodological factors, including the higher density of transporters (*e.g.*, sodium-glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) in rodents, shorter intestinal transit times in humans, and the greater complexity of food matrices in human diets compared to standardized animal diets (Faria et al., 2009).

The colon emerges as the central site of anthocyanin metabolism, where resident microbiota crucially transform these compounds. Anthocyanins that reach the colon undergo extensive microbial metabolism, resulting in the formation of a variety of phenolic acids with demonstrated biological activity. In addition, polyphenols can positively modulate microbial fermentation processes, indirectly influencing the production of short-chain fatty acids (SCFASs) (Hidalgo et al., 2012) (Fig. 2). Notably, butyrate production in humans exceeds animal models by 30–50 % (Czank et al., 2013), with profound implications for intestinal and systemic health. Microbial transformation yields structure-dependent metabolite profiles: cyanidin derivatives predominantly yield protocatechuic, vanillic, and p-coumaric acids (Chen et al., 2017); malvidin derivatives produce syringic and homovanillic acids (Boto-Ordóñez et al., 2014); and pelargonidin-3-glucoside derivatives generate tyrosol and p-hydroxybenzoic acid (de López Las Hazas et al., 2017).

Anthocyanin's modulatory effects on gut microbiota composition and function are well-documented. *In vitro* studies show that cyanidin-3-O-glucoside and peonidin-3-glucoside from black rice selectively promote beneficial species (*Bifidobacterium adolescentis*, *B. infantis*, *B. bifidum* and *Lactobacillus acidophilus*) while reducing pathogens (*Clostridium perfringens* and *Escherichia coli*) within 24 h (Zhu et al., 2018). Similarly, peonidin from purple sweet potato inhibits *Staphylococcus aureus* and *Salmonella typhimurium* (Sun et al., 2018). Human fecal cultures fermented with grape-derived anthocyanins increased *Bifidobacterium spp.* and *Lactobacillus spp.* while reducing *C. histolyticum* (Hidalgo et al., 2012), and red wine consumption elevated *Bifidobacterium*, *Eggerthella lenta*, and *Enterococcus* in middle-aged humans (Boto-Ordóñez et al., 2014). Additionally, the consumption of a functional soup containing 'Anthaplex', an anthocyanin-rich ingredient, significantly increased the abundance of lactic acid-producing bacteria—particularly *Bifidobacterium spp.*—in the feces of healthy volunteers (Wattanathorn et al., 2023).

In murine colorectal carcinogenesis models, blackberry anthocyanins enriched commensals (*Eubacterium rectale*, *Faecalibacterium prausnitzii*, *Lactobacillus spp.*) and suppressed *Desulfovibrio spp.* and *Enterococcus spp.* (Chen et al., 2018).

Animal studies demonstrate dose-dependency: high-dose black rice anthocyanins (100 mg/kg) shifted the Firmicutes/Bacteroidetes ratio (57.02 % / 30.88 % to 44.61 % / 42 %) and enriched *norank_f_Muribaculaceae* while reducing *Candidatus_Saccharimonas* (Sun et al., 2025). Cyanidin-3-rutinoside in high-fat-diet mice similarly increased *Akkermansia* and *Bacteroides* (Zhong et al., 2025). Human responses are more variable: elderberry juice increased *Ruminococcaceae*, *Faecalibacterium*, and *Bifidobacterium* in overweight adults within one week (Teets et al., 2024), whereas blueberry extract had minimal effects in healthy elderly (Wood et al., 2023). Obese individuals supplemented with *Vaccinium* anthocyanins and prebiotic fibers (inulin/FOS) showed increased Bacteroidetes, reduced Firmicutes/Actinobacteria, and elevated short-chain fatty acid (SCFAS) production (Hester et al., 2018). *In vitro*, cyanidin-3-glucoside-dimalonylated (C3GdM) anthocyanin from black corn cob enhanced microbial diversity in elderly (65–71 years) but not younger (22–28 years) samples, underscoring age- and baseline microbiota-dependent responses (Li et al., 2025).

SCFASs, the end products of microbial anthocyanin fermentation, play multifaceted roles in intestinal and systemic homeostasis (Fig. 2). Butyrate, a key metabolite, fuels colonocytes, strengthens the intestinal barrier *via* tight junction proteins (occludin, claudins), and stimulates goblet cell mucus secretion (Parada Venegas et al., 2019). SCFASs also act as immunomodulators by inhibiting histone deacetylases (HDACs) and activating G-protein coupled receptors 43/109 A (GPR43/GPR109A), promoting regulatory T-cell differentiation and suppressing pro-inflammatory pathways (Dalile et al., 2019). The resulting luminal acidification (pH ~5.5–6.5) inhibits pathogenic *Enterobacteriaceae* while favoring butyrate-producing commensals (Ríos-Covián et al., 2016). Additionally, SCFASs promote adipocyte lipogenesis and improve insulin sensitivity by reducing inflammation and enhancing glucagon-like peptide-1 / peptide YY (GLP-1/PYY) secretion, synergistically improving glycemic control (Chambers et al., 2018).

Despite this promise, challenges remain. Limited systemic bioavailability (<10 % as intact compounds) and interindividual variability necessitate innovative delivery systems (*e.g.*, purple potato anthocyanin nanoparticles), which enhance *Lactobacillus* while reducing *Actinobacteria* (Zang et al., 2024). Disparities between experimental doses (50–100 mg/kg in animals, ~500 mg/day human equivalents) and habitual intake (15–215 mg/day) underscore the need for rigorous dose-response studies.

Anthocyanins represent a bioactive class with remarkable potential to modulate gut microbiota and extend benefits beyond the gastrointestinal tract. Realizing this potential requires advances in: (1) bioavailability-enhancing technologies; (2) understanding interindividual response variability; and (3) evidence-based dietary recommendations accounting for diet-microbiota-host interactions. Future research must employ integrated multi-omics approaches and longitudinal designs to elucidate mechanisms and translate findings into personalized, accessible nutritional strategies for health promotion.

4. Biological properties

Anthocyanins are widely studied due to their diverse biological

effects (Table 1). Among them, their potent antioxidant properties stand out, neutralizing free radicals and protecting against cellular oxidative stress (Tena et al., 2020). Additionally, they exhibit anti-inflammatory activity by modulating the immune response and reducing the production of inflammatory mediators (Kim et al., 2016; Speer et al., 2020). They have also shown neuroprotective effects, proving promising in the prevention of neurodegenerative diseases (Santos et al., 2019). In the cardiovascular context, anthocyanins demonstrate cardioprotective effects through various markers of cardiovascular disease risk (Krga & Milenkovic, 2019). The interaction of anthocyanins with the intestinal microbiota has been studied, highlighting their potential to modulate microbial populations and influence intestinal health (Igwe et al., 2018; Tian et al., 2018). In addition to these effects, these bioactive compounds also demonstrate antimicrobial activity against foodborne pathogens such as *Escherichia coli* and *Salmonella* (Ma et al., 2019). This article will specifically review the antioxidant, anti-inflammatory, and antitumor activities of anthocyanins, deepening the understanding of their benefits for human health.

4.1. Antioxidant

The main mechanisms initiating the chronic disease process involve oxidative stress (Sies et al., 2022). Oxidative stress represents an imbalance between the production of ROS and the ability of the body to neutralize or repair them. These free radicals are unstable molecules containing unpaired electrons in their structure. These unpaired electrons can react with other molecules, damaging healthy cells in the body. This mechanism contributes to premature aging and the development of diseases such as cancer, heart disease, and neurodegenerative disorders (Dubois-Deruy et al., 2020; Singh & Manna, 2022; Tan et al., 2018; Yang et al., 2024).

To neutralize the harmful effects of free radicals it is crucial to maintain a balance between free radicals and antioxidants, their counterparts. This redox balance is essential for protecting cells and ensuring the proper functioning of the immune system (Lauridsen, 2018).

In this context, anthocyanins neutralizes free radicals, thereby preventing cellular damage (Migliorini et al., 2019; Tena et al., 2020; Vishnu et al., 2019). The specific mechanism of action can include: I) hydrogen atom transfer (HAT), stabilizing the free radical through hydrogen donation; II) electron donation to the free radical; III) chelation of metal ions, forming stable bonds; and IV) activation of superoxide dismutase (SOD) and glutathione peroxidase enzymes (Fig. 3) (Apak et al., 2016b; Fallah et al., 2020; Gulçin, 2011; Huang et al., 2005).

In addition, anthocyanins can exert antioxidant function by modulating cellular signaling pathways associated with oxidative stress and inflammation. For example, these compounds can modulate the expression of genes involved in antioxidant and anti-inflammatory responses, thereby aiding in maintaining redox balance and mitigating inflammation related to oxidative stress (Tena et al., 2020).

In anthocyanins the presence of hydroxyl groups in the B ring and the arylation of sugar residues by phenolic acids confer increased potential to neutralizing free radicals. Moreover, hydroxyl groups in the C ring enable anthocyanins to act as chelators of metal ions. This property inhibits lipid peroxidation induced by free radicals attacking lipids in cell membranes, preventing damage (Dangles & Fenger, 2018; Miguel, 2011; Navas et al., 2012).

This extract of *Hibiscus sabdariffa* L., tested at doses of 1000 and 2000 μg/mL of anthocyanins, demonstrated the ability to inhibit lipid peroxidation and neutralize superoxide and hydroxyl radicals. The authors emphasized the significant antioxidant potential of the anthocyanins present in the raw material. Furthermore, adding anthocyanins to the culture medium before fermentation enhanced the overall antioxidant efficacy of the fermented medium. This supplementation with anthocyanins also contributed to preserving the antioxidant activity of the fermented medium over six months of storage at 4 °C (Simionescu & Petrovici, 2024).

