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Effects of anthocyanins as nutraceuticals

KEYWORDS: anthocyanins, nutraceutical, neuro-protective, cardio-vascular protective, anti-inflammatory, hepatic health

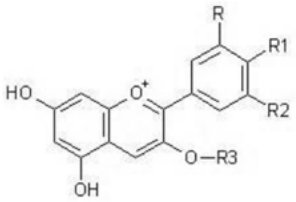
Abstract Anthocyanins are bioactive compounds with strong nutraceutical potential. They are a widely distributed class of flavonoids and can be defined as glycosides of anthocyanidins. Anthocyanins are usually present as pigmented compounds in fruits and vegetables including cherries, plums, strawberries, raspberries, blackberries, grapes, redcurrants, blackcurrants, vegetable roots, legumes and cereals. There are various reports about the biological properties and nutraceutical potential of anthocyanins. This review discusses some recent reports on the neuro-protective effects, cardio-vascular benefits, liver health improvement and anti-inflammatory effects associated with the consumption of anthocyanins compounds, either in pure or complex forms.

INTRODUCTION

It is a well established fact that diet has direct relationship with the health and most of the health promoting effects is associated with different kinds of nutrients and bioactive compounds present in fruits and vegetables. The magnitude of different types of chronic diseases that may be related to age including cardiovascular problems, neurological disorders, diabetes, and cancers is increasing. These facts lead the researchers and health practitioners to explore and recommend use of plant derived health promoting foods. The ability of plant derived products in reducing or preventing chronic health diseases is linked mostly to their non-nutrient secondary metabolites or phytochemicals which have been studied to exert wide range of actions in living organisms. The phytochemicals may not be as quick in action as the synthetic pharmaceuticals however a long term use can significantly improve the health of consumers. These include sulphur containing compounds, terpenoids (carotenoids, monoterpenes, and phytosterols), and different polyphenolic groups (anthocyanins, flavones, flavan-3-ols, isoflavones, stilbenoids, ellagic acid, etc.) (1). Consumers these days look for products in the market that contain bioactive compounds or extracts derived from plant or natural sources. Such products have close affiliation with the pharmaceuticals and hence cannot be classified simply as 'food' instead a new term that carries impression of being nutrients as well as pharmaceuticals, 'nutraceuticals', has been used for the identification of this class of product.
Nutraceuticals can be referred as supplements to normal diet and carry a bioactive agent derived or originated from a food, and usually contained in a non-food matrix. The purpose is to deliver certain kinds of bioactive compounds in quantities much higher than that obtained in regular food material. The objective of this review is to focus on up to date available information on anthocyanins from plant sources with relation to their biological effects and applications as nutraceuticals. Anthocyanins have been studied to possess different biological functions such as antioxidants, anti-inflammatory, antimicrobial and anti-carcinogenic activities. They have also been associated with vision enhancement, induction of apoptosis and neuro-protective effects (2). The objective of this review was to collect most resent information on structure, sources and health benefits imparted by anthocyanins.

STRUCTURE OF ANTHOCYANINS

Chemically anthocyanins are glycosides of anthocyanidins and usually present in pigmented compounds in fruits and vegetables. There are complex glycosylation patterns which result in many type of anthocyanins compounds however only some of the aglycones (anthocyanidins) types have been characterized. Anthocyanins are a widely distributed class of flavonoid compounds that are water-soluble and nontoxic pigments and being studied extensively because of their biological or antioxidant properties (3). The most common anthocyanins are based on six anthocyanidins that include cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin (4) and their structures are presented in Figure 1.



	R1	R2	R3
Peonidin	$-OCH_3$	-H	-OH
Malvidin	$-OCH_3$	$-OCH_3$	-OH
Petunidin	-OH	$-OCH_3$	-OH
Delphinidin	-OH	-OH	-OH
Luteolinidin	-OH	-H	-H
Cyanidin	-OH	-H	-OH
Pelargonidin	-H	-H	-OH

Figure 1. Structure of anthocyanins commonly obtained from different plant sources

The position and amount of hydroxylation and methoxylation in the B ring can be regarded as crucial for biological properties of anthocyanins (3). Delphinidin that contained three hydroxylations in the B ring possessed a higher antioxidant activity whereas the pelargonin had the lowest antioxidant activity among six common anthocyanidins. The pattern of glycosylation in anthocyanins can also affect their biological properties (5).