Another study investigated the association between anthocyanin supplementation and dyslipidemia in 169 participants. The findings indicated that higher doses of anthocyanins, such as 320 mg/day, significantly reduced oxidative stress and inflammation markers, including C-reactive protein (CRP). Interestingly, the study suggested that doses as low as 80 mg/day could also benefit participants (Zhang et al., 2020).

In a study, the antioxidant potential of anthocyanins from various *Rubus* species was evaluated based on their ability to inhibit lipid peroxidation. Methanolic extracts at 50 mg/mL exhibited 53 % inhibition for *R. jamaicensis* (blackberry), over 80 % for *R. racemosus* and *R. acuminatus* (black raspberries), while *R. idaeus* showed the highest inhibition, reaching approximately 90 %. In parallel, isolated anthocyanins from *R. idaeus* (red raspberry), specifically pelargonidin-3-glucoside and cyanidin-3-rutinoside, demonstrated 56 % and 60 % inhibition at a concentration of 10 mM, respectively. Additionally, the hexane extract of *R. jamaicensis* exhibited 74 % inhibition, while the ethyl acetate extracts of *R. acuminatus* and *R. rosifolius* (roseleaf raspberry) demonstrated 84 % and 66 % inhibition, respectively (Bowen-Forbes et al., 2010).

The methanolic extract of violet glutinous rice (*Oryza sativa* L.), with 0.35 μg/mg of cyanidin-3-O-glucoside in its composition, was evaluated for its antioxidant activity by different methodologies. The extract showed free radical scavenging activity, with an IC_{50} (inhibitory concentration 50 %) value of 32.31 ± 1.28 mg/mL, as well as inhibiting lipid peroxidation (IC_{50} = 57.40 ± 2.12 mg/mL) and demonstrating iron ion chelating capacity (IC_{50} = 85.05 ± 5.43 mg/mL). These results suggest that, despite the presence of cyanidin-3-O-glucoside, the extract has moderate potency, which may be related to the low concentration of the bioactive compound (Manosroi et al., 2020).

Crude and partially purified extracts obtained from black bean (*Phaseolus vulgaris* L.) hulls showed significant antioxidant activity. An IC_{50} value of 149 ± 2 μg/mL was observed in the crude extract and 40 ± 1 μg/mL in the partially purified extract. Similarly, in the ABTS [2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay, the IC_{50} values were 135 ± 7 μg/mL for the crude extract and 44 ± 5 μg/mL for the partially purified extract. These results indicate that purification of the extracts can concentrate bioactive compounds responsible for neutralizing free radicals, thereby increasing antioxidant efficacy (Kuasnei et al., 2025).

In human subjects, it was observed that the administration of capsules containing anthocyanins at doses higher than 80 mg/day, mainly delphinidin-3-O-glucoside and cyanidin-3-O-glucoside, was able to attenuate lipid peroxidation, as indicated by the reduction in the levels of the biomarker 8-isoprostane Prostaglandin F2α (8-isoPGF2α) (Guo et al., 2020). Additionally, the administration of 60–120 mL of cherry juice, containing 0.343 mg/mL of cyanidin-3-glucosylrutinoside and 0.143 mg/mL of cyanidin-3-rutinoside, in individuals with gout, demonstrated the ability to increase the expression of the nuclear factor erythroid 2–related factor 2 (NRF2) gene, a key regulator in the activation of endogenous antioxidant defenses (Brunetti et al., 2023).

These findings demonstrate the multifaceted antioxidant effects of anthocyanins in cellular, animal, and human models, which reinforces their therapeutic potential in mitigating damage related to oxidative stress and the progression of chronic diseases.

4.2. Anti-inflammatory

In the inflammatory process, the immune system reacts to harmful stimuli, such as infections or injuries, through a coordinated response that aims to eliminate the threat and promote tissue repair (Samad & Ruf, 2013). The organism's exposure to pathogenic agents, environmental toxins, or tissue injuries triggers this response, mediated by a complex cascade of events that includes the release of cytokines, chemokines, and inflammatory mediators (Vendrame & Klimis-Zacas,

Table 1
Analyses of the effects of anthocyanins from different sources and concentrations, detailing mechanisms of action.

Anthocyanin	Source	Effects	Mechanism	Model	Reference
Cyd-3-O-soph, Cyd-3-O-glc and Cyd-3-O-rut (10–50 μL in 1.5 mL and 10–40 μL in 1 mL)	Isolated from raspberry pomace (*Rubus idaeus* L.)	Anti-inflammatory and antioxidant	Inhibited the activity of LOX (IC_{50} 4,85 mg FW/mL) and COX-2 (IC_{50} 0,87 mg FW/mL); neutralized DPPH (12.92 mg FW/mL) and ABTS (3.85 mg FW/mL) free radicals, with a low correlation with ferric ion reduction (FRAP).	*In vitro*	Szymanowska & Baraniak, 2019
Dp3-Sam and Dp (50–200 μM – *in vitro;* 15 mM/kg body weight – *in vivo*)	Isolate of dried calyces of *Hibiscus sabdariffa*	Anti-inflammatory	Modulation of MEK/ERK and NF-κB signaling pathways *(in vitro*). Dp3-Sam and Dp reduced edema by 89.3 % and 96.3 % after 6 h, respectively. Pretreatment with Dp3-Sam and Dp decreased serum levels of IL-6, MCP-1, and TNF-α *(in vivo)*	*In vitro* (Cell RAW 264.7) and *in vivo* (mouse)	Sogo et al., 2015
Cyd-3-O-glc (4 mg per 100 g body weight)	Wild black mulberry (*Morus nigra* L.) extract	Anti-inflammatory	Reduction of edema by up to 80 % after 4 h and of leukocytes; inhibition of COX-2 expression (mRNA and protein).	*In vivo* (mouse)	Hassimotto et al., 2013
Cyd-3-O-glc (25 μM)	Commercial C3G 97 % HPLC	Anti-inflammatory	It significantly reduced NO and inhibited PGE2 and IL-8.	*In vitro* (Cell HT-29)	Serra et al., 2013
Cyd-3-glc and Peo-3-glc (50–200 μg/mL – *in vitro*) (100 mg/kg/day - *in vivo*).	Black rice extract (*Oryza sativa* L.)	Anti-inflammatory	*In vitro*: it reduced the cell viability of all cell lines, especially MDA-MB-453, in a dose-dependent manner. *In vivo*: it significantly suppressed tumor growth and angiogenesis, as well as reduced the expression of MMP-9 and MMP-2 and uPA.	*In vitro* (Cells MCF-7, MDA-MB-231, and MDA-MB-453) and *in vivo* (mouse)	Hui et al., 2010
Cyd-3-glc, Cyd-3-gal, Cyd-3-ara and Cyd-3-xyl (DPPH 0.2 mg/mL), (ORAC 1 mg/mL), (anti-inflammatory: 0.5–500 μg/mL)	*Aronia melanocarpa* (Michx.) Elliott extract (25 % anthocyanins).	Anti-inflammatory and antioxidant	It reduced the levels of IL-1β and TNF-α in RAW 264.7 cells. It attenuated the increase in lipid peroxidation (MDA). It neutralized DPPH free radicals and the peroxyl radical (ORAC).	*In vitro* (cell RAW 264.7)	Banach, Wiloch, Zawada, Cyplik, & Kujawski, 2020
Peo-3-glc (300 μM)	Isolated from purple corn (Jinnuo No.8).	Anti-inflammatory and antioxidant	It reduced the levels of IL-1β, IL-6, and TNF-α, and the expression of caspase-1 and NF-κB. It inhibited ROS and superoxide ions. It increased the levels of GSH. It improved the activity of the antioxidant enzymes SOD, GPX, and CAT.	*In vitro* (cell L02)	Hao et al., 2023
Commercial Cyd-3-glc, with a purity ≥98 %, 50 μM (*in vitro*) and 10 mg/kg (*in vivo*).	Commercial	Antioxidant	In the *in vitro* model, it attenuated the production of lipid peroxide and MDA. Elevated GSH levels were observed in both *in vivo* and *in vitro* models, whereas increased SOD activity was detected only *in vitro*. It reduced ROS in tissues and free iron in the bodies of rats with acute renal injury.	*In vitro* (Cells NRK-52E and HK-2) and *in vivo* (mouse)	Du et al., 2023
Del-3-glc and cyd cyd-3-glc (320 mg/day - *in vivo*) (0.1 a 50 mg/mL *in vitro*)	Commercial	Anti-inflammatory	Significant reduction of hs-CRP, VCAM-1, and IL-1β in humans with hypercholesterolemia; inhibition of CRP and sVCAM-1 in HepG2 cells.	*In vivo* (human) and *in vitro* (cell HepG2)	Zhu et al., 2013
Cyd-3-sam and del-3-sam	Extract of *Hibiscus sabdariffa* L. (1000–2000 μg/mL)	Antioxidant	Scavenged free radicals (DPPH and ABTS), reduced ferric ions (FRAP), and inhibited lipid peroxidation by >99 %	*In vitro*	Simionescu & Petrovici, 2024
Pel-3-glc and pel-3-rut (10 mM)	Extract of *Rubus rosifolius, R. acuminatus and R. jamaicensis* (50 mg/mL)	Antioxidant	The isolated anthocyanins showed 60 % and 56 % inhibition of lipid peroxidation, while the hexane extract of *R. jamaicensis* showed 74 %, the ethyl acetate extract of *R. acuminatus*, ≥ 80 %, and that of *R. rosifolius*, 66 % inhibition.	*In vitro*	Bowen-Forbes et al., 2010
Pel-3-glc and pel-3-rut	Extract of *Rubus rosifolius, R. acuminatus and R. jamaicensis* (100 mg/mL)	Anti-inflammatory	The hexane extract of *R. acuminatus* stood out with 71 % inhibition of COX-2, followed by *R. jamaicensis* and *R. racemosus*, which also showed COX-2 inhibition ranging from 18 to 33 %. Meanwhile, *R. rosifolius* and *R. racemosus* exhibited COX-1 inhibition of approximately 30–33 %.	*In vitro*	Bowen-Forbes et al., 2010
Mal-3,5-diglc, mal-3-glc, cyd-3-cou-5-diglc, del-3-cou-glc, pet-3-cou-glc and mal-3-cou-glc.	3D-printable juice gels made from Bordô, Isabel, and Concord grapes (100–1000 μg/mL)	Anti-inflammatory	Reduced NF-κB activation and the secretion of TNF-α and CXCL2/MIP-2 in LPS-stimulated macrophages.	*In vitro* (Cell RAW 264.7)	de Sartori et al., 2023
Pel-3-glc and pel-3-rut	Extract of *Rubus rosifolius, R. acuminatus and R. jamaicensis* (250 mg/mL)	Antitumor	The hexane extract of *Rubus jamaicensis* exhibited the highest antiproliferative activity, inhibiting 50 % of colon cancer cells, 24 % of breast, 54 % of lung, and 37 % of gastric cancer cells.	*In vitro* (Cells MCF-7, SF-268, NCI-H460, HCT-116 and AGS)	Bowen-Forbes et al., 2010
Peo-3-gal, peo-3-glc, peo-3-ara, cyd-3-ara, mal-3-glc and del-3-glc	Anthocyanin-rich juice - 942 mg/L de anthocyanins (330 mL/dia)	Antitumor and antioxidant	Reduced the migration of PANC-1, downregulated the expression of adhesion molecules such as β1-integrins and ICAM-1, and inhibited the FAK and NF-κB signaling	*In vitro* (plasma extracts isolated from humans).	Mostafa et al., 2023.

(*continued on next page*)

Table 1 (*continued*)

Anthocyanin	Source	Effects	Mechanism	Model	Reference
			pathways, resulting in decreased phosphorylation of NF-κB p65 and FAK. Additionally, it lowered the levels of ROS.		
Cyd-3-glc	Standardized extract of blueberry (*Vaccinium myrtillus*) containing 36 % anthocyanins (1 g/dia)	Antitumor	Inverse correlation between adiponectin and Ki67, along with increased IL-6 levels in individuals with high-grade dysplasia, suggesting a potential role of these biomarkers in disease progression.	*In vivo* (Humans with adenomatous polyps in the colon).	Macis et al., 2023
Cyd-3-glc	Crude extract of the black bean (*Phaseolus vulgaris*) seed coat (35,8 % C3G) (0–100 μM)	Antitumor and antioxidant	The maximum dose resulted in a 26.05 % reduction in HepG2 cell viability. Exhibited significant antioxidant properties, as evidenced by its ability to scavenge DPPH radicals, reduce ferric ions, and inhibit lipid peroxidation.	*In vitro* (Cell HepG2)	Zhang et al., 2024.
Medox – anthocyanin supplement (17 different purified natural anthocyanins) (40–320 mg/mL).	Norwegian wild blueberries and blackcurrants.	Anti-inflammatory and antioxidant	Significantly reduced serum levels of IL-6 and TNF-α in a dose-dependent manner, with a 40 % reduction in IL-6 and a 21 % reduction in TNF-α. Significantly increased SOD activity and reduced oxidative stress biomarkers, such as urinary 8-isoprostaglandin F2α, urinary 8-OHdG, and serum MDA, in a dose-dependent manner.	*In vivo* (Humans aged 35 to 70 years.)	Zhang et al., 2020
Freeze-dried fruits (blueberry 50 g; blackberry 62.9 g; blackcurrant 57.1 g) containing 470 mg of Cyd-3-glc equivalents (CGE) per kg.	Blueberry, blackberry e blackcurrant	Anti-inflammatory	The animals that received black currant exhibited a local anti-inflammatory effect in the epididymal adipose tissue due to a significant reduction in the expression of F4/80 mRNA, a macrophage marker, and in the number of CLS; however, the other sources used did not show significant anti-inflammatory changes.	*In vivo* (mouse)	Kim et al., 2016
Mv-3-glc e mv-3-gal (1–100 μM).	Anthocyanins isolated from Rabbiteye blueberry (*Vaccinium ashei*).	Anti-inflammatory	Both inhibited the increase in the production of MCP-1, ICAM-1, and VCAM-1 induced by TNF-α; decreased the degradation of IκBα and blocked the nuclear translocation of p65.	*In vitro* (Cell HUVEC)	Huang et al., 2014
Cyd-3-glc, cyd-3-rut, del-3-glc, mal-3-glc, pel-3-glc and peo-3-glc (10 to 250 μg/mL).	Açaí extract (*Euterpe oleracea* Mart.).	Anti-inflammatory	Significant reduction of NO, COX-2 expression, release of TNFα, and phosphorylation of NF-κB. Significantly attenuated the increase in p38-MAPK phosphorylation.	*In vitro* (Cell BV-2)	Poulose et al., 2012
Del-3-gal, del-3-glc, del-3-ara, cy-3-gal, and cy-3-glc (2.5, 5, 10, and 25 μg/mL).	Blueberry extract (*Vaccinium myrtillus* L.)	Anti-inflammatory	They inhibited the production and secretion of pro-inflammatory mediators such as TNF-α, IP-10, I-TAC, sICAM-1, and GRO-α.	*In vitro* (Cell T84)	Triebel et al., 2012
Cyd-3-glc: *In vitro*: extract (10, 20, 30 μg/mL) and isolated anthocyanin (1–5 μM); *in vivo*: extract (100 mg/kg) and isolated anthocyanin (5, 25 mg/kg).	Black rice extract (BR) and cyd-3-glc were isolated.	Anti-inflammatory	*In vitro:* Both reduced the production of pro-inflammatory cytokines TNF-α and IL-1β, inflammatory mediators NO and PGE2, and inhibited the phosphorylation of IκB-α, NF-κB, and activation of MAP kinases (ERK1/2, JNK1/2, and p38-MAPK). *In vivo:* They reduced exudate volume, leukocyte count, and inhibited the expression of TNF-α, IL-1β, PGE2, COX-2, and NF-κB activation in exudates.	*In vitro* (RAW 264.7) and *in vivo* (rat)	Min et al., 2010
Mal-3-glc, cyd-3-glc and del-3-rut	Blueberry (BBA), blackberry (BKA), and blackcurrant BCA) anthocyanin fraction (0–20 μg/mL).	Anti-inflammatory and antioxidant	The anthocyanin fractions reduced mRNA levels of IL-1β and TNFα, as well as TNFα secretion, by inhibiting NF-κB p65 nuclear translocation. The BKA fraction showed higher total antioxidant capacity, and all fractions decreased ROS levels in wild-type macrophages.	*In vitro* (Cells RAW 264.7 and BMMs)	Lee et al., 2014
Cyd-3-sam, cyd-3-glc and cyd-3-sam-5-glc	Extract of *Sambucus nigra* L. (0,1–100 %)	Antioxidant	It exhibited DPPH radical scavenging activity, reducing power, and lipid peroxidation inhibition, with IC_{50} values of 3.1 ± 0.1 mg/mL, 3.7 ± 0.2 mg/mL, and 9.4 ± 0.3 mg/mL, respectively.	*In vitro*	da Silva et al., 2019
Cyd-3-glc	Extract of *Lonicera caerulea* (blue honeysuckle) and *Vaccinium myrtillus* (blueberry) (5 to 50 mg/L)	Antioxidant e Anti-inflammatory	Significantly decreased the generation of RONS. Partially decreased the expression of IL-6 and the activity of caspases-3 and − 9 induced by UVB.	*In vitro* (Cell HaCaT)	Svobodová et al., 2009

Note. LOX: Lipoxygenase; FRAP: Ferric Reducing Antioxidant Power; COX-2: Cyclooxygenase-2; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FW/mL: Fresh Weight per Milliliter; MEK: Mitogen-Activated Protein Kinase Kinase; ERK: Extracellular Signal-Regulated Kinase; NF-κB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; Dp3-Sam: Delphinidin-3-sambubioside; Dp: Delphinidin; IL-6: Interleukin 6; MCP-1: Monocyte Chemoattractant Protein-1; TNF-α: Tumor necrosis factor-alpha; mRNA: Messenger Ribonucleic Acid; NO: Nitric Oxide; PGE2: Prostaglandin E2; IL-8: Interleukin-8; IC_{50}: Inhibitory Concentration 50 %; Interleukin 8; MDA-MB-453: Human Breast Cancer Cell Line; MMP-9: Matrix Metalloproteinase-9; MMP-2: Matrix Metalloproteinase-2; uPA: Urokinase Plasminogen Activator; IL-1β:

Interleukin 1 Beta; RAW 264.7: Mouse Macrophage Cell Line; MDA: Malondialdehyde; ROS: Reactive Oxygen Species; GSH: Glutathione; SOD: Superoxide Dismutase; GPX: Glutathione Peroxidase; ORAC: Oxygen Radical Absorbance Capacity; CAT: Catalase; hs-CRP: High-Sensitivity C-Reactive Protein; VCAM-1: Soluble Vascular Cell Adhesion Molecule-1; CRP: C-Reactive Protein; VCAM-1: Vascular Cell Adhesion Molecule-1; HepG2: Human Hepatocellular Carcinoma Cell Line; ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); COX-1: Cyclooxygenase-1; CXCL2/MIP-2: C-X-C Motif Chemokine Ligand 2 / Macrophage Inflammatory Protein 2; PANC-1: Human Pancreatic Cancer Cell Line; ICAM-1: Intercellular Adhesion Molecule-1; FAK: Focal Adhesion Kinase; p65: RelA (a subunit of NF-κB); Ki67: Ki-67 Antigen; Urinary 8-OHdG: Urinary 8-Hydroxy-2′-deoxyguanosine; F4/80 mRNA: F4/80 Macrophage Marker (mRNA); CLS: Crown-Like Structures; p38-MAPK: p38 Mitogen-Activated Protein Kinase; IP-10: Interferon Gamma-Inducible Protein 10; I-TAC: Interferon-Inducible T-Cell Alpha Chemoattractant; sICAM-1: Soluble Intercellular Adhesion Molecule-1; GRO-α: Growth-Regulated Oncogene Alpha; IκB-α: Inhibitor of Nuclear Factor Kappa B Alpha; MAP: Mitogen-Activated Protein; ERK1/2: Extracellular Signal-Regulated Kinases 1 and 2; JNK1/2: c-Jun N-terminal Kinases 1 and 2; RONS: Reactive Oxygen and Nitrogen Species; UVB: Ultraviolet B Radiation; μL: Microliter; mL: Milliliter; mg: Milligram; μM: Micromolar; Kg: Kilogram; mM: Millimolar; g: Gram; MCF-7: Human Breast Cancer Cell Line; SF-268: Human Glioblastoma Cell Line; NCI-H460: Human Non-Small Cell Lung Cancer Cell Line; HCT-116: Human Colorectal Carcinoma Cell Line; AGS: Human Gastric Cancer Cell Line; Cyd-3-ara: cyanidin-3-arabinoside; Cyd-3-cou-5-diglc: cyanidin-3-coumaroyl-5-diglucoside; Cyd-3-gal: cyanidin-3-galactoside; Cyd-3-glc: cyanidin-3-O-glucoside; Cyd-3-rut: cyanidin-3-O-rutinoside; Cyd-3-sam: cyanidin-3-sambubioside; Cyd-3-sam-5-glc: cyanidin-3-O-sambubioside-5-O-glucoside; Cyd-3-soph: cyanidin-3-O-sophoroside; Cyd-3-xyl: cyanidin-3-xyloside; Del-3-cou-glc: delphinidin-3-coumaroylglucoside; Del-3-glc: delphinidin-3-O-β-glucoside; Del-3-rut: delphinidin-3-rutinoside; Del-3-sam: delphinidin-3-sambubioside; Mal-3,5-diglc: malvidin-3,5-diglucoside; Mal-3-cou-glc: malvidin-3-coumaroylglucoside; Mal-3-glc: malvidin-3-glucoside; Pel-3-glc: pelargonidin-3-glucoside; Pel-3-rut: pelargonidin-3-rutinoside; Peo-3-ara: peonidin-3-arabinoside; Peo-3-gal: peonidin-3-galactoside; Peo-3-glc: peonidin-3-glucoside; Pet-3-cou-glc: petunidin-3-coumaroylglucoside; HPLC: High Performance Liquid Chromatography; BBA: Blueberry; BCA: blackcurrant; BKA: Blackberry.

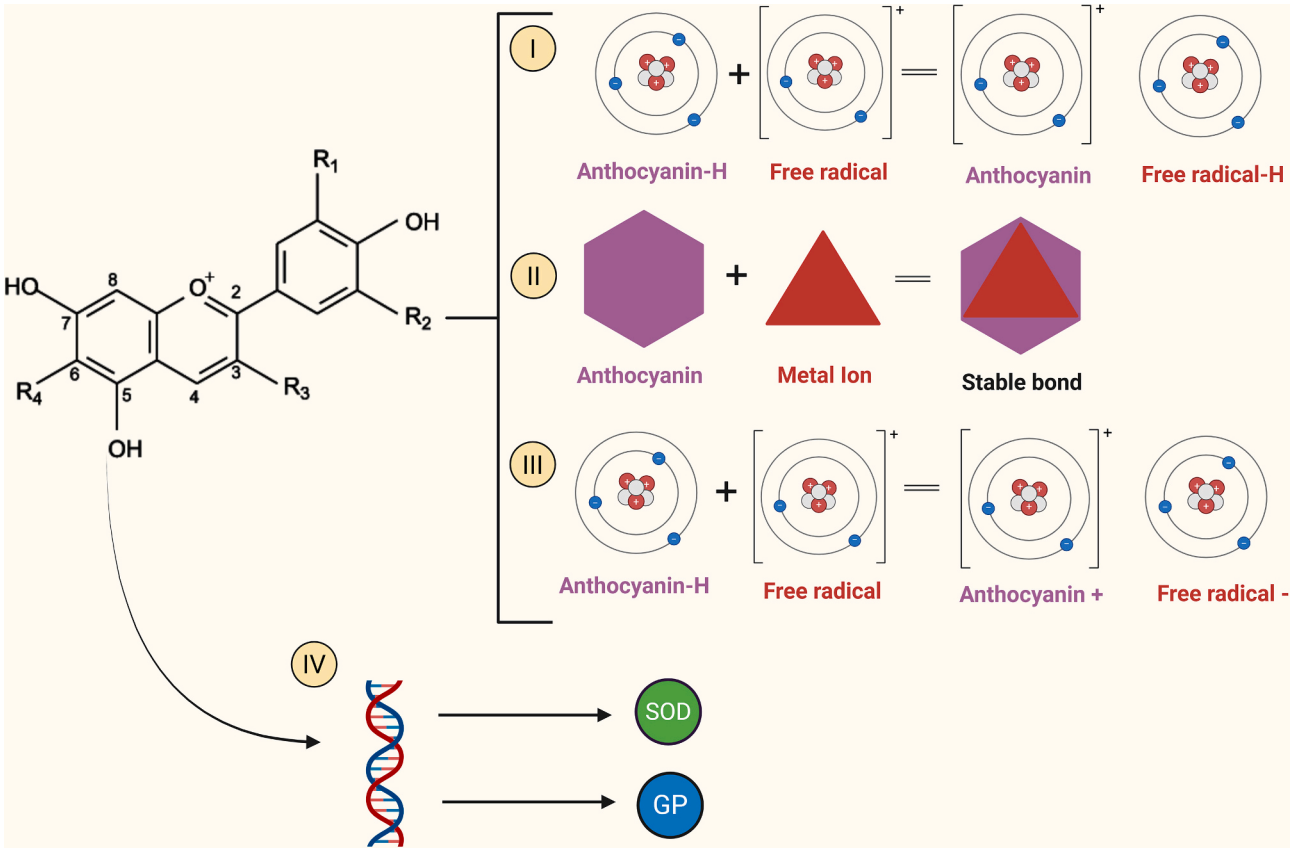

Fig. 3. Mechanisms of antioxidant activity of anthocyanins against free radicals and metal ions.
Fig. 3. Antioxidant mechanisms of anthocyanins. I) Hydrogen transfer from anthocyanin to the free radical; II) Anthocyanin chelates the ion, forming a stable bond; III) Anthocyanin donates an electron to the free radical; and IV) Anthocyanins can influence gene expression, including genes encoding antioxidant enzymes such as SOD and peroxide.

2015).

Anthocyanins emerge as alternative compounds in modulating cellular signaling pathways related to the inflammatory response. Studies have associated anthocyanins with the inhibition of pro-inflammatory cytokines, the reduction of cell adhesion molecule expression, and the suppression of inflammatory cell activation, such as macrophages (Kim et al., 2016; Speer et al., 2020). Moreover, evidence suggests that anthocyanins effectively reduce oxidative stress, a predominant factor in initiating chronic inflammation (Huang et al., 2014; Speer et al., 2020). Therefore, anthocyanins confer health benefits, especially in chronic inflammatory conditions (Bowen-Forbes et al., 2010; Kozłowska & Dzierżanowski, 2021; Vendrame & Klimis-Zacas, 2015).

Macrophages are generally used for *in vitro* clinical trials analyzing anti-inflammatory responses, as these cells are among the first responders recruited to infection sites or inflammation for microorganism elimination or tissue repair. Macrophage activation typically involves lipopolysaccharides (LPS) and/or interferon-gamma (IFN-γ) signaling. Hence, many assays utilize LPS to induce macrophage activation (Abbas et al., 2023; Hirayama et al., 2017; Rosadini & Kagan, 2017; Zhang, Pan, et al., 2021).

Macrophages perform several functions, including phagocytosis,

production of ROS, nitric oxide (NO), and proteolytic enzymes for microorganism eradication. Inducible nitric oxide synthase (iNOS) or nitric oxide synthase 2 (NOS2) in macrophages is responsible for NO production, which is regulated by pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β). NO, in turn, modulates the activity of these pro-inflammatory cytokines, potentially amplifying the inflammatory response (Jungi et al., 1996; MacMicking et al., 1997; Sharma et al., 2007; Zhang, Yang, & Ericsson, 2021). Under increased oxidative stress, NO can interact with the superoxide anion (O_2^-) to form peroxynitrite (ONOO-), a highly reactive molecule that causes DNA damage. Peroxynitrite contributes to inflammation and tissue damage, promoting noncommunicable diseases (Król & Kepinska, 2021; Preiser, 2012).

Furthermore, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), comprising subunits such as RelA (p65) and p50, remains inactive under normal conditions, bound to inhibitory proteins (IκB) in the cytoplasm. Inflammatory stimuli activate the IκB kinase complex (IKK), phosphorylating IκB and releasing NF-κB for translocation to the nucleus (Dorrington & Fraser, 2019; Kang et al., 2023; Lin et al., 1998; Yatim et al., 2015). In the nucleus, NF-κB acts as a transcription factor, binding to DNA and promoting pro-inflammatory gene expression.

The anti-inflammatory properties of anthocyanins have been widely investigated in *in vitro* studies and animal models. These studies suggest that anthocyanins can modulate several inflammatory pathways, including the inhibition of pro-inflammatory cytokine production, such as TNF-α and interleukins, as well as reducing the expression of cell adhesion molecules and pro-inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and iNOS (de Sartori et al., 2023; Lee et al., 2014; Poulose et al., 2012; Tsoyi et al., 2008; Vendrame & Klimis-Zacas, 2015).