SOURCES OF ANTHOCYANINS

There are different plant sources of anthocyanins and a summarized list of some of these sources and food products based on such sources is presented in Table 1 along with approximate proportion of anthocyanins in each case. Almost 600 anthocyanins compounds have been isolated and identified from different plant sources including fruits, vegetables, roots, legumes and cereals (4). There may also be many other sources of anthocyanins which have been either identified or need yet to be qualified. The consumption of natural sources rich in anthocyanins is important for its health benefits. Populations not aware about the nutraceutical potential of this particular phytochemical can also get its benefits due to presence of anthocyanins in routine human diet. The estimated consumption on daily basis is from 3 to 215 mg (6). The daily anthocyanins consumption was studied in Europe by Zamora-Ros et al. (7) and it was observed that total anthocyanins consumption by men was 19.3 to 64.88 mg/day, whereas it was 18.73 to 44.08 mg/day for women. The daily anthocyanins consumption in USA has been reported to be 180–215 mg/day. A recent study however showed an even lower consumption range from around 3 to 15 up to 150 mg/day in USA (4). It is recommendable therefore to increase the consumption of foods rich in anthocyanins in order to prevent certain chronic disorders which are being discussed in subsequent sections.

NUTRACEUTICAL EFFECTS OF ANTHOCYANINS

Anthocyanins are regarded as the most important types of flavonoids in plant based foods due to their strong potential as antioxidant and other valuable physicochemical and biological properties. These are primarily responsible for colour in leaves, stems, flowers, roots and fruits of different

Food material	Scientific names of plants	Anthocyanins contents (mg/100g)
Apples red delicious with peel	*Malus domestica*	4.99
Apples red delicious without peel	*Malus domestica*	2.97
Avocados	*Persea americana*	0.33
Bananas	*Musa paradisiaca*	7.39
Purple accai berries	*Euterpe oleracea*	320.93
Blackberries	*Rubus fruticosus*	100.61
Blueberries	*Vaccinium myrtillus*	163.3
Frozen blueberries	*Vaccinium myrtillus*	94.25
Cranberries	*Vaccinium oxycoccos*	103.7
Gooseberries	*Ribes uva-crispa*	9.51
Raspberries	*Rubus idaeus*	48.63
Black raspberries	*Rubus idaeus*	686.79
Red frozen raspberries	*Rubus idaeus*	24.22
Strawberries	*Fragaria X anan*	27.01
Frozen strawberries	*Fragaria X anan*	20.61
Black currents	*Ribes nigrum*	154.77
Chinese cabbage, cooked	*Brassica oleracea*	0.06
Red cabbage, cooked	*Brassica oleracea*	39.38
Red cabbage	*Brassica oleracea*	209.95
Sweet cherries	*Prunus cerasus*	33.43
Dates	*Phoenix dactylifera*	1.7
Eggplant	*Solanum melongena*	85.69
Eggplant, cooked	*Solanum melongena*	0.06
Grapes, red	*Vitis vinifera*	48.04
Mangoes	*Mangifera indica*	0.14
Nectarines	*Prunus persica, var. nectarine*	2.13
Almond	*Prunus dulcis*	2.44
Hazelnuts	*Corylus avellana*	6.71
Pecan nuts	*Carya illinoinensis*	18.02
Pistachio	*Pistacia vera L.*	7.33
Yellow peaches	*Prunus persica*	1.92
Peas, frozen	*Pisum sativum*	0.08
Onion	*Allium cepa*	9.56
Radish	*Raphanus sativus*	63.13
Kidney beans, red	*Phaseolus vulgaris*	6.68
Sweet potato, purple and cooked	*Ipomoea batatas*	11.52
Red wine vinegar	-	0.66
Red table wine	-	19.27
White wine	-	0.06
Red dessert wine	-	109.29

Table 1. Anthocyanins contents of some commonly consumed foods and beverages (6).

plants which may depend on pH structural characteristics of these compounds (3). Anthocyanins are involved in important roles in plant–animal interactions and can be regarded as antioxidants, phytoalexins or plant's chemical defense mechanism against different infections (8). Increased consumption of foods rich in anthocyanins has been shown to have potential health beneficial effects against different diseases such as cancer, aging, neurological diseases, inflammation, diabetes as well as reported to act as anti-bacterial agents (3).