Regarding the anti-inflammatory effects, anthocyanin doses of approximately 320 mg/day were associated with a reduction in circulating levels of the cytokine interleukin-6 (IL-6). In addition, a positive correlation was observed for interleukin-10 (IL-10) (Guo et al., 2020).

Furthermore, anthocyanins have been associated with the modulation of intracellular signaling pathways, such as NF-κB and MAP kinases, which play crucial roles in regulating the inflammatory response (Lee et al., 2014; Vendrame & Klimis-Zacas, 2015). Experimental models support these findings. Anthocyanin-rich extracts from *Aronia melanocarpa* were able to reduce the inflammatory response in RAW 264.7 cells and significantly decrease levels of pro-inflammatory cytokines such as TNF-α and IL-1β (Fig. 4) (Banach, Wiloch, Zawada, Cyplik, & Kujawski, 2020). Similarly, cherry juice rich in anthocyanins such as cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside was able to attenuate the expression of inflammatory genes including TNF and iNOS (Brunetti et al., 2023).

The anti-inflammatory effects of anthocyanins have also been demonstrated in humans. Anthocyanin sources such as red apple and *Aronia melanocarpa* have shown effects on inflammatory biomarkers in humans with hypercholesterolemia. The groups received 80 g of apple snack or aronia infusion containing 34.5 mg and 37.4 mg of anthocyanins (cyanidin-3-O-galactoside and cyanidin-O-arabinoside), respectively. There was a reduction in the levels of high-sensitivity C-reactive protein (hs-CRP) and IL-6, especially in men, and aronia consumption prevented P-selectin and intercellular adhesion molecule-1 (ICAM-1). These results suggest therapeutic potential in modulating inflammation intensity, with the apple dietary matrix possibly enhancing the effects of anthocyanins through synergy (Pedret et al., 2024).

In individuals with non-inflammatory fatty liver disease (NAFLD), supplementation at doses of 320 mg/day of anthocyanins for 12 weeks significantly reduced plasma levels of the pro-inflammatory cytokines

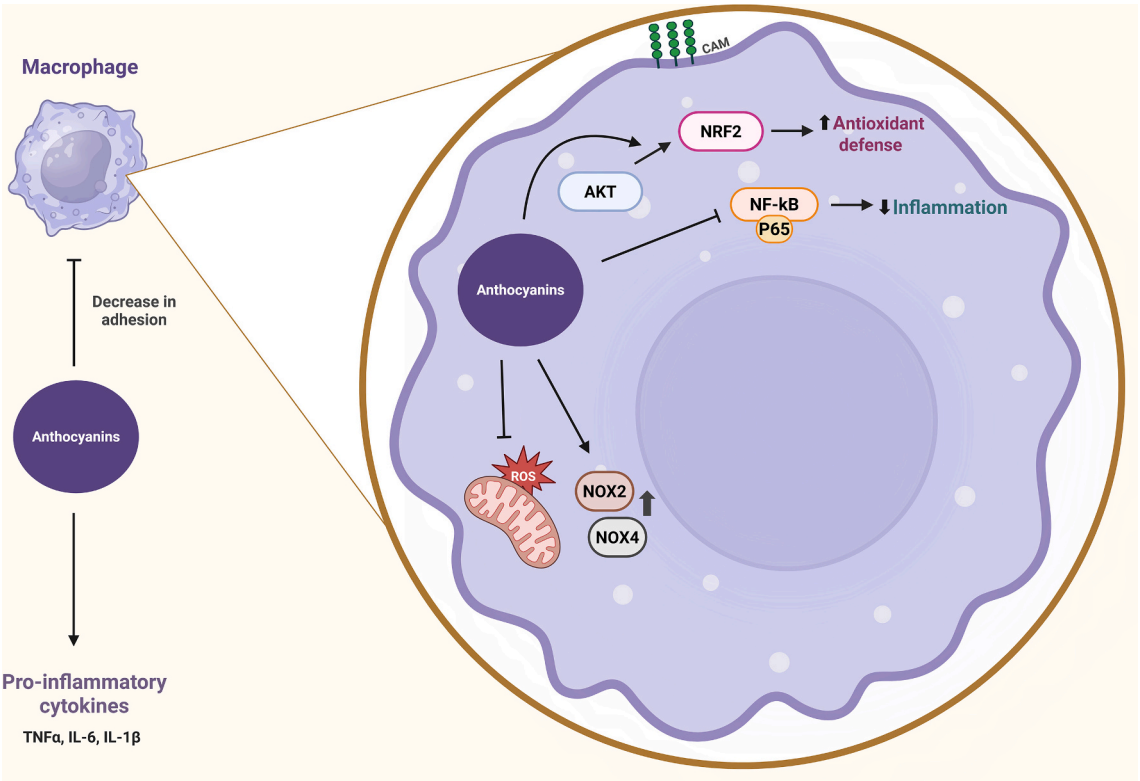

Fig. 4. Anthocyanin-Mediated modulation of inflammatory and oxidative pathways in macrophages.
Fig. 4. Molecular mechanisms involved in the anti-inflammatory activity of anthocyanins in macrophages. Anthocyanins reduce cell adhesion and the expression of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β). Intracellularly, these compounds inhibit mitochondrial ROS production and the expression of NADPH oxidases NOX2 and NOX4. In addition, they activate the AKT/NF2 signaling pathway, enhancing antioxidant defense and inhibiting the activation of the transcription factor NF-κB (p65), ultimately leading to a reduced inflammatory response.

IL-1β (−3.73 pg/mL *vs.* −1.33 pg/mL for placebo), interleukin-18 (IL-18) (−36.4 pg/mL *vs.* +6.9 pg/mL), and IL-6 (−2.83 pg/mL *vs.* +4.93 pg/mL), whereas changes in TNF-α were not statistically significant (−3.81 pg/mL *vs.* +1.38 pg/mL). Furthermore, anthocyanin supplementation significantly downregulated mRNA expression of caspase-1, IL-1β, and IL-18 in peripheral blood mononuclear cells (PBMCs), while mRNA levels of NOD-like receptor family, pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), TNF-α, and IL-6 remained unaltered (Zhu et al., 2021).

Moreover, administration of capsules containing extracts of 17 different anthocyanins from wild blueberry (*Vaccinium myrtillus*) and black currant (*Ribes nigrum*) at doses ranging from 80 to 320 mg/day reduced serum IL-6 by 20–40 % and TNF-α by 11–21 % in individuals with dyslipidemia over 12 weeks, with dose-dependent effects (Zhang et al., 2020).

In conclusion, anthocyanins have the potential to attenuate inflammation and can therefore be considered as promising dietary components in the prevention and control of inflammatory diseases.

4.3. Antitumor activity

Cancer is a disease characterized by the abnormal growth and development of cells in the body, which can give rise to cell masses called tumors, leading to functional impairments in tissues or organs. According to the World Health Organization (2019), cancer is one of the leading causes of death worldwide, causing 9.6 million deaths in 2018. According to the Pan American Health Organization, 40 % of cases could be avoided by controlling risk factors, and 30 % could be addressed with early diagnosis and treatment (Pan American Health Organization, 2020b).

Data from 2022 show that the most prevalent types of cancer are led by lung cancer, followed by breast cancer and colorectal cancer, with colorectal cancer being the second leading cause of death worldwide (International Agency for Research on Cancer (IARC), 2022; Pan American Health Organization, 2024). In 2022 alone, there were over 20 million new cancer cases globally, and this number is expected to increase to more than 35 million by 2050, representing a 77 % rise (Pan American Health Organization, 2024). One of the main risk factors is lifestyle, such as an unhealthy diet rich in saturated fats, food additives, and refined sugars (Pan American Health Organization, 2020b; World Health Organization, 2020). Therefore, research has focused on studies (Anandan et al., 2022; Bowen-Forbes et al., 2010; George & Abrahamse, 2019; Nova et al., 2023; Sousa & Conte-Junior, 2022) exploring food components associated with the prevention and treatment of NCDs, such as cancer (World Health Organization, 2020).

4.3.1. PI3K/AKT/mTOR

Recent clinical studies address the relationship between the consumption of fruits and vegetables rich in anthocyanins and the reduction of cancer risk, especially colorectal and breast cancer (Bars-Cortina, Sakhawat, Piñol-Felis, & Motilva, 2021). In triple-negative breast cancer (TNBC) cells, anthocyanins were shown to modulate multiple cancer-related processes, including apoptosis—through significant increases in cleaved caspase-3, cleaved caspase-8, and poly(ADP-ribose) polymerase (PARP)—and inhibition of cell proliferation, primarily *via* downregulation of the protein kinase B (Akt)/mechanistic target of rapamycin (mTOR) pathway. They also affected angiogenesis (Rabelo et al., 2023).

Similar results were found with anthocyanins (delphinidin-3,5-diglucoside, petunidin-3,5-diglucoside, delphinidin-3-O-glucoside, malvidin-3,5-diglucoside, petunidin-3-O-glucoside, and malvidin-3-O-glucoside) at 500 μg/mL, isolated from black bean (*Phaseolus vulgaris*) hulls, in lung adenocarcinoma (A549), mouse glioma (GL261) and rat glioma (C6) cells in which cell viability of less than 10 % was observed, demonstrating the antitumor capacity of anthocyanins. In particular, delphinidin, the main anthocyanin in the extract, exerts an antiangiogenic effect by inhibiting the expression of hypoxia-inducible factor 1 alpha (HIF-1α) through the blockade of extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)/Protein kinase B (Akt)/Mechanistic target of rapamycin (mTOR)/70-kDa ribosomal protein S6 kinase (p70S6K) signaling pathways, a mechanism that restricts neovascularization essential for tumor growth and progression (Kuasnei et al., 2025).

An *in vitro* study evaluated the antitumor activity of purified delphinidin in human colon carcinoma HCT116 cells, using normal HGF-1 fibroblasts as controls. Delphinidin selectively reduced HCT116 viability in a dose-dependent manner (IC_{50} 106 μM), while exerting no cytotoxic effects on HGF-1 cells at concentrations up to 100 μM. The compound disrupted mitochondrial membrane potential, triggering apoptosis characterized by upregulation of pro-apoptotic proteins (Bax, caspase-3, caspase-8, caspase-9, cytochrome *c*) and downregulation of anti-apoptotic proteins (Bcl-2, Bcl-XL). Mechanistically, delphinidin suppressed signal transducer and activator of transcription 3 (STAT3) phosphorylation and inhibited mitogen-activated protein kinase (MAPK) signaling specifically — p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinases 1 and 2 (ERK1/2)—, indicating that blockade of the janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) axis—alongside MAPK inhibition—plays a central role in its pro-apoptotic effects in colon cancer cells (Zhang, Yang, & Ericsson, 2021).

4.3.2. Human studies

A randomized, double-blind clinical trial was conducted with 35 individuals with adenomatous polyps. One group received a placebo, while the others received treatments with curcumin and associated anthocyanin, 1 g of each bioactive compound, for six weeks. Inflammatory and metabolic biomarkers such as adiponectin, blood glucose, and body mass index (BMI), which are related to the progression of colorectal cancer, were evaluated. Reduction in adiponectin, increased blood glucose, and BMI have been associated with a higher risk of colorectal cancer development. (Macis et al., 2023). Although the study used the combination of the two compounds, it is relevant to mention the trend of improvement in metabolic biomarkers related to colorectal cancer risk, indicating the potential contributory role of anthocyanins in this context.

4.3.3. FAK pathway and invasion

Another randomized, double-blind clinical trial involving 35 healthy individuals evaluated the effect of consuming fruit juice rich in anthocyanins (Mostafa et al., 2023). After ingesting the juice, the researchers analyzed the metabolites of anthocyanins present in the participants' blood plasma. These metabolites were then used in *in vitro* studies to evaluate their effects, particularly on pancreatic cancer cells such as PANC-1 cells. Notably, exploratory metabolomic analysis identified o-coumaric acid and peonidin-3-galactoside as specific metabolites inversely associated with PANC-1 cell migration, an effect attributed to the reduced phosphorylation of NF-κB and focal adhesion kinase (FAK), thereby highlighting their potential contribution to the anti-cancer effects observed.

4.3.4. In vitro cytotoxicity

Following the *in vitro* model, a similar study was conducted with 3 g of raw black bean powder extract containing approximately 35.8 % cyanidin-3-O-glucoside (C3G) in a human hepatoma cell line (HepG2) (Zhang et al., 2024). The authors showed that acylated anthocyanins significantly inhibited HepG2 cells in a dose-dependent manner. Furthermore, acylated anthocyanins showed low toxicity to normal liver cells, indicating potential selectivity to inhibit cancer cell growth without affecting healthy cells.

In one study, anthocyanin-rich pomegranate (*Punica granatum* L.) extract encapsulated in sphingosomes was used at concentrations ranging from 31.25 to 1000 μg/mL and its cytotoxic effect was analyzed

on three cell lines: breast adenocarcinoma (MCF-7), cervical carcinoma (HeLa), and colorectal carcinoma (HCT116) (AlMadalli et al., 2024). The authors observed a dose-dependent inhibition of cell growth in all cell lines tested, with over 90 % inhibition of the cell lines when the concentration reached 1000 μg/mL.

In general, different anthocyanins have demonstrated the ability to inhibit abnormal cell growth, induce apoptosis, and suppress invasion and metastasis in cellular and animal models, with some preliminary evidence in humans (Sood et al., 2024). The mechanism by which these compounds exert the antitumor effects includes 1) interfering with multiple cellular signaling pathways, such as the activation of the Adenosine monophosphate-activated protein kinase (AMPK) pathway, 2) modulation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) pathway, 3) Inhibition of STAT (Signal Transducer and Activator of Transcription) and STAT3, consequently inhibiting the JAK-STAT pathway (Janus Kinase–Signal Transducer and Activator of Transcription), thereby providing a comprehensive approach to colon and breast cancer prevention and treatment (Bars-Cortina, Sakhawat, Piñol-Felis, & Motilva, 2021). Furthermore, due to the anti-inflammatory and antioxidant effects of anthocyanins, these flavonoids have high potential in preventing diseases such as cancer by preventing cell DNA damage.

5. Methodologies used for screening the biological effects of anthocyanins

5.1. Antioxidant effect

The methodologies commonly used to analyze antioxidant activity *in vitro* are DPPH (1,1-diphenyl-2-picrylhydrazyl), FRAP (Ferric reducing antioxidant power), and ABTS [2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]. These assays typically involve measuring absorbance using a spectrophotometer (Oliveira, 2015).

It is important to emphasize that the results obtained from antioxidant assays such as DPPH, FRAP, and ABTS are not directly comparable, as each method relies on distinct chemical mechanisms. For instance, while DPPH and ABTS assays may involve both hydrogen atom transfer (HAT) and single electron transfer (SET) reactions, the FRAP assay is strictly based on a SET mechanism. These mechanistic differences mean that a compound may exhibit strong activity in one assay and weak activity in another, depending on its chemical structure, polarity, and reaction environment (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016a,b; Kedare & Singh, 2011; Rumpf, Burger and Schulze, 2023).

DPPH is a stable, purple free radical. This assay may involve three distinct mechanisms: HAT (hydrogen atom transfer), SET (sequential proton loss electron transfer) (Abramovič et al., 2018; Rumpf, Burger and Schulze, 2023; Sirivibulkovit et al., 2018). When an antioxidant compound is added to a DPPH solution, it can donate an electron or hydrogen atom to the radical, promoting its reduction to a colorless or light yellow molecule called diphenylpicrylhydrazine (DPPH-H). The decrease in absorbance of the solution, measured at a wavelength of 515–520 nm, is directly proportional to the radical scavenging capacity of the sample (Abramovič et al., 2018). Results are typically expressed as a percentage of inhibition or as IC_{50} values (concentration required to reduce the initial DPPH concentration by 50 %) using standards such as ascorbic acid, gallic acid, vanillic acid, quercetin, caffeic acid or Trolox (Brand-Williams et al., 1995; Sirivibulkovit et al., 2018).

The DPPH assay is widely used to evaluate the antioxidant activity of plant extracts and food samples (Gülçin & Alwasel, 2023; Sirivibulkovit et al., 2018). It is a simple, rapid, and low-cost method that allows the simultaneous analysis of approximately 20 samples, making it highly practical (Sirivibulkovit et al., 2018). Furthermore, it does not require heating, which is advantageous for thermosensitive compounds, helping to preserve their stability (Kedare & Singh, 2011).

However, several experimental factors can influence the results obtained, such as the type of solvent used, the pH of the solution, and the incubation time (Abramovič et al., 2018). Methanol is the most commonly employed solvent in this assay; however, its strong interaction with hydrogen atoms may hinder hydrogen atom transfer (HAT) mechanisms. The addition of water to the system can promote these mechanisms, thereby enhancing the observed antioxidant activity (Gülçin & Alwasel, 2023). Other solvents, such as acetone, ethyl acetate, hexane, and dichloromethane, may also be used depending on the polarity of the compounds being analyzed (Wołosiak et al., 2021).

The pH of the assay solution also exerts a significant influence (Gülçin & Alwasel, 2023). In alkaline environments, greater efficiency is observed in electron donation mechanisms (SET) compared to methanolic solutions. Nevertheless, pH control of the solution is not commonly addressed in studies, which may lead to overestimation or underestimation of the results, as well as hinder comparisons across different studies (Abramovič et al., 2018; Apak, Özyürek, Güçlü, & Çapanoğlu, 2016a,b).

Although many studies adopt 30 min as the standard reaction time, shorter or longer durations (such as 60 min) have also been reported (Kedare & Singh, 2011; Sirivibulkovit et al., 2018; Wołosiak et al., 2021). This variation can directly affect the results, as some compounds exhibit better linearity between absorbance and concentration with longer or shorter reaction times (Wołosiak et al., 2021). However, prolonged incubation may lead to secondary reactions, such as the degradation of previously oxidized antioxidants, thereby compromising the accuracy of the assay (Abramovič et al., 2018).

The Ferric reducing antioxidant power (FRAP) assay, measures the capacity antioxidants by the reduction reaction of the ferric-tripyridyltriazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+}), under acidic conditions (pH 3,6) (Apak et al., 2016b; Rumpf, Burger and Schulze, 2023). Furthermore, the assay requires incubation at 37 °C, a temperature still considered stable for anthocyanins (Oancea, 2021). The reaction results in the formation of an intense blue complex with maximum absorbance at 593 nm. The antioxidant potential of the sample is determined by comparing the reducing capacity of the sample with a standard curve prepared with Trolox, ascorbic acid or gallic acid. The results are expressed as μmol equivalents of the reference substance per gram of sample or can be presented as a graph comparing the curves of the sample and the reference substance. This assay is simple and inexpensive; however, it requires rigor regarding the incubation time of the solutions, as this factor can affect the results. In addition, the assay measures reducing capacity (SET mechanism), which is different from free radical scavenging (HAT mechanism) (Benzie & Strain, 1996; Munteanu & Apetrei, 2021; Rumpf, Burger and Schulze, 2023).

Finally, the ABTS or Trolox Equivalent Antioxidant Capacity (TEAC) test is based on the interaction of antioxidant substances with the cationic ABTS radical. The method works by reducing the ABTS− + radical, which has an intense blue-green color, and when an antioxidant substance is added to the solution, it donates electrons to the radical, reducing it to its nearly colorless neutral form (ABTS). As with the FRAP test, the result can be expressed in TEAC units. This reduction is directly proportional to the antioxidant capacity of the test substance and is quantified by measuring the decrease in absorbance at 734 nm. Furthermore, this assay also shows good correlation with the presence of bioactive compounds, such as phenolics and flavonoids, reinforcing its applicability in food and vegetable matrices (Sadeer et al., 2020). In addition, it is a simple and inexpensive method, classified as mixed (HAT/SET), but occurs mainly through the SET mechanism, and can be applied to both hydrophilic and lipophilic compounds. However, care must be taken when using the method with colored samples, as these can interfere with the color of the solution and affect the absorbance (Munteanu & Apetrei, 2021; Re et al., 1999; Rumpf, Burger and Schulze, 2023).