Neuro-protective effects

Polyphenolic compounds have been associated with their free radicals scavenging ability; however the fact that their peak concentrations in the brain are lower than endogenous glutathione levels has led to their possible ability to reduce neuro-degeneration through additional protective mechanisms (9). Based on this very assumption the polyphenols have been observed to exhibit different neuro-protective activities other than their abilities to scavenge free radicals (10). The neuro-protective effects include reduction of oxidative stress via effects on mitochondrial respiratory chain function and increase in inflammatory responses linked to glial activation (11). Recent epidemiological findings suggest that the consumption of berries (e.g. blueberries, strawberries) rich in anthocyanins may reduce the risk of Parkinson's disease (12). Brief descriptions of some of the recent examples of neuro-protective effects of anthocyanins in relation to the possible mechanisms are given in Table 2. Previous *in vivo* studies have shown that extracts from berries rich in anthocyanins can impart protection to brain function as a result of reduction in oxidative ischemic damage and memory enhancement (13). There was decrease in blood sugar levels in animals fed with anthocyanins and reduction in their body weight which means its ability to control diabetes and obesity (14). The rats which were fed with high quantity of lipids showed reduction in their total plasma cholesterol and of LDL-cholesterol levels after they were given chokeberry juice which was rich in anthocyanins hence capable of imparting cardio-vascular protective effects (15).

Source	Neuro-protective effects	Reported by
Anthocyanins from grape skin	Anthocyanins treatment with seven days prevented memory impairment in rats caused by caused by scopolamine administration. Anthocyanins were able to regulate cholinergic neurotransmission and restore the Na+, K+-ATPase and Ca2+-ATPase activities.	Gutierres et al. (16)
Anthocyanins from blue berries	Blueberry-enriched diet containing anthocyanins was studied as neuroprotectant in a rat model of light-induced retinopathy It showed protection of the outer nuclear layer of the retina in the Wistar rats	Tremblay et al. (17)
Anthocyanins from ripe Miquel (Rubus coreanus) fruit	The anthocyanins showed neuro-protective effects on PC-12 cells *in vitro* against oxidative stress in a dose-dependent manner. The major anthocyanins for their neuro-protective effects were identified as; cyanidin 3-O-sambubioside, cyanidin 3-O-glucoside, cyanidin 3-O-xylosylrutinoside, and cyanidin 3-O-rutinoside in increasing order of amounts.	Im et al. (18)
Anthocyanins rich extracts from berry fruits	Fruit extracts rich in anthocyanins showed neuro-protective activity and a number of individual anthocyanins interfered with rotenone neurotoxicity. It was suggested that anthocyanins rich botanical extracts may alleviate neuro-degeneration in Parkinson's disease via enhancement of mitochondrial function.	Strathearn et al. (11)

Table 2. Some recent examples of neuro-protective effects by anthocyanins

Cardio-vascular effects

The protective effects of anthocyanins against cardio-vascular disease have been revealed in various studies. These types of protective effects depend on the structural nature of anthocyanins and the degree of polymerization (19). These effects may include reduction of oxidative stress, improvement of endothelial dysfunction, mediation of vasodilatation, anti-inflammatory effects related to cardiac system, metabolic effects related to heart health and decrease in hypertension as shown in Table 3 (6). The commercially available chokeberry extract consumption along with statin for a period of six week in cardio-vascular patients led to a significant decrease in levels of serum iso-prostanes and oxidized LDL; increase in the level of adiponectin and reduction in blood pressure (20). Herrera-Arellano et al. (21) studied that *Hibiscus sabdariffa*-based nutraceutical product, enriched with anthocyanins was able to cause significant reductions in blood pressure and plasma angiotensin converting enzyme activity in hypertensive patients.