Due to the peculiarities of each of these methods, it is necessary to use more than one method in combination to analyze the antioxidant activity of compounds. Furthermore, they all play a fundamental role in the characterization of the antioxidant potential of substances and are

essential tools for research, in addition to being simple and inexpensive.

5.2. Anti-inflammatory effect

Clinical trials assessing the anti-inflammatory activity of flavonoids, such as anthocyanins, use methods to evaluate both the production of enzymes such as iNOS, which is responsible for NO synthesis, and cytokine levels. Studies have also shown that anthocyanins inhibit the activation of NF-κB, a key regulator of macrophage activation and cytokine production (Chen et al., 2023; Dorrington & Fraser, 2019; Liu et al., 2017).

Western blotting and enzyme-linked immunosorbent assay (ELISA) techniques are widely used to evaluate the modulation of inflammatory pathways after cell stimulation with LPS. Western blotting is an assay primarily used in studies investigating pathways related to inflammation, oxidative stress, and tumorigenesis. This method enables the detection, quantification, and characterization of specific proteins in complex biological samples and is particularly valuable for analyzing the expression and post-translational modifications (PTMs) of signaling molecules such as NF-κB, IκB, and MAPK (Bass et al., 2016; Kang et al., 2016; Mishra et al., 2017; Pillai-Kastoori, Schutz-Geschwender, & Harford, 2020). Western blotting can be used to detect phosphorylation of NF-κB inhibitor (IκB - Inhibitor of kappa B and nuclear translocation of the p65 subunit by analyzing cytoplasmic and nuclear protein fractions, particularly in the context of anti-inflammatory studies. This assay is distinguished by its versatility and specificity, stemming from the selective interaction between antibodies and antigens (Bass et al., 2016; Pillai-Kastoori, Schutz-Geschwender, & Harford, 2020). However, the technique also presents limitations: it is labor-intensive, susceptible to variability across experimental steps, and highly dependent on the quality of both the antibodies and the sample used. Issues such as limited reproducibility, signal saturation, inadequate reference proteins, challenges in transferring or detecting high-molecular-weight proteins, and the semi-quantitative nature of data interpretation can compromise the reliability of results. Therefore, the use of total protein staining as a loading control is recommended, as it offers a more robust alternative to traditional housekeeping proteins (Bass et al., 2016; Mishra et al., 2017; Pillai-Kastoori, Schutz-Geschwender, & Harford, 2020).

ELISA is one of the most widely used and well-validated methodologies for the quantification of cytokines and other inflammatory mediators in both clinical and experimental studies. This assay has become the gold standard for the measurement of individual cytokines due to its high specificity, sensitivity, and reproducibility. In its most common format, the sandwich ELISA employs two antibodies: a capture antibody, immobilized on the plate, and a detection antibody, conjugated to an enzyme. This dual binding interaction allows for the accurate detection of targets such as IL-6, TNF-α, IL-1β, and monocyte chemoattractant protein-1 (MCP-1), which are frequently modulated by anthocyanins in inflammation models (Kozłowska & Dzierżanowski, 2021; Leng et al., 2008).

ELISA allows the specific and sensitive identification and quantification of pro- and anti-inflammatory cytokines using pre-selected antibodies that specifically bind to the analytes of interest (Bartosh & Ylostalo, 2014; Chiswick et al., 2011; Förstermann, 2010; Liu et al., 2017; Maguire, O'Loughlin, & Minderman, 2015; Marino & Idris, 2019).

Because it is limited to the analysis of a single analyte per well, the ELISA assay requires larger sample volumes and becomes costly when multiple biomarkers are analyzed simultaneously. Additionally, the quality of the results is directly dependent on the specificity of the antibodies used and the technical proficiency of the operator. Variability among manufacturers, sensitivity to circulating carrier proteins, and a relatively narrow dynamic range can further affect the interpretation of cytokine levels, particularly at extreme concentrations. Although multiplex methods offer broader analytical coverage, ELISA remains superior in terms of robustness for individual cytokine quantification. As such, it is an indispensable tool for assessing the anti-inflammatory potential of compounds such as anthocyanins, especially when precise quantification of specific cytokine modulation is required (Kozłowska & Dzierżanowski, 2021; Leng et al., 2008).

Target cytokines for research include TNF-α, IL-1β, and IL-6, which are essential mediators of the inflammatory response and are mainly produced by macrophages. In addition, the production of the chemokine MCP-1 is also commonly analyzed (Bent et al., 2018; Chen et al., 2017; Fujiwara & Kobayashi, 2005; Singh et al., 2021; Tanaka et al., 2014; Zelová & Hošek, 2013).

These methods provide a concise framework for analyzing the anti-inflammatory potential of anthocyanins. By modulating key signaling pathways, such as NF-κB, and regulating the production of enzymes and cytokines critical to the inflammatory response, anthocyanins show promising therapeutic effects. The integration of protein analysis, gene expression, and cytokine quantification ensures a comprehensive understanding of the molecular mechanisms by which these bioactive compounds exert their immunomodulatory activities.

5.3. Antitumoral effect

In vitro analyses of the antitumor activity of anthocyanins include a variety of established methods. Cell viability assays, such as the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and the MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]) assay, evaluate cellular metabolic activity as an indirect marker of cell viability (Riss et al., 2016). The MTT assay is based on the ability of metabolically active cells to reduce the yellow tetrazolium salt (MTT), which is water-soluble, into insoluble violet formazan crystals. This reaction is mediated by mitochondrial and cytosolic enzymes, such as dehydrogenases and oxidoreductases, which use redox cofactors like NAD(*P*)H. MTT crosses the cell and mitochondrial membranes and is internalized by viable cells. The amount of formazan produced is quantified by spectrophotometry, usually at 570 nm, indirectly reflecting cell viability or metabolic activity (Ghasemi et al., 2021).

This assay is widely used to assess the cytotoxicity and antiproliferative effects of bioactive compounds, such as anthocyanins, being useful for calculating the half-maximal inhibitory concentration (IC_{50}) and in studies of antitumor activity and compound safety (Ghasemi et al., 2021; Rampersad, 2012; Stepanenko & Dmitrenko, 2015). Its popularity stems from its simplicity, low cost, good reproducibility, and the ability of MTT to penetrate cells without additional reagents, unlike other tetrazolium salts such as MTS, XTT, and WST-1 (Ghasemi et al., 2021).

Nevertheless, MTT measures metabolic activity, not cell viability directly. Cells undergoing programmed cell death may still reduce MTT, leading to an overestimation of viability. Factors such as cell density, incubation time, reagent concentration, and the accumulation or extrusion of formazan crystals can also influence the results and compromise analysis, especially if supernatant washing is performed improperly. Still, the MTT assay remains a robust tool for studying compounds like anthocyanins, provided its limitations are properly managed (Ghasemi et al., 2021).

The use of complementary methods is recommended for a more accurate assessment of the cellular effects of these phenolic compounds (Stepanenko & Dmitrenko, 2015).

Alternatively, the Alamar Blue assay, which is based on the reduction of resazurin to resorufin by metabolically active cells, is widely used to assess cell viability, metabolic activity and cytotoxicity in different cell types, is widely used due to its high sensitivity and low toxicity (Rampersad, 2012). The principle of this assay lies in the reduction of resazurin, a blue dye, into resorufin, a pink-colored compound, by metabolically active cells. This conversion occurs predominantly through the action of mitochondrial enzymes involved in cellular metabolism, although more recent evidence indicates that cytosolic and microsomal enzymes also contribute to this process (Nakayama et al.,

1997; Rampersad, 2012). The intensity of the resulting fluorescence or absorbance is directly proportional to cellular metabolic activity, allowing for the indirect quantification of viable cell numbers (Al-Nasiry et al., 2007; Rampersad, 2012).

As a non-destructive assay, Alamar Blue enables kinetic monitoring of cell cultures over time, setting it apart from terminal methodologies such as the MTT assay or trypan blue exclusion. Moreover, it stands out for its ease of execution, low cost, and high sensitivity. However, the reduction of resazurin may not exclusively reflect mitochondrial function, as it can also occur in non-proliferative cells or cells in early stages of cell death, potentially compromising data interpretation, as similarly observed with the MTT assay (Al-Nasiry et al., 2007; Nakayama et al., 1997; Rampersad, 2012). In this context, additional concerns arise when plant-derived compounds such as anthocyanins are tested, since these flavonoids can directly reduce tetrazolium salts or interfere with absorbance/fluorescence, producing false-positive results. To minimize such biases, studies recommend the use of no-cell compound-only blanks, solvent controls, absorbance correction, and cross-validation with orthogonal non-metabolic assays such as the sulforhodamine B (SRB) assay, trypan blue exclusion, or ATP-based assays (Bruggisser et al., 2002; Karakas et al., 2017; Somayaji & Shastry, 2021).

Apoptosis assays, including annexin V/propidium iodide (PI) staining coupled with flow cytometry, and caspase-3 and caspase-9 activation assays are used to differentiate apoptotic cells from necrotic cells (Kumar et al., 2021; Robinson et al., 2023; Telford, 2018). One of the most established methods for distinguishing apoptosis from necrosis involves the combination of annexin V and propidium iodide (PI). Annexin V is a protein with high affinity for phosphatidylserine, a phospholipid normally located on the inner leaflet of the plasma membrane. During the early stages of apoptosis, phosphatidylserine is translocated to the outer surface of the membrane, becoming accessible for annexin V binding. Conversely, PI is a DNA-binding dye that can only penetrate cells with compromised membranes, such as necrotic cells or cells in late-stage apoptosis. This dual staining assay is sensitive and enables quantitative data acquisition, making it applicable across various cell lines (Robinson et al., 2023).

Although these methods provide robust discrimination based on membrane integrity and metabolic function, they do not directly assess the biochemical pathways involved in programmed cell death. Therefore, complementary assays detecting the activation of caspases, particularly caspase-3 and caspase-9, can be employed to confirm the underlying mechanisms. These enzymes are key executioners of the intrinsic apoptotic pathway and serve as reliable biochemical markers of programmed cell death. It is important to emphasize that factors such as dye selection, fixation effects, and spectral compatibility must be carefully considered, as they can affect the accuracy and interpretation of data obtained by flow cytometry (Robinson et al., 2023).

In addition, molecular techniques such as quantitative polymerase chain reaction (qPCR), Western blotting, and ELISA are often used to investigate changes in gene and protein expression associated with apoptosis, cell cycle regulation, and inflammatory responses (Kari et al., 2022). qPCR provides a sensitive and precise method for quantifying specific mRNA transcripts, thereby indicating changes in gene expression levels (Sanders et al., 2014). Western blotting allows for the qualitative and semi-quantitative detection of specific proteins by separating them by size, transferring them to a membrane, and then probing with specific antibodies. This technique is crucial for assessing protein abundance, post-translational modifications like phosphorylation, and the cleavage of proteins involved in pathways such as apoptosis (*e.g.*, caspases) or cell cycle regulation (*e.g.*, cyclins) (Mishra et al., 2023). ELISA is a highly sensitive and specific immunoassay used for the identification and quantification of various analytes, including cytokines and other secreted proteins, in biological samples (Leng et al., 2008). To further characterize cellular responses, especially in the context of cancer cell behavior, intracellular ROS assays are used to assess the oxidative stress status of cells. These assays often utilize fluorescent probes that react with ROS, allowing for the measurement of their levels, as modulation of ROS is closely related to survival and apoptotic processes in cancer cells (Wu et al., 2019). Colony formation assays are used to determine the long-term antiproliferative effects of anthocyanin treatments. These assays assess the ability of single tumor cells to proliferate indefinitely and form macroscopic colonies, providing a direct measure of their reproductive viability and survival after treatment (Anwar et al., 2016).

Furthermore, to evaluate the metastatic potential of cancer cells, wound healing assays (scratch assays) and cell migration assays are important. A wound healing assay is a straightforward and cost-effective method to measure collective cell migration *in vitro*. It involves creating a "scratch" in a confluent cell monolayer, and then monitoring the cells' ability to migrate into and close this gap over time. This technique is particularly useful for assessing the capacity of cells to move into an empty space, mimicking aspects of tissue repair and tumor invasion (Pinto et al., 2016; Rodriguez et al., 2005). Cell migration assays, such as Transwell assay, provide a more quantitative assessment of individual cell migration. These assays typically involve cells migrating through a porous membrane towards a chemoattractant, allowing for the quantification of migrated cells and providing insights into directed cell movement, a critical step in metastasis (Justus et al., 2023).

In addition, the sulforhodamine B (SRB) assay, a colorimetric method that quantifies the total protein content of cells, provides a reliable assessment of long-term cell proliferation and cytotoxicity in response to anthocyanins (Anwar et al., 2016; Orellana & Kasinski, 2016). Together, these complementary methods provide a comprehensive assessment of the antitumor properties of anthocyanins and contribute to the elucidation of their therapeutic potential and mechanisms of action.

6. Challenges for industrial application

Anthocyanins exhibit promising bioactive effects; however, their industrial application remains limited due to chemical instability, low bioavailability, and degradation that can generate undesirable flavors and odors. Moreover, conventional extraction techniques often result in low yields and require long, costly processes, whereas advanced methods, although more efficient, may further increase costs (Anusha Siddiqui et al., 2022; Zannou et al., 2023).

Strategies such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) stand out for their speed, selectivity, lower solvent consumption, and better preservation of functional properties. These methods are capable of disrupting cellular structures and releasing anthocyanins such as cyanidin-3-O-glucoside (C3G), pelargonidin-3-glucoside, and peonidin-3-glucoside (Zannou et al., 2023; Zahed et al., 2023; Anusha Siddiqui et al., 2022). More advanced techniques, including supercritical CO_2 extraction (SFE-CO_2) and pressurized fluid extraction (PFE), combine rapidity, selectivity, and the use of eco-friendly solvents, providing higher yields than traditional methods, although in some cases their efficiency may be lower than that of UAE and MAE in terms of yield and processing time (Anusha Siddiqui et al., 2022; Zannou et al., 2023).

Furthermore, nanoencapsulation has emerged as a promising tool to overcome limitations related to instability and low bioavailability. It offers protection against light, oxygen, pH, temperature, and humidity, while enabling controlled and targeted release of anthocyanins (Shishir et al., 2018; Zannou et al., 2023). Various nanocarriers have been explored, including proteins (casein, ferritin nanocages, WPI, albumin), polysaccharides (pectin, chitosan, β-cyclodextrin), lipids (liposomes, niosomes, micelles), and exosomes (Shen et al., 2022; Zannou et al., 2023). These structures enhance chemical and thermal stability, promote more efficient absorption and tissue distribution, prolong gastrointestinal half-life, and in some cases, potentiate biological effects. Among them, pectin- and lysozyme-based nanostructures and lipid-based systems demonstrate greater consistency in terms of protection,

bioavailability, and tissue distribution, while polysaccharides such as chitosan allow for controlled and targeted release, particularly in the colon (Rosales et al., 2024; Shen et al., 2022; Zannou et al., 2023). Collectively, these approaches represent promising solutions for optimizing the use of anthocyanins in therapeutic and food applications, overcoming limitations of stability, absorption, and yield.

7. Conclusion, limitations and future perspectives

In conclusion, anthocyanins are secondary metabolites found in various fruits, vegetables, flowers, and seeds of plants, renowned for their antioxidant, anti-inflammatory, and antitumor properties. These compounds play a crucial role in mitigating oxidative stress, modulating inflammatory pathways, and inhibiting tumor growth by scavenging free radicals, influencing cellular signaling, and regulating gene expression associated with inflammation and carcinogenesis.

Despite these beneficial effects, several challenges hinder the broader application of anthocyanins, particularly in industrial contexts. One of the main obstacles is their low chemical stability. Anthocyanins are unstable compounds that are highly susceptible to degradation when exposed to factors such as high temperatures, alkaline pH, oxygen, light, and enzymatic activity (Cheng et al., 2023). These variables significantly affect the quality, stability, and biological effect of natural anthocyanin extracts, limiting their effectiveness in scientific and industrial applications.

Another critical challenge lies in their limited bioavailability. Once ingested, anthocyanins face degradation in the acidic environment of the stomach and show restricted absorption in the small intestine, although this may be partially improved by enzymatic action from the intestinal microbiota (Kumkum et al., 2024; Shen et al., 2022). Additionally, the extraction of anthocyanins is often inefficient due to the complexity of the plant matrix, and improper extraction techniques can accelerate degradation even further.

In addition to these challenges, many of the primary sources of anthocyanins—such as berries and other fruits—are also important components of the human diet. Because these natural sources are in high demand for food consumption, their use for industrial anthocyanin extraction is limited, which contributes to higher production costs and increased market competition. Consequently, there is growing interest in exploring alternative sources, such as certain forage grasses (*e.g.*, purple elephant grass), which may provide a more sustainable and cost-effective option for large-scale anthocyanin production.

To address the issue of anthocyanin bioavailability, often limited by their susceptibility to degradation and poor absorption, various technological strategies are under development. These include controlled-release systems, which regulate the gradual delivery of anthocyanins over time to maintain sustained levels; nanoparticles, which, with their nanoscale dimensions, offer improved solubility, protection against degradation, and enhanced cellular uptake due to their small size and diverse material composition; and encapsulation methods, utilizing carriers like liposomes (phospholipid vesicles that protect and improve cellular delivery by mimicking cell membranes) or biopolymers (natural polymers like chitosan or alginate that form protective matrices), are employed to shield anthocyanins from adverse conditions and enhance their intestinal absorption. These diverse systems differ primarily in their structural composition, release kinetics, and specific mechanisms for improving stability and delivery. Additionally, the combination of anthocyanins with other bioactive compounds or the design of synergistic formulations is being explored to optimize their functional potential (Kumkum et al., 2024; Shen et al., 2022).

Understanding the precise mechanisms of action of anthocyanins remains a critical area of investigation. Continued research is essential to fully elucidate their antioxidant, anti-inflammatory, and antitumor effects, thus paving the way for the development of innovative therapeutic strategies. Future studies should focus on determining optimal dosages, administration routes, and target populations to maximize the preventive and adjuvant roles of anthocyanins in combating non-communicable diseases.

CRediT authorship contribution statement

Érika de Fátima dos Santos: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Yasmin Neves Vieira Sabino:** Writing – review & editing, Supervision, Conceptualization. **Elisa Nassif Montenegro:** Writing – original draft, Conceptualization. **Cinthia Alvim Faria:** Visualization, Conceptualization. **Thaís Costa de Almeida:** Writing – review & editing. **Jacy Gameiro:** Writing – review & editing, Supervision, Conceptualization. **Sheila Cristina Potente Dutra Luquetti:** Writing – review & editing, Supervision, Conceptualization. **Alessandra Barbosa Ferreira Machado:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization. **Juarez Campolina Machado:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Ethics statement

This manuscript is a narrative review that synthesizes and discusses data from previously published studies. As it is not original research with human participants, animals or personal data, it has not been submitted for ethics committee approval. All the sources included in this review are duly cited and come from scientific literature and have been reviewed by all the contributors to this review.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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