Hepatic health benefits

The liver plays vital role in different metabolic and detoxification activities against different materials entering human body. Different factors such as toxic chemicals, alcohol intake and viral diseases result in liver damage and liver malfunction. Such diseases are common now days and become one of the important health concerns (22). Hou et al. (23) observed that anthocyanins-rich black rice bran extract containing cyanidin-3-glucoside (Cy-3-G) and peonidin-3-glucoside, can impart significant benefits on liver health, and that Cy-3-G was predominant anthocyanins in black rice bran exerting this effect. The antioxidant potential of anthocyanins was the main reason of black rice bran extract to exert hepatic health benefits.

Naturally occurring anthocyanins in pigmented extract obtained from purple sweet potato was tested for its effectiveness in improving the fasting blood glucose level, glucose and insulin tolerance through reduction of ROS and restoration of glutathione (GSH) contents (24). This extract was observed to prevent the endoplasmic reticulum stress in livers of high fat diet treated mice. Anthocyanins restored, to a notable extent, the impairment of the insulin receptor substrate-1/phosphoinositide 3 kinase/protein kinase B (Akt) insulin signaling in the livers of mice. In was concluded that anthocyanins from purple sweet potato can impart protection against high fat diet induced hepatic insulin resistance and the main mechanisms were reported to be the decrease in ROS level and resistance to ROS-mediated endoplasmic reticulum stress.

Anti-inflammatory effects

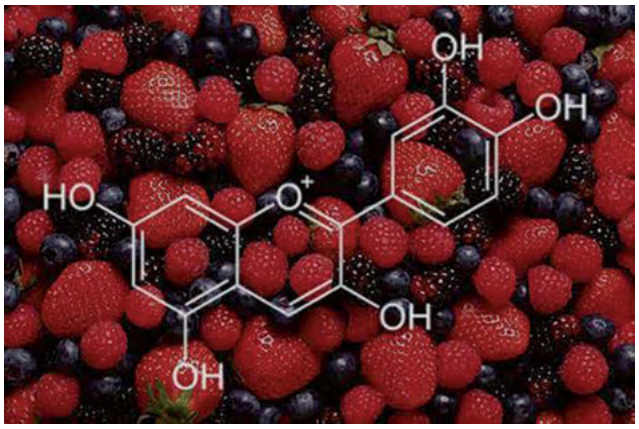
Inflammation can be regarded as a part of a complex series of physiological reactions to a

Main mechanisms	Effects induced by anthocyanins
Metabolic effects	Anthocyanins decreases LDL-cholesterol, increase HDL-cholesterol, prevent LDL oxidation, improve fecal expression of acidic and neutral sterols, change and improve the sugar and lipid metabolism, enhance insulin resistance, reduce NFκB levels, suppress iNOS and COX2 expression
Oxidative stress	Prevention of oxidative stress by anthocyanins is accomplished by superoxide radicals and hydroxyl radicals scavenging, improvement of antioxidant enzyme activity, ROS reduction and NO reduction
Cardiomyocytes and the endothelium	Anthocyanins improves endothelial dysfunction and vasodilation
Anti-inflammatory effects	There is decrease in P-selection, MCP-1, TNF-α and IL-10 expression, reduction in VEGF and ICAM-1 expression on endothelial cells, reduction of VLA-4, CD40 and CD36 expression on monocytes as a result of consumption of anthocyanins
Anti-ischemic and hypertension effects	Anthocyanins decrease the magnitude of ischemia–reperfusion injury and reduce hypertension

Table 3. Health benefits of anthocyanins in relation to cardio-vascular protective effects (6).

stimulus that may be a pathogen, harmful substance and damaged cell. The symptoms of inflammatory condition are pain, redness, irritation, swelling and function loss. Although, inflammation is an attempt to protect the body from harmful organisms or to remove the injurious stimuli for the initiation of natural healing, however the progressive destruction of the tissue can also become chronic that can result into a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer. Due to these reasons, inflammation is normally closely regulated by the body and treatment strategies are adopted for timely eradication. Anti-inflammatory activity may refer to the ability of a drug to reduce inflammation or pain caused due to inflammation (25).

One of the significant health benefits associated with the consumption of anthocyanins is cancer chemoprevention and have been found to impart both *in vivo* and *in vitro* anti-inflammatory effects without any potent toxic effects (26). Different studies have recently been carried out to verify, improve and test the anti-inflammatory properties of anthocyanins in different model systems including both *in vivo* and *in vitro*. A study carried out by Fernande et al. (4), showed that anthocyanins methylated metabolites such as delphinidin-3-glucoside, cyanidin-3-glucoside and petunidin-3-glucoside obtained as result of enzymatic hemi-synthesis, retained significant antioxidant properties. The antiproliferative activities of the metabolites were also compared with the parental anthocyanins in three cancer cell lines by sulforhodamine B assay. Both the parental anthocyanins and their metabolites showed the anti-inflammatory potential against cancer cell lines. In another study the polyphenolic extracts from coloured grapes which were particularly rich in anthocyanins demonstrated the ability to decrease the release of pro-inflammatory cytokines from activated human peripheral blood leucocytes (27). It was further observed that these extracts from grapes significantly reduced arthritis scores and cachexia in rats, and such decrease was more prominent in rats which received continuous low doses over a longer period in comparison to those treated with high doses five times only. The ability of anthocyanins to reduce inflammation can also be related to their ability for treatment of gastric ulcer. In a recent study carried out by Kim et al. (28) *in vitro* and *in vivo* experiments were conducted to demonstrate the palliative effects of anthocyanins on gastric ulcer in rats (ulcer was induced by naproxen dosage). It was observed that ROS produced by naproxen were effectively reduced by anthocyanins treatment which relieved the oxidative stress. The treatment using anthocyanins also showed significant reduction in lipid peroxidation products and increments in the quantities of antioxidant enzymes which included catalase, superoxide dismutase, and glutathione peroxidase. It was concluded that gastric ulcer therapy using anthocyanins is an effective approach in rats. Decendit et al. (26) observed that malvidin-3-O-b glucoside which a major anthocyanins compounds in grape has no toxicity on human peripheral blood mononuclear cells. It reduced the transcription of genes that are responsible for encoding the inflammatory mediators. This was demonstrated by the inhibition of TNFa, IL1, IL-6 and iNOS-derived nitric oxide (NO) secretions from activated macrophages. Anthocyanins also significantly reduced the inflammatory cachexia and arthritic paw scores in rats at both therapeutic and preventive levels. It was also reported that malvidin-3-O-b glucoside has a strong potential to be considered as an anti-inflammatory agent through *in vitro* and *in vivo*, trials.



DIRECTIONS FOR ACTION

In general the quality of a nutraceutical is assessed by using different *in vitro* assays. However these assays may not be enough to elaborate the health benefits of a nutraceutical unless qualified using well designed *in vivo* assays. There are also labels on nutraceutical products that show recommended doses however the scientific evidence regarding such recommendations is still lacking. The daily recommended dose of dietary anthocyanins may vary from well above 100 mg and go up to 1000 mg. It is also essential that consumers are well aware about the risks associated with over dosage of such compounds. It is worth

mentioning that higher doses of such compounds may not be beneficial for increasing the activity or effects. The optimal quantities of specific polyphenolic compound required to yield certain amounts of specific metabolites for desired health effects, still need to be explored (1). The present review focused on some of the major health benefits associated with anthocyanins; however they may also be useful in prevention and cure of other diseases and in the manufacture of functional foods. The potential of these compounds as natural colorants for different food products also needs to be elaborated.

In spite of the knowledge accumulated in the recent few years, more scientific work is required on the nature and detection of possible anthocyanins derivatives formed *in vivo*: metabolites and breakdown products originated under physiological conditions or from the colonic micro-flora activity, as well as their tissue distribution. There may be also be many more health beneficial properties of anthocyanins which still need to be explored along with the discovery of new sources and best techniques for the optimal recovery of high quality anthocyanins from plant source. More efforts are needed in future to conduct systematic studies for development of nutraceutical products based on anthocyanins with easy availability, acceptability and faster benefits for improving human health.

ACKNOWLEDGEMENTS

This study was financially supported by King Saud University, Deanship of Scientific Research, College of Food & Agricultural Sciences, Research Center (Riyadh, Saudi Arabia).

REFERENCES AND NOTES

1. J.C. Espin, M.T. Garcia-Conesa et al., *Phytochem*, **68**, pp. 2986–3008 (2007).
2. G. Mazza, *Acta Hortic*, **744**, pp. 117-26 (2007).
3. P. Jing, S. Zhao, et al., Food Chem, **145**, pp. 365–371(2014).
4. I. Fernandes, F. Marques et al., *Food Chem*, **141**: pp. 2923–2933 (2013).
5. K. Azuma, A. Ohyama et al., *J Agric Food Chem*, **56**, pp. 10154–10159 (2008).
6. M.J. Kruger, N. Davies et al., *Food Res Int*, **59**, pp. 41–52 (2014).
7. R. Zamora-Ros, V. Knaze et al., *Br J Nutr*, **106**, pp.1090-1099 (2011)
8. E.V. Petersson, A. Puerta et al., *Electrophoresis*, **29**, pp. 2723–2730 (2008).
9. D. Del-Rio, A. Rodriguez-Mateos et al., *Antioxid Redox Signal*, **18**, pp. 1818–1892 (2013).
10. J.X. Song, S.C. Sze et al., *J Ethnopharmacol*, **139**, pp. 698–711 (2012).
11. K.E. Strathearn, G.G. Yousef, *Brain Res*, **1555**, pp. 60–77 (2014).
12. X. Gao, A. Cassidy et al., *Neurol*, **78**, pp. 1138–1145 (2012).
13. D. Barros, O.B. Amaral et al., *Pharmacol Biochem Behavior*, **84**, pp. 229–234 (2006).
14. H. Guo, W. Ling et al., *Plant Food Hum Nutr*, **62**, pp. 1–6 (2007).
15. S. Valcheva-Kuzmanova, K. Kuzmanov et al., *Plant Food Hum Nutr*, **62**, pp. 19–24 (2007).
16. J.M. Gutierres, F.B. Carvalho et al., *Int J Devl Neuroscience*, **33**, pp. 88–97 (2014).
17. F. Tremblay, J. Waterhouse et al., *J Nutr Biochem*, **24**, pp. 647–655 (2013).
18. S.E. Im, T.G. Nam et al., *Food Chem*, **139**, pp. 604–610 (2013)
19. M. Walter, E. Marchesan, *Brazilian Arch Biol Technol*, **54**, pp. 371–377 (2011).
20. M. Naruszewicz, I. Laniewska et al., *Atherosclerosis*, **194**, pp. 179-84 (2007).
21. A. Herrera-Arellano, J. Miranda-Sanchez et al., *Planta Med*, **73**, 6–12 (2007).
22. M. Tanaka, F. Katayama et al., *J Epidemiol*, **21**, pp. 401–416 (2011).
23. F. Hou, R. Zhanga et al., *J Funct Food*, **5**, pp. 1705–1713 (2013)
24. Z.F. Zhang, J. Lu et al., *J Nutr Biochem*, **24**, pp. 1008–1018 (2013).
25. Wikipedia, http://en.wikipedia.org/wiki/Inflammation (2014).
26. A. Decendit, M. Mamani-Matsuda et al., *Biochem Pharmacol*, **86**, pp. 1461–1467 (2013)
27. M.D. Mossalayi, J. Rambert et al., *Phytomed*, http://dx.doi.org/10.1016/j.phymed.2013.08.015 (2013)
28. S.J. Kim, J.M. Kim et al., *J Funct Food*, http://dx.doi.org/10.1016/j.jff.2013.12.028 (2014)